HAL
open science

Genetic diagnosis and identification of novel genes in neuromuscular disorders using next generation sequencing

Nasim Vasli Poursaeed

To cite this version:

Nasim Vasli Poursaeed. Genetic diagnosis and identification of novel genes in neuromuscular disorders using next generation sequencing. Human genetics. Université de Strasbourg, 2012. English. NNT : 2012STRAJ055 . tel-01124073

HAL Id: tel-01124073

https://theses.hal.science/tel-01124073

Submitted on 6 Mar 2015

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Institut de Génétique et de Biologie Moléculaire et cellulaire
CNRS-INSERM-Université de Strasbourg

THÈSE

Présentée en vue de l'obtention du grade de

Docteur de l'université de Strasbourg

Discipline : Sciences du vivant
Aspects moléculaires et cellulaires de la biologie

Par

Nasim VASLI

Genetic diagnosis and identification of novel genes in neuromuscular disorders using next generation sequencing

Soutenue le 17 Décembre 2012, devant la commission d'examen :

Mr. Le Docteur Jocelyn Laporte	Co-directeur de Thèse
Mr. Le Professeur Jean-Louis Mandel	Co-directeur de Thèse
Mme. Le Professeur Hélène Dollfus	Rapporteur interne
Mr. Le Docteur Christophe Béroud	Rapporteur externe
Mr. Le Docteur Richard Redon	Rapporteur externe

Acknowledgements

I would like to thank my jury members, Professor Hélène Dollfus, Dr. Christophe Béroud, Dr. Richard Redon and Professor Jamel Chelly for accepting to read and to evaluate my PhD work.

My biggest thank is for my major supervisor, Dr Jocelyn Laporte. I've never forgotten His helps from even the days before coming to France and starting my PhD until the last days in the lab. I've learnt many things from him and I had very nice days in his lab during past four years. I would like to thank him for giving me the opportunity to work with him in his team on a very exciting project and helping me for my future life.

The second person that I would like to thank is my co-supervisor, Professor Jean-Louis Mandel. For me he is a live genetics text book and I've learnt many things from him. I would like to thank him for helping and supporting me to come to France, to continue my PhD project and my future life.

I would like to thank my friends and colleges in MTM team. We had very nice days together and I've never forgotten our lab meetings, several non-scientific activities and birthday parties in atrium of third floor of IGBMC.

I would like to acknowledge Bernard Jost, Serge Vicaire and Muriel Philipps in Microarray and Sequencing Platform of IGBMC. I would like to thank especially Bernard who was really helpful person and he was always accessible for answering to my questions.

I would like to thank warmly Stephanie Le Gras in sequencing and bioinformatics platform of IGBMC. During past four years, Stephanie was one of the persons who helped me a lot in bioinformatic aspects of my project. She was a very kind and helpful person. Although it was not her responsibility to help me for the projects that were performed in private companies, she put a lot of efforts in helping me and I've never forgotten her helps.

I sincerely thank Dr. Christos Gavriilidis for being helpful for correction of my thesis manuscript.

I would like to thank Dr Valérie Biancalana from Hôpital civil of Strasbourg who was really helpful for performing experiments and giving us the clinical data of our patients.

I would like to thank Dr. Jean Muller for giving me the access to ALAMUT software and helping us for VaRank program.

I sincerely thank all people from IGBMC, clinicians, our collaborators and patients who helped me to work on my PhD work during these four years and to finish it.

And finally I would like to thank my family, especially my lovely husband. Without him and his supports, this PhD project could not be possible.

Abbreveations

aCGH: array comparative genomic hybridization

CNM: Centronuclear myopathy

CNV: Copy number variation

cPAL: combinatorial probe anchor-ligation

DNB: DNA nano-balls

HTS: High-throughput sequencing

Indel: Insertions-Deletions

MAPKKK: MAP kinase kinase kinase

MLPA: multiplex ligation-dependent probe amplification

MPS: Massively parallel sequencing

NGS: next generation sequencing

NMD: Neuromuscular disorder

NMJ: Neuromuscular junction

PI domain: Phosphoinositide- binding domain

SNP: Single-nucleotide polymorphism

SNV: Single Nucleotide Variation

SVA: Sequence Variant Analyzer

SV: Structural variation

XLMTM: X-linked myotubular myopathy

List of figures

Figure 1: Schematic representation of affected structures in NMDs. 1

Figure 2: Some clinical and histopathological features in patients with XLMTM. 6

Figure 3: Some clinical and histopathological features in patients with AD *DNM2* -related CNM. 8

Figure 4: Some clinical and histopathological features in patients with AR *BIN1* -related CNM. 9

Figure 5: Some clinical and histopathological features in patients with *RYR* - related CNM. 12

Figure 6: Solid-phase capture and enrichment. 16

Figure 7: Solution-phase capture and enrichment. 17

Figure 8: Microdroplet PCR. 18

Figure 9: Illumina sequencing technology. 19

Figure 10: Sequencing by ligation method using in SOLiD sequencer from Applied Biosystems. 20

Figure 11: Pyrosequencing using in Roch/454 platform. 22

Figure 12: Semiconductor sequencing using in Ion torrent platform. 23

Figure 13: The complete genomics sequencing technology. 25

Figure 14: Good quality and bad quality DNAs. 31

Figure 15: Number of targeted nucleotides in 3 different captures kits. 32

Figure 16: Number of unique overlapping reads or coverage, using 3 different kits. 33

Figure 17: Numbers of non- covered, totally covered and targeted regions, using 3 different kits. 34

Figure 18: Number of detected SNVs and Indels, using 3 capture kits. 35

Figure 19: Quality score for each nucleotide in reads with 40 base pairs length. 36

Figure 20: Micro deletion detection. 38

Figure 21: coverage for targeted nucleotides and exons. 52

Figure 22: The NGS reads, direct sequencing result and implicated domains and amino acid conservation through evolution for patient C (AHJ97) with c.490_491del, p.Met164ValfsX24 variant in *ZAK*. 55

Figure 23: Expression level of ZAK in different tissues. 57

Figure 24: RT-PCR results for AHJ97 (first lane for each couple of primers) and two control negatives without any variations in ZAK (second and third lanes for each couple of primers). 58

Figure 25: Sequence coverage for targeted nucleotides for AHJ97. 63

Figure 26: Pedigree of ABJ family. 68

Figure 27: Sequence coverage for targeted nucleotides in ABJ75 and ABJ79. 69

Figure 28: The variations in *PDE4DIP* gene detected by exome sequencing. 72

Figure 29: *PDE4DIP* variations segregation in ABJ family. 73

Figure 30: Sanger sequencing results for *MYO5B* and *TTN* genes in 314-1 and his parents. 81

Figure 31: Position of missense, GTT>CTT, V>L change in exon 168 of *TTN*. 81

Figure 32: The predictions showing the disruption of donor splice site due to missense, GTT>CTT, V>L change in exon 168 of *TTN*. 82

Figure 33: Sanger sequencing results for *CLIP1* and *FLYWCH1*genes in 314-1 and his parents. 84

Figure 34: comparison between whole genome and exome sequencing. 100

Figure 35: Schematic filtration workflow for genome as well as exome datasets. 101

List of tables

Table 1- Different types of neuromuscular disorders in three affected structures and number of known genes and chromosomal loci with unidentified genes. 2

Table 2- Implicated genes in CNM. 4

Table 3- List of papers showing the gene identification in myopathies by NGS. 28

Table 4- Some studies using different strategies for finding causal genes/mutations. 43

Table 5- List of genes selected for targeted sequencing of 76 genes. 51

Table 6- Statistics regarding to NGS reads for targeted sequencing of 76 genes. 51

Table 7- Statistics regarding to nucleotide coverage for targeted sequencing of 76 genes. 52

Table 8- Statistics regarding to NGS reads for targeted sequencing of 2500 genes. 53

Table 9- Number of different types of exonic variants in patients C, D and F for targeted sequencing of 2500 genes. 54

Table 10- Number of exonic variants after filtration in patients C, D and F for targeted sequencing of 2500 genes. 54

Table 11- Statistics regarding to NGS reads for trio sequencing. 62

Table 12- Statistics regarding to nucleotide sequencing coverage for trio sequencing. 62

Table 13- Statistics regarding to detected SNVs and Indels. 63

Table 14- Two homozygous variants detected in AHJ97. 64

Table 15- Sporadic cases sequenced in different sequencing platforms. 66

Table 16- Statistics regarding to NGS reads, depth of coverage and detected SNVs and Indels in CNM sporadic cases. 66

Table 17- Common genes with different variations in at least four CNM sporadic samples. 67

Table 18- Statistics regarding to NGS reads for ABJ79 and ABJ75. 68

Table 19- Statistics regarding to nucleotide coverage for ABJ79 and ABJ75. 69

Table 20- Statistics regarding to detected SNVs and Indels. 70

Table 21- Compound heterozygous variations in ABJ75 and ABJ79. 71

Table 22- Variations in *PDE4DIP* detected and shown in new version of Exome variant Server. 73

Table 23- Statistics regarding to genome and exome coverages for ABJ79 and ABJ68. 74

Table 24- Statistics regarding to numbers of detected variations in ABJ79 and ABJ68. 75

Table 25- Compound heterozygous variations in ABJ75. 76

Table 26- Statistics regarding to genome and exome coverages for AIZ family. 78

Table 27- Statistics regarding to numbers of detected variations in AIZ family. 78

Table 28- Compound heterozygous variations in 314-1. 80

Table 29- Muscle expression of the proteins and presence of variations in Exome Variant Server, NHLBI Exome Sequencing (http://evs.gs.washington.edu/EVS/) for detected variations in *MYO5B* and *TTN*. 82

Index

Acknowledgements

List of abbreviations

List of figures

List of Tables

1- Introduction 1

1-1- Neuromuscular disorders 1

1-2-Centronuclear myopathies 4

1-2-1- X-linked *MTM1*-related CNM (myotubular myopathy) 5

1-2-2- Autosomal dominant *DNM2*-related CNM 7

1-2-3- Autosomal recessive *BIN1*-related CNM 8

1-2-4- *MTMR14*-related CNM 10

1-2-5- *RYR1*-related CNM 11

1-3-Biological questions and aims 12

1-3-1- Aim 1: Gene identification 12

1-3-1-2- Previous methods for gene identification 13

1-3-1-3- Massively parallel sequencing 14

1-3-1-3-2- Template preparation & barcoding 15

1-3-1-3-3- DNA enrichment 15

1-3-1-3-4- High-throughput sequencing 18

1-3-1-3-5- Data analysis 27

1-3-1-4- Massively parallel sequencing for gene identification 28

1-3-2- Aim 2: Improving diagnostic of NMD using NGS 29

Review article: Impacts of massively parallel sequencing for genetic diagnosis of neuromuscular disorders 30

2- Materials and methods 31

2-1- DNA quality control for NGS 31

2-2- Capture kits 32

2-3- Data analysis workflow 35

2-3-1- Sequence quality control 36

2-3-2- Sequence alignment 37

2-3-3- Variant calling and annotation 37

2-3-4- Variant filtration 39
2-3-4-1- Filtration and ranking based on frequency 39
2-3-4-2- Filtration and ranking based on function 40
2-3-4-3- Filtering the “Black genes” and sequencing errors 40
2-3-4-4- Filtration and ranking based on effect 41
2-3-4-5- Filtration and ranking based on conservation 41
2-3-4-6- Filtration and ranking based on inheritance mode 41
2-3-4-6-1- Recessive mode of inheritance 42
2-3-4-6-2- Dominant mode of inheritance 42
2-3-4-7- Filtration and ranking based on gene function, tissue expression profile and cellular localization 43
3- Results 44
Results for aim 1: Gene identification in CNM 44
3-1- Patient selection 44
Publication 1: Novel molecular diagnostic approaches for X-linked centronuclear (myotubular) myopathy reveal intronic mutations 45
A- Introduction 46
B- Aim of study
C- Results
D- Conclusion
E- Original paper
Publication 2: Myotubular myopathy caused by multiple abnormal splicing variants in the MTM1 RNA in a patient with a mild phenotype 47
A- Introduction 48
B- Aim of study
C- Results
D- Conclusion
E- Original paper
Publication 3: Altered Splicing of the BIN1 Muscle-Specific Exon in Humans and Great Danes with Highly Progressive Centronuclear Myopathy 49
A- Introduction 50
B- Aim of study

C- Results
D- Conclusion
E- My contribution
F- Original paper
3-2-Targeted sequencing 51
3-2-1- NGS for 76 selected genes 51
3-2-2- NGS for 2500 selected genes 53
3-2-2-1- Results for patient C (AHJ97) 54
3-2-2-2- Results for patient D (34263) 58
3-2-2-3- Results for patient F (AHH42) 59
3-3- Exome sequencing 59
Publication 4: An integrated diagnosis strategy for congenital myopathies 60
A- Introduction 61
B- Aim of study
C- Results
D- Conclusion
E- Original paper
3-3-1- Trio sequencing for AHJ97 62
3-3-1-1- Recessive scenario due to homozygous change 64
3-3-1-2- Recessive scenario due to compound heterozygous changes 64
3-3-1-3- *De novo* scenario 65
3-3-2- Exome sequencing for CNM sporadic cases 65
3-3-3- Exome sequencing for ABJ family 68
3-3-3-1- Recessive scenario due to compound heterozygous changes 70
3-3-3-2- Recessive scenario due to homozygous change 73
3-4- Whole genome sequencing 74
3-4-1- Whole genome sequencing for ABJ family 74
3-4-1-1- Recessive scenario due to compound heterozygous changes 76
3-4-1-2- Recessive scenario due to homozygous change 77
3-4-2- Whole genome sequencing for AIZ family (314-1 and parents) 78
3-4-2-1- Recessive scenario due to compound heterozygous changes 80
3-4-2-2- Recessive scenario due to homozygous change 83

3-4-2-3- *De novo* scenario 83

Results for aim 2: Improving diagnostic of NMD using NGS 85

Publication 5: Next generation sequencing for molecular diagnosis of neuromuscular diseases 86

A- Introduction 87

B- Aim of study

C- Results

D- Conclusion

E- Original paper

4- Discussion and perspectives 88

4-1- Aim 1: Gene identification in CNM 88

4-1-1- Mutations in known genes 88

4-1-2- Targeted sequencing 89

4-1-3- Exome sequencing 92

4-1-4- Whole genome sequencing 96

4-1-4-1- Whole genome sequencing for ABJ family 96

4-1-4-2- Whole genome sequencing for AIZ family (314-1 and parents) 97

4-2- Aim 2: Improving diagnostic of NMD using NGS 98

4-3- General discussion 99

4-4- Perspectives 102

5- Conclusion 103

References

French summary

1- Introduction

1-1-Neuromuscular disorders

Neuromuscular disorders (NMD) are genetic diseases affecting muscles and nerves as well as the link region between a nerve and a muscle fiber termed as the neuromuscular junctions (NMJ) (Figure 1). They are rare and often severe with different age of onset from childhood to adulthood, with significant burden to the patients, their families and the public health system (Emery, 1991). The main symptoms in most of NMDs are muscle fatigue and weakness as well as muscular atrophy (wasting of muscles). Some NMDs present muscle pain and cramps. Different types of NMDs with number of related genes are shown in table 1. In this table the categories of "other neuromuscular disorders" and "cardiomyopathies" are not listed. As there are different classifications for NMDs, the classification from the muscle gene table created by Professor Jean Claude Kaplan (http://musclegenetable.org/) was used here (Kaplan, 2011).

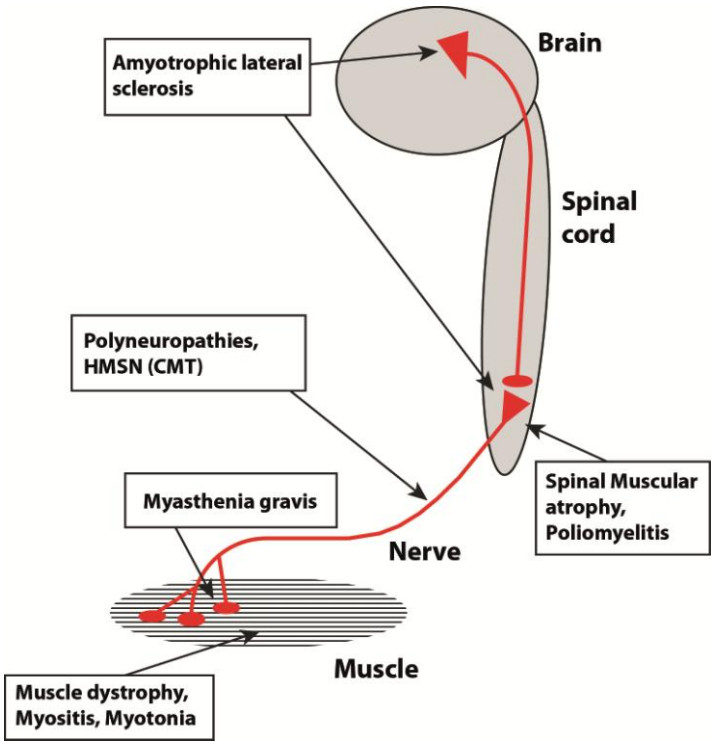


Figure 1: Schematic representation of affected structures in NMDs. Different types of NMDs and related affected structures (Brain, spinal cord, muscle and NMJs) are shown by arrows.

Reference: http://www.neuromuscular-disease.com/neuromuscular-disorders/

Table 1- Different types of NMDs in three affected structures and number of known genes and chromosomal loci with unidentified genes.

Reference: (http://musclegenetable.org/) and (Kaplan, 2011).

Affected structures	Types of NMD	No. of known genes	No. of chromosomal loci with unknown gene
Muscle	A- Myopathies (Congenital- Distal- Metabolic- Other)	67	7
	B- Dystrophies	36	2
	C- Myotonic syndromes	5	1
	D- Ion channel Muscle diseases	9	-
	E- Malignant Hyperthermia	2	4
Nerve	A- Hereditary motor and sensory neuropathies	37	9
	B- Spinal muscular atrophy & myotrophic lateral sclerosis	23	6
	C- Hereditary paraplegias	21	20
	D- Hereditary ataxias	39	15
NMJ	Myasthenic syndromes	13	1

NMDs are one of the most genetically heterogonous disorders with more than 300 implicated genes. This genetic heterogeneity can be seen, for instance in Charcot-Marie-Tooth neuropathies, also called Hereditary motor and sensory neuropathies, with more than 30 causative genes (Saporta et al, 2011) and more than 40 loci (http://www.molgen.ua.ac.be/CMTMutations) or in autosomal recessive limb girdle muscular dystrophy with 15 implicated genes, so far (Laing, 2012). This high degree of genetic heterogeneity presents a problem for molecular genetics diagnosis as it could be time-consuming and costly to test the different implicated genes in diagnostic laboratories.

in different neuromuscular disorders several of the largest human genes are mutated such as mutations in Dystrophin *(DMD,* MIM #300377) with 79 exons spanning more than 2.3 Mb (Tennyson et al, 1995), Titin (*TTN,* MIM #188840) with 363 exons spanning more than 280 kb (Bang et al, 2001), Nebulin (*NEB,* MIM #161650) with 183 exons spanning more than 249 kb (Donner et al, 2004) and Ryanodine receptor 1 (*RYR1,* MIM #180901) with 106 exons spanning more than 153 kb (Phillips et

al, 1996). These genes are mainly expressed in muscle, precluding cDNA analysis if such tissue is not available. While these genes are known to be implicated in diseases, they might not be fully tested or only the mutation hot spot regions may be analyzed at first (Andersen et al, 2009).

Clinical heterogeneity represents another challenge in diagnosis of NMDs. For example, mutations in the Caveolin 3 gene (*CAV3,* MIM #601253) have been detected in four different skeletal muscle disease phenotypes: rippling muscle disease, limb-girdle muscular dystrophy, hyperCKemia and distal myopathy (Gazzerro et al, 2011). Patients can have an overlap of these symptoms, or the same mutation can cause diverse clinical phenotypes with different severities. Thus it is sometimes difficult to suggest the best candidate genes to direct molecular diagnosis.

Another reason precluding the suggestion of the best molecular diagnosis approach is the fact that a large number of patients display unspecific clinical and histopathological features. For instance, reviews of cases with congenital myopathies that are usually sub-classified into nemaline, core or centronuclear myopathies, reveal no specific phenotypes in almost half of them whereas other patients have diverse but overlapping clinical and histopathological manifestations (Nance et al, 2012). Moreover, when a sequence variation is identified in one of the prioritized genes in diagnostic laboratories, further investigation might be stopped, which may lead in some cases to omission of the real disease-causing mutation. Conversely, when no change is identified, patients undergo additional time-consuming, costly and sometimes painful tests to orient the clinical diagnosis and prioritize other candidate genes (Prior, 2010).

The last obstacle is the presence of genes that have not been identified in different NMDs yet. For instance mutations in several genes, including *SOD1* (MIM #147450) (Rosen et al, 1993), *SETX* (MIM #608465) (Chen et al, 2004), *ALS2* (MIM #606352) (Hadano et al, 2001; Yang et al, 2001), *TARDBP* (MIM #605078) (Gitcho et al, 2009), *FUS* (MIM #137070) (Kwiatkowski et al, 2009), *ANG* (MIM #105850) (Greenway et al, 2004; Hayward et al, 1999) and *C9orf72* (MIM #614260) (DeJesus-Hernandez et al, 2011; Renton et al, 2011) are identified in Amyotrophic lateral sclerosis (ALS), a neurodegenerative disorder characterized by the loss of motor neurons in brain and spinal cord. However, for a significant number of ALS patients the causative mutation remains unknown, suggesting the involvement of other genes. More generally, about 40% of patients with NMDs do not have a genetic diagnosis. In table 1, the number of chromosomal loci with unidentified genes relating to different NMDs is shown.

1-2-Centronuclear myopathies

Centronuclear myopathies (CNM) are inherited neuromuscular disorders and a type of congenital myopathies, characterized mainly by the presence of central and one or more internalized nuclei in muscle fibers with different severities and age of onset (Fujimura-Kiyono et al, 2008; Jungbluth et al, 2008). Today, for comprehensive assessment of CNM, clinical, histopathological and if it is possible muscle MRI features should be evaluated prior to genetic molecular testing (Biancalana et al, 2012).

Mutations in patients with classical CNM have been identified in three genes (Table 2): Myotubularin (*MTM1,* MIM #300415) (Laporte et al, 1996), Bridging integrator 1 (*BIN1,* MIM #601248) (Nicot et al, 2007) and Dynamin 2 (*DNM2,* MIM #602378) (Bitoun et al, 2005).

Mutations in two other genes in patients with partially similar CNM phenotypes have been detected. Two heterozygous variations were reported in the Myotubularin-related 14 (*MTMR14,* MIM #611089) in patients with sporadic CNM, including one patient with an additional *DNM2* heterozygous change (Tosch et al, 2006). Whether these variations are disease-causing mutations or implicating in phenotype modifications, remains to be determined.

Ryanodine receptor 1 (*RYR1,* MIM #180901) mutations were mainly detected in patients with partially similar CNM features such as central nuclei and radial arrangement of sarcoplasmic strands in muscle biopsy with autosomal recessive inheritance (Bevilacqua et al, 2011; Jungbluth et al, 2007; Wilmshurst et al, 2010).

The prevalence of CNM (age < 18 years) has been estimated to be less than 1 in 100,000 (Amburgey et al, 2011) distributed between the *MTM1* (45%), *DNM2* (15%), *RYR1* (10-15%) and *BIN1* (<5%) (Biancalana et al, 2012). About 20% of CNM patients do not have any mutations in these five implicated genes. Disease-causing mutation(s)/gene(s) in these patients need to be identified.

Table 2- Implicated genes in CNM.

Reference: (http://www.ncbi.nlm.nih.gov/omim)

Gene	Gene MIM No.	Locus	Name	Phenotype MIM No.
MTM1	300415	Xq28	Myotubular myopathy 1 gene	310400
DNM2	602378	19p13.2	Dynamin 2 gene	160150
BIN1	601248	2q14.3	Bridging integrator 1	255200
MTMR14	611089	3p25.3	Myotubularin-related 14	160150
RYR1	180901	19q13.1	Ryanodine receptor 1	255200

1-2-1- X-linked *MTM1*-related CNM (myotubular myopathy)

X-linked CNM (myotubular myopathy) or XLMTM is caused by mutations in the *MTM1* gene with prenatal or neonatal onset. The term of myotubular myopathy was introduced for the first time, by Spiro et al in 1966 due to similar appearance of affected muscle fibers and fetal myotubes (Spiro et al, 1966), since the nuclei are presented at the center of fetal muscle fibers whereas at birth, the majority of the nuclei are positioned at the periphery beneath of the muscle cells. In MTM1 knockout mice a myopathy with centralized nuclei was seen after four weeks of age indicating the role of myotubularin in muscle maintenance rather than myogenesis so nuclear centralization is not caused due to myogenesis arrest (Buj-Bello et al, 2002). Myotubularin is a phospholipid phosphatase that dephosphorylates phosphatidylinositol 3-phosphate (PtDIns3p) and PtDIns(3,5)p2, two second messengers with an important role in membrane trafficking (North, 2008).

Clinical description

The affected new born males are presented often with severe hypotonia and muscle weakness, ophthalmoplegia (paralysis of one or more extraocular muscles which are responsible for eye movements), respiratory insufficiency requiring ventilation support and difficulties in swallowing requiring tube feeding (Figure 2a). The majority of patients die within the first months of life but a small proportion of patients survive until adulthood (Biancalana et al, 2003; Chanzy et al, 2003). The majority of heterozygous females (carriers) are asymptomatic but a few cases show mild muscle weakness due to random or skewed X-chromosome inactivation (Hammans et al, 2000; Tanner et al, 1999a).

Pathology

The morphological analysis of muscle biopsies shows a high number of small and round myofibers with predominance of type I fibers (slow twitch) and centralized nuclei. Central area of the fibers with decreased ATPase reaction and increased oxidative enzyme activity and glycogen staining due to mitochondrial aggregates and glycogen particles with peripheral halo due to reduced oxidative enzyme activity are seen as well (Figure 2b, c, d) (Fujimura-Kiyono et al, 2008; Romero, 2010).

Molecular screening

Today, direct sequencing of coding regions and flanked intronic sequences of genomic DNA is the routine method for the *MTM1* molecular screening. Some of mutations in the *MTM1* are only detected by RNA or protein analysis from cell lines (lymphoblasts, fibroblasts or myoblasts) or muscle biopsies, using real time-PCR and Western blot, respectively (Tosch et al, 2010). For detection of

dosage anomalies, a multiplex ligation-dependent probe amplification (MLPA) kit (SALSA MLPA kit p309-A1 MTM1, MRC, Holland) is also available. Using these methods, more than 400 mutations including missenses, nonsenses, splice sites, insertions and small and large deletions were reported in patients with XLMTM (Biancalana et al, 2003; de Gouyon et al, 1997; Herman et al, 2002; Hu et al, 1996; Laporte et al, 2000; Laporte et al, 1997; Tanner et al, 1999b; Trump et al, 2011; Tsai et al, 2005).

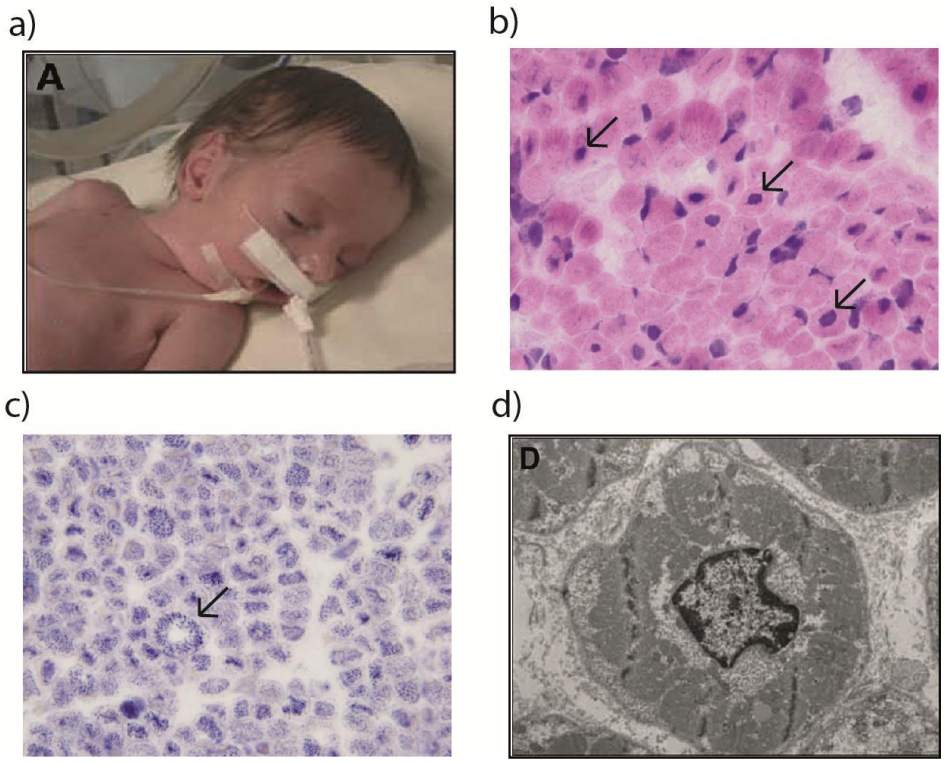


Figure 2: Some clinical and histopathological features in patients with XLMTM. a) Male infant with severe XLMTM. Note to severe hypotonia, ptosis and respiratory insufficiency requiring ventilation support. b, c) Muscle biopsy stained with Haematoxylin and Eosin (H&E) (b) and Nicotinamide adenosine dinucleotide – tetrazolium reductase (NADH-TR) (c). Central nuclei and pale halo are shown by arrows. d) Electron microscopic picture shows muscle fibers with a centralized nucleus surrounded by an area devoid of myofibrils and filled with glycogen granules.

Reference a, d: Romero N and Bitoun M. Centronuclear myopathies. Seminars in pediatric neurology.

Reference b, c: (Vasli et al, 2012)

1-2-2- Autosomal dominant *DNM2*-related Centronuclear myopathy

The autosomal dominant form of CNM is caused by mutations in *DNM2* gene (Bitoun et al, 2005). Disease severity tends to be milder in comparison with other CNM forms (Jeannet et al, 2004; Wallgren-Pettersson et al, 1995). Neonatal, late-onset childhood and adult-onset with mild CNM as well as an intermediate form in adults with early onset were reported so far (Bitoun et al, 2009; Bitoun et al, 2005). This gene encodes a large GTPase protein involved in membrane trafficking from plasma membrane and Golgi with a role in formation and fission of budding vesicles, endocytosis, actin assembly and centrosome cohesion (Jones et al, 1998; Jungbluth et al, 2008; North, 2008; Thompson et al, 2004; Unsworth et al, 2007).

Clinical description

In young adult patients, motor milestones are delayed especially when they walk and climb stairs. They also show moderate muscle weakness associated with ophthalmoplegia and ptosis. Achilles tendon contractures are also often observed. Pediatric patients have generalized weakness, moderate facial weakness with open mouth, hypotonia, ophthalmoplegia and ptosis (Fischer et al, 2006; Jeannet et al, 2004; Susman et al, 2010) (Figure 3a).

Pathology

The morphological analysis of muscle biopsies shows significant number of internalized and centralized nuclei, type I muscle fiber predominance, less rounded and more polygonal fibers, hypotrophy and a radial arrangement of sarcoplasmic strands with oxidative enzyme reaction. In electron microscopy pictures the internuclear spaces are occupied with mitochondria, Golgi complex, rough endoplasmic reticulum and glycogen particles (Figure 3b, c, d) (Bitoun et al, 2009; Fujimura-Kiyono et al, 2008; Jeannet et al, 2004; Romero, 2010).

Molecular screening

Similar to the *MTM1,* for molecular screening of *DNM2,* coding regions and flanked intronic sequences are analyzed by direct sequencing. Dynamin 2 has five domains: 1) A GTPase domain 2) A middle domain 3) A pleckstrin homology domain 4) A GTPase effector domain and 5) A C-terminal proline rich domain (Fujimura-Kiyono et al, 2008). Heterozygous changes in the middle domain are associated with mild phenotype and adolescent onset (Fischer et al, 2006; Schessl et al, 2007) whereas heterozygous changes in the pleckstrin homology domain are associated with a more severe presentation with neonatal onset (Bitoun et al, 2007). Mutations in the pleckstrin homology domain

of *DNM2* are also detected in autosomal dominant Charcot-Marie-Tooth neuropathies (Bitoun et al, 2008; Fabrizi et al, 2007; Zuchner et al, 2005).

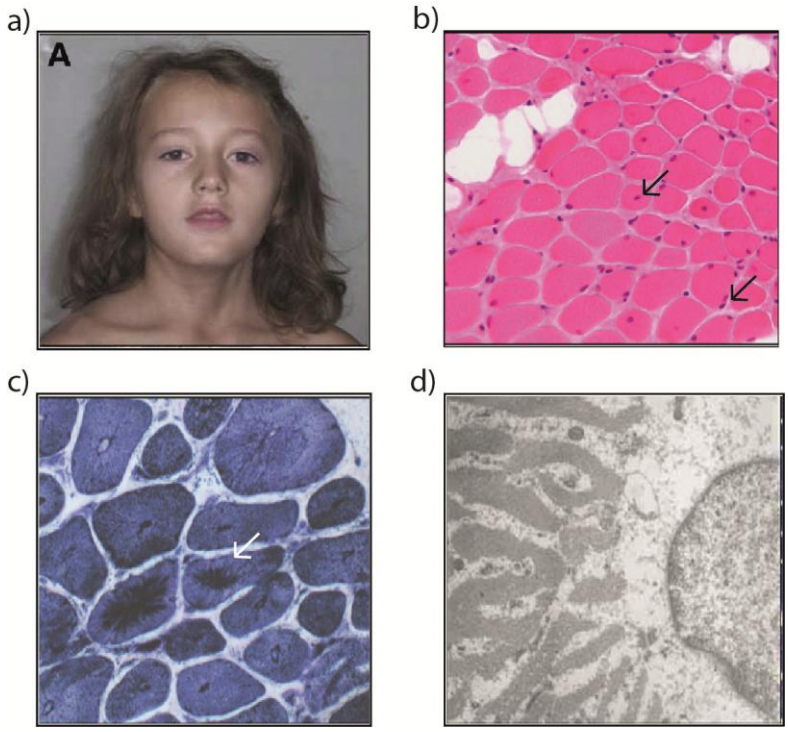


Figure 3: Some clinical and histopathological features in patients with AD *DNM2*-related CNM. a) A girl with *DNM2*-related CNM. Note to open mouth, ptosis and mild facial weakness. b, c) Muscle biopsy stained with Haematoxylin and Eosin (H&E) (b) and Nicotinamide adenosine dinucleotide – tetrazolium reductase (NADH-TR) (c). Central and internalized nuclei and sarcoplasmic radial strands are shown by arrows. d) Electron microscopic picture shows muscle fiber with a centralized nucleus and radial strands.

Reference a, d: Romero N and Bitoun M. Centronuclear myopathies. Seminars in pediatric neurology.

Reference b, c: (Susman et al, 2010).

1-2-3- Autosomal recessive *BIN1*-related Centronuclear myopathy

The autosomal recessive form of CNM is caused by mutations in the bridging integrator 1 (*BIN1*) gene (Nicot et al, 2007). In general, age of onset and severity in AR *BIN1*-related CNM is between the X-liked and the autosomal dominant forms (Jungbluth et al, 2008). This gene encodes the Amphiphysin 2 protein which is involved in membrane remodeling and tabulation, by interacting with the DNM2 (Nicot et al, 2007).

Clinical description

A large clinical variability was seen in CNM patients with mutations in the *BIN1* from severe to moderate phenotypes (Claeys et al, 2010; Mejaddam et al, 2009). Delayed motor milestones and difficulties to walk and climb stairs since childhood are common features in these patients. Ptosis and ophthalmoplegia, muscular atrophy and diffuse and facial weakness are also frequently present (Figure 4a) (Romero, 2010).

Pathology

The morphological analysis of muscle biopsies shows a lot of rounded hypotrophic (small) type I fibers with central nuclei in majority of them, clusters of central nuclei, increase in connective tissues and fibroadipose replacement and a radial arrangement of sarcoplasmic strands less frequent than *DNM2*-related biopsies. The electron microscopy pictures show centrally-placed nuclei and an internuclei region occupied by an amorphous material containing glycogen particles, sarcotubular structures and mitochondria (Figure 4b, c, d) (Mejaddam et al, 2009; Romero, 2010; Toussaint et al, 2011).

Molecular screening

Similar to the *MTM1* and *DNM2,* for molecular screening of *BIN1,* the coding regions and flanked intronic sequences are analyzed by direct sequencing (Biancalana et al, 2012).

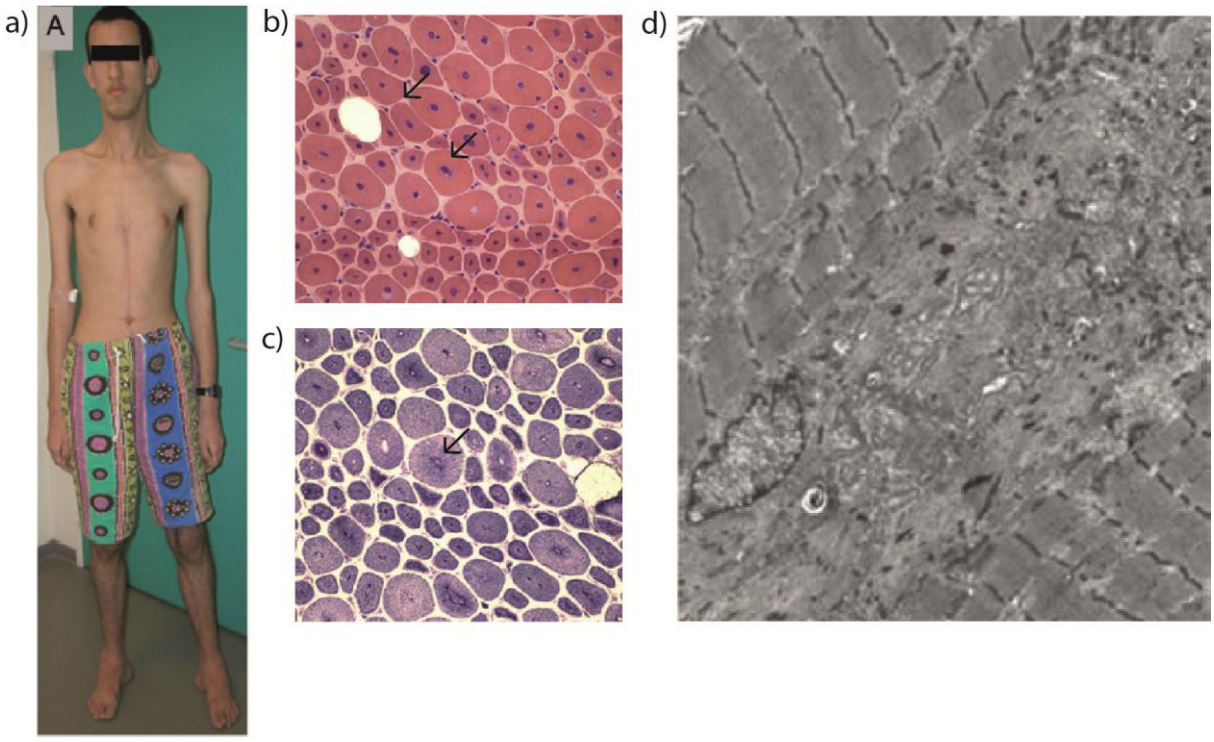


Figure 4: Some clinical and histopathological features in patients with AR *BIN1*-related CNM. a) A boy with *BIN1*-related CNM. Note to facial diplegia, elongated face and generalized muscle atrophy. b, c) Muscle biopsy stained with Haematoxylin and Eosin (H&E) (b) and Nicotinamide adenosine dinucleotide – tetrazolium reductase (NADH-TR) (c). Central and internalized nuclei and sarcoplasmic radial strands are shown by arrows. d) Electron microscopic picture shows amorphous material in the center of the fiber containing glycogen particles, sarcotubular structures, nucleus and mitochondria.

Reference a: (Claeys et al, 2010)

Reference b, c& d: (Romero, 2010)

1-2-4- *MTMR14*-related Centronuclear myopathy

Two heterozygous missense variants (R336Q-Y462C) that affect the enzymatic function were identified in the *MTMR14* gene (*hJUMPY*) in two sporadic cases of centronuclear myopathy with early onset, so far. One of these patients had an additional *DNM2* heterozygous change (Tosch et al, 2006). This protein is a phosphoinositide phosphatase that may regulate the level of phosphoinositides in skeletal muscle. Whether these variations are disease-causing mutations or implicating in phenotype modifications remains to be determined.

Clinical description

The first patient described with heterozygous R336Q variation in *MTMR14* was a 12-year-old boy at the time of publication (2006), with a neonatal hypotonia and hypoxia, stable or slowly progressive disease and ophthalmoparesis. The second patient with heterozygous Y462C variation in *MTMR14* and heterozygous E368K variation in *DNM2*, was a 36-year-old woman at the time of publication (2006), with a neonatal hypotonia followed by a stable muscle involvement and ophthalmoparesis (Alonso et al, 1981; Tosch et al, 2006; Zanoteli et al, 1998).

Pathology

The histological criteria seen in two patients were centralized nuclei, type I fiber predominance, an increase of oxidative enzyme activity in the center of the fibers and absence of other structural abnormalities such as nemaline bodies (Tosch et al, 2006).

Molecular screening

All the coding exonic regions and exon-intron boundaries were analyzed by direct sequencing and SSCP by Tosch et al.

1-2-5- *RYR1*-related Centronuclear myopathy

Several variations (dominant, compound heterozygous or homozygous) were detected in the *RYR1* gene in patients with CNM phenotype but without mutations in the three main genes implicated in CNM with neonatal age of onset (Bevilacqua et al, 2011; Jungbluth et al, 2007; Wilmshurst et al, 2010). This gene encodes the skeletal muscle ryanodine receptor, which is a calcium channel in sarcoplasmic reticulum as well as a bridging structure connecting the sarcoplasmic reticulum and transverse tubule (Fill et al, 1989).

Clinical description

This group of patients shows axial and proximal weakness with diverse severities, facial weakness, ophthalmoparesis and ptosis as well as contractures (Figure 5a) (Romero, 2010).

Pathology

The morphological analysis of muscle biopsies in these patients shows a significant number of internalized and centralized nuclei, some fibers with a fuchsinophilic granular precipitate as purple dusty fibers, large areas devoid of ATPase activity and type I fiber predominance (Wilmshurst et al, 2010). Large zones of myofibrillar disorganization, Z-disc streaming with accumulation of Z-disc proteins and depleted mitochondria are frequently observed by electron microscopy (Figure 5b, c, d) (Wilmshurst et al, 2010).

Molecular screening

As this gene is one of the largest genes in the human genome with 106 exons, in most of the laboratories, if muscle biopsies are accessible, the cDNA or protein is analyzed by RT-PCR or Western blot, as the amount of functional RYR1 protein is often reduced (Clarke et al, 2010; Monnier et al, 2001; Wilmshurst et al, 2010). If muscle biopsies are not available, the coding exons with hot spot regions of mutations are analyzed first. In some studies, all 106 exons and intron-exon boundaries were analyzed by direct sequencing (Clarke et al, 2010; Klein et al, 2012).

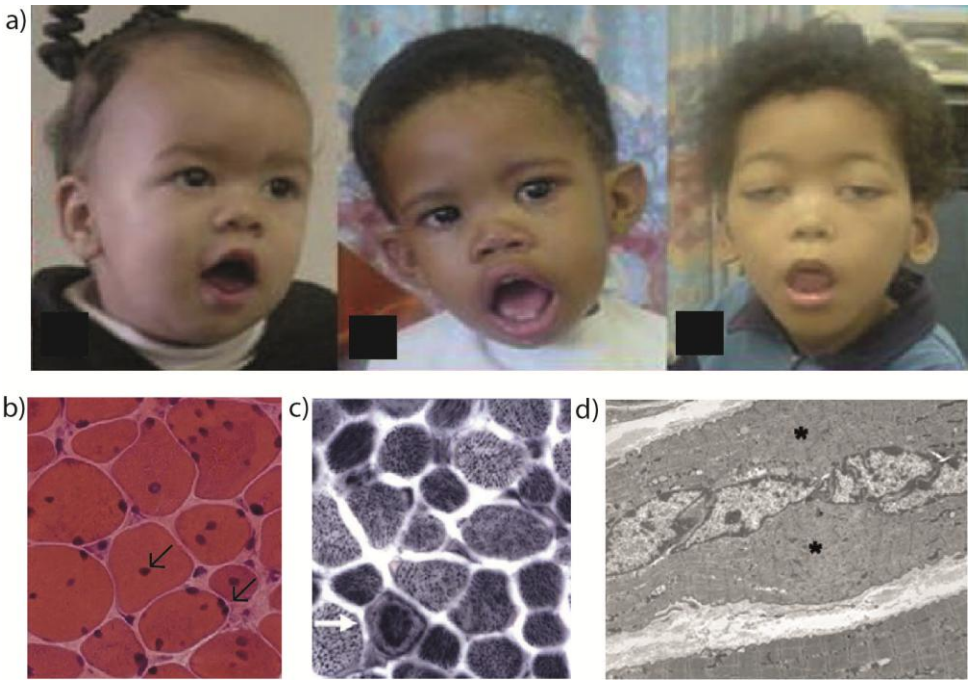


Figure 5: Some clinical and histopathological features in patients with *RYR*1-related CNM. a) Patients with *RYR1*-related CNM. Note to facial problems with inverted V-shaped mouth and ptosis. b, c) Muscle biopsy stained with Haematoxylin and Eosin (H&E) (b) and Nicotinamide adenosine dinucleotide – tetrazolium reductase (NADH-TR) (c). Central and internalized nuclei and purple dusty appearance are shown by arrows. d) Electron microscopy picture shows the zone of myofibrillar disorganization, central nuclei aligned in chains and Z-disc streaming.

Reference a, c& d: (Wilmshurst et al, 2010)

Reference b: (Romero, 2010)

1-3-Biological questions and aims

About 20% of patients with CNM do not have mutations in genes mentioned above (Biancalana et al, 2012) thus it is likely that there is or there are other genes implicated in this disorder. The first biological question and aim of my PhD project was to find this/these gene(s) using massively parallel sequencing and the second aim was to test if massively parallel sequencing can be used as routine technique in molecular diagnosis of neuromuscular disorders.

1-3-1- Aim 1: Gene identification

The identification of genes responsible for monogenic disorders enables molecular diagnosis of affected patients, and carrier and prenatal testing. Gene identifications lead to a better

understanding of pathophysiological role of the related proteins and disease pathways, which can serve as a starting point for developing therapeutic approaches (Antonarakis & Beckmann, 2006). Traditional gene identification approaches and novel strategies will be mentioned below.

1-3-1-2- Previous methods for gene identification

Until three years ago, several disease causing genes were identified by Sanger sequencing of candidate genes in patients. These candidates were selected due to their similar predicted protein functions to the physiology of the disease, or based on positional mapping results pointing to these genes in a genomic region or they resemble genes associated with similar diseases (Botstein & Risch, 2003). The most successful strategy was selecting candidates based on positional mapping results, as this technique can be applied in an unbiased manner and it does not rely on prior medical or biological knowledge. The most important genetic mapping approaches rely on following methods:

A- Linkage analysis, where the probability of whether two loci are likely to lie near each other on a chromosome and are therefore likely to be inherited together is calculated statistically and shown by LOD score. A LOD score of 3 or more is generally taken to show that the two loci are linked and are close to one another. This technique was used successfully, for instance, in identification of cystic fibrosis gene (Kerem et al, 1989; Riordan et al, 1989).

B- Karyotyping, where the changes in chromosomes numbers and structures are analyzed. This technique was used successfully, for instance, in identification of *NSD1* in patients with Sotos syndrome, a rare genetic disorder characterized by excessive physical growth during first years of life, mild mental retardation, delayed motor, hypotonia and speech impairment (Kurotaki et al, 2002).

C- Homozygosity mapping, where the genes that cause recessive traits are localized in homozygous regions in affected individuals in consanguineous families (Lander & Botstein, 1987). This technique was used successfully, for instance, in identification of gene implicated in retinoblastoma, the common intraocular cancer of childhood (Lee et al, 1987).

D- Copy number variation (CNV) analysis, where the CNVs in patient's samples are analyzed with different techniques such as fluorescent in situ hybridization, array comparative genomic hybridization (aCGH) or single nucleotide polymorphism (SNP) array technologies. In aCGH technique fluorescently labeled patient and control DNAs are hybridized to an array then differences in the hybridization signals of gains and losses in patient DNA versus control

DNA can be detected and can be used for identifying abnormal regions in the genome (Kirchhoff et al, 2001). This technique was used successfully, for instance, in identification of the gene implicated in CHARGE syndrome, a disorder characterized by hear defects, retarded growth and development, ear anomalies and genital hypoplasia (Vissers et al, 2005).

E- SNP-based association analysis, where typically, the association between SNPs and traits such as diseases, are analyzed. This technique was used successfully, for instance, in identification of the gene implicated in inflammatory bowel disease, a chronic, relapsing inflammatory disorder of the gastrointestinal tract (Duerr et al, 2006).

Although the above mentioned techniques were successful in disease gene identifications, these mapping approaches normally do not reduce the number of candidate genes sufficiently for follow-up by direct sequencing, when the disease locus remains very large. This is especially the case when these mapping approaches are applied to a single patient or family with a small number of informative relatives. One solution to reduce the number of candidate genes to manageable number is the combination of data from multiple unrelated but phenotypically similar patients and families, but this carries a risk that patients with similar phenotypes have disease-causing mutations in different genes.

For rare genetic disorders that occur often sporadically, and for which neither an associated-based approach nor a family-based approach can be used, these approaches will be unhelpful. For these disorders, an unbiased gene identification approach that can be used in small cohorts or single patients is necessary. Recent advances in massively parallel sequencing technologies have changed the disease-causing gene identification. In next part, this approach and its application will be mentioned.

1-3-1-3- Massively parallel sequencing

In an industrial, high-throughput manner, Sanger sequencing was used for the sequencing of the first human genome, which was completed in 2004 through the Human Genome Project (HGP), a 13-year effort with an estimated cost of $2.7 billion (Consortium, 2004). In 2008, by comparison, a human genome was sequenced for approximately $1.5 million in five months (Wheeler et al, 2008).

Massively parallel sequencing (MPS), also called next generation sequencing (NGS) or high-throughput sequencing (HTS) is a new sequencing technology for sequencing a large amount of genes at once. Using this technique, target genes or all protein coding parts of the genome (whole exome) or whole genome can be sequenced in few hours/days. In targeted sequencing, a number of

target genes or chromosomal regions are captured and sequenced. This technique is useful when the aim of the study is analysis of the genes or the regions of interests. When there is not enough data to point out to the specific genes or regions of interests and as about 1-2% of the human genome is protein-coding regions, split across 180,000 exons, and since about 85% of disease-causing mutations have been found in these regions (Botstein & Risch, 2003), almost all protein coding parts of the genome which is called exome, are captured and sequenced (Ng et al, 2009). The last possibility is to sequence all parts of the genome including exons, introns, UTRs, etc, known as whole genome sequencing.

This sequencing technology is based on a combination of template preparation, sequencing and imaging and data analysis (Metzker, 2010).

1-3-1-3-2-Template preparation & barcoding

DNA and library preparations are the first steps in MPS process. Two to twenty microgram of high quality genomic DNA is randomly sheared into smaller sizes by sonication or nebulization. There is no capturing step for whole genome sequencing, however, if the aim is to sequence a portion of genomic DNA which can be the protein-coding part of the genome (whole exome) or regions of interest such as coding parts of selective genes (targeted sequencing), capturing the target sequences should be done. The capture can be done in solid or solution bases (Mamanova et al, 2010). Several applications such as Illumina, Agilent and Nimblegen capture kits with technical differences such as using RNA baits (Agilent) or DNA baits (Illumina and Nimblegen) with almost similar performance can be used. Several DNA can also be pooled and sequenced together. The bar-coding and pooling steps can be done either before or after capturing, using a unique DNA tag sequence per sample (Craig et al, 2008). In this way it is possible to sequence a few genes in many patients or many genes from a few patients.

1-3-1-3-3- DNA enrichment

There is no enrichment step for whole genome sequencing (Wheeler et al, 2008); however if the aim is to sequence a portion of genomic DNA which can be the protein-coding part of the genome (exome) or regions of interest such as selected genes or a genomic region linked to a disease, such targeted sequencing includes the enrichment of the target sequences.

This enrichment or capture can be done on microarray or in solution (Mamanova et al, 2010). In solid-phase enrichment, high-density primers targeting the sequences of interest are covalently attached to the slide array (Fedurco et al, 2006) (Figure 6), while in solution-phase enrichment,

primers are generated on beads (Dressman et al, 2003) (Figure 7). Several solutions such as Illumina, Agilent and Nimblegen capture kits can be used; they have technical differences such as using RNA baits (Agilent) or DNA baits (Illumina and Nimblegen) with almost similar performance (Clark et al, 2011).

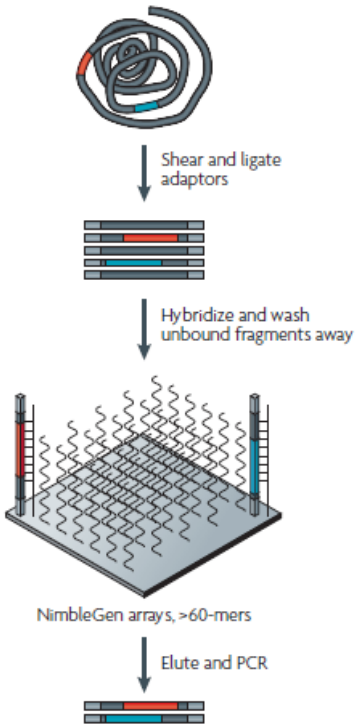


Figure 6: Solid-phase capture and enrichment. Adaptor sequences are ligated to fragmented genomic DNA. After stringent washing to remove non-selected fragments, the enriched targets are eluted, PCR amplified and sequenced.

Reference: (Metzker, 2010)

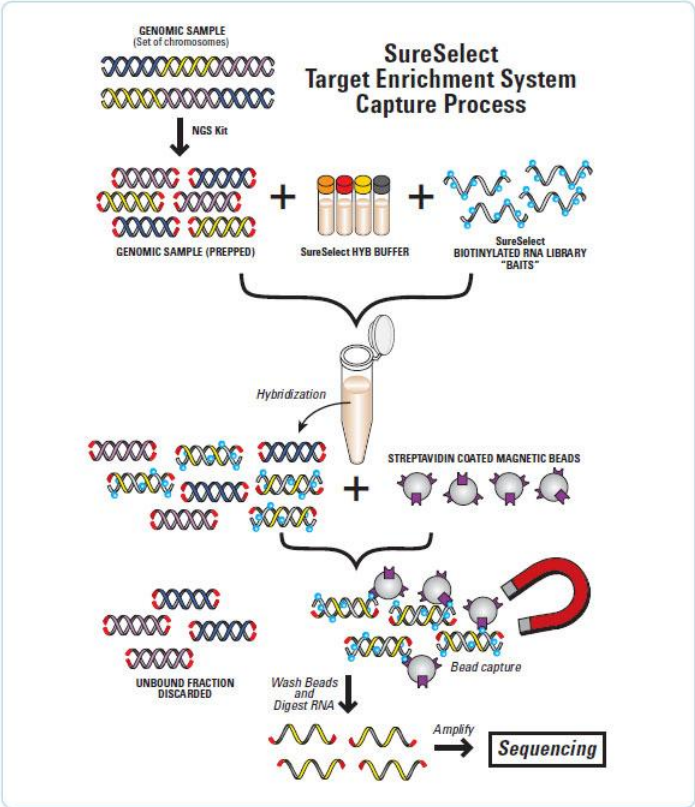


Figure 7: Solution-phase capture and enrichment. The genomic DNA is sheared, fragments with appropriate sizes are selected and incubated with RNA nucleotides or baits. RNA bait-DNA hybrids are then separated from complex mixture by incubation with streptavidin-labeled magnetic beads and captures onto a strong magnet. The beads are washed and RNAs are digested, thus the remaining nucleotides are the targeted DNA of interest. Then a few cycles of PCR are performed. The targeted sample is ready to load onto the sequencing machine.

Reference:
http://www.genomics.agilent.com/GenericB.aspx?pagetype=Custom&subpagetype=Custom&pageid=3083

In sequencing platforms using single molecule template the amount of starting DNA is less and there is not any PCR step which eliminates artificially created mutations in clonally amplification and AT or GC rich amplification bias. In these platforms either the template, primer or polymerase enzyme is immobilized on a solid support before NGS reaction (Metzker, 2010).

Another enrichment method is the RainStorm microdroplet-based technology from Raindance technologies which is a multiplex PCR method, using microdroplets containing PCR components

loaded on microfluidic chip to compartmentalize the PCR reactions by single primer pairs (Tewhey et al, 2009) (Figure 8).

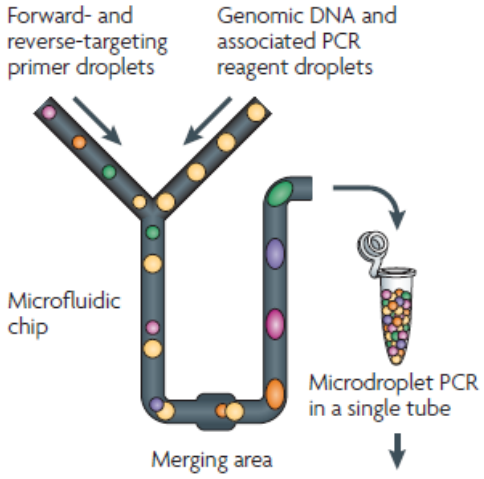


Figure 8: Microdroplet PCR. Forward and reverse primers are distributed into a channel in a microfluidic chip as aqueous droplets. The fragmented genomic DNA and PCR reagents are separately distributed as aqueous droplets too. Both droplets pair together at a 1/1 ratio and pass through a merging area and collected into a single tube for thermal cycling.
Reference: (Metzker, 2010)

1-3-1-3-4- High-throughput sequencing

Sequencing and imaging steps are different in sequencing platforms as well. Illumina/Solexa uses clonally amplified templates coupled with cyclic reversible termination method with four fluorescent colors. First, one fluorescently modified nucleotide complementary to the template sequence is incorporated. After washing and imaging for detection of the incorporated nucleotide, a cleavage step which cleaves fluorescent dye and inhibitor group is performed, and the last step is washing. These steps are done in a cyclic manner, 72 or 100 times or more, depend on the sequencing fragment size (Bentley et al, 2008) (Figure 9).

SOLiD (Sequencing by Oligonucleotide Ligation and Detection) sequencer from Applied Biosystems, is another NGS machine that works based on sequencing by ligation, using DNA ligase and a cleavable two-base-encoded probe which is a sequence with two oligonucleotides combined with a particular dye (Valouev et al, 2008) (Figure 10).

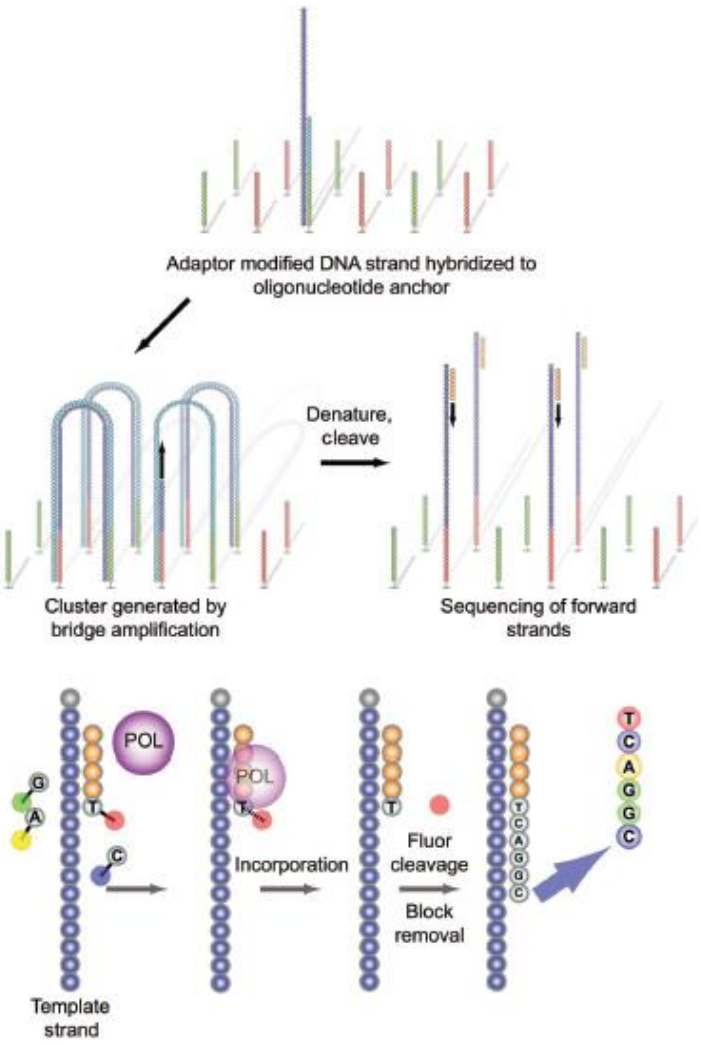
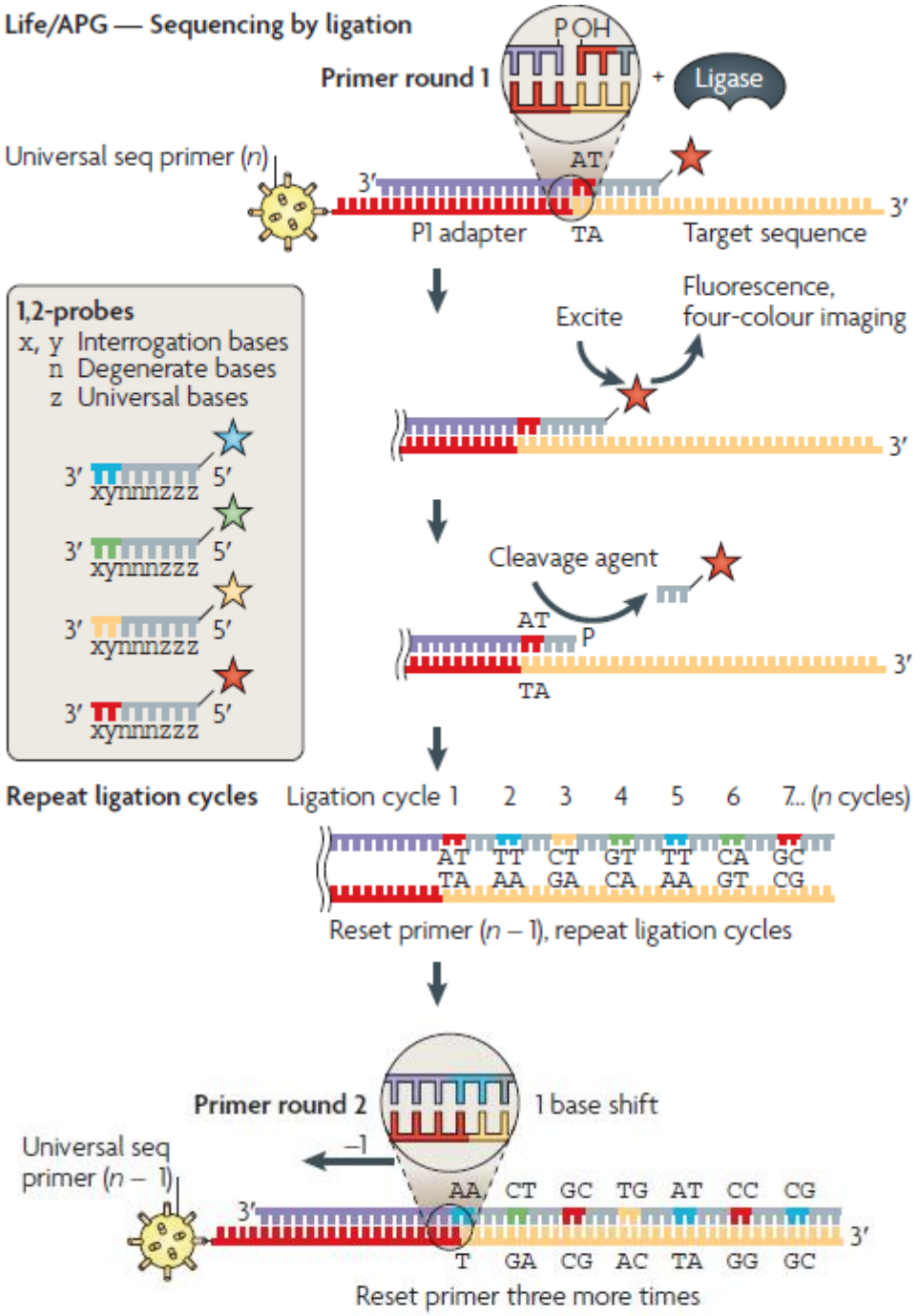


Figure 9: Illumina sequencing technology. Genomic DNA is fragmented randomly and adaptors are ligated to both ends of fragments. Single strand fragments are bound randomly to the surface of the flow cell channels. Bridge amplification is started by adding unlabeled nucleotides and enzyme. Nucleotides are incorporated by enzyme to make double-stranded bridges. Single-strand templates anchored to the surface are made by denaturation. Several million dense clusters of double-stranded DNA are generated in each channel of the flow cell. Four labeled reversible terminators, DNA polymerase and primers are added to initiate the sequencing cycle. The emitted fluorescence from each cluster is captured after laser excitation and the nucleotide is identified. The sequencing cycles are repeated to identify the nucleotides in a fragment, one base at a time. The data are aligned and compared to a reference genome, and nucleotide differences are identified.

Reference: (Voelkerding et al, 2009)


Life/APG — Sequencing by ligation
Primer round 1
P OH
Ligase
Universal seq primer (n)
3′
AT
TA
P1 adapter
Target sequence
1,2-probes
x, y Interrogation bases
n Degenerate bases
z Universal bases
3′ xynnnzzz 5′
Excite
Fluorescence, four-colour imaging
Cleavage agent
AT
P
TA
Repeat ligation cycles
Ligation cycle 1 2 3 4 5 6 7... (n cycles)
AT TT CT GT TT CA GC
TA AA GA CA AA GT CG
Reset primer (n – 1), repeat ligation cycles
Primer round 2
1 base shift
Universal seq primer (n – 1)
–1
AA CT GC TG AT CC CG
T GA CG AC TA GG GC
Reset primer three more times

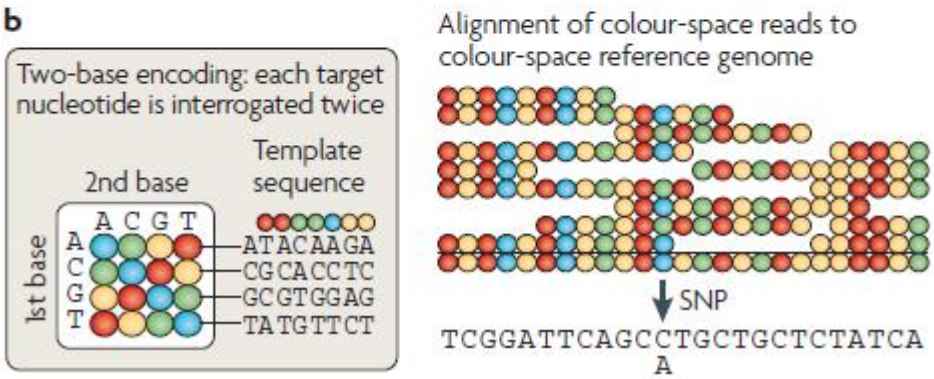

b
Two-base encoding: each target nucleotide is interrogated twice
2nd base
Template sequence
A C G T
1st base
A
C
G
T
ATACAAGA
CGCACCTC
GCGTGGAG
TATGTTCT
Alignment of colour-space reads to colour-space reference genome
SNP
TCGGATTCAGCCTGCTGCTCTATCA
A

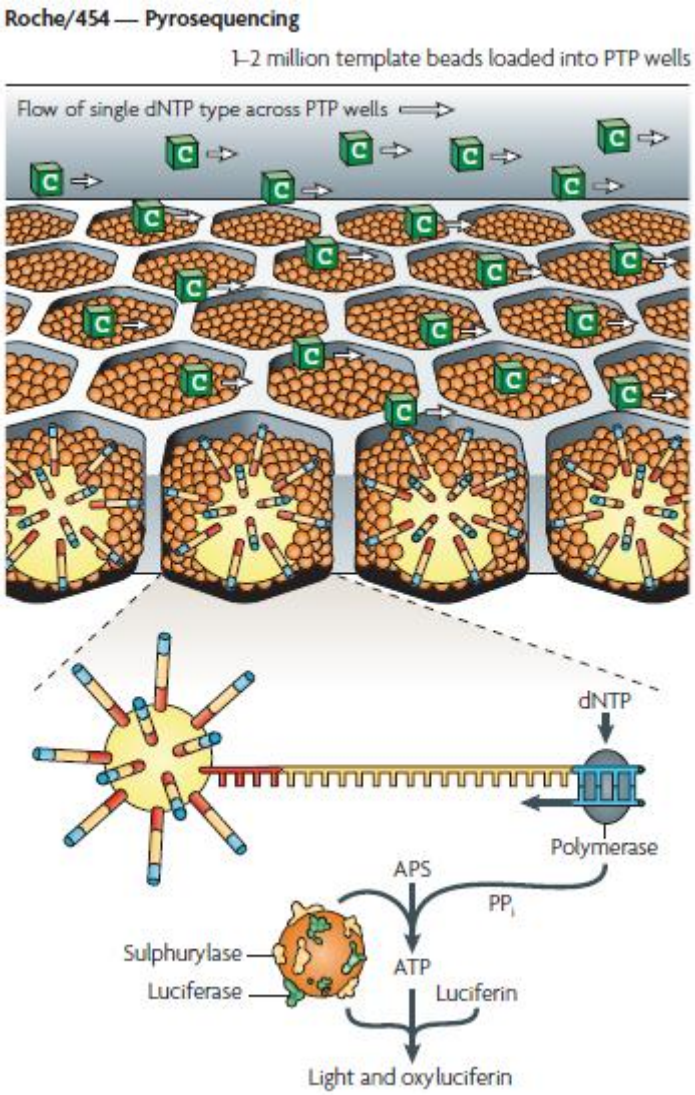
Figure 10: Sequencing by ligation method using in the SOLiD sequencer from Applied Biosystems. This technique is based on sequencing by ligation. The ligation is performed, using specific 8-mer probes. These probes are eight bases in length with a fluorescent dye at the 5' end, a free hydroxyl group at the 3' end, and a cleavage site between the fifth and sixth nucleotide. The first two bases (starting at the 3' end) are complementary to the nucleotides being sequenced. Bases 3 through 5 are able to pair with any nucleotides on the template sequence. Bases 6-8 are cleaved off, along with the fluorescent dye, as the reaction continues. Cleavage of the fluorescent dye and bases 6-8 leaves a free 5' phosphate group ready for further ligation. In this manner, positions n+1 and n+2 are correctly base-paired followed by n+6 and n+7 being correctly paired, etc. The composition of bases n+3, n+4 and n+5 remains undetermined until further rounds of the sequencing reactions are performed. The sequencing step is basically composed of five rounds and each round consists of about 5-7 cycles. Addition of a P1-complementary universal primer initiates each sequencing round. This primer has, for example, n nucleotides and its 5'-end matches exactly with the 3'-end of the P1. In each cycle, 8-mer probes are added and ligated according to their first and second bases. Then, the remaining unbound probes are washed out, the fluorescent signal from the bound probe is measured, and the bound probe is cleaved between its fifth and sixth nucleotide. Finally the primer and probes are all reset for the next round. In the next round a new universal primer anneals the position n-1 (its 5'-end matches to the base exactly before the 3'-end of the P1) and the subsequent cycles are repeated similar to the first round. The remaining three rounds will be performed with new universal primers annealing positions n-2, n-3 and n-4 relative to the 3'-end of P1. A complete reaction of five rounds allows the sequencing of about 25 base pairs of the template from P1.

The data are represented as colors. Each color indicates two bases and one of the bases in the sequence needs to be known: this base is incorporated in the sequence in the last (fifth) round of step 5. This known base is the last nucleotide of the 3'-end of the known P1. Therefore, since each color represents two nucleotides in which the second base of each dinucleotides unit constitutes the first base of the following dinucleotides, knowing just one base in the sequence will lead us to interpret the whole sequence.

Reference: (Metzker, 2010) & http://www.appliedbiosystems.com/absite/us/en/home/applications-technologies/solid-next-generation-sequencing.html

The HeliScope single-molecule sequencer of Helicos BioSciences works with one fluorescent color (Cy5), using cyclic reversible termination approach (Braslavsky et al, 2003).

Roche/454 is another sequencing platform working based on pyrosequencing. In this sequencing by synthesis method, a pyrophosphate (PPi) is released after incorporation of one dNTP. This released pyrophosphate is converted into detectable light through a series of enzymatic reactions (Ronaghi et al, 1996; Ronaghi et al, 1998) (Figure 11).



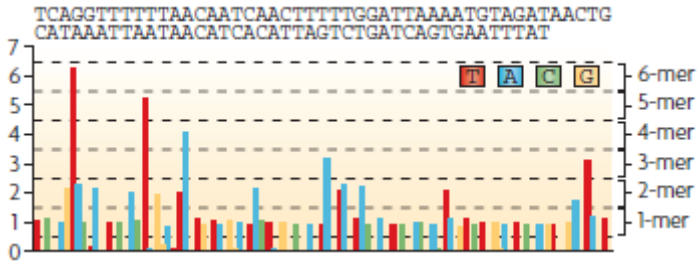
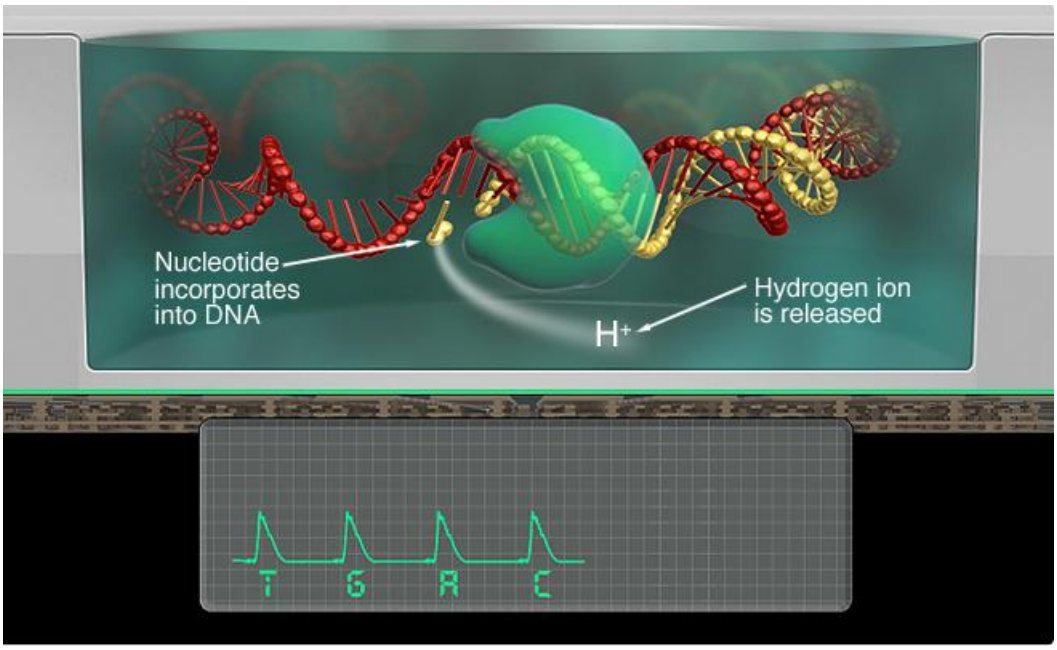


Figure 11: Pyrosequencing using in Roch/454 platform. DNA-amplified beads and the beads coupled with sulphurylase and luciferase are loaded into PicoTiter Plate (PTP) wells. When one dNTP is incorporated, a ppi (inorganic pyrophosphate) is released. This ppi is gone through a series of enzymatic reactions in order to generate a light which is detectable by a high-resolution charge-coupled device (CCD) camera. The light is recorded as a series of peaks called a flowgram.

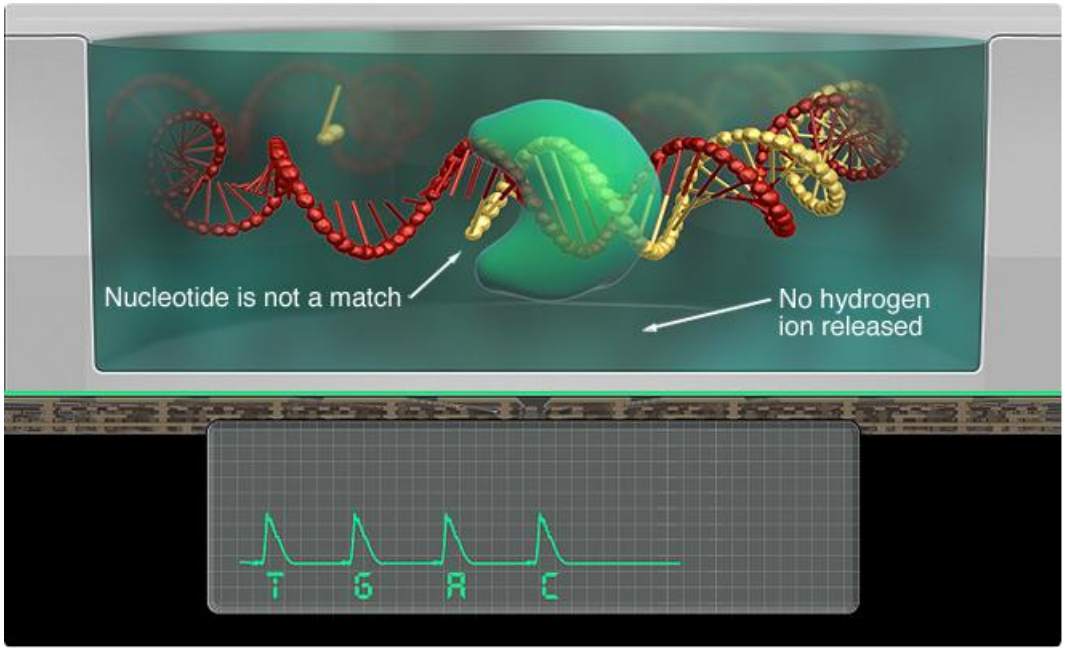
Reference: (Metzker, 2010)

The Ion Proton from Ion torrent is another NGS machine. In this semiconductor sequencing machine, the release of a proton after incorporation of a nucleotide into the DNA by the polymerase leads to a change in pH. This change is detected by voltage change detection. If the nucleotide is not incorporated, the voltage will not change and if two identical nucleotides are incorporated, the voltage will be double (Flusberg et al, 2010) (Figure 12).

a)



b)



c)

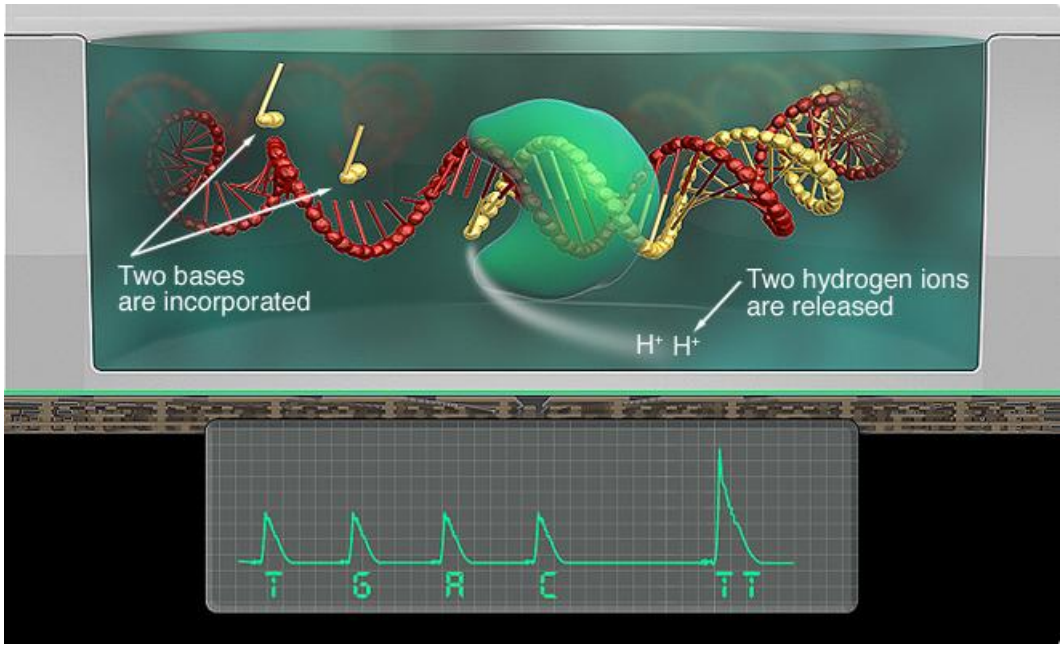


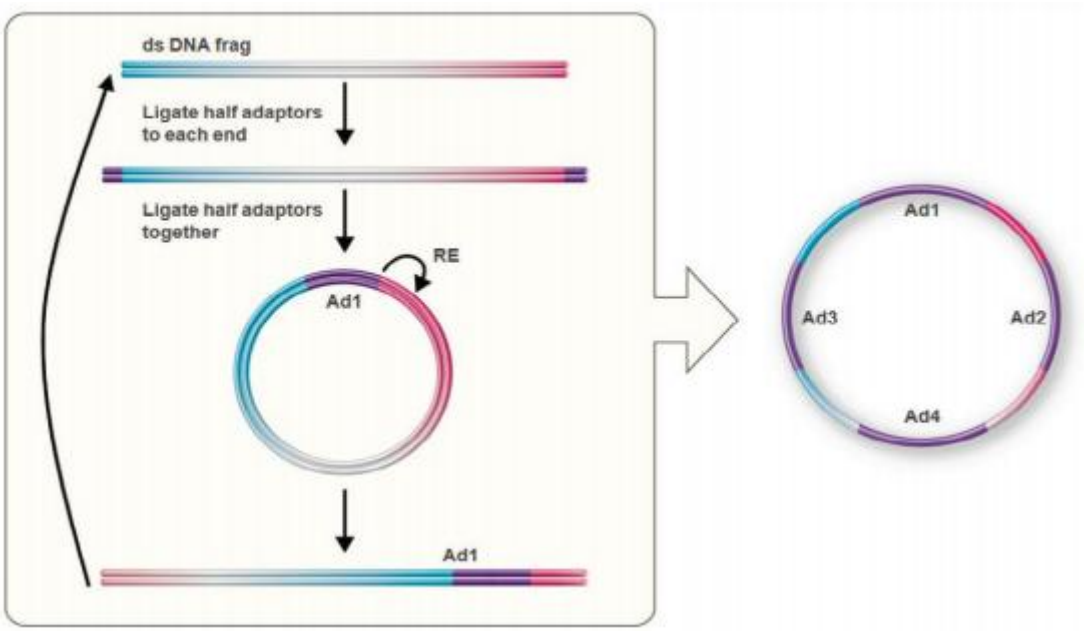
Figure 12: Semiconductor sequencing using in Ion torrent platform. a) When one nucleotide is incorporated into a DNA, a hydrogen ion is released. The charge of this ion will change the pH of the solution which will be detected by ion sensors by detection of voltage change. The sequencer machine will convert the chemical information to digital information. b) The sequencer then sequentially floods the chip with one nucleotide after another. If the next nucleotide that floods the

chip is not a match, no voltage change will be recorded and no base will be called. c) If two identical nucleotides incorporate, the voltage will be double and the chip will record two identical bases called.

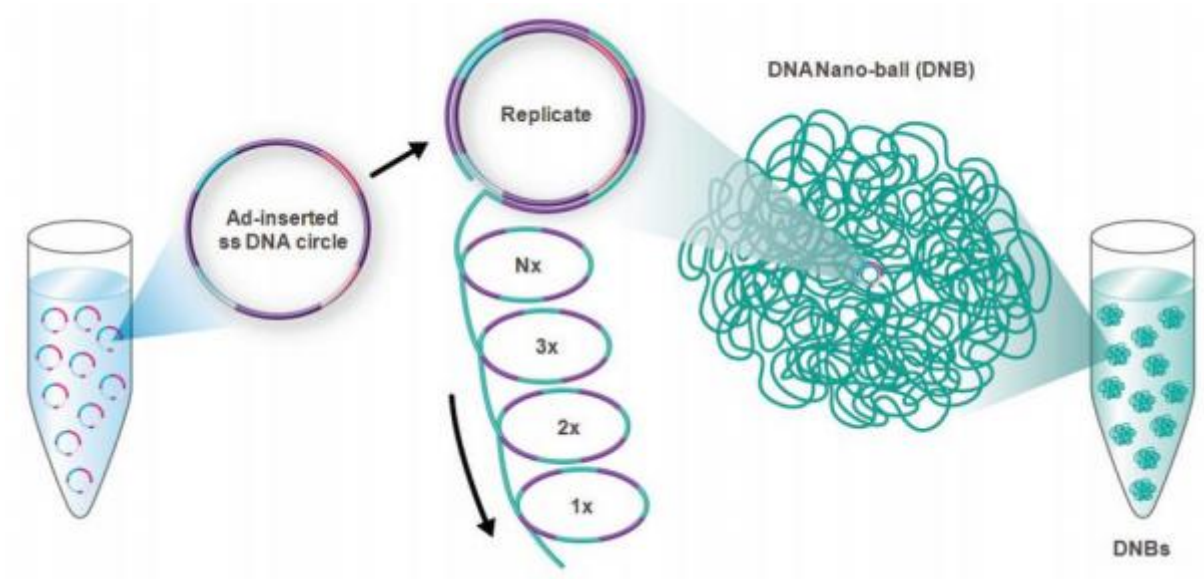
Reference:http://fr-fr.invitrogen.com/site/fr/fr/home/Products-and Services/Applications/Sequencing/Semiconductor-Sequencing/Semiconductor-Sequencing-Technology.html

For human whole genome sequencing, apart from the above mentioned sequencing platforms, Complete Genomics offers a non-commercialized solution. In that case, DNA nano-balls (DNB) containing hundreds of copies of the 70 bases of DNA fragments are sequenced, using combinatorial probe anchor-ligation (cPAL) technology where fluorescent molecules are attached to each nucleotide in each DNB by a ligase enzyme and by imaging the fluorescence, the sequence of nucleotides are subsequently determined (Drmanac et al, 2010) (Figure 13).

a)



b)



c)

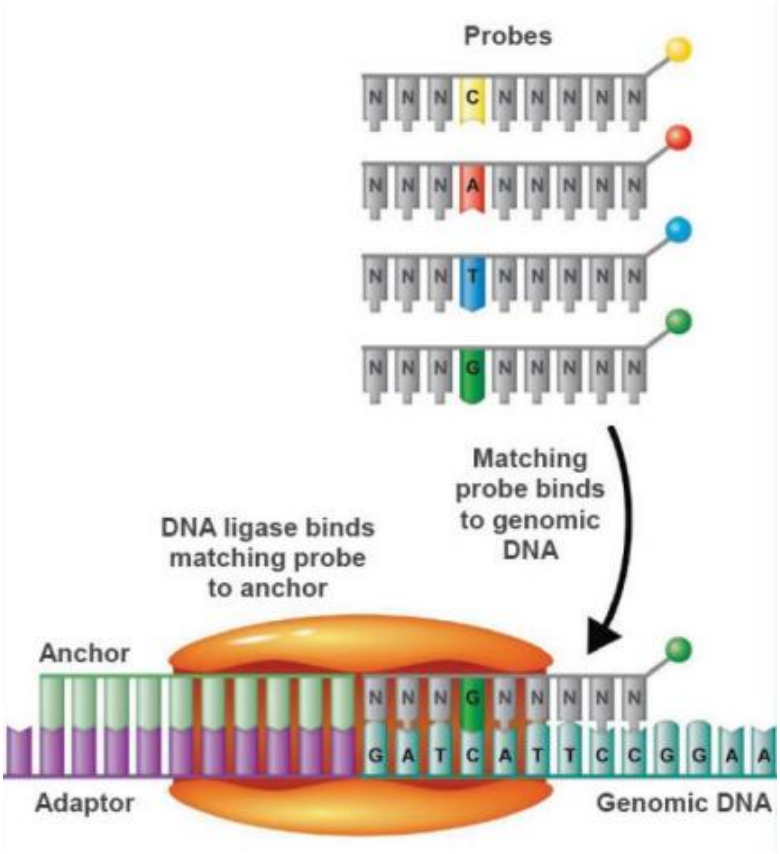


Figure 13: The complete genomics sequencing technology. a) Multiple adaptor library construction process: paired-end DNA libraries consist of genomic DNA fragments with adaptors interspersed within the genomic DNA at regular intervals. The adaptors act as initiating points for reading up to 10

bases from each adaptor-genomic DNA junction. Complete Genomics uses a dedicated library construction process to insert four adaptors into each DNA fragment. A four-adaptor approach supports 70-base reads (35 bases per paired-end). The read length may be increased by inserting more adaptors.

b) DNA nano-ball formation: Starting with a small circular DNA template consisting of approximately 80 bases of genomic DNA and four synthetic adaptors, a head to-tail concatemer consisting of more than 200 copies of the circular template is generated. One milliliter (ml) of reaction volume generates over 10 billion DNBs, sufficient for sequencing an entire human genome.

c) Combinatorial probe anchor-ligation (cPAL): cPAL uses pools of probes labeled with four distinct dyes (one per base) to read the positions adjacent to each adaptor. Each read position has a separate pool of probes. This approach allows 10 contiguous bases to be read from each end of an adaptor. Ligating the matching probes with the adjacent anchors improves the full-match specificity of the probe binding as compared to hybridization without ligation. After each base is read, the entire anchor-probe complex is washed away. The next anchor is then hybridized, and the next probe is ligated to the anchor.

Reference: http://www.completegenomics.com/

1-3-1-3-5- Data analysis

The final steps in next generation sequencing are genome alignment, variant calling and data analysis. After sequencing, the generated reads are aligned to the human reference genome and the variations are detected using different softwares and algorithms including BWA (Li & Durbin, 2009; Trapnell & Salzberg, 2009) for alignment and Samtools (Li et al, 2009) for variant calling. For filtering and scoring the variants and detection of disease-causing variations, there are several strategies depending on mode of inheritance and number of affected/non-affected sequenced cases (Gilissen et al, 2012). Several public websites display polymorphisms and allele frequencies such as dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/), 1000 genomes (http://www.1000genomes.org/) or Exome variant server (http://evs.gs.washington.edu/EVS/) that covers SNPs but no indels. Many softwares are available to filter sequence variants, such as ALAMUT software (Interactive Biosoftware, Rouen, France); Cartagenia benchlab tool (http://www.cartagenia.com/); SIMPLEX (http://simplex.i-med.ac.at) (Fischer et al, 2012) or ANNOVAR (http://www.openbioinformatics.org/annovar/) (Wang et al, 2010b). Different filtering strategies will be discussed in material and method section.

1-3-1-4- Massively parallel sequencing for gene identification

In 2009, Ng et al published a paper where they identified mutations in *MYH3* gene in 4 patients with Freeman-Sheldon syndrome, also called distal arthrogryposis type 2A, a rare autosomal dominant disorder, in 4 patients by doing exome sequencing (Ng et al, 2009). This paper was the first study that used NGS for finding disease-causing mutations in Mendelian disorders. The same team in 2010 described the first application of exome sequencing to discover the gene in a Mendelian disorder, Miller syndrome (Ng et al, 2010b). They found mutations in a gene, *DHODH*, in four affected patients from 3 independent families. In the same year, Lupski et al published the first paper in NMDs, using NGS, where they showed the result of whole genome sequencing of a patient from a family with a recessive form of Charcot-Marie-Tooth neuropathy (Lupski et al, 2010). They prioritized sequence variants in genes known to be implicated in Charcot-Marie-Tooth neuropathies and found the compound heterozygous mutations p.Arg954X and p.Tyr169His in the *SH3TC2* gene as a cause of the neuropathy. Since then several papers have been published using massively parallel sequencing for detection of causative mutations/genes in different Mendelian disorders as well as NMDs. Studies where they used massively parallel sequencing for detection of new genes implicated in myopathies are shown in table 3.

Table 3- List of papers showing the gene identification in myopathies by NGS.

Disease	Sequenced regions	Sequencing machine	Detected new gene	Date of publication	Reference
Congenital myopathy	Exome	Illumina HiSeq 2000	*CCDC78*	August 2012	(Majczenko et al, 2012)
Congenital nemaline myopathy	Targeted sequencing of 15 genes	SOLiD 4	*RYR1*	March 2012	(Kondo et al, 2012)
Myofibrillar myopathy	Exome	Illumina HiSeq 2000	*DES*	March 2012	(Hedberg et al, 2012)
A dominantly-inherited myopathy	Exome	Illumina HiSeq 2000	*DNAJB6*	February 2012	(Harms et al, 2012)
A recessive congenital myopathy	Targeted sequencing of 85 genes	Illumina GAIIx	*MEGF10*	November 2011	(Logan et al, 2011)

1-3-1- Aim 2: Improving diagnostic of NMD using NGS

The second aim of my PhD project was to test if massively parallel sequencing can be used as routine technique, in molecular diagnosis of neuromuscular disorders. As mentioned above, genetic and clinical heterogeneity, unspecific clinical features, implication of several often large genes requiring many techniques are the main drawbacks in routine NMD molecular diagnostic laboratories. Despite many technical improvements in molecular diagnosis of NMDs, due to different obstacles and problems, it will be necessary to use new and improved technology to overcome most of the challenges such as using massively parallel sequencing to sequence target genes or to do whole exome or whole genome sequencing.

This section was written as a review paper and it is under revision.

Review article

Impacts of massively parallel sequencing for genetic diagnosis of neuromuscular disorders

Nasim Vasli and Jocelyn Laporte

Aim of study/paper: a review paper discussing the problems of routine diagnostic techniques for NMD molecular genetic diagnosis and the advantages of using NGS as routine technique in molecular genetic diagnostic laboratories.

2012, Acta Neuropathologica

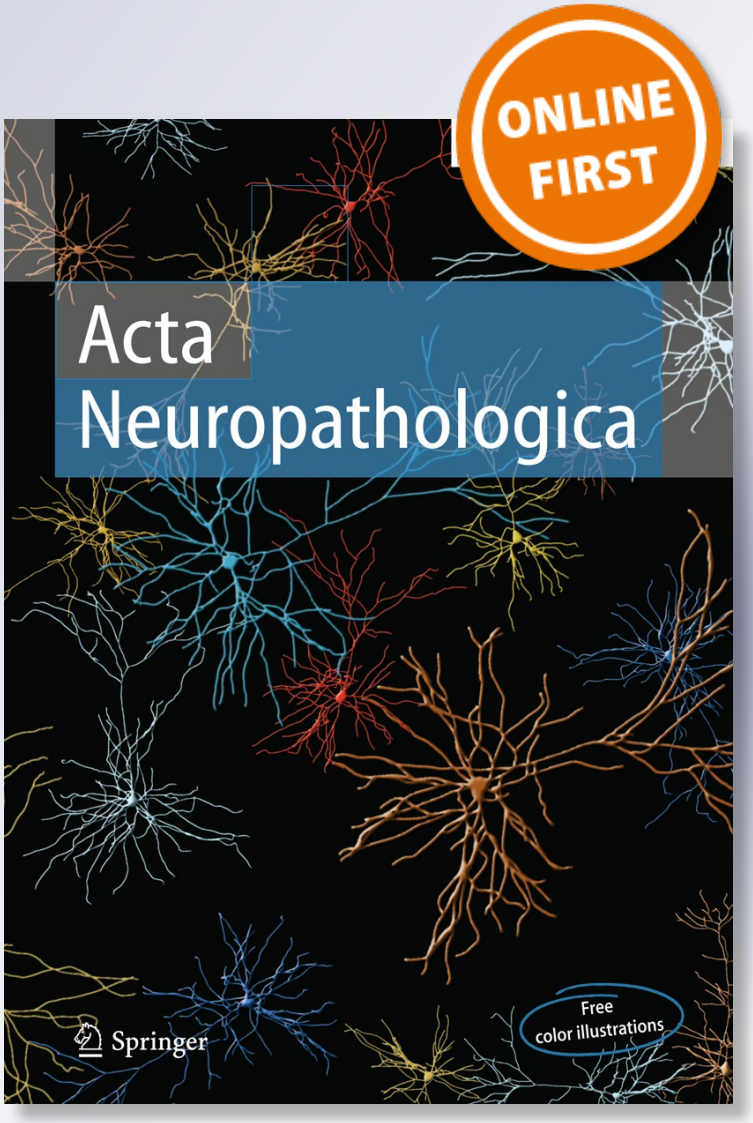
Impacts of massively parallel sequencing for genetic diagnosis of neuromuscular disorders

Nasim Vasli & Jocelyn Laporte

Acta Neuropathologica
Pathology and Mechanisms of Neurological Disease

ISSN 0001-6322

Acta Neuropathol
DOI 10.1007/s00401-012-1072-7



Springer

Your article is protected by copyright and all rights are held exclusively by Springer-Verlag Berlin Heidelberg. This e-offprint is for personal use only and shall not be self-archived in electronic repositories. If you wish to self-archive your work, please use the accepted author's version for posting to your own website or your institution's repository. You may further deposit the accepted author's version on a funder's repository at a funder's request, provided it is not made publicly available until 12 months after publication.

Springer

Author's personal copy

Acta Neuropathol
DOI 10.1007/s00401-012-1072-7

REVIEW

Impacts of massively parallel sequencing for genetic diagnosis of neuromuscular disorders

Nasim Vasli · Jocelyn Laporte

Received: 8 October 2012 / Revised: 27 November 2012 / Accepted: 28 November 2012
© Springer-Verlag Berlin Heidelberg 2012

Abstract Neuromuscular disorders (NMD) such as neuropathy or myopathy are rare and often severe inherited disorders, affecting muscle and/or nerves with neonatal, childhood or adulthood onset, with considerable burden for the patients, their families and public health systems. Genetic and clinical heterogeneity, unspecific clinical features, unidentified genes and the implication of large and/or several genes requiring complementary methods are the main drawbacks in routine molecular diagnosis, leading to increased turnaround time and delay in the molecular validation of the diagnosis. The application of massively parallel sequencing, also called next generation sequencing, as a routine diagnostic strategy could lead to a rapid screening and fast identification of mutations in rare genetic disorders like NMD. This review aims to summarize and to discuss recent advances in the genetic diagnosis of neuromuscular disorders, and more generally monogenic diseases, fostered by massively parallel sequencing. We remind the challenges and benefit of obtaining an accurate genetic diagnosis, introduce the massively parallel sequencing technology and its novel applications in diagnosis of patients, prenatal diagnosis and carrier detection, and discuss the limitations and necessary improvements. Massively parallel sequencing synergizes with clinical and pathological investigations into an integrated diagnosis approach. Clinicians and pathologists are crucial in patient selection and interpretation of data, and persons trained in data management and analysis need to be integrated to the diagnosis pipeline. Massively parallel sequencing for mutation identification is expected to greatly improve diagnosis, genetic counseling and patient management.

Keywords Neuromuscular disorders · Diagnosis · Mutation · Next generation sequencing · Neuropathy · Myopathy

N. Vasli · J. Laporte (✉)
IGBMC (Institut de Génétique et de Biologie Moléculaire et Cellulaire), 1, rue Laurent Fries, BP 10142,
67404 Illkirch, France
e-mail: jocelyn@igbmc.fr

N. Vasli · J. Laporte
Inserm, U964, Illkirch, France

N. Vasli · J. Laporte
CNRS, UMR7104, Illkirch, France

N. Vasli · J. Laporte
Université de Strasbourg, Illkirch, France

N. Vasli · J. Laporte
Collège de France, chaire de génétique humaine, Illkirch, France

Neuromuscular disorders

Inherited neuromuscular disorder (NMD) is a wide term covering different genetic disorders affecting muscles (different types of myopathies and dystrophies, ion channel muscle diseases and malignant hyperthermia), nerves (Charcot–Marie–Tooth neuropathies also called hereditary motor and sensory neuropathies, amyotrophic lateral sclerosis, hereditary ataxias and spinal muscular atrophies) and neuromuscular junctions (myasthenic syndromes [41]) (http://musclegenetable.fr/) [44]. Muscle weakness, twitching, cramps and numbness are common features in several NMDs. These disorders are rare and often severe, affecting children and adults with considerable burden to the patients, their families and public health systems [25].

Published online: 07 December 2012

Author's personal copy

Provisions in genetic diagnosis laboratories

The most important criterion for using a test in standard health care is whether the test will lead to a better outcome for the patient [97]. The clinical test should address the specific problem. The aim of a clinical genetic service is to make genetic diagnosis and estimation of transmission risk for an affected patient and to provide genetic counseling and preventive and therapeutic guidance for family members [31]. Anonymity and confidentiality are important standards in genetic diagnosis. Data should be reliable and diagnosis should be cost-effective. The turnaround time for test results is another important issue in genetics diagnosis.

Challenges in molecular diagnosis

Genetic heterogeneity

NMDs are one of the most genetically heterogeneous disorders class with more than 300 implicated genes. This genetic heterogeneity can be seen, for instance in Charcot–Marie–Tooth (CMT) neuropathies with more than 30 causative genes [83] and more than 40 loci (http://www.molgen.ua.ac.be/CMTMutations) or in autosomal recessive limb girdle muscular dystrophy with 15 genes implicated to date [49]. This high degree of genetic heterogeneity is problematic for molecular genetics diagnosis as it could be time-consuming and costly to test the different implicated genes in diagnostic laboratories. The rarity of mutations in some genes explains the fact that samples should be sent to several specialized laboratories to cover all candidate genes.

Implication of large genes

Several of the largest human genes are mutated in neuromuscular disorders such as *DMD* (MIM #300377) spanning more than 2.3 Mb with 79 exons [86], *TTN* (MIM #188840) with 363 exons with an open reading frame spanning more than 100 Kb, *NEB* (MIM #161650) with 183 exons [22] and *RYR1* (MIM #180901) with 106 exons [73]. These genes are also mainly expressed in muscle, precluding cDNA analysis if such tissue is not available. While these genes are known to be implicated in diseases, they might not be fully tested or only the mutation hot spot regions may be analyzed at first [4].

Clinical heterogeneity

Clinical heterogeneity is another parameter in diagnosis of NMDs. For example, mutations in the Caveolin 3 gene (MIM #601253) have been detected in four different skeletal muscle disease phenotypes: rippling muscle disease, limb-girdle muscular dystrophy, hyperCKemia and distal myopathy [28]. Patients can have an overlap of these symptoms, or the same mutation can cause diverse clinical phenotypes with different severities. Thus, it is sometimes difficult to suggest the best candidate genes to direct molecular diagnosis.

Unspecific clinical features

Another reason precluding the suggestion of the best molecular diagnosis approach is the fact that a large number of patients display unspecific clinical and histopathological features. For instance, reviews of cases with congenital myopathies that are usually sub-classified into nemaline, core or centronuclear myopathies reveal no specific signs in almost half of them, whereas other patients have diverse but overlapping clinical and histopathological manifestations [64]. Moreover, when a sequence change is found in one of the prioritized genes in diagnostic laboratories, further investigation might be stopped, which may lead in some cases to missing the real disease-causing mutation. Conversely, when no change is found, patients undergo additional time-consuming, costly and sometimes painful tests to precise the clinical diagnosis and prioritize other genes [75].

Requirements for several approaches

Different types of mutations are detected in patients with NMDs. For example, 60–65 % of patients with Duchenne and Becker muscular dystrophies (DMD and BMD) have deletions in the dystrophin gene, *DMD* (MIM #300377), 5–15 % have duplications, and the rest have point mutations or small insertions–deletions [1, 63]. Thus, diagnostic laboratories should apply different techniques to detect disease-causing mutations in patients, which is laborious, expensive, necessitates different platforms and increases turnaround time [76].

Unidentified genes

The last obstacle is the unidentified genes in different NMDs. For instance, mutations in several genes, including *SOD1* (MIM #147450) [81], *SETX* (MIM #608465) [14], *ALS2* (MIM #606352) [35, 96], *TARDBP* (MIM #605078) [30], *FUS* (MIM #137070) [48], *ANG* (MIM #105850) [33, 39] and *C9orf72* (MIM #614260) [19, 79] can cause amyotrophic lateral sclerosis (ALS), a neurodegenerative disorder characterized by the loss of motor neurons in brain and spinal cord. However, for a significant number of ALS patients, the causative mutation remains unknown, suggesting the involvement of other genes. More generally,

Author's personal copy

about 40 % of patients with NMDs do not have a genetic diagnosis.

Benefits of definitive molecular diagnosis

Identification of related disease-causing mutations helps to confirm the clinical findings and provide an accurate diagnosis. The knowledge of the mutation and mutated gene usually improves disease management, and allows for inclusion into therapeutic trials. Genetic counseling becomes possible, as carrier status determination and prenatal diagnosis can decrease the risk of recurrence in affected families. In addition, finding the disease-causing mutation permits potential phenotype–genotype correlations and a better understanding of the underlying pathophysiological mechanisms, a pre-requisite for the development of specific therapeutic approaches.

Routine molecular techniques in genetics diagnostic laboratories

Today, several techniques are used in routine diagnostic to find causative mutations. The most common method is PCR amplification of coding/exonic sequences of candidate genes from genomic DNA, followed by Sanger sequencing of PCR products. This approach is the gold standard for detection of point mutations and small insertions–deletions. If a gene is large and mutations are spread throughout the entire gene, using this technique is extremely time-consuming, expensive and laborious. An alternative approach is reverse transcription PCR (RT-PCR) followed by Sanger sequencing of the entire cDNA [34, 59] which needs the access to specific tissues such as muscle for the muscle-specific genes. Such tissues may not be available all the times. Multiplex PCR and long-range PCR (LR-PCR) are other PCR-based techniques. Using multiplex PCR of only 19 exons of the *DMD* gene, about 98 % of deletions could be detected in patients with DMD/BMD [6, 13, 70]. With the MLPA technique in patients with sporadic amyotrophic lateral sclerosis, it was shown that SMN1 duplications are associated with ALS susceptibility whereas SMN1 deletions and SMN2 copy number status are not associated with ALS [10]. In patients with spinal muscular atrophy (SMA), a neuromuscular disorder characterized by degeneration and loss of alpha-motor neurons in the anterior horn of the spinal cord, LR-PCR was used to detect deletions of the *SMN1* gene (MIM #600354) [3].

For detection of repeat expansions, a common type of mutation in NMDs, several methods are used, such as repeat primed PCR (RP-PCR) and Southern blot [32, 95]. In 56 out of 76 patients with myotonic dystrophy type 2 (DM2), a neuromuscular disorder characterized by myotonia and muscle dysfunction, and 25 out of 378 patients with spinocerebellar ataxia type 8, a slowly progressive neurodegenerative disorder, repeat expansions were detected using RP-PCR [46].

Comparative genomic hybridization (CGH) array is a method of choice for detection of large rearrangements and copy number variations. Using this technique, genomic rearrangements were detected in dysferlin (*DYSF*, MIM #603009) and calpain-3 (*CAPN3*, MIM # 114240) genes, implicated in two forms of limb-girdle muscular dystrophy types 2B and 2A, respectively [5].

Indirect diagnostic techniques such as monitoring the presence of a protein by Western blot [88] or enzymatic activity are other available approaches; however, they do not provide a specific knowledge of the mutation.

Massively parallel sequencing technology

As mentioned above, genetic and clinical heterogeneity, unspecific clinical features, implication of large genes and the necessity to apply multiple techniques are the main drawbacks in routine molecular diagnostic laboratories. Massively parallel sequencing (MPS), also called next generation (NGS) or high-throughput sequencing (HTS), allows to sequence target genes and regions, exome or whole genome, and is revolutionizing the molecular diagnosis as it now permits large-scale parallel sequencing and can be used to detect several types of mutations. The exome represents about 1–2 % of the genome but harbor 85 % of disease-causing mutations [15].

Template preparation and barcoding

The massively parallel sequencing technology is based on a combination of template preparation, sequencing, imaging/recording and data analysis [61]. DNA and library preparations are the first steps. Two to twenty microgram of high quality genomic DNA is randomly sheared into smaller size molecules by sonication or nebulization. Several DNA can also be pooled and sequenced together. The barcoding and pooling step can be done either before or after capture of specific targeted sequences, using a unique DNA tag sequence per sample [18]. In this way, it is possible to sequence a few genes in many patients or many genes from a few patients.

Targeted sequence enrichment

There is no enrichment step for whole genome sequencing [94]; however, if the aim is to sequence a portion of

Author's personal copy

genomic DNA which can be the protein-coding part of the genome (exome) or regions of interest such as selected genes or a genomic region linked to a disease, such targeted sequencing includes the enrichment of the target sequences.

This enrichment or capture can be done on microarray or in solution [60]. In solid-phase enrichment, high-density primers targeting the sequences of interest are covalently attached to the slide array [25], while in solution-phase enrichment, primers are generated on beads [20]. Several solutions such as Illumina, Agilent and Nimblegen capture kits can be used; they have technical differences such as using RNA baits (Agilent) or DNA baits (Illumina and Nimblegen) with almost similar performance [17]. Other enrichment methods include RainStorm microdroplet-based technology from Raindance technologies which is a multiplex PCR method using microdroplets containing PCR components loaded on microfluidic chip to compartmentalize the PCRs by single primer pairs [87].

Sequencing

For some of the sequencing platforms such as Helicos BioSciences, single molecule template is used, whereas in others such as Illumina, SOLiD and Roche/454, clonally amplified DNA is required to detect the signal produced by the incorporation of nucleotides [57]. Thus, for sequencing platforms using single molecule templates, the amount of starting DNA is lower and there is no PCR amplification step that could create artificial mutations and AT or GC-rich amplification bias.

In all the MPS platforms, either templates, primers or polymerase enzyme are immobilized on a solid support before the sequencing reaction [61]. Sequencing and recording steps are different [54]. Illumina technology is based on clonally amplified templates coupled with cyclic reversible termination method with four fluorescent colors. First, one fluorescently modified nucleotide complementary to the template sequence is incorporated. After washing and imaging for detection of the incorporated nucleotide, a cleavage step removes the fluorescent dye and a novel incorporation step is performed. These steps are done in a cyclic manner, 72 or 100 times or more [9]. It is possible to sequence from both extremities of the DNA template (paired-end sequencing). HeliScope single-molecule sequencer of Helicos BioSciences works with one fluorescent color (Cy5) using a cyclic reversible termination approach [12]. SOLiD (Sequencing by Oligonucleotide Ligation and Detection) sequencer from Applied Biosystems is another MPS technology based on sequencing by ligation using DNA ligase and a cleavable two-base-encoded probe consisting of two nucleotides combined with a particular dye [91]. Roche/454 sequencing platform is based on pyrosequencing. In this sequencing by synthesis method, a pyrophosphate (PPi) is released after incorporation of one dNTP. This released pyrophosphate is converted into detectable light through a series of enzymatic reaction [80]. Ion Torrent commercializes semiconductor sequencing machines where the release of a proton after incorporation of a nucleotide leading to change in pH is detected by voltage change. If two nucleotides are incorporated, the voltage will be double [82]. For human whole genome sequencing, apart from the above mentioned sequencing platforms, Complete Genomics offers a non-commercialized solution. In that case, DNA nanoballs containing hundreds of copies of a short DNA fragment are sequenced using combinatorial probe anchor-ligation (cPAL) where fluorescent molecules are attached to each nucleotide by a ligase enzyme [23] (Table 1).

Data analysis

The final steps in massively parallel sequencing are genome alignment, variant calling and data analysis. The generated sequence reads are aligned to a human reference genome such as UCSC assembly hg18/NCBI 36 or hg19/GRCH 37, and the variations are detected using different programs including BWA [50, 89] for alignment and Samtools [51] for variant calling. For filtering and scoring the variants and detection of disease-causing mutations, there are several strategies depending on the mode of inheritance and number of affected/non-affected sequenced cases [29]. Sequencing several affected and eventually non-affected individuals from the same family improves the filtering; variations common in affected and absent in non-affected persons will be prioritized. Several public websites display polymorphisms and allele frequencies such as dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/), 1000 genomes (http://www.1000genomes.org/) or the Exome Variant Server, NHLBI Exome Sequencing (http://evs.gs.washington.edu/EVS/) which covers SNPs but no indels. Many softwares are available to filter sequence variants, such as ALAMUT software (Interactive Biosoftware, Rouen, France), Cartagenia benchlab (http://www.cartagenia.com/); SIMPLEX (http://simplex.i-med.ac.at) [27] or ANNOVAR (http://www.openbioinformatics.org/annovar/) [93], and most integrate tools to predict the pathogenicity of the variations on RNA splicing and protein function such as SIFT [65], Polyphen [2, 77] or NNSPLICE (Fig. 1).

Massively parallel sequencing for NMD diagnosis

In 2010, Lupski et al. [58] published the first paper using massively parallel sequencing technology in neuromuscular diseases, where they showed the result of whole genome sequencing of a patient with a recessive form of Charcot–

Author's personal copy

Table 1 Different massively parallel sequencing platforms and their principles

Sequencing machine	Company	How does it work	Read length	Website
HiSeq2000	Illumina	Cyclic reversible termination method with four fluorescent colors	2 × 100 bp	http://www.illumina.com/systems/hiseq_systems.ilmn
HeliScope Single Molecule Sequencer	Helicos BioSciences Corporation	Cyclic reversible termination with one fluorescent color	25–55 bp	http://www.helicosbio.com/
SOLiD v4	Applied Biosystems	Sequencing by ligation	50 + 35 bp/ 50 + 50 bp	http://www.appliedbiosystems.com/
454 GS FLX	Roche	Pyrosequencing	700 bp	http://www.454.com/
Ion Proton Sequencer	Ion Torrent/Life Technologies	Ion semiconductor sequencing	200 bp	http://www.iontorrent.com/
Complete Genomics' sequencing instrument	Complete Genomics	Combinatorial probe anchor-ligation (cPAL) technology	35-base mate pair reads	http://www.completegenomics.com/

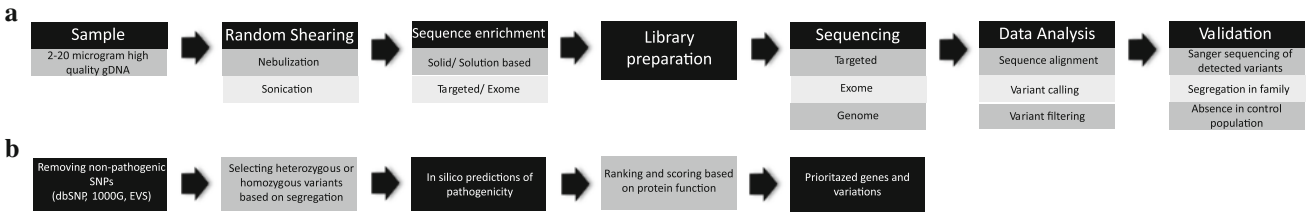


Fig. 1 Workflow of massively parallel sequencing and analysis. **a** 2–20 µg of genomic DNA is sheared randomly and targeted sequences are enriched following capture protocols in solid or liquid phases. Targeted genes/regions or exome can be enriched, or the whole genome can be sequenced. Amplified or single molecules are prepared for sequencing according to each platform. Output sequences are used for mapping to a reference genome, variation calling and filtering, using different algorithms and softwares. The last step is validation, where the interesting variations are confirmed by Sanger sequencing from the starting DNA, segregation of the variation in the family investigated, and a healthy control population checked. Sequencing of additional patients with similar phenotypes to identify more mutated patients with variations in the same gene, and functional studies could be done to confirm the implication of the gene or the pathogenicity of the variation if not previously known. **b** Different steps of filtering are shown. From the list of called variations, the known non-pathogenic variations are removed by comparison with dbSNP, 1,000 genomes and Exome Variant Server databases. Based on disease inheritance mode, homozygous or heterozygous changes are selected. Next, in silico prediction of pathogenecity and effect on splicing can be tested using different programs such as PolyPhen, SIFT or NNSPLICE. Scoring and ranking of the different variations/genes based on expression profile and function using different databases such as GENATLAS or Genecards provide a list of prioritized genes and variations

Marie–Tooth neuropathy. They prioritized sequence variants in genes known to be implicated in Charcot–Marie–Tooth neuropathies and found the compound heterozygous mutations p.Arg954X and p.Tyr169His in the *SH3TC2* gene as the causes of the neuropathy. Since then, several studies have been published using massively parallel sequencing for detection of causative mutations in different NMDs. Through exome sequencing, Montenegro et al. [62] found a missense variation, p.Val95Met, in the *GJB1* gene, a known gene in CMT neuropathies, in a family with CMT. Sanger sequencing confirmed this change and validated the complete co-segregation within the family.

In our recent publication, we showed the efficacy of massively parallel sequencing in molecular diagnosis of patients with different NMDs by capturing and sequencing 267 genes implicated in NMDs [92]. We could retrieve successfully all known mutations, detect and precise a large deletion linked to DMD, and identify novel disease-causing mutations in patients awaiting molecular diagnosis since more than 15 years. Other targeted sequencing focused on subsets of NMD genes. 25 patients with Duchenne or Becker muscular dystrophies (DMD/BMD) with known or unknown mutations were sequenced with the Illumina genome analyzer using a capture kit targeting the whole genomic sequence of muscular dystrophy-related genes after DNA barcoding [53]. The authors could detect disease-causing mutations in 24 patients out of 25 and concluded that this technology is useful for diagnosis of patients with DMD/BMD. Hoischen et al. [42] reported the validation of an array based sequence capture of seven genes and two loci related to the autosomal recessive form of ataxias, by sequencing these genes in five patients with known mutations and two unaffected persons using a one-quarter Roche GS FLX Titanium sequencing run. Other examples were dedicated to other specific disease classes [36, 45] (Table 2).

Author's personal copy

Such targeted parallel sequencing of all candidate genes is especially appropriate for disorders with high genetic heterogeneity like NMDs, and should ease the identification of allelic diseases, i.e. different diseases caused by mutations of the same gene. In addition, for large genes, such as *TTN*, which are difficult to fully test by conventional Sanger sequencing routinely even if known to be implicated in disorders, different studies have shown the effectiveness of massively parallel sequencing in variant detection of such a large gene [68, 72, 92]. Although there are some limitations in massively parallel sequencing as described below, important issues in genetic diagnosis laboratories such as cost and turnaround time can be resolved using massively parallel sequencing. Transfer of these technologies to diagnosis laboratories will benefit from the constantly dropping costs and increasing output. Reliable data can be produced if the depth of sequence coverage is high enough. In Fig. 2, an example of MPS achieved data is shown.

Possible applications of massively parallel sequencing in diagnosis

Diagnosis of affected individuals

Ideal strategies for diagnostic laboratories are methods that are simple with high accuracy and low error rate in a short run time; they should also be cost-effective with easy data analysis and interpretation [20]. Massively parallel sequencing is a useful alternative or complementary technique for molecular diagnosis. Routine use of massively parallel sequencing leads to a rapid screening and fast identification of mutations in rare genetic disorders through

Table 2 First reports using high-throughput sequencing for mutation and gene identification (rows 1–3), for neuromuscular diseases (row 4) and other recent examples using this strategy (rows 5–15)

Disease	Sequenced region	Sequencing machine	What was found	Publication year	Reference
Different disorders	Genome	Roche 454	Several putative mutations	2008	[94]
Freeman-sheldon syndrome (distal arthrogryposis type 2A)	Exome	Genome Analyzer IIx	Different mutations in *MYH3*	2009	[67]
Miller syndrome	Exome	Genome Analyzer IIx	Different mutations in *DHODH*	2010	[66]
Charcot–Marie–Tooth neuropathy	Genome	SOLiD	Compound heterozygous in *SH3TC2*	2010	[58]
Limb-girdle congenital myasthenic syndrome with tubular aggregates	Exome	Illumina HiSeq 2000 and Genome Analyzer IIx	Compound heterozygous in *DPAGT1*	2012	[7]
Hereditary myopathy with early respiratory failure	Exome	Illumina HiSeq 2000	Missense in *TTN*	2012	[69, 72]
Heterogeneous neuromuscular diseases	Targeted sequencing of 267 genes	Illumina Genome Analyzer IIx	Mutations in different genes detected in 12 patients	2012	[92]
Hereditary sensory autonomic neuropathy	Exome	Illumina HiSeq 2000	A deleterious mutation in *DST*	2012	[24]
Spinal muscular atrophy	Targeted sequencing of 73 genes in the 14q32 linkage interval	Illumina Genome Analyzer IIx	Missense in *DYNC1H1*	2012	[36]
Emery–Dreifuss muscular dystrophy	Exome	Illumina HiSeq 2000	Missense (homozygous) in *LMNA*	2012	[43]
Duchenne muscular dystrophy	Targeted sequencing of 79 exons of *DMD*	Illumina HiSeq 2000	Nonsense in *DMD*	2012	[55]
Nemaline myopathy	Targeted sequencing of 15 genes	SOLiD 4	Two missenses in *RYR1*	2012	[45]
Limb-girdle muscular dystrophy	Exome	Illumina HiSeq 2000	Missense in *DNAJB6*	2012	[37]
Genes associated with muscle disease and spastic paraplegia	Exome	Illumina Genome Analyzer IIx	Deleterious *CAPN3* mutation in myopathy	2012	[21]
Benign Samaritan myopathy	Exome	Illumina Genome Analyzer IIx	Allelic disease to RYR1 myopathies	2012	[11]

Author's personal copy

a

VariantID	Gene	TranscriptID	TranscriptLength	Chr	Start	End	Ref	Mut	HomHet	Coverage	ReadWithVar
X_149767058_DEL_4_1	MTM1	NM_000252.2	3421	X	149767058	149767061	AAAG	-	het	212.0	87.0
1_156848947_SNV_G_1	NTRK1	NM_002529.3	2638	1	156848947	156848947	T	G	het	52.0	16.0
12_863505_INS_3_1	WNK1	NM_001184985.1	11204	12	863505	863506	-	CTT	het	187.0	73.0

VariantID	VarType	CodingEffect	VarLocation	Exon	Intron	gNomen	cNomen	pNomen	rsID	rsValidation	hgmdID
X_149767058_DEL_4_1	DEL	Frameshift	exon	4	NA	g.149767058_149767061del	c.141_144del	p.Glu48Leufs*24	NA	NA	NA
1_156848947_SNV_G_1	SNV	Synonymous	exon	15	NA	g.156848947T>G	c.1839T>G	p.=	NA	NA	NA
12_863505_INS_3_1	INS	NA	intron	1	1	g.863506_863508dup	c.759+16_759+18dup	p.?	NA	NA	NA

b

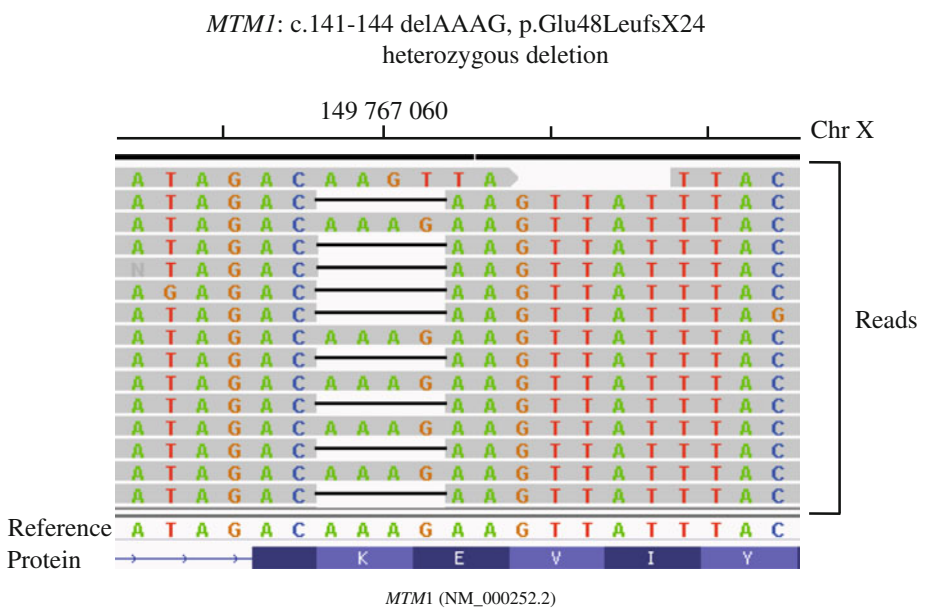


Fig. 2 An example of massively parallel sequencing data for a patient with myotubular myopathy. **a** The table shows three different variations: the *first row* is the disease-causing mutation which is a deletion of four nucleotides in the *MTM1* gene. The *second row* is a synonymous change and the *third* is an intronic insertion. **b** Deletion of four nucleotides at the beginning of exon 4 in the *MTM1* gene displayed with the integrative genomics viewer (IGV)

sequencing of either the exons or the genomic sequence of all genes or a subset of genes. Nowadays, with benchtop sequencers such as Ion Torrent PGM (Ion Torrent, Guilford, CT), MiSeq (Illumina Inc., San Diego, CA) and 454 GS Junior (454 Life Sciences, Roche, Branford, CT), these new technologies can be transferred easily to clinics for mutation identification in patients, carrier status determination and prenatal diagnosis. The cost of whole genome sequencing of a DNA without interpretation is about 6,500 USD and exome sequencing is offered for about 1,000 USD (an example: http://www.edgebio.com). High throughput screening at low cost and low complexity can be addressed by targeted sequencing rather than complete sequencing of exomes or genomes, combined with multiplexing of barcoded samples. The cost for this strategy depends on the number of mixed samples and the size of targeted regions and is generally less than 1,000 USD per sample. Moreover, targeted and exome sequencing are starting to be proposed on a routine diagnosis basis (for examples: http://www.bcm.edu/geneticlabs/ or http://genetics.emory.edu/egl/). Financing this cost depends on specific countries regulation and insurance policies.

Massively parallel sequencing will probably replace most of the genetic screening methods but will not substitute for clinical and histopathological investigations. While until now, clinical and pathological diagnoses were used to orient genetic screening, massively parallel sequencing may now be used on a first intention to better orient clinical tests that could be invasive, costly and necessitate patient travel. Although biopsies might be less needed for establishing the molecular diagnosis, they will be necessary for understanding the pathogenesis of a disease. Thus, massively parallel sequencing will promote a more efficient integrated diagnosis encompassing clinic, histopathology and molecular analyses.

Author's personal copy

A rapid and accurate molecular diagnosis will have important impacts on patients as it will improve disease management, may lead to inclusion into therapeutic trials, will help genetic counseling and will reduce further unhelpful investigations.

Prenatal diagnosis

This technology is also applicable to noninvasive prenatal diagnostic by massively parallel sequencing of fetal DNA present in the maternal plasma. The circulating cell-free fetal DNA is assessed using a set of differentially methylated markers [71] or by exome or genome sequencing compared to parents DNAs [26]. Currently, this approach is used for detection of severe monogenic disorders, fetal chromosomal aneuploidies and determination of blood groups [56]. Such approach will avoid the need for invasive and risky procedures such as chorionic villus sampling. Comprehensive diagnosis of any Mendelian disorders such as NMDs early in pregnancy might permit early termination of pregnancy in an ethically acceptable way. It can have a significant impact on the reproductive decision making and pregnancy management as it has been shown that families with increased risk of having a child with a severe disorder tend to have fewer children [78].

Carrier detection

The massively parallel sequencing approach might also apply to families without previous cases of severe genetic disorders. It would be technically possible to determine the carrier risks of individuals for any Mendelian disorders and predict for example, for which recessive diseases both parents are carriers of a heterozygous mutation [8]. Based on this knowledge, parents would have the possibility to test the fetus by sequencing the gene(s) with heterozygous mutations, or performing in vitro fertilization (IVF) and selecting non-affected zygotes. This approach will not predict de novo mutation, another source of disease-causing mutations.

Massively parallel sequencing limitations and improvements for clinical use

Although massively parallel sequencing is an appropriate alternative technique to use in diagnostic laboratories, some issues need to be addressed.

False negatives

One challenge in the use of massively parallel sequencing for diagnosis is the detection of different types of mutations, especially repeat expansions or structural variations. Some of the most common NMDs are due to repeat expansions; these mutations may be missed leading to false negative results. Increasing sequence coverage [40], improving bioinformatics algorithms and softwares and novel sequencing technologies may solve this problem in order to propose an exclusion diagnosis. Alternatively, a combination of different methods can be proposed. Detection of structural variations will benefit from whole genome sequencing. Another obstacle is the incomplete coverage of commercial exome capture libraries. Although new versions of capture kits are released consistently, none of them captures all coding parts of all genes [47]. This issue can be addressed by targeted sequencing of genes and regions of interest and improving the capturing process.

False positives

Another drawback is the high error rates in massively parallel sequencing compared to Sanger sequencing. Artificial mutations can be produced during templates amplification or sequencing, leading to false positive results. Thus, Sanger sequencing of interesting variants detected by massively parallel sequencing is an essential validation step which is increasing the cost and turnaround time. This issue can be addressed by improving capturing and sequencing approaches to increase variant coverage, and thus leads to achieve reliable data. In addition, better data filtering protocols can reduce the pool of false positives.

Volume of data

Massively parallel sequencing generates a high volume of data which becomes problematic for data management, analysis and storage in diagnostic laboratories [74]. As the genetic test results should be kept at least 5 years or even 10–20 years [85], substantial investment in infrastructure and informatics is needed. Cloud computing can be a solution for reducing the cost of expensive computing infrastructure [20, 84]. Importantly, decreasing costs in massively parallel sequencing outpaces the increase in calculation power and storage capacity of computers. As sequencing becomes cheaper than data storage of the corresponding sequence output, re-sequencing of a patient DNA might be more cost-effective than saving original data [38]. In other words, DNA is becoming the cheapest storage support.

Variants filtering and mutation identification

As a high number of variations are detected by massively parallel sequencing, it is difficult to distinguish between

Author's personal copy

individual, rare and non-pathogenic variations without clinical significance versus disease-causing mutations. Defining the complete list of polymorphisms in different populations will require sequencing a large part of the world populations [52]. A recent study hypothesized that 27 % of published mutations appear to be sequencing errors, common polymorphisms, or have a lack of pathogenicity evidence [8]. This issue can be addressed by further analysis and validation such as in silico predictions of pathogenicity, detection of mutations in the same gene in unrelated individuals with the same disorder and absence in a control population, co-segregation in affected families and finally functional studies [90]. Multiple criteria should be combined in order to prove the pathogenicity of the variation.

Incidental findings

With massively parallel sequencing, incidental findings are an issue and unsolicited/unexpected information can be problematic, especially for unreported diseases. Diagnostic laboratories should prepare informative, complete and precise consent and result reports to use massively parallel sequencing for diagnosis application. Guidelines should be established and implemented by national committees in order to determine whether the analysis and reports should be selective or complete and what is the best way to cope with the ethical issues [16, 31].

Trained people

Having enough trained people for data analysis and information transfer to patients will be a key point for the future. Specific trainings are needed for interpreting genetic data for rare and common diseases and eventually genetic predispositions, and how to transfer this information to patients. Ideally a team of bioinformaticians, scientists, geneticists and clinicians will collaborate together in order to provide an accurate and accessible results to patients. Development of robust, easy to use and practical softwares in the clinical setting and comprehensive mutations and polymorphisms databases are necessary. For a full understanding of genetic variations, considerable amount of genetic data from various populations should be gathered and interpreted. Recurrent reanalysis of the sequencing data will be needed while our understanding of genetic variations improves. Well-trained clinicians and pathologists in neuromuscular disorders and bioinformaticians will have crucial roles in the selection of patients and interpretation of the obtained data.

Conclusion

Although there are some limitations and challenges using massively parallel sequencing for diagnosis, this technology appears mature enough for a routine approach in clinics. As the cost of massively parallel sequencing declines consistently and the technology improves continually leading to obtaining faster and more accurate data, it is strongly anticipated that this recent technology will complement clinical and pathological investigations and will greatly improve diagnosis and management of Mendelian disorders.

Acknowledgments We thank Pr. Jean-Louis Mandel and Dr. Valérie Biancalana for discussions. This study was supported by the Institut National de la Santé et de la Recherche Médicale (INSERM), the Centre National de la Recherche Scientifique (CNRS), University of Strasbourg (UdS), Collège de France, Agence Nationale de la Recherche (ANR-11-BSV1-026), Muscular Dystrophy Association (186985), and the Myotubular Trust.

References

1. Abbs S, Bobrow M (1992) Analysis of quantitative PCR for the diagnosis of deletion and duplication carriers in the dystrophin gene. J Med Genet 29(3):191–196
2. Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR (2010) A method and server for predicting damaging missense mutations. Nat Methods 7(4):248–249. doi:10.1038/nmeth0410-248
3. Alias L, Bernal S, Fuentes-Prior P, Barcelo MJ, Also E, Martinez-Hernandez R, Rodriguez-Alvarez FJ, Martin Y, Aller E, Grau E, Pecina A, Antinolo G, Galan E, Rosa AL, Fernandez-Burriel M, Borrego S, Millan JM, Hernandez-Chico C, Baiget M, Tizzano EF (2009) Mutation update of spinal muscular atrophy in Spain: molecular characterization of 745 unrelated patients and identification of four novel mutations in the SMN1 gene. Hum Genet 125(1):29–39. doi:10.1007/s00439-008-0598-1
4. Andersen PS, Havndrup O, Hougs L, Sorensen KM, Jensen M, Larsen LA, Hedley P, Thomsen AR, Moolman-Smook J, Christiansen M, Bundgaard H (2009) Diagnostic yield, interpretation, and clinical utility of mutation screening of sarcomere encoding genes in Danish hypertrophic cardiomyopathy patients and relatives. Hum Mutat 30(3):363–370. doi:10.1002/humu.20862
5. Bartoli M, Negre P, Wein N, Bourgeois P, Pecheux C, Levy N, Krahn M (2012) Validation of comparative genomic hybridization arrays for the detection of genomic rearrangements of the calpain-3 and dysferlin genes. Clin Genet 81(1):99–101. doi:10.1111/j.1399-0004.2011.01708.x
6. Beggs AH, Koenig M, Boyce FM, Kunkel LM (1990) Detection of 98 % of DMD/BMD gene deletions by polymerase chain reaction. Hum Genet 86(1):45–48
7. Belaya K, Finlayson S, Slater CR, Cossins J, Liu WW, Maxwell S, McGowan SJ, Maslau S, Twigg SR, Walls TJ, Pascual Pascual SI, Palace J, Beeson D (2012) Mutations in DPAGT1 cause a limb-girdle congenital myasthenic syndrome with tubular aggregates. Am J Hum Genet 91(1):193–201. doi:10.1016/j.ajhg.2012.05.022
8. Bell CJ, Dinwiddie DL, Miller NA, Hateley SL, Ganusova EE, Mudge J, Langley RJ, Zhang L, Lee CC, Schilkey FD, Sheth V, Woodward JE, Peckham HE, Schroth GP, Kim RW, Kingsmore

Author's personal copy

SF (2011) Carrier testing for severe childhood recessive diseases by next-generation sequencing. Sci Transl Med 3(65):65ra64. doi:10.1126/scitranslmed.3001756

9. Bentley DR, Balasubramanian S, Swerdlow HP, Smith GP, Milton J, Brown CG, Hall KP, Evers DJ, Barnes CL, Bignell HR, Boutell JM, Bryant J, Carter RJ, Keira Cheetham R, Cox AJ, Ellis DJ, Flatbush MR, Gormley NA, Humphray SJ, Irving LJ, Karbelashvili MS, Kirk SM, Li H, Liu X, Maisinger KS, Murray LJ, Obradovic B, Ost T, Parkinson ML, Pratt MR, Rasolonjatovo IM, Reed MT, Rigatti R, Rodighiero C, Ross MT, Sabot A, Sankar SV, Scally A, Schroth GP, Smith ME, Smith VP, Spiridou A, Torrance PE, Tzonev SS, Vermaas EH, Walter K, Wu X, Zhang L, Alam MD, Anastasi C, Aniebo IC, Bailey DM, Bancarz IR, Banerjee S, Barbour SG, Baybayan PA, Benoit VA, Benson KF, Bevis C, Black PJ, Boodhun A, Brennan JS, Bridgham JA, Brown RC, Brown AA, Buermann DH, Bundu AA, Burrows JC, Carter NP, Castillo N, Chiara ECM, Chang S, Neil Cooley R, Crake NR, Dada OO, Diakoumakos KD, Dominguez-Fernandez B, Earnshaw DJ, Egbujor UC, Elmore DW, Etchin SS, Ewan MR, Fedurco M, Fraser LJ, Fuentes Fajardo KV, Scott Furey W, George D, Gietzen KJ, Goddard CP, Golda GS, Granieri PA, Green DE, Gustafson DL, Hansen NF, Harnish K, Haudenschild CD, Heyer NI, Hims MM, Ho JT, Horgan AM, Hoschler K, Hurwitz S, Ivanov DV, Johnson MQ, James T, Huw Jones TA, Kang GD, Kerelska TH, Kersey AD, Khrebtukova I, Kindwall AP, Kingsbury Z, Kokko-Gonzales PI, Kumar A, Laurent MA, Lawley CT, Lee SE, Lee X, Liao AK, Loch JA, Lok M, Luo S, Mammen RM, Martin JW, McCauley PG, McNitt P, Mehta P, Moon KW, Mullens JW, Newington T, Ning Z, Ling Ng B, Novo SM, O'Neill MJ, Osborne MA, Osnowski A, Ostadan O, Paraschos LL, Pickering L, Pike AC, Chris Pinkard D, Pliskin DP, Podhasky J, Quijano VJ, Raczy C, Rae VH, Rawlings SR, Chiva Rodriguez A, Roe PM, Rogers J, Rogert Bacigalupo MC, Romanov N, Romieu A, Roth RK, Rourke NJ, Ruediger ST, Rusman E, Sanches-Kuiper RM, Schenker MR, Seoane JM, Shaw RJ, Shiver MK, Short SW, Sizto NL, Sluis JP, Smith MA, Ernest Sohna Sohna J, Spence EJ, Stevens K, Sutton N, Szajkowski L, Tregidgo CL, Turcatti G, Vandevondele S, Verhovsky Y, Virk SM, Wakelin S, Walcott GC, Wang J, Worsley GJ, Yan J, Yau L, Zuerlein M, Mullikin JC, Hurles ME, McCooke NJ, West JS, Oaks FL, Lundberg PL, Klenerman D, Durbin R, Smith AJ (2008) Accurate whole human genome sequencing using reversible terminator chemistry. Nature 456(7218):53–59. doi:10.1038/nature07517
10. Blauw HM, Barnes CP, van Vught PW, van Rheenen W, Verheul M, Cuppen E, Veldink JH, van den Berg LH (2012) SMN1 gene duplications are associated with sporadic ALS. Neurology 78(11):776–780. doi:10.1212/WNL.0b013e318249f697
11. Bohm J, Leshinsky-Silver E, Vassilopoulos S, Le Gras S, Lerman-Sagie T, Ginzberg M, Jost B, Lev D, Laporte J (2012) Samaritan myopathy, an ultimately benign congenital myopathy, is caused by a RYR1 mutation. Acta Neuropathol 124(4):575–581. doi:10.1007/s00401-012-1007-3
12. Braslavsky I, Hebert B, Kartalov E, Quake SR (2003) Sequence information can be obtained from single DNA molecules. Proc Natl Acad Sci USA 100(7):3960–3964. doi:10.1073/pnas.0230489100
13. Chamberlain JS, Gibbs RA, Ranier JE, Nguyen PN, Caskey CT (1988) Deletion screening of the Duchenne muscular dystrophy locus via multiplex DNA amplification. Nucleic Acids Res 16(23):11141–11156
14. Chen YZ, Bennett CL, Huynh HM, Blair IP, Puls I, Irobi J, Dierick I, Abel A, Kennerson ML, Rabin BA, Nicholson GA, Auer-Grumbach M, Wagner K, De Jonghe P, Griffin JW, Fischbeck KH, Timmerman V, Cornblath DR, Chance PF (2004) DNA/RNA helicase gene mutations in a form of juvenile amyotrophic lateral sclerosis (ALS4). Am J Hum Genet 74(6):1128–1135. doi:10.1086/421054
15. Choi M, Scholl UI, Ji W, Liu T, Tikhonova IR, Zumbo P, Nayir A, Bakkaloglu A, Ozen S, Sanjad S, Nelson-Williams C, Farhi A, Mane S, Lifton RP (2009) Genetic diagnosis by whole exome capture and massively parallel DNA sequencing. Proc Natl Acad Sci USA 106(45):19096–19101. doi:10.1073/pnas.0910672106
16. Christenhusz GM, Devriendt K, Dierickx K (2012) To tell or not to tell? A systematic review of ethical reflections on incidental findings arising in genetics contexts. Eur J Hum Genet. doi:10.1038/ejhg.2012.130
17. Clark MJ, Chen R, Lam HY, Karczewski KJ, Euskirchen G, Butte AJ, Snyder M (2011) Performance comparison of exome DNA sequencing technologies. Nat Biotechnol 29(10):908–914. doi:10.1038/nbt.1975
18. Craig DW, Pearson JV, Szelinger S, Sekar A, Redman M, Corneveaux JJ, Pawlowski TL, Laub T, Nunn G, Stephan DA, Homer N, Huentelman MJ (2008) Identification of genetic variants using bar-coded multiplexed sequencing. Nat Methods 5(10):887–893. doi:10.1038/nmeth.1251
19. DeJesus-Hernandez M, Mackenzie IR, Boeve BF, Boxer AL, Baker M, Rutherford NJ, Nicholson AM, Finch NA, Flynn H, Adamson J, Kouri N, Wojtas A, Sengdy P, Hsiung GY, Karydas A, Seeley WW, Josephs KA, Coppola G, Geschwind DH, Wszolek ZK, Feldman H, Knopman DS, Petersen RC, Miller BL, Dickson DW, Boylan KB, Graff-Radford NR, Rademakers R (2011) Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron 72(2):245–256. doi:10.1016/j.neuron.2011.09.011
20. Desai AN, Jere A (2012) Next-generation sequencing: ready for the clinics? Clin Genet 81(6):503–510. doi:10.1111/j.1399-0004.2012.01865.x
21. Dias C, Sincan M, Cherukuri PF, Rupps R, Huang Y, Briemberg H, Selby K, Mullikin JC, Markello TC, Adams DR, Gahl WA, Boerkoel CF (2012) An analysis of exome sequencing for diagnostic testing of the genes associated with muscle disease and spastic paraplegia. Hum Mutat 33(4):614–626. doi:10.1002/humu.22032
22. Donner K, Sandbacka M, Lehtokari VL, Wallgren-Pettersson C, Pelin K (2004) Complete genomic structure of the human nebulin gene and identification of alternatively spliced transcripts. Eur J Hum Genet 12(9):744–751. doi:10.1038/sj.ejhg.5201242
23. Drmanac R, Sparks AB, Callow MJ, Halpern AL, Burns NL, Kermani BG, Carnevali P, Nazarenko I, Nilsen GB, Yeung G, Dahl F, Fernandez A, Staker B, Pant KP, Baccash J, Borcherding AP, Brownley A, Cedeno R, Chen L, Chernikoff D, Cheung A, Chirita R, Curson B, Ebert JC, Hacker CR, Hartlage R, Hauser B, Huang S, Jiang Y, Karpinchyk V, Koenig M, Kong C, Landers T, Le C, Liu J, McBride CE, Morenzoni M, Morey RE, Mutch K, Perazich H, Perry K, Peters BA, Peterson J, Pethiyagoda CL, Pothuraju K, Richter C, Rosenbaum AM, Roy S, Shafto J, Sharanhovich U, Shannon KW, Sheppy CG, Sun M, Thakuria JV, Tran A, Vu D, Zaranek AW, Wu X, Drmanac S, Oliphant AR, Banyai WC, Martin B, Ballinger DG, Church GM, Reid CA (2010) Human genome sequencing using unchained base reads on self-assembling DNA nanoarrays. Science 327(5961):78–81. doi:10.1126/science.1181498
24. Edvardson S, Cinnamon Y, Jalas C, Shaag A, Maayan C, Axelrod FB, Elpeleg O (2012) Hereditary sensory autonomic neuropathy caused by a mutation in dystonin. Ann Neurol 71(4):569–572. doi:10.1002/ana.23524
25. Emery AE (1991) Population frequencies of inherited neuromuscular diseases–a world survey. Neuromuscul Disord 1(1):19–29
26. Fan HC, Gu W, Wang J, Blumenfeld YJ, El-Sayed YY, Quake SR (2012) Non-invasive prenatal measurement of the fetal genome. Nature 487(7407):320–324. doi:10.1038/nature11251
27. Fischer M, Snajder R, Pabinger S, Dander A, Schossig A, Zschocke J, Trajanoski Z, Stocker G (2012) SIMPLEX: cloud-

Author's personal copy

enabled pipeline for the comprehensive analysis of exome sequencing data. PLoS ONE 7(8):e41948. doi:10.1371/journal.pone.0041948
28. Gazzerro E, Bonetto A, Minetti C (2011) Caveolinopathies: translational implications of caveolin-3 in skeletal and cardiac muscle disorders. Handb Clin Neurol 101:135–142. doi:10.1016/B978-0-08-045031-5.00010-4
29. Gilissen C, Hoischen A, Brunner HG, Veltman JA (2012) Disease gene identification strategies for exome sequencing. Eur J Hum Genet 20(5):490–497. doi:10.1038/ejhg.2011.258
30. Gitcho MA, Bigio EH, Mishra M, Johnson N, Weintraub S, Mesulam M, Rademakers R, Chakraverty S, Cruchaga C, Morris JC, Goate AM, Cairns NJ (2009) TARDBP 3′-UTR variant in autopsy-confirmed frontotemporal lobar degeneration with TDP-43 proteinopathy. Acta Neuropathol 118(5):633–645. doi:10.1007/s00401-009-0571-7
31. Godard B, Kaariainen H, Kristoffersson U, Tranebjaerg L, Coviello D, Ayme S (2003) Provision of genetic services in Europe: current practices and issues. Eur J Hum Genet 11(Suppl 2):S13–S48. doi:10.1038/sj.ejhg.5201111
32. Goto K, Nishino I, Hayashi YK (2006) Rapid and accurate diagnosis of facioscapulohumeral muscular dystrophy. Neuromuscul Disord 16(4):256–261. doi:10.1016/j.nmd.2006.01.008
33. Greenway MJ, Alexander MD, Ennis S, Traynor BJ, Corr B, Frost E, Green A, Hardiman O (2004) A novel candidate region for ALS on chromosome 14q11.2. Neurology 63(10):1936–1938. pii:63/10/1936
34. Guis S, Figarella-Branger D, Monnier N, Bendahan D, Kozak-Ribbens G, Mattei JP, Lunardi J, Cozzone PJ, Pellissier JF (2004) Multiminicore disease in a family susceptible to malignant hyperthermia: histology, in vitro contracture tests, and genetic characterization. Arch Neurol 61(1):106–113. doi:10.1001/archneur.61.1.106
35. Hadano S, Hand CK, Osuga H, Yanagisawa Y, Otomo A, Devon RS, Miyamoto N, Showguchi-Miyata J, Okada Y, Singaraja R, Figlewicz DA, Kwiatkowski T, Hosler BA, Sagie T, Skaug J, Nasir J, Brown RH Jr, Scherer SW, Rouleau GA, Hayden MR, Ikeda JE (2001) A gene encoding a putative GTPase regulator is mutated in familial amyotrophic lateral sclerosis 2. Nat Genet 29(2):166–173. doi:10.1038/ng1001-166
36. Harms MB, Ori-McKenney KM, Scoto M, Tuck EP, Bell S, Ma D, Masi S, Allred P, Al-Lozi M, Reilly MM, Miller LJ, Jani-Acsadi A, Pestronk A, Shy ME, Muntoni F, Vallee RB, Baloh RH (2012) Mutations in the tail domain of DYNC1H1 cause dominant spinal muscular atrophy. Neurology 78(22):1714–1720. doi:10.1212/WNL.0b013e3182556c05
37. Harms MB, Sommerville RB, Allred P, Bell S, Ma D, Cooper P, Lopate G, Pestronk A, Weihl CC, Baloh RH (2012) Exome sequencing reveals DNAJB6 mutations in dominantly-inherited myopathy. Ann Neurol 71(3):407–416. doi:10.1002/ana.22683
38. Hastings R, de Wert G, Fowler B, Krawczak M, Vermeulen E, Bakker E, Borry P, Dondorp W, Nijsingh N, Barton D, Schmidtke J, van El CG, Vermeesch J, Stol Y, Carmen Howard H, Cornel MC (2012) The changing landscape of genetic testing and its impact on clinical and laboratory services and research in Europe. Eur J Hum Genet 20(9):911–916. doi:10.1038/ejhg.2012.56
39. Hayward C, Colville S, Swingler RJ, Brock DJ (1999) Molecular genetic analysis of the APEX nuclease gene in amyotrophic lateral sclerosis. Neurology 52(9):1899–1901
40. Herdewyn S, Zhao H, Moisse M, Race V, Matthijs G, Reumers J, Kusters B, Schelhaas HJ, van den Berg LH, Goris A, Robberecht W, Lambrechts D, Van Damme P (2012) Whole-genome sequencing reveals a coding non-pathogenic variant tagging a non-coding pathogenic hexanucleotide repeat expansion in C9orf72 as cause of amyotrophic lateral sclerosis. Hum Mol Genet 21(11):2412–2419. doi:10.1093/hmg/dds055
41. Hill M (2003) The neuromuscular junction disorders. J Neurol Neurosurg Psychiatry 74(Suppl 2):ii32–ii37
42. Hoischen A, Gilissen C, Arts P, Wieskamp N, van der Vliet W, Vermeer S, Steehouwer M, de Vries P, Meijer R, Seiqueros J, Knoers NV, Buckley MF, Scheffer H, Veltman JA (2010) Massively parallel sequencing of ataxia genes after array-based enrichment. Hum Mutat 31(4):494–499. doi:10.1002/humu.21221
43. Jimenez-Escrig A, Gobernado I, Garcia-Villanueva M, Sanchez-Herranz A (2012) Autosomal recessive Emery-Dreifuss muscular dystrophy caused by a novel mutation (R225Q) in the lamin A/C gene identified by exome sequencing. Muscle Nerve 45(4):605–610. doi:10.1002/mus.22324
44. Kaplan JC (2011) The 2012 version of the gene table of monogenic neuromuscular disorders. Neuromuscul Disord 21(12):833–861
45. Kondo E, Nishimura T, Kosho T, Inaba Y, Mitsuhashi S, Ishida T, Baba A, Koike K, Nishino I, Nonaka I, Furukawa T, Saito K (2012) Recessive RYR1 mutations in a patient with severe congenital nemaline myopathy with ophthalomoplegia identified through massively parallel sequencing. Am J Med Genet A 158A(4):772–778. doi:10.1002/ajmg.a.35243
46. Krysa W, Rajkiewicz M, Sulek A (2012) Rapid detection of large expansions in progressive myoclonus epilepsy type 1, myotonic dystrophy type 2 and spinocerebellar ataxia type 8. Neurol Neurochir Pol 46(2):113–120. doi:18490
47. Ku CS, Cooper DN, Polychronakos C, Naidoo N, Wu M, Soong R (2012) Exome sequencing: dual role as a discovery and diagnostic tool. Ann Neurol 71(1):5–14. doi:10.1002/ana.22647
48. Kwiatkowski TJ Jr, Bosco DA, Leclerc AL, Tamrazian E, Vanderburg CR, Russ C, Davis A, Gilchrist J, Kasarskis EJ, Munsat T, Valdmanis P, Rouleau GA, Hosler BA, Cortelli P, de Jong PJ, Yoshinaga Y, Haines JL, Pericak-Vance MA, Yan J, Ticozzi N, Siddique T, McKenna-Yasek D, Sapp PC, Horvitz HR, Landers JE, Brown RH Jr (2009) Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. Science 323(5918):1205–1208. doi:10.1126/science.1166066
49. Laing NG (2012) Genetics of neuromuscular disorders. Crit Rev Clin Lab Sci 49(2):33–48. doi:10.3109/10408363.2012.658906
50. Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25(14):1754–1760. doi:10.1093/bioinformatics/btp324
51. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R (2009) The sequence alignment/map format and SAMtools. Bioinformatics 25(16):2078–2079. doi:10.1093/bioinformatics/btp352
52. Li Y, Vinckenbosch N, Tian G, Huerta-Sanchez E, Jiang T, Jiang H, Albrechtsen A, Andersen G, Cao H, Korneliussen T, Grarup N, Guo Y, Hellman I, Jin X, Li Q, Liu J, Liu X, Sparso T, Tang M, Wu H, Wu R, Yu C, Zheng H, Astrup A, Bolund L, Holmkvist J, Jorgensen T, Kristiansen K, Schmitz O, Schwartz TW, Zhang X, Li R, Yang H, Wang J, Hansen T, Pedersen O, Nielsen R (2010) Resequencing of 200 human exomes identifies an excess of low-frequency non-synonymous coding variants. Nat Genet 42(11):969–972. doi:10.1038/ng.680
53. Lim BC, Lee S, Shin JY, Kim JI, Hwang H, Kim KJ, Hwang YS, Seo JS, Chae JH (2011) Genetic diagnosis of Duchenne and Becker muscular dystrophy using next-generation sequencing technology: comprehensive mutational search in a single platform. J Med Genet 48(11):731–736. doi:10.1136/jmedgenet-2011-100133
54. Liu L, Li Y, Li S, Hu N, He Y, Pong R, Lin D, Lu L, Law M (2012) Comparison of next-generation sequencing systems. J Biomed Biotechnol 2012:251364. doi:10.1155/2012/251364
55. Liu MJ, Xie M, Mao J, Li H, Yan WH, Chen Y (2012) Application of next-generation sequencing technology for genetic diagnosis of Duchenne muscular dystrophy. Zhonghua Yi Xue Yi

Author's personal copy

Chuan Xue Za Zhi 29(3):249–254. doi:10.3760/cma.j.issn.1003-9406.2012.03.001
56. Lo YM, Chiu RW (2012) Genomic analysis of fetal nucleic acids in maternal blood. Annu Rev Genomics Hum Genet. doi:10.1146/annurev-genom-090711-163806
57. Loman NJ, Misra RV, Dallman TJ, Constantinidou C, Gharbia SE, Wain J, Pallen MJ (2012) Performance comparison of benchtop high-throughput sequencing platforms. Nat Biotechnol 30(5):434–439. doi:10.1038/nbt.2198
58. Lupski JR, Reid JG, Gonzaga-Jauregui C, Rio Deiros D, Chen DC, Nazareth L, Bainbridge M, Dinh H, Jing C, Wheeler DA, McGuire AL, Zhang F, Stankiewicz P, Halperin JJ, Yang C, Gehman C, Guo D, Irikat RK, Tom W, Fantin NJ, Muzny DM, Gibbs RA (2010) Whole-genome sequencing in a patient with Charcot–Marie–Tooth neuropathy. N Engl J Med 362(13):1181–1191. doi:10.1056/NEJMoa0908094
59. Lynch PJ, Krivosic-Horber R, Reyford H, Monnier N, Quane K, Adnet P, Haudecoeur G, Krivosic I, McCarthy T, Lunardi J (1997) Identification of heterozygous and homozygous individuals with the novel RYR1 mutation Cys35Arg in a large kindred. Anesthesiology 86(3):620–626
60. Mamanova L, Coffey AJ, Scott CE, Kozarewa I, Turner EH, Kumar A, Howard E, Shendure J, Turner DJ (2010) Target-enrichment strategies for next-generation sequencing. Nat Methods 7(2):111–118. doi:10.1038/nmeth.1419
61. Metzker ML (2010) Sequencing technologies - the next generation. Nat Rev Genet 11(1):31–46. doi:10.1038/nrg2626
62. Montenegro G, Powell E, Huang J, Speziani F, Edwards YJ, Beecham G, Hulme W, Siskind C, Vance J, Shy M, Zuchner S (2011) Exome sequencing allows for rapid gene identification in a Charcot–Marie–Tooth family. Ann Neurol 69(3):464–470. doi:10.1002/ana.22235
63. Muntoni F, Torelli S, Ferlini A (2003) Dystrophin and mutations: one gene, several proteins, multiple phenotypes. Lancet Neurol 2(12):731–740 pii:S1474442203005854
64. Nance JR, Dowling JJ, Gibbs EM, Bonnemann CG (2012) Congenital myopathies: an update. Curr Neurol Neurosci Rep 12(2):165–174. doi:10.1007/s11910-012-0255-x
65. Ng PC, Henikoff S (2003) SIFT: predicting amino acid changes that affect protein function. Nucleic Acids Res 31(13):3812–3814
66. Ng SB, Buckingham KJ, Lee C, Bigham AW, Tabor HK, Dent KM, Huff CD, Shannon PT, Jabs EW, Nickerson DA, Shendure J, Bamshad MJ (2010) Exome sequencing identifies the cause of a Mendelian disorder. Nat Genet 42(1):30–35. doi:10.1038/ng.499
67. Ng SB, Turner EH, Robertson PD, Flygare SD, Bigham AW, Lee C, Shaffer T, Wong M, Bhattacharjee A, Eichler EE, Bamshad M, Nickerson DA, Shendure J (2009) Targeted capture and massively parallel sequencing of 12 human exomes. Nature 461(7261):272–276. doi:10.1038/nature08250
68. Nowak KJ (2012) Trusting new age weapons to tackle titin. Brain 135(Pt 6):1665–1667. doi:10.1093/brain/aws123
69. Ohlsson M, Hedberg C, Bradvik B, Lindberg C, Tajsharghi H, Danielsson O, Melberg A, Udd B, Martinsson T, Oldfors A (2012) Hereditary myopathy with early respiratory failure associated with a mutation in A-band titin. Brain 135(Pt 6):1682–1694. doi:10.1093/brain/aws103
70. Oudet C, Hanauer A, Clemens P, Caskey T, Mandel JL (1992) Two hot spots of recombination in the DMD gene correlate with the deletion prone regions. Hum Mol Genet 1(8):599–603
71. Palomaki GE, Deciu C, Kloza EM, Lambert-Messerlian GM, Haddow JE, Neveux LM, Ehrich M, van den Boom D, Bombard AT, Grody WW, Nelson SF, Canick JA (2012) DNA sequencing of maternal plasma reliably identifies trisomy 18 and trisomy 13 as well as Down syndrome: an international collaborative study. Genet Med 14(3):296–305. doi:10.1038/gim.2011.73
72. Pfeffer G, Elliott HR, Griffin H, Barresi R, Miller J, Marsh J, Evila A, Vihola A, Hackman P, Straub V, Dick DJ, Horvath R, Santibanez-Koref M, Udd B, Chinnery PF (2012) Titin mutation segregates with hereditary myopathy with early respiratory failure. Brain 135(Pt 6):1695–1713. doi:10.1093/brain/aws102
73. Phillips MS, Fujii J, Khanna VK, DeLeon S, Yokobata K, de Jong PJ, MacLennan DH (1996) The structural organization of the human skeletal muscle ryanodine receptor (RYR1) gene. Genomics 34(1):24–41. doi:10.1006/geno.1996.0238
74. Pop M, Salzberg SL (2008) Bioinformatics challenges of new sequencing technology. Trends Genet 24(3):142–149. doi:10.1016/j.tig.2007.12.006
75. Prior TW (2010) Perspectives and diagnostic considerations in spinal muscular atrophy. Genet Med 12(3):145–152. doi:10.1097/GIM.0b013e3181c5e713
76. Prior TW, Bridgeman SJ (2005) Experience and strategy for the molecular testing of Duchenne muscular dystrophy. J Mol Diagn 7(3):317–326. doi:10.1016/S1525-1578(10)60560-0
77. Ramensky V, Bork P, Sunyaev S (2002) Human non-synonymous SNPs: server and survey. Nucleic Acids Res 30(17):3894–3900
78. Raymond FL, Whittaker J, Jenkins L, Lench N, Chitty LS (2010) Molecular prenatal diagnosis: the impact of modern technologies. Prenat Diagn 30(7):674–681. doi:10.1002/pd.2575
79. Renton AE, Majounie E, Waite A, Simon-Sanchez J, Rollinson S, Gibbs JR, Schymick JC, Laaksovirta H, van Swieten JC, Myllykangas L, Kalimo H, Paetau A, Abramzon Y, Remes AM, Kaganovich A, Scholz SW, Duckworth J, Ding J, Harmer DW, Hernandez DG, Johnson JO, Mok K, Ryten M, Trabzuni D, Guerreiro RJ, Orrell RW, Neal J, Murray A, Pearson J, Jansen IE, Sondervan D, Seelaar H, Blake D, Young K, Halliwell N, Callister JB, Toulson G, Richardson A, Gerhard A, Snowden J, Mann D, Neary D, Nalls MA, Peuralinna T, Jansson L, Isoviita VM, Kaivorinne AL, Holtta-Vuori M, Ikonen E, Sulkava R, Benatar M, Wuu J, Chio A, Restagno G, Borghero G, Sabatelli M, Heckerman D, Rogaeva E, Zinman L, Rothstein JD, Sendtner M, Drepper C, Eichler EE, Alkan C, Abdullaev Z, Pack SD, Dutra A, Pak E, Hardy J, Singleton A, Williams NM, Heutink P, Pickering-Brown S, Morris HR, Tienari PJ, Traynor BJ (2011) A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron 72(2):257–268. doi:10.1016/j.neuron.2011.09.010
80. Ronaghi M, Karamohamed S, Pettersson B, Uhlen M, Nyren P (1996) Real-time DNA sequencing using detection of pyrophosphate release. Anal Biochem 242(1):84–89. doi:10.1006/abio.1996.0432
81. Rosen DR, Siddique T, Patterson D, Figlewicz DA, Sapp P, Hentati A, Donaldson D, Goto J, O'Regan JP, Deng HX et al (1993) Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 362(6415):59–62. doi:10.1038/362059a0
82. Rothberg JM, Hinz W, Rearick TM, Schultz J, Mileski W, Davey M, Leamon JH, Johnson K, Milgrew MJ, Edwards M, Hoon J, Simons JF, Marran D, Myers JW, Davidson JF, Branting A, Nobile JR, Puc BP, Light D, Clark TA, Huber M, Branciforte JT, Stoner IB, Cawley SE, Lyons M, Fu Y, Homer N, Sedova M, Miao X, Reed B, Sabina J, Feierstein E, Schorn M, Alanjary M, Dimalanta E, Dressman D, Kasinskas R, Sokolsky T, Fidanza JA, Namsaraev E, McKernan KJ, Williams A, Roth GT, Bustillo J (2011) An integrated semiconductor device enabling non-optical genome sequencing. Nature 475(7356):348–352. doi:10.1038/nature10242
83. Saporta AS, Sottile SL, Miller LJ, Feely SM, Siskind CE, Shy ME (2011) Charcot–Marie–Tooth disease subtypes and genetic testing strategies. Ann Neurol 69(1):22–33. doi:10.1002/ana.22166

Author's personal copy

84. Sboner A, Mu XJ, Greenbaum D, Auerbach RK, Gerstein MB (2011) The real cost of sequencing: higher than you think! Genome Biol 12(8):125. doi:10.1186/gb-2011-12-8-125
85. Schwartz MK (1999) Genetic testing and the clinical laboratory improvement amendments of 1988: present and future. Clin Chem 45(5):739–745
86. Tennyson CN, Klamut HJ, Worton RG (1995) The human dystrophin gene requires 16 hours to be transcribed and is cotranscriptionally spliced. Nat Genet 9(2):184–190. doi:10.1038/ng0295-184
87. Tewhey R, Warner JB, Nakano M, Libby B, Medkova M, David PH, Kotsopoulos SK, Samuels ML, Hutchison JB, Larson JW, Topol EJ, Weiner MP, Harismendy O, Olson J, Link DR, Frazer KA (2009) Microdroplet-based PCR enrichment for large-scale targeted sequencing. Nat Biotechnol 27(11):1025–1031. doi:10.1038/nbt.1583
88. Tosch V, Vasli N, Kretz C, Nicot AS, Gasnier C, Dondaine N, Oriot D, Barth M, Puissant H, Romero NB, Bonnemann CG, Heller B, Duval G, Biancalana V, Laporte J (2010) Novel molecular diagnostic approaches for X-linked centronuclear (myotubular) myopathy reveal intronic mutations. Neuromuscul Disord 20(6):375–381. doi:10.1016/j.nmd.2010.03.015
89. Trapnell C, Salzberg SL (2009) How to map billions of short reads onto genomes. Nat Biotechnol 27(5):455–457. doi:10.1038/nbt0509-455
90. Tucker EJ, Mimaki M, Compton AG, McKenzie M, Ryan MT, Thorburn DR (2011) Next-generation sequencing in molecular diagnosis: NUBPL mutations highlight the challenges of variant detection and interpretation. Hum Mutat 33(2):411–418. doi:10.1002/humu.21654
91. Valouev A, Ichikawa J, Tonthat T, Stuart J, Ranade S, Peckham H, Zeng K, Malek JA, Costa G, McKernan K, Sidow A, Fire A, Johnson SM (2008) A high-resolution, nucleosome position map of *C. elegans* reveals a lack of universal sequence-dictated positioning. Genome Res 18(7):1051–1063. doi:10.1101/gr.076463.108
92. Vasli N, Bohm J, Le Gras S, Muller J, Pizot C, Jost B, Echaniz-Laguna A, Laugel V, Tranchant C, Bernard R, Plewniak F, Vicaire S, Levy N, Chelly J, Mandel JL, Biancalana V, Laporte J (2012) Next generation sequencing for molecular diagnosis of neuromuscular diseases. Acta Neuropathol 124(2):273–283. doi:10.1007/s00401-012-0982-8
93. Wang K, Li M, Hakonarson H (2010) ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res 38(16):e164. doi:10.1093/nar/gkq603
94. Wheeler DA, Srinivasan M, Egholm M, Shen Y, Chen L, McGuire A, He W, Chen YJ, Makhijani V, Roth GT, Gomes X, Tartaro K, Niazi F, Turcotte CL, Irzyk GP, Lupski JR, Chinault C, Song XZ, Liu Y, Yuan Y, Nazareth L, Qin X, Muzny DM, Margulies M, Weinstock GM, Gibbs RA, Rothberg JM (2008) The complete genome of an individual by massively parallel DNA sequencing. Nature 452(7189):872–876. doi:10.1038/nature06884
95. Wijmenga C, Hewitt JE, Sandkuijl LA, Clark LN, Wright TJ, Dauwerse HG, Gruter AM, Hofker MH, Moerer P, Williamson R et al (1992) Chromosome 4q DNA rearrangements associated with facioscapulohumeral muscular dystrophy. Nat Genet 2(1):26–30. doi:10.1038/ng0992-26
96. Yang Y, Hentati A, Deng HX, Dabbagh O, Sasaki T, Hirano M, Hung WY, Ouahchi K, Yan J, Azim AC, Cole N, Gascon G, Yagmour A, Ben-Hamida M, Pericak-Vance M, Hentati F, Siddique T (2001) The gene encoding alsin, a protein with three guanine-nucleotide exchange factor domains, is mutated in a form of recessive amyotrophic lateral sclerosis. Nat Genet 29(2):160–165. doi:10.1038/ng1001-160ng1001-160
97. Zimmern RL, Kroese M (2007) The evaluation of genetic tests. J Public Health (Oxf) 29(3):246–250. doi:10.1093/pubmed/fdm028

2- Materials and methods

2-1- DNA quality control for next generation sequencing

For next-generation sequencing, quantification and quality assessment of genomic and fragmented DNA as starting materials, as well as DNA sequencing libraries are essential to achieve highest quality of sequencing data. Two to twenty microgram of genomic DNA, extracted from blood, saliva or cell lines is used for random shearing. The DNA quantification can be done by micro-volume spectrophotometers such as NanoDrop (Thermo scientific, USA) or The Qubit® 2.0 Fluorometer (Life technologies, USA). Sometimes, there is a big difference in detected amount of DNA, using these two techniques. The Qubit® 2.0 Fluorometer seems to be more accurate than NanoDrop.

The quality of gDNA can be checked by loading two-hundred nanogram of genomic DNA on an agarose gel. If no smear due to DNA degradation or small band at the bottom of the gel due to RNA contaminations is seen, the quality of DNA is good enough for using in NGS. In Figure 14 the examples of good quality and bad quality DNAs are shown.

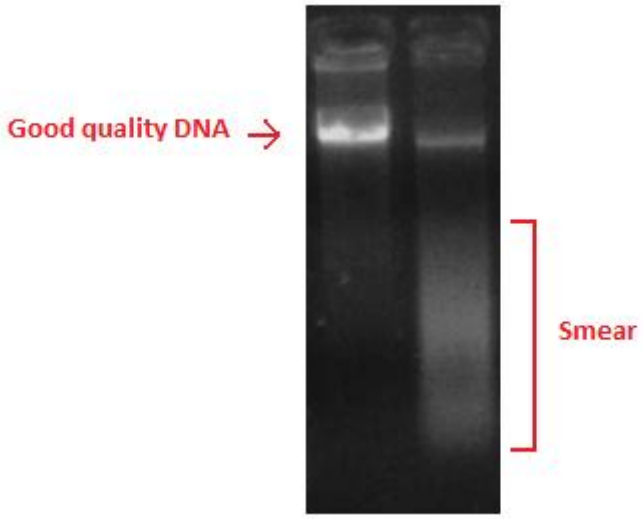
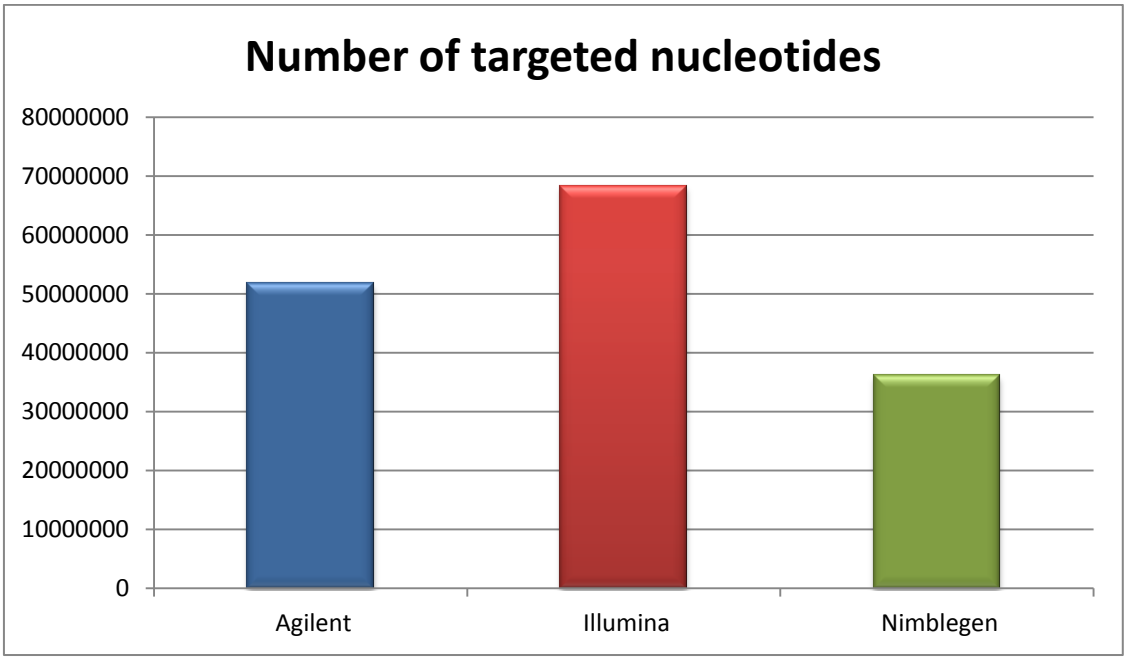


Figure 14: Good quality and bad quality DNAs. If electrophoresis of gDNA on agarose gel shows the smear or small products at the bottom of the gel, the quality of DNA is not good for later using in NGS. If a single band with high size is detected, this DNA is good enough for using in NGS.

2-2- Targeted sequence capture

Illumina, Agilent and Nimblegen capture kits are the most used kits in NGS. In Illumina and Nimblegen kits, DNA baits are used whereas Agilent works based on RNA baits. As the quality and quantity of achieved sequencing data can be different, using different kits for exome capturing; we did whole exome sequencing for one DNA sample, using three different capture kits, TruSeq Exome Enrichment kit (v1.3.0) from Illumina, SeqCap EZ Human Exome Library v2.0 from Nimblegen and Agilent SureSelect Human All Exon 50 Mb kit, following by NGS in one lane of Illumina HiSeq 2000 sequencer machine to determine the best exome enrichment kit.

Number of targeted nucleotides for capturing is different in 3 capture kits (Figure 15). It seems that TruSeq Exome Enrichment kit from Illumina captures more nucleotides than two other kits.



Kit	Number of captured nucleotides
Agilent	51756122
Illumina	68317278
Nimblegen	36172938

Figure 15: Number of targeted nucleotides in 3 different captures kits. As shown in graph, Illumina captures 68 Mb, Agilent captures 51 Mb and Nimblegen captures 36 Mb. In terms of capturing more target nucleotides, Illumina works better.

The number of unique overlapping reads, also called coverage is another issue to show the efficacy of capture kits, if the NGS is done using the same DNA and the same sequencer machine. Using one lane of Illumina HiSeq 2000 per captured DNA with different kits, i.e. 3 lanes in total, the coverage of targeted regions were higher, using Agilent SureSelect Human All Exon 50 Mb kit (Figure 16). The numbers of non-covered and totally covered regions are also important and as shown in Figure 17, numbers of targeted and totally covered regions are higher using Agilent SureSelect Human All Exon 50 Mb kit (Figure 17).

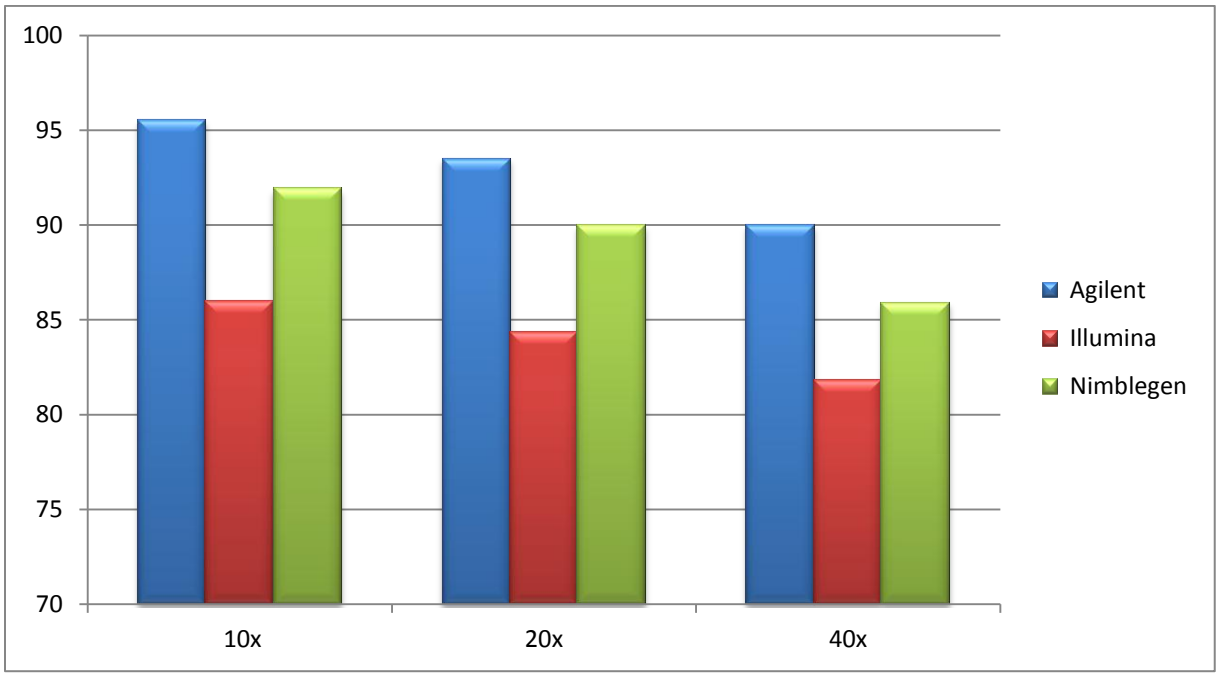
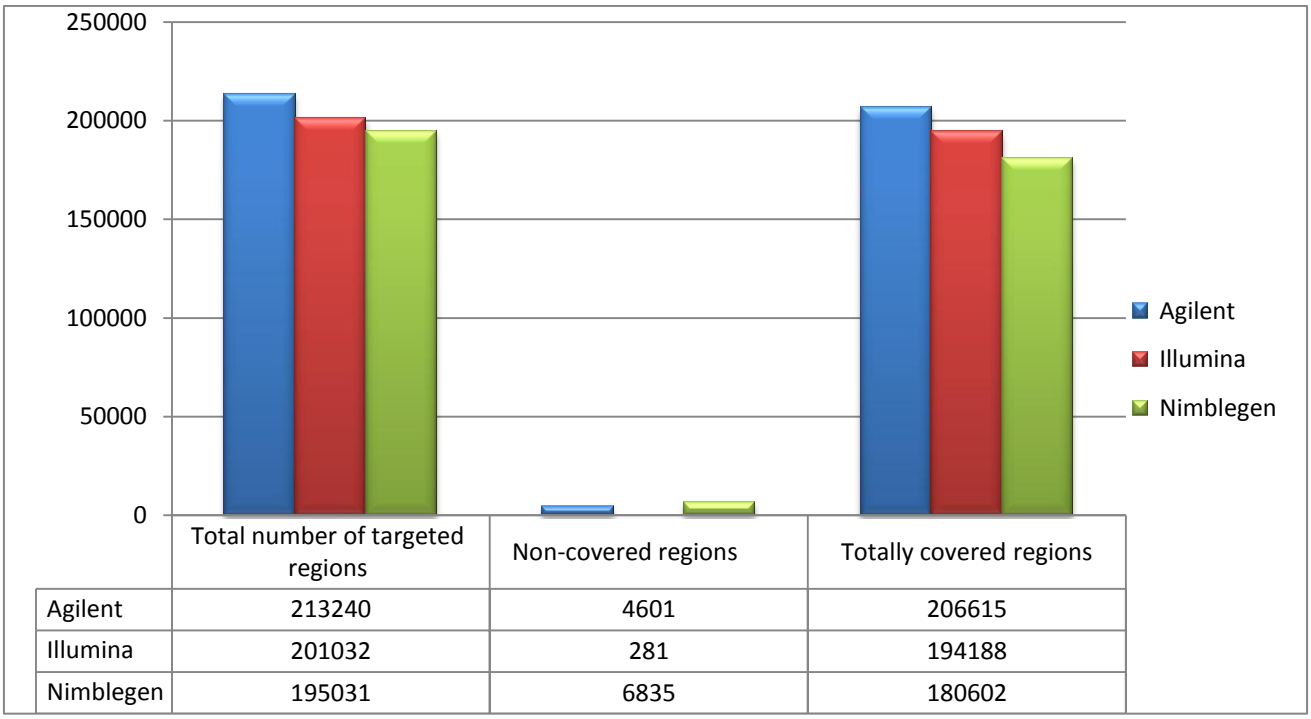


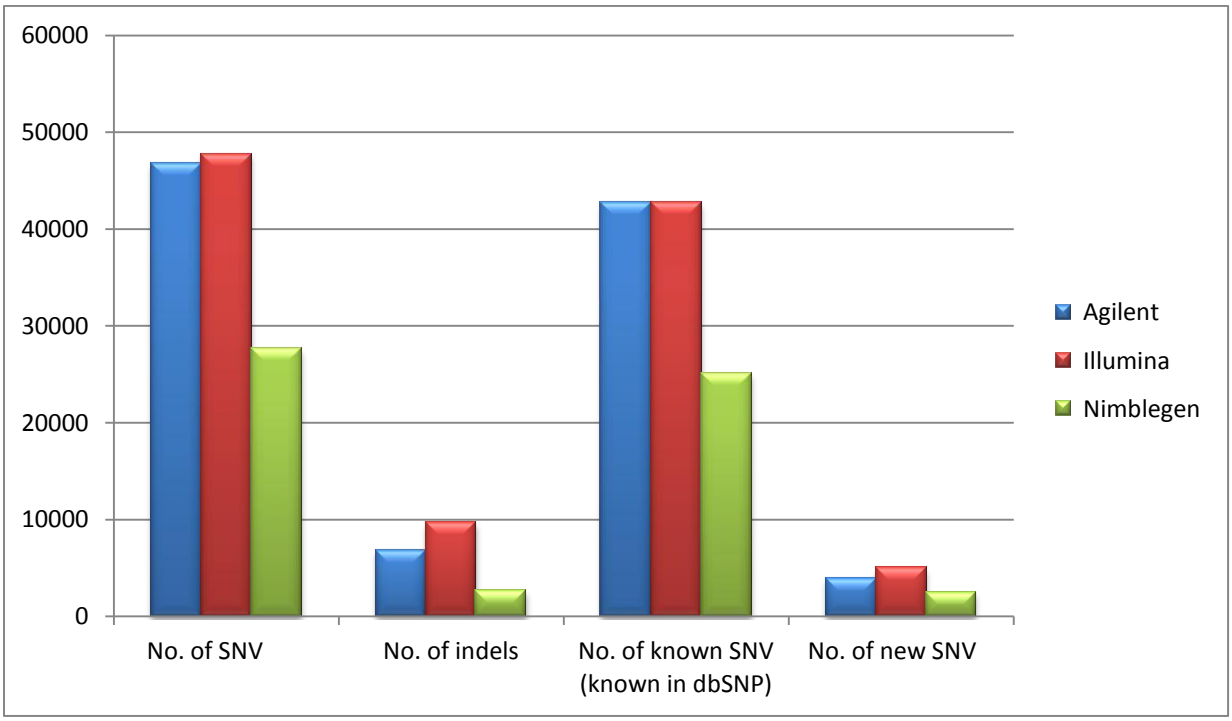
Figure 16: Number of unique overlapping reads or coverage, using 3 different kits. The percentage of nucleotides with 10x coverage or more is about 95, using Agilent SureSelect Human All Exon 50 Mb kit, whereas for TruSeq Exome Enrichment kit (v1.3.0) from Illumina is about 85 and for SeqCap EZ Human Exome Library v2.0 from Nimblegen is about 92. For 20x coverage and 40x, the percentage of covered nucleotides is consistent with 10x coverage results, and it is higher, using Agilent. Thus, in terms of Coverage, Agilent works better.



	Total number of targeted regions	Non-covered regions	Totally covered regions
Agilent	213240	4601	206615
Illumina	201032	281	194188
Nimblegen	195031	6835	180602

Figure 17: Numbers of non-covered, totally covered and targeted regions, using 3 different kits. As shown in graph, number of targeted regions as well as totally covered regions are higher, using Agilent SureSelect Human All Exon 50 Mb kit. Although, number of non-covered regions is less, using TruSeq Exome Enrichment kit (v1.3.0) from Illumina, as the number of totally covered regions is 194188 which is less than 206615, using Agilent SureSelect Human All Exon 50 Mb kit, capturing the targeted regions with Agilent will lead to cover more regions.

The last comparison was done by looking to the number of detected single nucleotide variations (SNV) and insertions-deletions (Indels) (Figure 18). The numbers of detected SNVs & Indels as well as numbers of known (using dbSNP132) and new SNVs & Indels are higher, using TruSeq Exome Enrichment kit (v1.3.0) from Illumina. Whether these variations are PCR/sequencing errors or real, remain to be verified, as the detected variations did not confirm by direct sequencing. However, some variations detected by previous essays and confirmed by direct sequencing were used as positive controls (data will be shown in results section).



Kit	No. of SNV	No. of indels	No. of known SNV (known in dbSNP)	No. of new SNV
Agilent	46739	6802	42800	3939
Illumina	47772	9713	42770	5002
Nimblegen	27605	2698	25086	2519

Figure 18: Number of detected SNVs and Indels, using 3 capture kits. As shown in graph, the numbers of detected SNVs & Indels as well as numbers of known (using dbSNP132) and new SNVs & Indels are higher, using TruSeq Exome Enrichment kit (v1.3.0) from Illumina.

Performing these comparisons, it seems that capturing the exome, using Agilent SureSelect Human All Exon 50 Mb kit, will lead to have more and better covered targeted regions.

2-3- Data analysis workflow

After sequencing, the raw data is used for sequence quality control, mapping or alignment to reference genome, variant calling and annotation and the last step is variant filtration.

2-3-1- Sequence quality control

For checking the quality of raw sequencing data coming from NGS, there are some tools and programs such as FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) or FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/). Using these programs, a quick impression of whether the data has any problems which needs to assess more analysis is achieved. For instance, the Phred quality score for each nucleotide in a read can be calculated and shown by graph, using FastQC Program (Figure 19). Phred quality score is defined as -10*log10 (*p*), where *p* is the base-calling error probability (Yu et al, 2012). Low quality bases are the ones with Phred quality score ranging from 5-15 and high quality bases are the ones with Phred quality score ranging from 30-40.

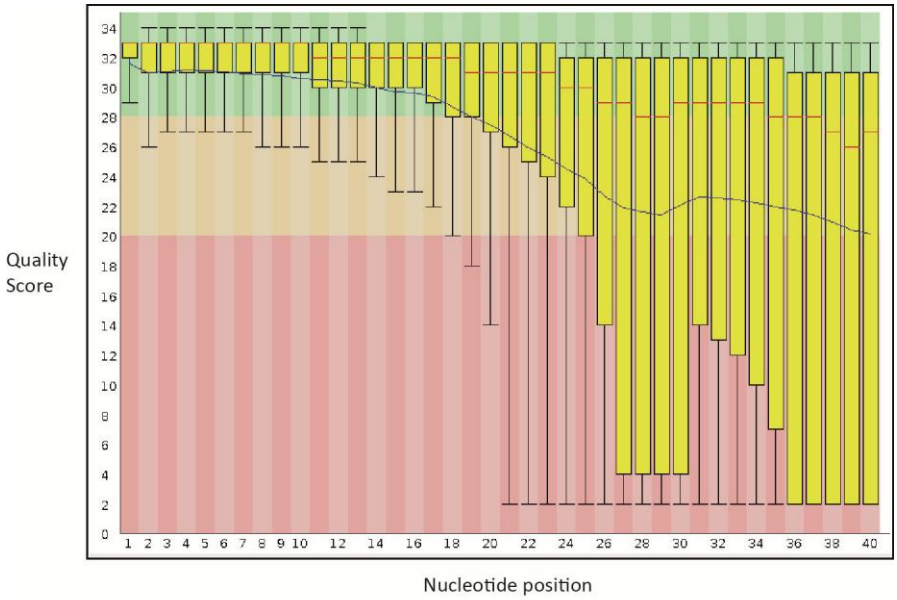


Figure 19: Quality score for each nucleotide in reads with 40 base pairs length. Normally, the quality score more than 20 is used as threshold for showing the reads with high quality. The best quality scores are between 30 and 40.

Reference:

http://www.bioinformatics.babraham.ac.uk/projects/fastqc/bad_sequence_fastqc/fastqc_report.html#M1

2-3-2- Sequence alignment

After sequencing, mapping or alignment of the reads onto a reference genome such as human reference genome (UCSC assembly hg18/NCBI 36 or hg19/GRCH 37) is required. There are a number of tools for aligning short reads. The most popular ones are MAQ (Li et al, 2008a), SOAP2 (Li et al, 2008b) and the new version of that, SOAP3 (Liu et al, 2012), Bowtie (Langmead et al, 2009) and BWA (Li & Durbin, 2009). For aligning 70 million read pairs, equivalent to the throughput of one lane of the Illumina HiSeq 2000, with four mismatches maximum, the alignment to the human genome as the reference takes > 3.5 h, using the fastest existing alignment tool (Liu et al, 2012). In a study, the comparison analysis between four mapping tools, BWA, Bowti, SOAP2 and Novoalign was done and it was shown that for sequencing data with relatively good quality, all four programs performed similarly (Yu et al, 2012). After mapping, for unique reads generation, reads with the same start position and strand are filtered out, using Picard (http://picard.sourceforge.net/). Reads that mapped to several positions in the genome are also filtered out, using SAMtools (Li et al, 2009).

2-3-3- Variant calling and annotation

One of the most used tool for variant calling and annotation is SAMtools (http://samtools.sourceforge.net). SAMtools is a software package for doing different analysis in the SAM/BAM (Sequence Alignment/Map) format. Using this software, converting other alignment formats, sorting and merging alignments, calling SNVs and short indels and removing PCR duplicates can be performed easily (Li et al, 2009). The criteria that we used for calling a variant were: A coverage threshold of at least 3 reads that cover a nucleotide, variant must be seen in both directions/strands, reads showing the reference nucleotide must be seen in at least one direction/strand and a threshold of at least 3 reads supporting the SNV/indel change. A homozygous SNV/indel was called when >85% of aligned reads showed the variant and a heterozygous SNV/indel was called if 20-85% of reads showed the variation. For micro deletion detection, we used a coverage-based method where the number of reads in a sliding window of 20nt was computed across the genome for each sample and then this coverage was compared to the coverage of the same window in three randomly selected samples (Figure 20).

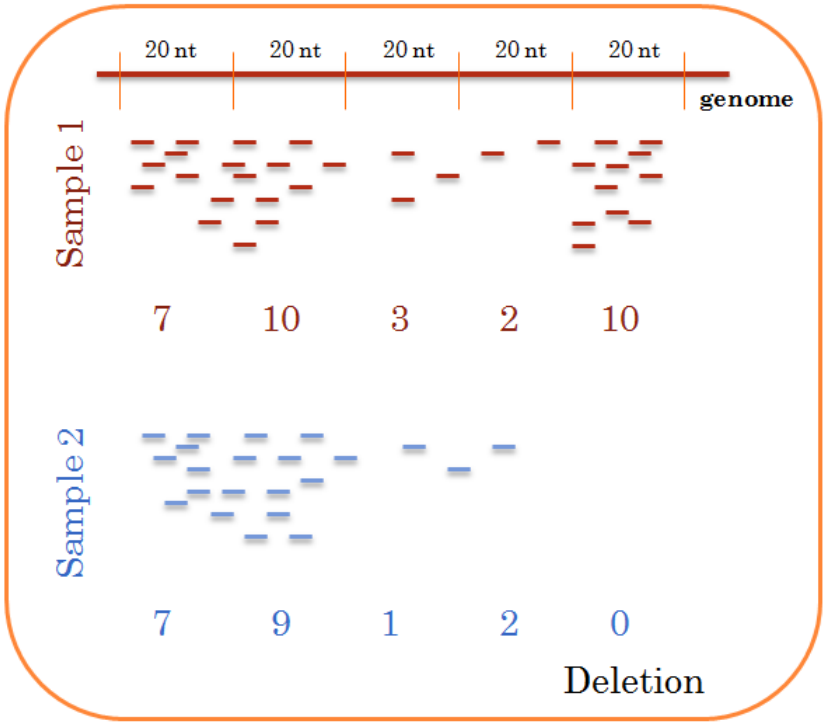


Figure 20: Micro deletion detection. A coverage-based method where the number of reads in a sliding window of 20nt is computed across the genome for each sample and then this coverage is compared to the coverage of the same window in a randomly selected sample. In the first window from right, there is a deletion in sample 2, as the depth of coverage in sample 1 is 10 reads whereas the depth of coverage is 0 in sample 2.

For variant annotation and displaying polymorphisms and allele frequencies, several tools and programs can be used such as Database of Single Nucleotide Polymorphisms (dbSNP Build ID: 129 to 135) (http://www.ncbi.nlm.nih.gov/SNP/), 1000 genomes - A Deep Catalog of Human Genetic Variation (http://www.1000genomes.org/), Exome Variant Server, NHLBI Exome Sequencing (http://evs.gs.washington.edu/EVS/), Online Mendelian Inheritance in Man (OMIM) (http://www.omim.org/) (McKusick, 2007), Human Gene Mutation Database (HGMD) (www.hgmd.cf.ac.uk/) (Stenson et al, 2009), Ensembl Genome project (www.ensembl.org), UCSC Genome Browser (http://genome.ucsc.edu/), ALAMUT software (Interactive Biosoftware, Rouen, France), Cartagenia benchlab tool (http://www.cartagenia.com/), SIMPLEX (http://simplex.i-med.ac.at) (Fischer et al, 2012) or ANNOVAR (http://www.openbioinformatics.org/annovar/)(Wang et al, 2010b).

2-3-4- Variant filtration

Identification of disease-causing genes/variations among the variants generated by NGS requires the separation of variations with potential high pathogenicity from variants with low probability for disease causation. To detect causal mutations in an individual after NGS, the most straightforward method is to search for known mutations and disease-associated genes, like those listed in databases such as Online Mendelian Inheritance in Man (OMIM) (http://www.omim.org/) (McKusick, 2007) or Human Gene Mutation Database (HGMD) (www.hgmd.cf.ac.uk/) (Stenson et al, 2009). This is of most utility in well-studied diseases and can be used in molecular diagnosis, i.e. finding novel and known mutations in previously identified genes. For instance, in a study by whole genome sequencing of a patient from a family with a recessive form of Charcot-Marie-Tooth neuropathy, compound heterozygous variants in the *SH3TC2* gene, a known gene in CMT neuropathies, were detected (Lupski et al, 2010).

For disorders without known mutations or disease-associated genes different filtration strategies are required for detection of pathogenic mutations. In general, the following assumptions are made for monogenic, Mendelian diseases: (i) the disease is genetically homogeneous, i.e. unrelated affected individuals with similar phenotypes have mutations in the same gene (ii) a single mutation is adequate to cause the disorder for dominant inheritance and one homozygous or at least two heterozygous changes in the same gene for recessive diseases, which would (iii) be rare, and most likely private to affected individuals, and (iv) they are probably coding and (v) highly penetrant (Ng et al, 2010c).

2-3-4-1- Filtration and ranking based on frequency

Rare disorders, by definition, have an individual incidence of less than 1/2000 in the population (www.eurordis.org). It is expected that mutations underlying these disorders have rare frequencies and probably private to affected individuals. Thus for NGS data from patients with rare disorders, normally the variations with high frequency (> 5% or even 1% for really rare diseases) in population are filtered out in first step of filtration. The frequency of variations can be found or estimated, using Database of Single Nucleotide Polymorphisms (dbSNP Build ID: 129 to 135) (http://www.ncbi.nlm.nih.gov/SNP/), 1000 genomes - A Deep Catalog of Human Genetic Variation (http://www.1000genomes.org/) and Exome Variant Server, NHLBI Exome Sequencing (http://evs.gs.washington.edu/EVS/) where in the last database the NGS data is comprised of a set of 2203 African-Americans and 4300 European-Americans unrelated individuals, totaling 6503 samples (13,006 chromosomes) in June 2012. It is important to note

that the phenotypic information is not always available or accessible for the samples used in these datasets, and it is possible that the pathogenic mutations, especially for disorders with recessive mode of inheritance presenting as heterozygous variation in normal carriers, are present in these datasets, as well.

2-3-4-2- Filtration and ranking based on variation type

Variations that change protein sequences, i.e. missense and nonsense changes, coding indels and splice sites changes as well as very rare or novel changes are checked first. The main rational for this is that these variants tend to be of larger effect than non-coding variants, and also because it is difficult to predict and check the effect of synonymous and non-coding variants. Thus, for most of the studies, synonymous and non-coding variants, i.e. variants in UTRs, introns and intergenic regions are ignored unless the predictions show that the variation, especially the synonymous one, can alter canonical splice sites. In general, the loss-of-function variations (nonsense, splice and frameshift changes) are ranked first, since there are low number of such mutations in any genome (<50) and they seem to be more harmful.

2-3-4-3- Filteration the "Black genes" and sequencing errors

False-positive variants must be filtered out from the list of variations generated by NGS. These false-positive variants are normally the variants in highly polymorphic genes, positions at which the reference genome contains the minor alleles, positions difficult for mapping leading to misalignments (Fuentes Fajardo et al, 2012) and sequencing errors. There are some genes such as members of olfactory and taste receptor gene families in which several variations are detected in them in any exome or genome datasets. A list of such genes can be found in literature (Fuentes Fajardo et al, 2012) and it is used to filtering out such genes from the NGS data. For removing the sequencing errors, normally variants with at least 8 reads with Phred quality score greater than 20 with at least 20% of reads showing the change are kept and other variants are removed.

2-3-4-4- Filtration and ranking based on effect

Variants can also be ranked by potential effect on protein structures and functions. Several predicting tools are available such as SIFT (Ng & Henikoff, 2003) and PolyPhen (Adzhubei et al, 2010; Ramensky et al, 2002). The potential damaging variants are ranked first, as the rational is that the mutations which are disruptive to proteins are more likely to be pathogenic. Although predictions of such tools are not correct all the time, and these tools have limitations in their specificity and sensitivity, if this ranking is used in conjunction with other rankings, it will be helpful.

2-3-4-5- Filtration and ranking based on conservation

Nucleotide and amino acid conservation scores can be helpful for variant ranking, as well. The variations in most conserved regions are ranked first, as the rational is that the mutations in conserved regions of genome or proteins are more likely to be pathogenic. Several algorithms and tools such as phastCons (Siepel et al, 2005) or PhyloP (http://compgen.bscb.cornell.edu/phast/help-pages/phyloP.txt) can be used. Both phastCons and phyloP conservation scores were computed separately for three groups of organisms: 8 primates, 31 placental mammals, and 44 vertebrates. The two conservation scores are informative in different ways. The phyloP score is a separate measurement of conservation at each base, ignoring neighboring bases in its calculation. The phastCons score takes neighboring bases into account, estimating the probability that each nucleotide belongs to a conserved element (Rhead et al, 2010).

2-3-4-6- Filtration and ranking based on inheritance mode

If the inheritance mode of the disorder is predicted based on family pedigree, the variants can be ranked and prioritized, using this criterion. The number of affected and unaffected individuals that would be sequenced by NGS for finding causal mutation can be also determined, using this criterion. Several strategies can be used for finding causal mutations by looking to inheritance mode and combining the individuals sequenced data. These strategies will be discussed below and some examples are listed in table 4.

2-3-4-6-1- Recessive mode of inheritance

For a pedigree with a rare recessive inherited disorder and consanguinity, the first hypothesis is that the disease is caused by a homozygous variant inherited from both parents and this variant most probably is in a large stretch of a homozygous region. These homozygous regions can be detected by SNP microarrays (Walsh et al, 2010) or the exome data itself (Becker et al, 2011) and the variants in these stretches are ranked first. If the NGS data from unaffected parents are available, those homozygous variants in patients that are found as heterozygous in both parents will be ranked first.

For a pedigree with probable recessive mode of inheritance without any indication of consanguinity, sequencing of only one affected patient (if the sample from other family members are not available) and searching for homozygous and especially compound heterozygous variants, is the most probable hypothesis. If the sample from other family members are available, sequencing of more affected patients and even non-effected individuals are helpful. By this way, common variations in affected patients that are not present in non-affected family members (or present as heterozygous for common homozygous variations in affected individuals, or just one of the two compound heterozygous variations presents in non-affected family members) will be ranked first.

2-3-4-6-2- Dominant mode of inheritance

For a pedigree with probable dominant mode of inheritance, sequencing multiple affected and also non-affected family members to exclude private benign variations is the best strategy. By choosing the most distantly affected family members, the amount of common benign variants will be minimum (Gilissen et al, 2012). Another strategy is sequencing both parents and the affected child, known as trio sequencing, and prioritizing the heterozygous variations in affected child that are absent in both parents.

If the samples from multiple affected individuals or parents are not available or there are few patients or even one affected individual present in a family, NGS data from multiple unrelated individuals with similar phenotypes can be overlapped and common genes can be prioritized, if the genetic heterogeneity is absent in the interested disorder.

2-3-4-7- Filtration and ranking based on gene function, tissue expression profile and cellular localization

Information on gene function in relation to phenotype and what is known about its pathophysiology, tissue expression profile of the candidate gene and cellular localization of related protein using different databases such as GENATLAS or Genecards can be helpful as well in further prioritization.

Table 4- Some studies using different strategies for finding causal genes/mutations.

Adapted from: (Gilissen et al, 2012)

Sequenced cases	Assumptions	Examples
Multiple affected within a single family	Fully penetrant mutation segregating with the disorder	(Johnson et al, 2010; Krawitz et al, 2010; Norton et al, 2011; Wang et al, 2010a; Zuchner et al, 2011)
Multiple affected with a dominant disorder	The disorder is monogenic and all patients suffer from the same disorder	(Albers et al, 2011; Hoischen et al, 2010; Isidor et al, 2011; Simpson et al, 2011b; Vissers et al, 2011)
Single affected from consanguine family	Homozygous mutation within a homozygous stretch	(Barak et al, 2011; Bilguvar et al, 2010; Bolze et al, 2010; Caliskan et al, 2011; O'Sullivan et al, 2011)
Single affected with a recessive disorder	A single rare homozygous or two rare compound heterozygous mutations	(Gilissen et al, 2010; Gotz et al, 2011; Murdock et al, 2011; Musunuru et al, 2010; Pierce et al, 2010)
Single sporadic affected	De novo mutation in patient	(O'Roak et al, 2011; Vissers et al, 2010; Xu et al, 2011)
Single affected with a dominant disorder without additional family	The causative gene or mutation shares features with known genes/mutations	(Byun et al, 2010; Erlich et al, 2011; Haack et al, 2010; Ozgul et al, 2011; Worthey et al, 2011)

3- Results

Results for aim 1: Gene identification in CNM

3-1- Patient selection

For finding new genes implicated in CNM, patients with CNM phenotypes without mutations in three classical genes, *MTM1, BIN1* and *DNM2,* and enough amount of DNA for performing NGS were selected. To have a panel of patients with the above mentioned criteria, the coding exons and flanking intronic sequences of genomic DNA were analyzed by direct sequencing of three genes mentioned above. MTM1 protein level was also examined by Western blot, when patient cell lines (lymphoblasts, fibroblasts or myoblasts) were available, for detection of intronic variations in *MTM1*. A number of new mutations in any of the three known genes were detected and complementary studies were done to prove the pathogenicity of the identified mutations. These data were published or submitted in peer-reviewed journals which are listed below.

If no mutations were found in the three classical genes, the patients' DNA would be used for performing NGS in order to find new gene(s) potentially implicated in CNM.

Publication 1

Novel molecular diagnostic approaches for X-linked centronuclear (myotubular) myopathy reveal intronic mutations

Valérie Tosch*, Nasim Vasli*, Christine Kretz, Anne-Sophie Nicot, Claire Gasnier, Nicolas Dondaine, Denis Oriot, Magalie Barth, Hugues Puissant, Norma B. Romero, Carsten G. Bönnemann, Betty Heller, Gilles Duval, Valérie Biancalana, Jocelyn Laporte

* These authors are contributed equally to this study

2010, Neuromuscul Disord, **20**(6)**:** 375-381

A- Introduction

XLMTM is the most frequent and severe form of CNM. For disease causing mutation detection, it is important to provide molecular diagnosis approaches that detect the largest number of mutations. Today, direct sequencing of coding regions and flanking intronic sequences of genomic DNA is the first routine method for the *MTM1* molecular screening.

B- Aim of study

Introduction of novel myotubularin antibodies and re-assess the status of patients without mutations in *MTM1* exons in order to facilitate the molecular diagnosis of patients with XLMTM.

C- Results

We characterized 5 patients with X-linked centronuclear myopathy. We identified the disease causing mutations and their consequence on protein level and RNA splicing by combining direct Western blotting, RT-PCR and genomic DNA sequencing. We also reported the characterization of a deep intronic mutation leading to an abnormal exonisation.

D- Conclusion

Novel anti-myotubularin antibodies allow a rapid detection of myotubularin from cell and muscle extracts by direct Western blotting. With these novel antibodies the enrichment step by immunoprecipitation is not required. They can be used for direct Western blotting to detect both exonic and non-exonic mutations and should be able to detect most of *MTM1* changes using a variety of cell types and muscle tissue. However, this approach does not provide information on the causative mutations. Furthermore, RT-PCR followed by sequencing of the PCR products represents a useful approach to detect or/and confirm both exonic mutations and splice alterations due to intronic mutations.

Neuromuscular Disorders 20 (2010) 375–381

Contents lists available at ScienceDirect

Neuromuscular Disorders

journal homepage: www.elsevier.com/locate/nmd

Novel molecular diagnostic approaches for X-linked centronuclear (myotubular) myopathy reveal intronic mutations

Valérie Tosch [a,b,c,d,e,1], Nasim Vasli [a,b,c,d,e,1], Christine Kretz [a,b,c,d,e], Anne-Sophie Nicot [a,b,c,d,e], Claire Gasnier [f], Nicolas Dondaine [f], Denis Oriot [g], Magalie Barth [h], Hugues Puissant [h], Norma B. Romero [i], Carsten G. Bönnemann [j], Betty Heller [b,c,d], Gilles Duval [b,c,d], Valérie Biancalana [a,b,c,d,e,f], Jocelyn Laporte [a,b,c,d,e,*]

[a] Department of Neurobiology and Genetics, IGBMC (Institut de Génétique et de Biologie Moléculaire et Cellulaire), Illkirch, France
[b] Inserm, U964, Illkirch, France
[c] CNRS, UMR7104, Illkirch, France
[d] Université de Strasbourg, Illkirch, France
[e] Collège de France, chaire de génétique humaine, Illkirch, France
[f] Laboratoire Diagnostic Génétique, Faculté de Médecine, CHRU, Strasbourg, France
[g] Service de Réanimation et néonatalogie, CHU, Poitiers, France
[h] Service de génétique, CHU, Angers, France
[i] Unité de Morphologie Neuromusculaire, Institut de Myologie, Inserm UMRS 974, CNRS UMR 7215, Université Paris 6, CHU Pitié-Salpêtrière, Paris, France
[j] Division of Neurology, The Children's Hospital of Philadelphia, Pennsylvania Muscle Institute, University of Pennsylvania School of Medicine, Philadelphia, PA, USA

ARTICLE INFO

Article history:
Received 16 October 2009
Received in revised form 20 February 2010
Accepted 30 March 2010

Keywords:
Centronuclear myopathy
Myotubular myopathy
Myotubularin
Intronic mutations
MTM1
XLMTM

ABSTRACT

X-linked centronuclear myopathy (XLMTM), also called myotubular myopathy, is a severe congenital myopathy characterized by generalized hypotonia and weakness at birth and the typical histological finding of centralization of myo-nuclei. It is caused by mutations in the *MTM1* gene encoding the 3-phosphoinositides phosphatase myotubularin. Mutations in dynamin 2 and amphiphysin 2 genes lead to autosomal forms of centronuclear myopathy (CNM). While XLMTM is the most frequent and severe form of CNM, no mutations are found in about 30% of patients by sequencing all *MTM1* exons. Moreover, the impact of *MTM1* sequence variants is sometimes difficult to assess. It is thus important to devise a complete molecular diagnostic strategy that includes analysis of the myotubularin transcript and protein expression. We therefore developed novel antibodies against human myotubularin and showed that they are able to detect the endogenous protein by direct Western blot from muscle samples and from cultured cells. In conjunction with RT-PCR analysis we validated the consequences of missense and splice mutations on transcript integrity and protein level. We also detected and characterized a novel deep intronic mutation consisting of a single nucleotide change that induces exonisation of a conserved intronic sequence. Patients with centronuclear myopathy and no molecular diagnosis should be investigated for *MTM1* defects at the cDNA and protein level.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

X-linked centronuclear myopathy (XLMTM), also called myotubular myopathy, is a rare congenital myopathy. XLMTM and more generally centronuclear myopathies (CNM) are characterized by muscle weakness associated with a predominance of type I hypotrophic fibers and numerous centrally located nuclei on muscle biopsy [1,2]. Patients with XLMTM display severe generalized hypotonia at birth accompanied by respiratory insufficiency requiring ventilator support. While the life expectancy is usually strongly reduced, up to 40% of patients survive into childhood and even adulthood, most of them requiring some degree of respiratory support [3–6]. XLMTM primarily affects males, and myopathic manifestations in female carriers have been found to be associated with skewed X-inactivation, with or without chromosomal rearrangements [7–9]. XLMTM is due to mutation in the *MTM1* gene encoding the 3-phosphoinositides phosphatase myotubularin [10]. Phosphoinositides are second messengers that flag specific membrane subdomains. Myotubularin is thought to regulate membrane traffic and T-tubules maintenance in animal models like *Caenorhabditis elegans*, Zebrafish and mice [11–13]. About 400 patients with *MTM1* mutations widespread through the gene have been reported so far [4,14–19]. Myotubularin is ubiquitously

* Corresponding author at: IGBMC, 1 rue Laurent Fries, B.P. 10142, 67404 Illkirch, France. Tel.: +33 388653412; fax: +33 388653201.
E-mail address: jocelyn@igbmc.fr (J. Laporte).
[1] Contributed equally to this study.

0960-8966/$ - see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.nmd.2010.03.015

expressed and a specific protein isoform is present in muscles [10,20].

Other forms of centronuclear myopathies include autosomal dominant and recessive forms, and sporadic cases. Most autosomal dominant cases are associated with mutations in the large GTPase dynamin 2 (DNM2) while mutations in the *BIN1* gene encoding the membrane remodeling protein amphiphysin 2 have been associated with a few autosomal recessive consanguineous cases [21,22]. In addition, sequence variants in the Ryanodine receptor (RYR1) and in another 3-phosphoinositides phosphatase (hJUMPY, also called MTMR14) were recently associated with sporadic cases [23,24]. These autosomal forms are usually less severe than XLMTM [25,26] but it is to note that some specific *BIN1* and *DNM2* mutations can be associated with neonatal hypotonia and weakness [22,27].

As XLMTM is the most frequent and severe form of centronuclear myopathies, it is important to provide molecular diagnosis approaches that detect the largest number of mutations. The routine molecular diagnosis is done by sequencing exons and intron–exon junctions from genomic DNA. RT-PCR analysis from muscle cDNA has been also used to identify *MTM1* mutations and confirm the effect of splice mutations in 12 cases [18,19,28,29]. Monitoring of myotubularin level by Western blot using previously described antibodies necessitated an immunoprecipitation step to be able to detect the protein, and showed that most *MTM1* mutations including missense mutations are associated with a lower protein level compared to controls [30].

In order to facilitate the molecular diagnosis of XLMTM and detect and validate non-exonic mutations, we developed novel myotubularin antibodies and re-assess the status of patients without mutations in *MTM1* exons. We characterize here 5 patients with X-linked centronuclear myopathy. We identify the disease-causing mutations and their consequence on protein level and RNA splicing by combining direct Western blotting, RT-PCR and genomic DNA sequencing. We also report the characterization of a deep intronic mutation leading to abnormal exonisation.

2. Materials and methods

2.1. Patients and murine cells and tissues

Six control cells (3 fibroblast and 3 lymphoblast cell lines) were derived from individuals unaffected by known neuromuscular disorders. Three previously characterized patient cells were used as mutated controls: lymphoblastoid cells from patients 1 (89-441; deletion of exons 1–13 in *MTM1*) and 3 (CF88; p.Ser376Asn in *MTM1*), and fibroblast cells from patient 2 (H31; complete genomic deletion of *MTM1*) [30]. Patients cells analyzed were from nine clinically diagnosed CNM patients, based on muscle weakness and a characteristic CNM histopathology (abnormal proportion of centralized nuclei, predominance of type I fibers and variation in size) and directed to us for molecular diagnosis. Myotonic dystrophy has been excluded in all patients by Triplet repeat primed-PCR, Quantitative Multiplex PCR of Short fluorescent Fragments (QMPSF) or Southern blot. Patients 4, 5 and 6 had severe neonatal hypotonia and deceased by one year of age, while patient 8 is a 6 years old boy able to sit and on night-time ventilatory support, and patient 7 is a 8 years old boy who had hypotonia at birth and respiratory assistance during 3 days, and later presented proximal weakness, walking difficulties, lordosis, strabismus and learning difficulties. Appropriate written consent was received from patients or their legal representant for molecular study in a diagnosis setting, and such molecular diagnosis investigations were validated by on-site ethical committees. Some cell lines were established by the DNA and Cell Bank of Généthon (Evry, France). A summary of patients' clinical and molecular data is provided in Table 1.

Mouse C2C12 cells were from the European collection of cell cultures (ECACC, 91031101) and skeletal muscle biopsies taken from quadriceps of 5 weeks old wild-type and *Mtm1* knock-out mice [31].

2.2. Antibodies and Western blotting

New Zealand white female rabbits were immunized against two human myotubularin sequences: R2630 and R2631 against the N-terminal peptide MASASTSKYNSHSLE, R2826 and R2827 against the C-terminal peptide SQMMPHVQTHF. Peptides were coupled to ovalbumin as a protein carrier prior to immunization. Sera were immuno-purified using the immunizing peptides coupled to a Sulfolink column (Pierce, Rockford, IL, USA). For Western blot, cells and muscle extracts were prepared in extraction buffer (50 mM Tris pH 7.8, 10% glycerol, 1 mM EDTA, 50 mM KCl, 1% SDS, 2% Triton X-100, 1 mM sodium orthovanadate), homogenized through 25G needles and cycles of liquid nitrogen freezing, then centrifuged. Aliquots were loaded onto 8% SDS–polyacrylamide gels, electrotransferred onto nitrocellulose and blotted with anti-myotubularin antibodies (dilutions ranging from 1:300 to 1:500) and mouse anti-GAPDH antibodies (diluted 1:10,000; Millipore, Billerica, MA, USA). Goat secondary antibodies were anti-mouse or anti-rabbit coupled to peroxidase (1:10,000; Jackson ImmunoResearch Inc., Baltimore, USA) and signals were detected with SuperSignal Pico (Pierce).

2.3. RT-PCR and genomic DNA sequencing

Total RNA was extracted from cultured cells using Tri reagent (Molecular Research Center Inc., Cincinnati, OH, USA) and reverse transcribed using Superscript (Invitrogen, Carlsbad, CA, USA). *MTM1* and *HPRT* cDNAs were subsequently amplified by PCR. *MTM1* specific primers were ATGGCTTCTGCATCAACTTC (5′–3′ direction) with TGGAATTCGATTTCGGGAC for fragment 1 (678nt) while we also used the forward primer GCAGCCGAGCAGCCTGGC in the non-coding exon 1 (729nt) for some PCR experiments, GTTCCGTATCGTGCCTCAG with GGAGAACGGTCAGCATCGG for fragment 2 (698nt), AGAATGGATAAGTTTTGGAC with TTATTTCGA GCTCTAATGCG for fragment 3 (622nt). HPRT primers were CCAA AGATGGTCAAGGTCGC and CTGCTGACAAAGATTCACTGG (745nt).

Genomic DNA prepared from cultured cells or fresh blood using standard procedures was amplified by PCR with primers GCTTGAAACAAAACAGTTCCTG and CCCATTCTCACAAATGCACA located in intron 7. RT-PCR and PCR products were purified using Multiscreen HTS 96-wells plates (Millipore) and sequenced with ABI PRISM BigDye Terminator cycle kits on a 3130XL Genetic Analyzer (Applied Biosystems, Carlsbad, CA, USA).

2.4. Sequence analysis

Reference sequences used were: human *MTM1* cDNA (U46024), human genomic *MTM1* (NG_008199) and mouse genomic *Mtm1* (NT_039706). The genomic structure of human *MTM1* was published elsewhere [32]. Sequence alignment was performed with BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and prediction of splice site strength with MaxEntScan (MES, http://genes.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq.html), NNSPLICE (http://www.fruitfly.org/seq_tools/splice.html), and Human Splicing Finder (http://www.umd.be/HSF/).

3. Results

3.1. Validation of novel myotubularin antibodies

Previously characterized anti-myotubularin antibodies allowed the detection of myotubularin from human cells after immunopre-

Table 1
Summary of clinical and molecular data.

Patients	Codes	Clinical severity	*MTM1* mutation	Cells/tissues[a]	MTM1 protein level[b]	*MTM1* RNA analysis
1	89-441	Severe, additional hypospadias	Deletion exons 1–13	Lym	Not detectable	n.d.
2	H31	Severe, additional hypospadias	Deletion all MTM1	Fib	Not detectable	Absent
3	CF88	Severe	c.1127G>A (p.Ser376Asn)	Lym	Normal	n.d.
4	GU15	Severe	c.582+1334A>G	Lym	Not detectable	Intron 7 partial exonisation
5	AHW88	Severe	c.(1644+3)_(1644+6)delAAGT	Fib	n.d.	Exon 14 skipping
6	AEK28	Severe	c.868-13T>A	Muscle, Lym	Not detectable	Splicing defects of exon 10
7	AHY60	Mild, 8 years old	c.575A>G (p.Tyr192Cys)	Lym	Lower than controls	n.d.
8	AAS96	Intermediate, 6 years old	n.d.	Fib	Not detectable	Lower than controls[c]
9	AIF27	Prenatal diagnosis	c.1493T>A (p.Leu498Stop)	Chor villus	n.d.	c.1493T>A in RNA sequence

Patients 1–3 were reported elsewhere [16].
Classification of severity is based on ventilation support [6]. All patients with a severe phenotype died before one year of age.
MTM1 mutations according to GenBank U46024.
n.d., not determined.
[a] Lym, lymphoblastoid cells; Fib, fibroblasts; Chor villus, chorionic villus.
[b] Ref. [30] and this study. Not detectable means that the protein level is below the detection threshold of the method.
[c] Semi-quantitative.

cipitation as an enrichment step [30]. Here we generated novel rabbit polyclonal antibodies against human myotubularin. Most of the depicted experiments are with the R2826 antibody while the other antibodies gave similar results. By direct Western blot on murine skeletal muscle extracts, these antibodies detect a band at the expected size (about 65 kDa), that is absent in *Mtm1* KO sample (Fig. 1A). We concluded they detect myotubularin and cross-react with the mouse protein. Myotubularin can also be detected from cell extracts of murine C2C12 myotubes, human fibroblasts, human lymphoblasts (Fig. 1B), human embryonic kidney epithelial cells, human mammary epithelial cells, culture of chorionic villi cells and amniotic cells (not shown), suggesting these antibodies can be used onto extracts from different cell origin.

3.2. Myotubularin level in cells and tissues from XLMTM patients

We monitored the level and size of myotubularin from lymphoblast and fibroblast cell lines derived from controls and CNM

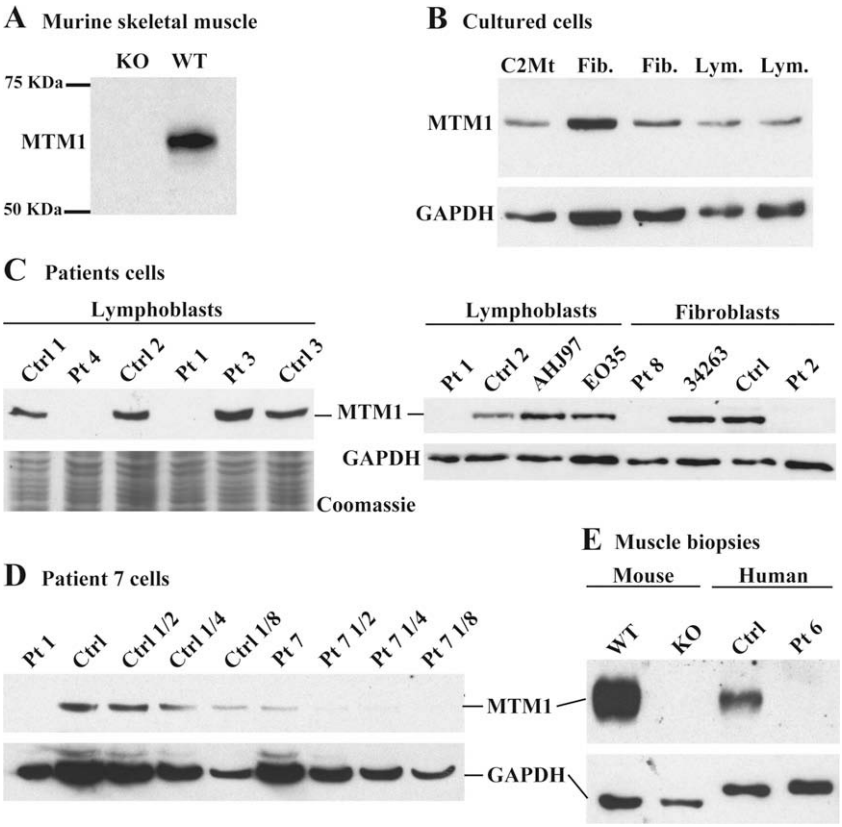


Fig. 1. Detection of myotubularin by direct Western blot from cultured cells and muscle biopsies. (A) Skeletal muscle extract from wild-type (WT) and *Mtm1* knock-out (KO) mice immunoblotted with anti human myotubularin antibody. Myotubularin appears at 65 kDa. (B) Myotubularin immunoblot on different cell lines: murine C2C12 myotubes (C2Mt), human fibroblasts (Fib) and lymphoblasts (Lym). (C) Different controls cells (Ctrl) and CNM patients (Pt) cells were analyzed for the presence of myotubularin. Cells from patient 1 and patient 2 are derived from patients with *MTM1* deletions. Cells AHJ97, EO35 and 34263 are derived from CNM patients in whom no subsequent *MTM1* abnormalities or mutations were found. Note that myotubularin is not detectable in cells from patients 4 and 8. (D) Serial dilution of patient 7 cells extracts showing that myotubularin is still detectable at expected size although in a much lower amount, compared to controls cells. (E) Skeletal muscle biopsies from wild-type (WT) and *Mtm1* knock-out (KO) mice and from human control and patient 6 analyzed by immunoblotting: myotubularin is not detectable in *Mtm1* KO mouse and patient 6. Loading control was anti-GAPDH or a Coomassie staining for panel (C).

patients (Table 1 for a summary). Myotubularin was below detection level in cells from patients with large *MTM1* deletions (patients 1 and 2), while a missense mutation p.Ser376Asn in the phosphatase active site did not change the protein level (patient 3; Fig. 1C; [30]). Myotubularin was not detectable in cells from patient 4 and 8, in whom no *MTM1* mutation was previously identified by DNA sequencing of exons and intron–exon junctions, suggesting that the disease is nevertheless linked to myotubularin deficiency (Fig. 1C; [30]). Prior to DNA sequencing, we noted that myotubularin level was lower in cells from patient 7 compared to control. Semi-quantitative analysis compared to GAPDH level suggested a decrease of about 7 times compared to control (Fig. 1D). Subsequent DNA sequencing revealed a c.575A>G mutation leading to a missense change p.Tyr192Cys. Lastly, these antibodies can be used to detect myotubularin from muscle biopsies (Fig. 1E). Muscle biopsy from the CNM patient 6 with a c.868-13T>A sequence variant in intron 9 did not show any detectable myotubularin. Similar results were obtained on this muscle extract either with N-terminal or C-terminal directed antibodies, suggesting unstability of RNA or protein as the molecular mechanisms, rather than production of a truncated protein.

In conclusion, novel anti-myotubularin antibodies allow a rapid detection of myotubularin from cell and muscle extracts by direct Western blot.

3.3. Detection of abnormal MTM1 splicing

To analyze further the molecular mechanism leading to myotubularin deficiency in cells from patients 4, 6 and 8, we next established RT-PCR analysis from cultured cells, covering the *MTM1* coding sequence into three overlapping fragments (Fig. 2A). RT-PCR amplification for patient 8 and sequencing of the products showed a normal *MTM1* cDNA sequence, although PCR amplification gave a much lower level compared to the control cells in two independent experiments (not shown). The intronic variant c.868-13T>A found in patient 6 is predicted to create an acceptor splice site with similar strength compared to the endogenous site, by Human Splicing Finder. RT-PCR analysis of lymphoblast cells from this patient showed abnormal products that correspond to the use of three different acceptor sites for exon 10, the normal site and two other sites leading to predicted out-of-frame translation (Fig. 2C). This experiment confirms that the intronic variant c.868-13T>A is the causative mutation leading to the lower protein level seen in Fig. 1E. Screening other patient cells, including chorionic villus and amniotic liquid cells, revealed abnormal PCR products for cells from patients 4 and 5 (Fig. 2B). Patient 5 DNA showed a c.(1644+3)_(1644+6)delAAGT variation in intron 14 and sequencing of the RT-PCR products reveal skipping of exon 14 (not shown). In cells from patient 4, an insertion of about 100nt was predominantly present in the N-terminal part of the *MTM1* cDNA (Fig. 2D and E). Subsequent sequencing of the aberrant transcript from patient 4 revealed the insertion of a 94nt sequence between exon 7 and exon 8, bearing stop codons in all three possible frames; this abnormal sequence inclusion is responsible for the lack of myotubularin protein as seen on Fig. 1C.

RT-PCR followed by sequencing of the PCR products thus represents a useful approach to detect or/and confirm both exonic mutations and splice alterations due to intronic mutations.

3.4. Characterization of a deep intronic MTM1 mutation

Comparison of the 94nt insertion in patient 4 *MTM1* cDNA showed that it matches a region located in the middle of *MTM1* intron 7. Sequencing this region on genomic DNA from both cultured

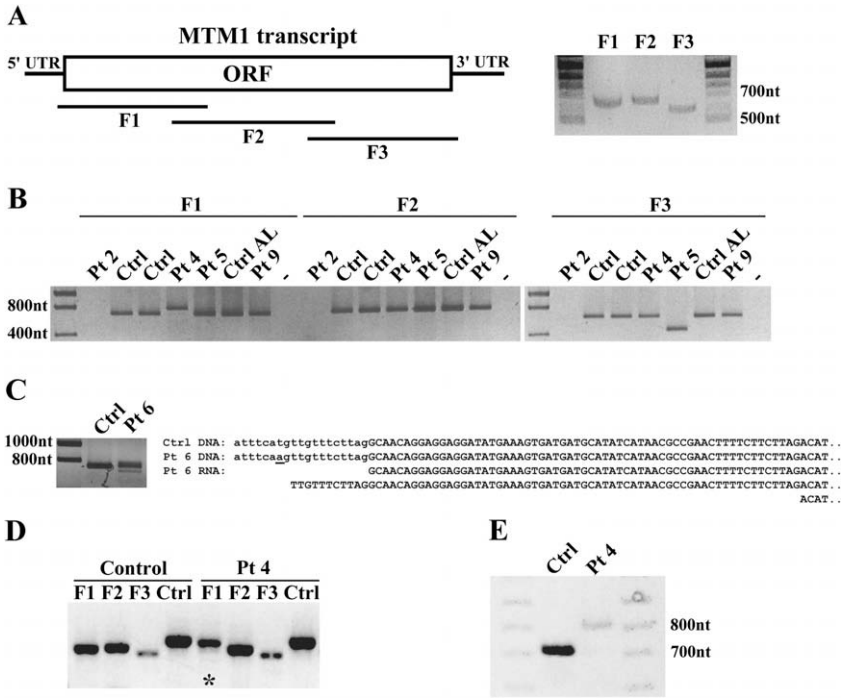


Fig. 2. Detection of *MTM1* transcript abnormalities in patients with X-linked centronuclear myopathy. (A) *MTM1* cDNA was amplified in three overlapping fragments (F1, F2, F3) covering the coding sequence, from cultured immortalized fibroblasts. (B) RT-PCR amplification products from control cells (Ctrl), control amniotic liquid cells (Ctrl AL), patient 2 (Pt 2; complete deletion of the *MTM1* gene) and several CNM cells. "–" is a control without cDNAs. Note aberrant products size from patients 4 and 5. Cells from patient 9 were from chorionic villus and sequencing lead to the identification of a c.1493T>A mutation (p.Leu498Stop). (C) RT-PCR from patient 6 lymphoblasts detected aberrant fragments. Sequencing of these products showed that the c.868-13T>A mutation (underlined) lead to the usage of three different acceptor sites, that were predicted with similar scores by Human Splicing Finder. Intronic and exonic sequences are in small and upper cases respectively. (D) and (E), a focus on patient 4 cells. (D) RT-PCR amplification products from control and patient 4 cells showing an aberrant size of fragment 1 (indicated by a star) while other fragments were found at expected size. RT-PCR control gene was HPRT (Ctrl). (E) A longer migration of PCR fragment 1 from control (Ctrl) and patient 4 cells: the insertion in patient 4 *MTM1* cDNA was estimated to be about 100nt in length.

cells and blood sample derived from the patient revealed a point mutation A>G at position −1 from the start of this insertion (Fig. 3A and B). This c.582+1334 A>G mutation is located 1.3 kb distal from exon 7 donor site and 0.9 kb proximal from exon 8 acceptor site. The sequence of this insertion, called exon 7′, is well conserved in mouse intron 7 with 89% identity, a score similar to *MTM1* exons (ranging from about 87% to 90% identity), and the surrounding 400nt sequence shares about 84% identity between mouse and human. However, exon 7′ is not conserved in human *MTMR1*, a close *MTM1* homologue located 20 kb distal on the X chromosome [33]. Together with our RT-PCR data, *in silico* analysis using MES and NNSPLICE demonstrated the creation of a functional acceptor splice site with very high score of 9.7 and 1.0, respectively. A cryptic donor site downstream of exon 7′ is also predicted by these programs (AG/GTACA, NNSPLICE score of 0.9; Fig. 3C) but is used only in the context of the c.582+1334 A>G mutation. No EST that includes exon 7′ sequence has been described.

We conclude that a single intronic point mutation creates a functional acceptor site that was used together with an existing cryptic donor site to induce the exonisation of a conserved intronic sequence.

4. Discussion

We have characterized novel anti-myotubularin antibodies that have been used, in parallel with RT-PCR amplification, to assign molecular diagnosis in XLMTM patients (Table 1 for a summary). For the 5 patients characterized here, we first detected MTM1 defects by Western blotting and RT-PCR, prior to the identification of the mutation in 4 of them, by sequencing the RNA or the genomic DNA. Using these approaches, we validated several potential splice mutations and uncover a novel kind of disease-causing mutation for XLMTM, sequence exonisation.

We previously used specific myotubularin antibodies and immunoprecipitation to assess the level of myotubularin from cultured cell lines and showed that most of the mutations including missense correlate with a lower myotubularin level [30]. With these novel antibodies the enrichment step by immunoprecipitation is not required. They can be used for direct Western blotting to detect both exonic and non-exonic mutations and should be able to detect most patients using a variety of cell types and muscle tissue. Taken together the present study and published data, only two out of 22 different *MTM1* mutations tested did not correlate with a lower myotubularin level [30]. This was the case of the p.Ser376-Asn missense in patient 3 and of the in-frame deletion of the glycine at position 294. However, this approach does not provide information on the causative mutation. Indeed, for cells from patient 8 with no detectable myotubularin and no mRNA sequence abnormalities in fibroblasts, it is difficult to conclude between a non-exonic *MTM1* mutation that impacts on mRNA level or a mutation in another gene regulating *MTM1* expression. Nevertheless, Western blotting is useful to confirm the impact of splice muta-

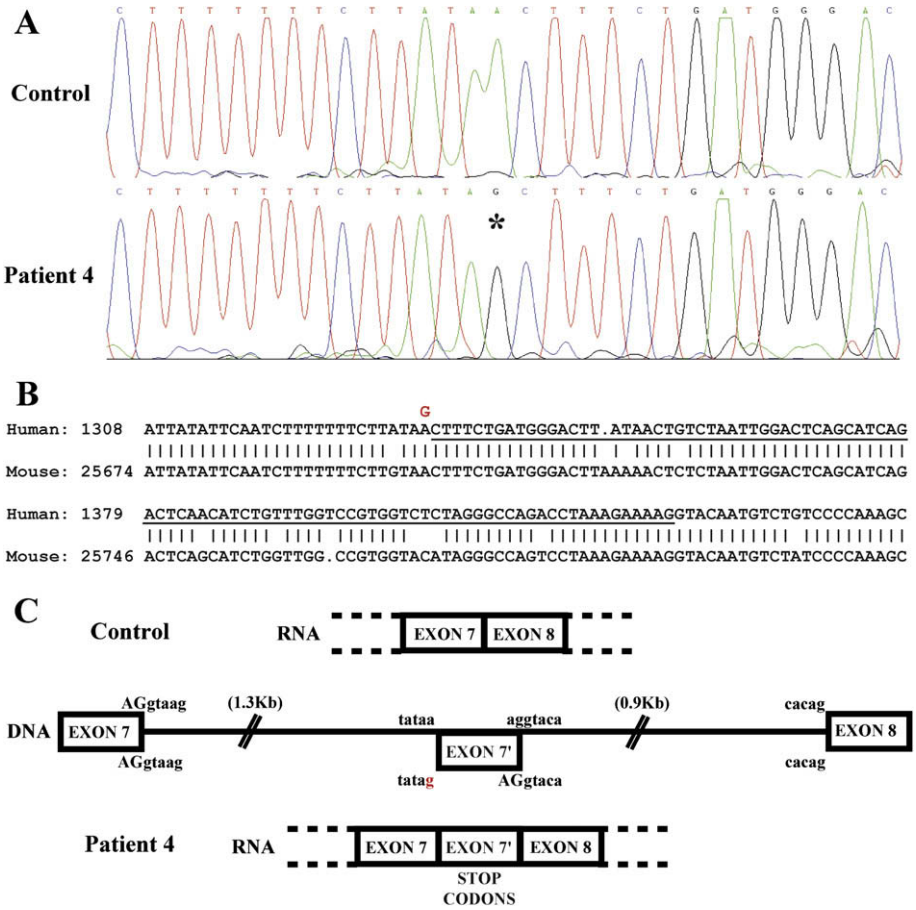


Fig. 3. Intronic sequence exonisation in patient 4 cells. (A) Electropherograms of a region of intron 7 from control and patient 4 genomic DNA, showing an A to G transition indicated by a star. (B) The sequence variation is found within a conserved sequence in intron 7, when comparing human and mouse genomic sequences, numbered from the first base of intron 7. The intronic exonised sequence is underlined and the A to G transition in patient 4 is depicted. Note that a conserved donor splice consensus sequence is pre-existing while the transition A to G creates a sequence matching an acceptor splice site consensus. (C) Schematic representation of *MTM1* splicing at exons 7 and 8, in control (above) and patient 4 cells (below). Splice sites sequences are indicated; intronic and exonic sequences are in small and upper cases, respectively. The exonised sequence (exon 7′ in patient 4) contains stop codons in all three frames.

tions, for example for patient 6, and can be used from cultured cells and muscle biopsies as a rapid approach to orient molecular diagnosis towards *MTM1* sequencing. Not all patients referred for MTM1 testing may have XLMTM or any form of CNM. Myotubularin level is detectable in cells from 3 patients with *BIN1* mutations and 5 patients with *DNM2* mutations, linked to autosomal forms of CNM (unpublished). Further developments may address the use of circulating blood cells and ELISA or FACS analysis.

RT-PCR allowed the confirmation of intronic variant found by genomic DNA sequencing (for e.g. patient 5) and detect both exonic and intronic mutation, the later if they have an effect on splicing. Importantly, the specific mutation can be identified by sequencing the *MTM1* cDNA, allowing subsequent genetic counseling and prenatal diagnosis.

In cells from patient 4, we detected the exonisation of a conserved intronic sequence. Such mutation mechanism was described for other diseases [34]. While RT-PCR analysis of *MTM1* was previously used efficiently to diagnose XLMTM [18,19], this study uncovered the first deep intronic mutation to our knowledge. In this case, a single point mutation was sufficient to create an acceptor site that activates a cryptic donor site. The high conservation of the exonised sequence and cryptic splice sites suggests that exon 7′ may have been used but was lost during evolution both in human and mouse, and accumulated stop codons. Indeed, this mutation was not detectable by direct sequencing of exons and intron–exon junctions, the approach used in routine for diagnosis. While Western blotting oriented the diagnosis, RT-PCR analysis was necessary to confirm and characterize this mutation in *MTM1*.

This work underlies the importance of using Western blotting and RT-PCR analysis in addition to genomic DNA sequencing, as a number of XLMTM patients do have non-exonic *MTM1* mutations. Up to now, about 19% of intronic mutations were found in the *MTM1* gene. The vast majority were located in close proximity to the exons and detected by the routine sequencing of splice sites and coding exons. Our approach should allow the detection of deep intronic mutations that were not targeted by the routine sequencing; their proportion will be determined once this approach will be applied to a large cohort of patients. *MTM1* cDNA sequencing may replace genomic DNA analysis if cells are treated with puromycin to inhibit potential non-sense mRNA decay and allow the detection of premature stop codons, especially when applied to heterozygous female carrier. As female carriers also express a normal allele, puromycin treatment will be necessary to detect the mutated RNA in addition to the normal RNA. We propose that myotubularin detection and RNA analysis can be performed on suspected XLMTM patients prior to the classical exon sequencing, if cultured cells are available. This was the case for example in patients 6 and 7 for whom we first identified protein and RNA abnormalities before identifying the mutations. Concerning patients for whom the classical genomic DNA sequencing method did not identified an exonic mutation (as in patient 4), cells or muscle biopsies should be collected and myotubularin level and RNA integrity analyzed. In addition, such analysis can confirm the impact of intronic variants and thus the molecular diagnosis. These approaches should allow the molecular diagnosis of additional XLMTM patients.

Conflict of interest

None of the authors has any conflict of interest or financial disclosure to declare.

Acknowledgements

We thank Julie Kretz and Elodie Klein for technical assistance, Serge Vicaire and Ingrid Colas for DNA sequencing, and Jon Andoni Urtizberea and Jean-Louis Mandel for help and support. Some cells lines were established by the Généthon DNA and Cell Bank (Evry, France). This study was supported by grants from the institute National de la Santé et de la Recherche Médicale (INSERM), the Centre National de la Recherche Scientifique (CNRS), University of Strasbourg (UdS), Collège de France, the Association Française contre les Myopathies (AFM), Fondation Recherche Médicale (FRM DEQ20071210538), Agence Nationale de la Recherche (ANR 06 MRAR 023) and the E-rare Program.

References

[1] Jungbluth H, Wallgren-Pettersson C, Laporte J. Centronuclear (myotubular) myopathy. Orphanet J Rare Dis 2008;3:26.
[2] Pierson CR, Tomczak K, Agrawal P, Moghadaszadeh B, Beggs AH. X-linked myotubular and centronuclear myopathies. J Neuropathol Exp Neurol 2005;64(7):555–64.
[3] Bevilacqua JA, Bitoun M, Biancalana V, Oldfors A, Stoltenburg G, Claeys KG, et al. "Necklace" fibers, a new histological marker of late-onset MTM1-related centronuclear myopathy. Acta Neuropathol 2009;117(3):283–91.
[4] Biancalana V, Caron O, Gallati S, Baas F, Kress W, Novelli G, et al. Characterisation of mutations in 77 patients with X-linked myotubular myopathy, including a family with a very mild phenotype. Hum Genet 2003;112(2):135–42.
[5] Herman GE, Finegold M, Zhao W, de Gouyon B, Metzenberg A. Medical complications in long-term survivors with X-linked myotubular myopathy. J Pediatr 1999;134(2):206–14.
[6] McEntagart M, Parsons G, Buj-Bello A, Biancalana V, Fenton I, Little M, et al. Genotype–phenotype correlations in X-linked myotubular myopathy. Neuromuscul Disord 2002;12(10):939–46.
[7] Dahl N, Hu LJ, Chery M, Gilgenkrantz S, Nivelon-Chevallier A, Gouyon JB, et al. Myotubular myopathy in a girl with a deletion at Xq27-q28 and unbalanced X inactivation assigns the MTM1 gene to a 600-kb region. Am J Hum Genet 1995;56:1108–15.
[8] Kristiansen M, Knudsen GP, Tanner SM, McEntagart M, Jungbluth H, Muntoni F, et al. X-inactivation patterns in carriers of X-linked myotubular myopathy. Neuromuscul Disord 2003;13(6):468–71.
[9] Tanner SM, Orstavik KH, Kristiansen M, Lev D, Lerman-Sagie T, Sadeh M, et al. Skewed X-inactivation in a manifesting carrier of X-linked myotubular myopathy and in her non-manifesting carrier mother. Hum Genet 1999;104(3):249–53.
[10] Laporte J, Hu LJ, Kretz C, Mandel JL, Kioschis P, Coy JF, et al. A gene mutated in X-linked myotubular myopathy defines a new putative tyrosine phosphatase family conserved in yeast. Nat Genet 1996;13(2):175–82.
[11] Al-Qusairi L, Weiss N, Toussaint A, Berbey C, Messaddeq N, Kretz C, et al. T-tubule disorganization and defective excitation–contraction coupling in muscle fibers lacking myotubularin lipid phosphatase. Proc Natl Acad Sci USA 2009;106(44):18763–8.
[12] Dang H, Li Z, Skolnik EY, Fares H. Disease-related myotubularins function in endocytic traffic in *Caenorhabditis elegans*. Mol Biol Cell 2004;15(1):189–96.
[13] Dowling JJ, Vreede AP, Low SE, Gibbs EM, Kuwada JY, Bonnemann CG, et al. Loss of myotubularin function results in T-tubule disorganization in zebrafish and human myotubular myopathy. PLoS Genet 2009;5(2):e1000372.
[14] de Gouyon BM, Zhao W, Laporte J, Mandel JL, Metzenberg A, Herman GE. Characterization of mutations in the myotubularin gene in twenty six patients with X-linked myotubular myopathy. Hum Mol Genet 1997;6(9):1499–504.
[15] Herman GE, Kopacz K, Zhao W, Mills PL, Metzenberg A, Das S. Characterization of mutations in fifty North American patients with X- linked myotubular myopathy. Hum Mutat 2002;19(2):114–21.
[16] Laporte J, Biancalana V, Tanner SM, Kress W, Schneider V, Wallgren-Pettersson C, et al. MTM1 mutations in X-linked myotubular myopathy. Hum Mutat 2000;15(5):393–409.
[17] Laporte J, Guiraud-Chaumeil C, Vincent MC, Fardeau M, Samson F, Mandel JL. Mutations in the MTM1 gene implicated in X-linked myotubular myopathy. ENMC International Consortium on Myotubular Myopathy. European Neuromuscular Center. Hum Mol Genet 1997;6(9):1505–11.
[18] Tanner SM, Schneider V, Thomas NS, Clarke A, Lazarou L, Liechti-Gallati S. Characterization of 34 novel and six known MTM1 gene mutations in 47 unrelated X-linked myotubular myopathy patients. Neuromuscul Disord 1999;9(1):41–9.
[19] Tsai TC, Horinouchi H, Noguchi S, Minami N, Murayama K, Hayashi YK, et al. Characterization of MTM1 mutations in 31 Japanese families with myotubular myopathy, including a patient carrying 240 kb deletion in Xq28 without male hypogenitalism. Neuromuscul Disord 2005;15:245–52.
[20] Laporte J, Blondeau F, Gansmuller A, Lutz Y, Vonesch JL, Mandel JL. The PtdIns3P phosphatase myotubularin is a cytoplasmic protein that also localizes to Rac1-inducible plasma membrane ruffles. J Cell Sci 2002;115(Pt. 15):3105–17.
[21] Bitoun M, Maugenre S, Jeannet PY, Lacene E, Ferrer X, Laforet P, et al. Mutations in dynamin 2 cause dominant centronuclear myopathy. Nat Genet 2005;37(11):1207–9.

[22] Nicot AS, Toussaint A, Tosch V, Kretz C, Wallgren-Pettersson C, Iwarsson E, et al. Mutations in amphiphysin 2 (BIN1) disrupt interaction with dynamin 2 and cause autosomal recessive centronuclear myopathy. Nat Genet 2007;39(9):1134–9.
[23] Jungbluth H, Zhou H, Sewry CA, Robb S, Treves S, Bitoun M, et al. Centronuclear myopathy due to a de novo dominant mutation in the skeletal muscle ryanodine receptor (RYR1) gene. Neuromuscul Disord 2007;17(4):338–45.
[24] Tosch V, Rohde HM, Tronchere H, Zanoteli E, Monroy N, Kretz C, et al. A novel PtdIns3P and PtdIns(3,5)P2 phosphatase with an inactivating variant in centronuclear myopathy. Hum Mol Genet 2006;15(21): 3098–106.
[25] Jeannet PY, Bassez G, Eymard B, Laforet P, Urtizberea JA, Rouche A, et al. Clinical and histologic findings in autosomal centronuclear myopathy. Neurology 2004;62(9):1484–90.
[26] Wallgren-Pettersson C, Clarke A, Samson F, Fardeau M, Dubowitz V, Moser H, et al. The myotubular myopathies: differential diagnosis of the X linked recessive, autosomal dominant, and autosomal recessive forms and present state of DNA studies. J Med Genet 1995;32:673–9.
[27] Bitoun M, Bevilacqua JA, Prudhon B, Maugenre S, Taratuto AL, Monges S, et al. Dynamin 2 mutations cause sporadic centronuclear myopathy with neonatal onset. Ann Neurol 2007;62(6):666–70.
[28] Nishino I, Minami N, Kobayashi O, Ikezawa M, Goto Y, Arahata K, et al. MTM1 gene mutations in Japanese patients with the severe infantile form of myotubular myopathy. Neuromuscul Disord 1998;8:453–8.
[29] Tanner SM, Laporte J, Guiraud-Chaumeil C, Liechti-Gallati S. Confirmation of prenatal diagnosis results of X-linked recessive myotubular myopathy by mutational screening, and description of three new mutations in the MTM1 gene. Hum Mutat 1998;11(1):62–8.
[30] Laporte J, Kress W, Mandel JL. Diagnosis of X-linked myotubular myopathy by detection of myotubularin. Ann Neurol 2001;50(1):42–6.
[31] Buj-Bello A, Laugel V, Messaddeq N, Zahreddine H, Laporte J, Pellissier JF, et al. The lipid phosphatase myotubularin is essential for skeletal muscle maintenance but not for myogenesis in mice. Proc Natl Acad Sci USA 2002;99(23):15060–5.
[32] Laporte J, Guiraud-Chaumeil C, Tanner SM, Blondeau F, Hu LJ, Vicaire S, et al. Genomic organization of the MTM1 gene implicated in X-linked myotubular myopathy. Eur J Hum Genet 1998;6(4):325–30.
[33] Kioschis P, Rogner UC, Pick E, Klauck SM, Heiss N, Siebenhaar R, et al. A 900-kb cosmid contig and 10 new transcripts within the candidate region for myotubular myopathy (MTM1). Genomics 1996;33(3):365–73.
[34] Dehainault C, Michaux D, Pages-Berhouet S, Caux-Moncoutier V, Doz F, Desjardins L, et al. A deep intronic mutation in the RB1 gene leads to intronic sequence exonisation. Eur J Hum Genet 2007;15(4):473–7.

Publication 2

Myotubular myopathy caused by multiple abnormal splicing variants in the MTM1 RNA in a patient with a mild phenotype

Nasim Vasli, Vincent Laugel, Johann Bohm, Béatrice Lanes, Valérie Biancalana, Jocelyn Laporte

2012, Eur J Hum Genet, **20**(6)**:** 701-704

A- Introduction

Centronuclear myopathies are rare congenital myopathies with X-linked and autosomal inheritance. XLMTM is the most severe form, characterized by severe hypotonia at birth and respiratory insufficiency associated with a poor prognosis. Most patients have a short life span and harbor mutations in the *MTM1* gene encoding the phosphoinositide phosphatase myotubularin.

B- Aim of study

Detection and characterization of a novel mutation in the *MTM1* gene.

C- Results

We reported the first *MTM1* mutation leading to the complex splicing rearrangements in a patient with mildly progressive myotubular myopathy.

D- Conclusion

Systematic analysis of the protein level and transcript integrity as a first screening for CNM patients is a suitable, fast, reliable and cost-effective diagnostic approach if cultured cells or tissues are available, as most *MTM1* mutations lead to a detectable decrease in the protein level.
In addition, sequencing the RNA species is seldom performed but the analysis of RNA by RT-PCR following by sequencing is an important step to characterize the precise impact of detected splice variants, and in some cases to detect deep intronic mutations. Similar complex pathogenic splicing is expected to exist in most genetic diseases.

European Journal of Human Genetics (2012), 1–4
© 2012 Macmillan Publishers Limited All rights reserved 1018-4813/12
www.nature.com/ejhg

SHORT REPORT

Myotubular myopathy caused by multiple abnormal splicing variants in the *MTM1* RNA in a patient with a mild phenotype

Nasim Vasli[1,2,3,4], Vincent Laugel[5], Johann Böhm[1,2,3,4], Béatrice Lannes[6], Valérie Biancalana[1,2,3,4,7] and Jocelyn Laporte*[,1,2,3,4]

Mutations impacting on the splicing of pre-mRNA are one important cause of genetically inherited diseases. However, detection of splice mutations, that are mainly due to intronic variations, and characterization of their effects are usually not performed as a first approach during genetic diagnosis. X-linked recessive myotubular myopathy is a severe congenital myopathy due to mutations in the *MTM1* gene encoding myotubularin. Here, we screened a male patient showing an unusually mild phenotype without respiratory distress by western blot with specific myotubularin antibodies and detected a strong reduction of the protein level.The disease was subsequently linked to a hemizygous point mutation affecting the acceptor splice site of exon 8 of *MTM1*, proven by protein, transcript and genomic DNA analysis. Detailed analysis of the *MTM1* mRNA by RT-PCR, sequencing and quantitative PCR revealed multiple abnormal transcripts with retention of a truncated exon 8, and neighboring exons 7 and 9 but exclusion of several other exons, suggesting a complex effect of this mutation on the splicing of non-adjacent exons. We conclude that the analysis of RNA by RT-PCR and sequencing is an important step to characterize the precise impact of detected splice variants. It is likely that complex splice aberrations due to a single mutation also account for unsolved cases in other diseases.

European Journal of Human Genetics advance online publication, 18 January 2012; doi:10.1038/ejhg.2011.256

Keywords: splice mutation; MTM1; myotubular myopathy; centronuclear myopathy; myotubularin; congenital myopathy

INTRODUCTION

Centronuclear myopathies are rare congenital myopathies with X-linked and autosomal inheritance. X-linked recessive myotubular myopathy (XLMTM; OMIM # 310400) is the most severe form, characterized by severe hypotonia at birth and respiratory insufficiency associated with a poor prognosis. Most patients have a short life span and harbor mutations in the *MTM1* gene encoding the phosphoinositide phosphatase myotubularin.[1,2]

To date, molecular diagnosis of about 400 patients with *MTM1* mutation have been reported, mainly based on direct sequencing of exons and intron–exon junctions.[3,4] Some of the mutations were found to affect the canonical acceptor (AG) or donor (GT) splice sites, involving alteration or skipping of the mutated exon but an impact on other exons has not been reported.[5–7]

Here, we report the detection and characterization of a novel mutation affecting the acceptor splice site of exon 8 in the *MTM1* gene, inducing splicing defects of non-adjacent exons, and leading to multiple aberrant transcripts.

PATIENTS AND METHODS

Patient

The male patient was born at 38-weeks' gestation. Fetal movements were decreased during pregnancy. He required oxygen administration in the first 2 h of life but did not need endotracheal intubation or mechanical ventilation. Major general hypotonia was present from birth with a narrow and elongated face, high-arched palate, decreased spontaneous movements, weak grasping reflex, weak cry and poor sucking. Left congenital torticollis and mild knee contractures were also present from birth. Motor milestones were delayed and he needed continued physiotherapy. He had numerous mild respiratory infections during the first 2 years although no sign of respiratory distress was ever noted. At 5 years of age, generalized muscle weakness is still present and facial weakness with dysarthria and chewing difficulties are still prominent. Muscle biopsy (quadriceps) showed the typical centronuclear myopathies pattern of small rounded muscle fibers with central nuclei (Figure 1).

Molecular biology procedures

Genomic DNA of the patient and his mother was isolated from blood samples or cultured lymphoblastoid cells using standard procedures. Molecular investigations were performed with the informed consent of the family.

For western blot, myotubularin was detected with the C-terminal R2827 anti-myotubularin polyclonal antibody as described.[8] Equal loading was monitored by Coomassie staining and a mouse monoclonal anti-GAPDH antibody (Millipore, Billerica, MA, USA).

Total RNA was extracted from lymphoblastoid cells or muscle using Tri reagent (Molecular Research Center Inc., Cincinnati, OH, USA),

[1]Department of Translational Medicine and Neurogenetics, IGBMC (Institut de Génétique et de Biologie Moléculaire et Cellulaire), Illkirch, France; [2]Inserm, U964, Illkirch, France; [3]CNRS, UMR7104, Illkirch, France; [4]University of Strasbourg, Illkirch, France; [5]Department of Pediatrics, Strasbourg University Hospital, Strasbourg, France; [6]Department of Pathology, Strasbourg University Hospital, Strasbourg, France; [7]Genetic diagnostic laboratory, Faculty of Medicine, CHRU, Strasbourg, France
*Correspondence: Dr J Laporte, Department of Translational Medicine and Neurogenetics, IGBMC (Institut de Génétique et de Biologie Moléculaire et Cellulaire), 1 rue Laurent Fries, B.P. 10142, 67404 Illkirch, France. Tel: +33 388653412; Fax: +33 388653201. E-mail: jocelyn@igbmc.fr
Received 5 August 2011; revised 6 December 2011; accepted 7 December 2011

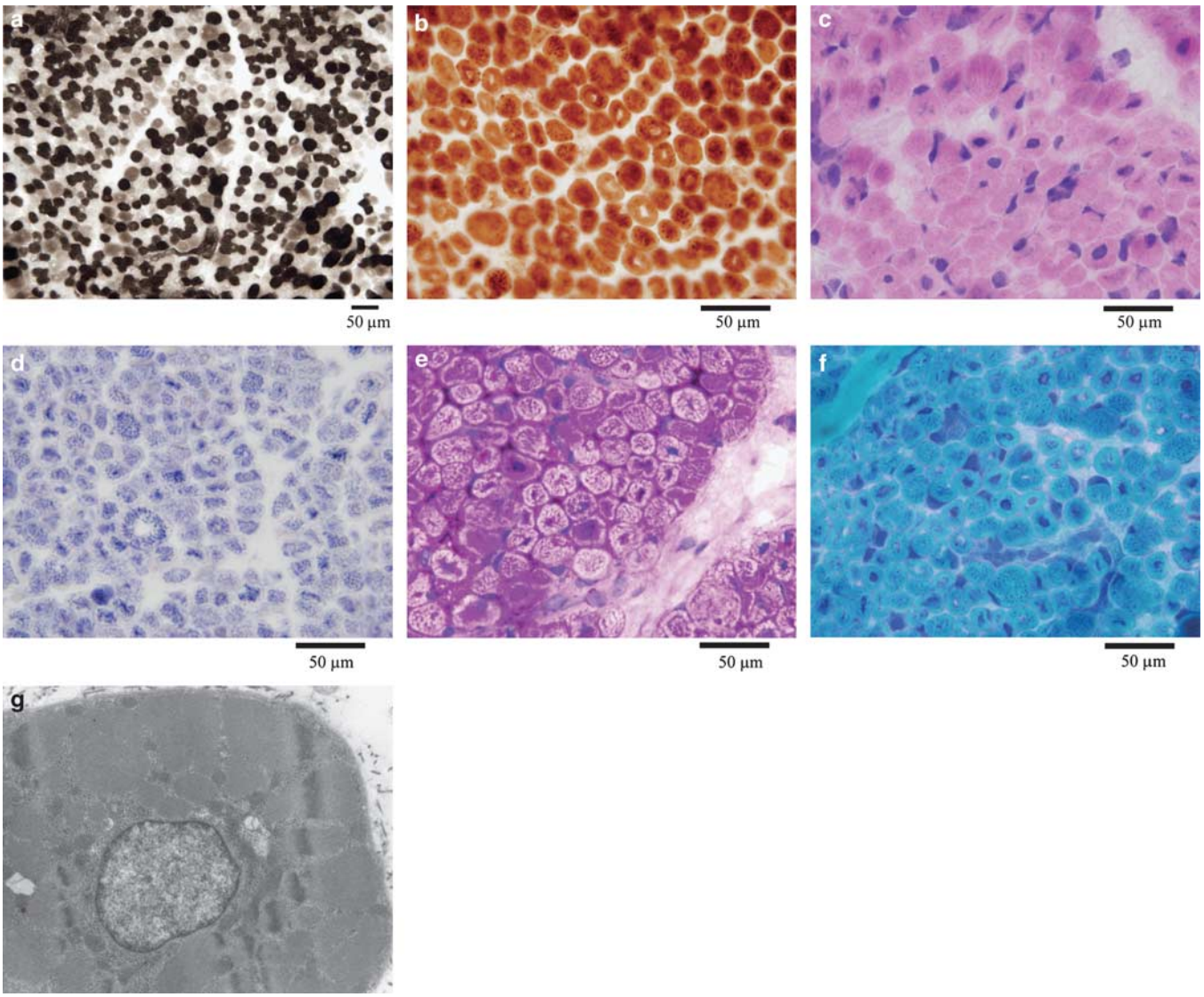


Figure 1 Histological analysis of the patient's muscle biopsy stained with ATPase, pH=4.5 (**a**), Cytochrome oxidase (cox) (**b**), Haematoxylin and Eosin (**c**), Nicotinamide adenosine dinucleotide – tetrazolium reductase (NADH-TR) (**d**) Periodic Acid Schiff (PAS) (**e**) and Gomori Trichrome (**f**). (**g**) Electron microscopic picture shows muscle fibers with a centralized nucleus surrounded by an area devoid of myofibrils and filled with glycogen granules. Myofibrils are of different size.

reverse transcribed and amplified using *MTM1* specific primers in three overlapping fragments covering the entire *MTM1* coding sequence, as previously reported.[8] Resulting cDNAs were cloned into the pGEM–T Easy vector (Promega, Madison, WI, USA) and sequenced (GATC; http://www.gatc-biotech.com/). Real-time RT-PCR was performed using a Lightcycler 480 (Roche Diagnostics, Meylan, France) with primers in the ubiquitous exons 12 and 14 and *RPLP0* and *GAPDH* mRNAs as standards. All exons and intron–exon boundaries of *MTM1*, *BIN1* and *DNM2* were sequenced from genomic DNA. Primer sequences are available upon request.

RESULTS

Unlike most XLMTM patients, our patient (ACJ7) showed a mild disease progression and no respiratory distress. To assess whether he has XLMTM or a milder autosomal form, and as a first and cost-effective diagnostic approach, we performed western blot on a muscle biopsy sample and lymphoblasts. The amount of MTM1 protein was below the detection level in the patient's lymphoblasts as compared with control cells (Figure 2a), indicating myotubularin deficiency as the disease cause. To decipher whether the disease-associated mutation is in the *MTM1* gene or in other genes regulating *MTM1* transcription, we analyzed the RNA of *MTM1* extracted from lymphoblastoid cells and a muscle biopsy. RT-PCR from lymphoblasts revealed the presence of abnormal RNA molecules with variable length of the 5' region (Figure 2b). We sequenced 68 and 56 cDNA clones extracted from the patient lymphoblastoid cells and muscle biopsy, respectively. Sanger sequencing identified five different frequent aberrant transcripts in lymphoblasts cDNA and five aberrant transcripts in muscle cDNA, compared with 50 independent cDNA clones sequenced from lymphoblasts of a healthy control (Figure 2c). We also detected other aberrant transcripts in single clone (data not shown) as well as the normal *MTM1* transcript (18 clones from lymphoblast cDNA and 12 from muscle cDNA). All aberrant clones had an abnormal 5′ part of exon 8, starting at cryptic acceptor sites at

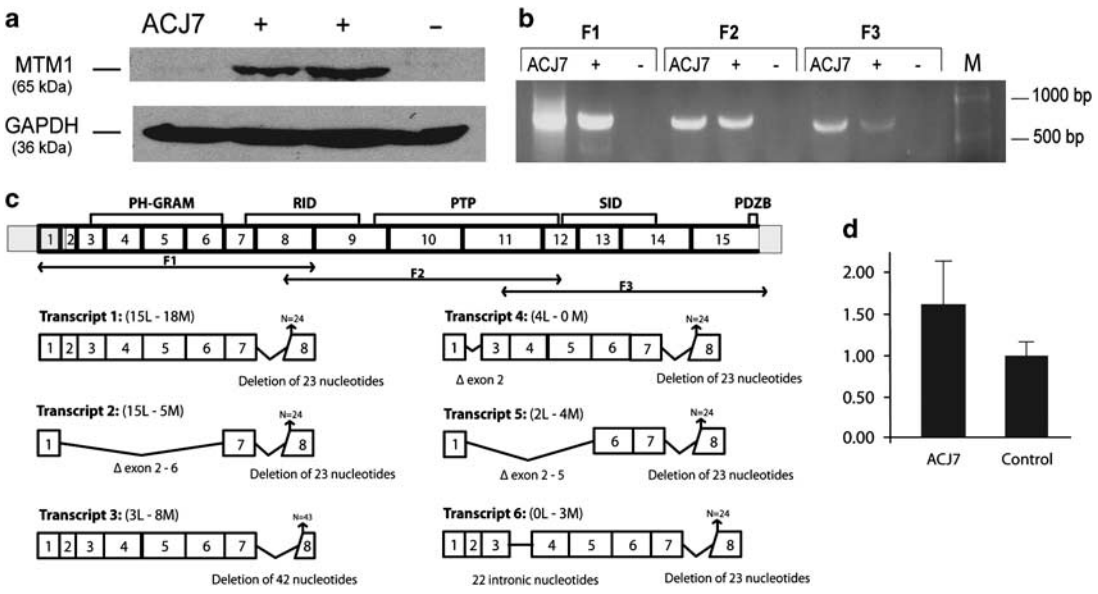


Figure 2 Detection and characterization of the *MTM1* splice mutation. (**a**) Western blot to asses the myotubularin level in cells from the patient (ACJ7), positive (+, cells from a healthy individual) and negative (–, cells from a XLMTM patient with an entire *MTM1* gene deletion) controls. (**b**) RT-PCR covering the *MTM1* coding sequence was performed in three overlapping fragments (F1: exons 1–8; F2: exons 8–12; F3: exons 11–15) using RNA extracts from the patient's lymphoblastoid cells and controls. (**c**) The *MTM1* gene has 15 exons drawn to scale with start and stop codons in exon 2 and 15, respectively. The five protein domains are: PH-GRAM (Pleckstrin homology, Glucosyltransferase, Rab-like GTPase activators and Myotubularins), RID (Rac-induced recruitment domain), PTP (Protein Tyrosine Phosphatase-like), SID (SET-interacting domain) and PDZB (PSD-95/Dlg/ZO-1 binding site). F1, F2 and F3 indicate the overlapping PCR fragments in (**b**). The abnormal transcripts and number of cDNA clones are shown below. L indicates the number of lymphoblastoid clones and M indicates the number of muscle clones. (**d**) The amount of *MTM1* RNA is not significantly different between ACJ7 patient cells and three controls as seen by quantitative RT-PCR in three independent experiments, using *RPLO* and *GAPDH* as standard genes. 2ΔCt method; *P*-value=0.134.

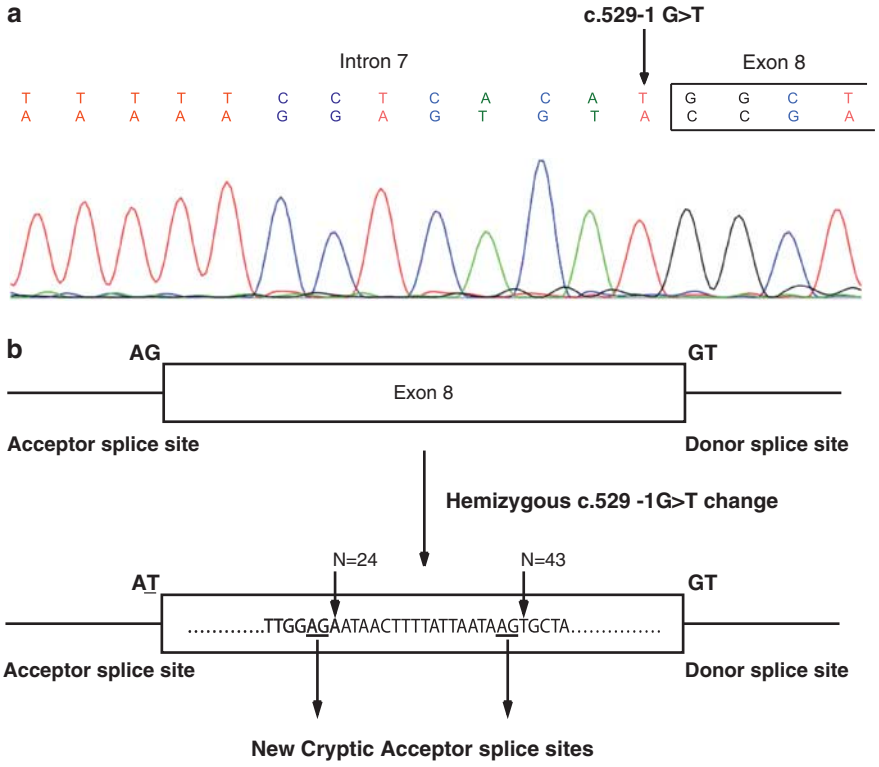


Figure 3 Genomic mutation and mechanism. (**a**) Electropherogram of the hemizygous c.529-1G>T change in intron 7 of *MTM1* (GenBank U46024). (**b**) Schematic representation of normal *MTM1* exon 8 splice sites and the cryptic acceptor splice sites found to be alternatively used in cells from the patient.

exon 8 positions 2, 24 (highly preferred) and 43, predicted by the human splice site finder program.[9] This suggested a mutation in the endogenous acceptor site of exon 8. Unexpectedly, most cDNA molecules displayed aberrant exclusion of non-adjacent exons but all retained exons 1 and 7.

The detected abnormal *MTM1* trancripts in our patient are not due to an overall decrease in RNA level and the amplification of illegitimate splicing background, as quantitative RT-PCR revealed a normal level of *MTM1* RNA (Figure 2d).

In accordance to the strong decrease in MTM1 protein and the aberrant splicing variants detected by RT-PCR, sequencing of the patient's genomic DNA revealed a novel hemizygous c.529-1G>T mutation modifying the physiological acceptor splice site from AG to AT in intron 7, leading to the use of cryptic acceptor splice sites in exon 8 (Figures 3a and b).The mother of the patient is a healthy heterozygous carrier of the mutation.

DISCUSSION

We report the first *MTM1* mutation leading to complex splicing rearrangements in a patient with mildly progressive myotubular myopathy.

Mature mRNAs are assembled by correct identification and joining of exons. The inaccurate attachement of exons leads to the generation of aberrant mRNAs, and unstable, truncated or deleterious proteins.[10] To date, some *MTM1* mutations were shown to have an impact on splicing and the majority of patients with splice site mutations presented with a severe phenotype, although a few cases show mild or moderate phenotypes.[4–7,11] In muscle and lymphoblastoid cells from the patient described in the present study, the majority of the analyzed *MTM1* cDNAs is predicted to lead either to the absence of the starting methionine in exon 2 or to the disruption of the coding frame. Indeed MTM1 protein level was strongly decreased although a weak normal sized band was detected when overloading the patient sample (not shown), probably sustaining the mild phenotype. Systematic analysis of the protein level and transcript integrity as a first screening, is a suitable, fast, reliable and cost-effective diagnostic approach if cultured cells or tissues are available, as most *MTM1* mutations lead to a detectable decrease in the protein level.[8,12] In addition, sequencing the RNA species is seldom performed but the present example shows that it represents a necessary approach to characterize the impact of the causative mutation.

In the analyzed patient, the acceptor site mutation in exon 8 was linked to variable exclusion of non-adjacent exons 2 to 6, whereas the total level of *MTM1* RNA was not compromised. Although unlikely, we cannot exclude the possibility of a second intronic variation that can induce this phenomenon. It is not known how the splice site mutation can have an impact on the inclusion/exclusion of non-adjacent exons. Similarly, the presence of multiple abnormal splicing variants in the CSA gene has been previously reported in a patient with Cockayne syndrome, although the causative mutation was not identified in that case.[13] A number of diseases associated with mutations that affect pre-mRNA splicing have been described before,[14,15] including intronic mutations leading to inclusion of cryptic exons.[8,16]

We conclude that the analysis of RNA by RT-PCR and sequencing is an important step to characterize the precise impact of detected splice variants, and in some cases to detect deep intronic mutation. Similar complex pathogenic splicing is expected to exist in most genetic diseases.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

The authors thank Charlotte Fugier and Nicolas Dondaine for technical assistance, Jean-Louis Mandel for support and the DNA and Cell Bank of Généthon (Evry, France) for cell lines. This study was supported by INSERM, CNRS, University of Strasbourg, Collège de France, and by grants from Association Française contre les Myopathies. JB was supported by the Deutsche Forschungsgemeinschaft (DFG).

1 Jungbluth H, Wallgren-Pettersson C, Laporte J: Centronuclear (myotubular) myopathy. *Orphanet J Rare Dis* 2008; **3**: 26.
2 Laporte J, Hu LJ, Kretz C *et al*: A gene mutated in X-linked myotubular myopathy defines a new putative tyrosine phosphatase family conserved in yeast. *Nat Genet* 1996; **13**: 175–182.
3 Laporte J, Biancalana V, Tanner SM *et al*: MTM1 mutations in X-linked myotubular myopathy. *Hum Mutat* 2000; **15**: 393–409.
4 Tsai TC, Horinouchi H, Noguchi S *et al*: Characterization of MTM1 mutations in 31 Japanese families with myotubular myopathy, including a patient carrying 240 kb deletion in Xq28 without male hypogenitalism. *Neuromuscul Disord* 2005; **15**: 245–252.
5 Nishino I, Minami N, Kobayashi O *et al*: MTM1 gene mutations in Japanese patients with the severe infantile form of myotubular myopathy. *NeuromusculDisord* 1998; **8**: 453–458.
6 Tanner SM, Laporte J, Guiraud-Chaumeil C, Liechti-Gallati S: Confirmation of prenatal diagnosis results of X-linked recessive myotubular myopathy by mutational screening, and description of three new mutations in the MTM1 gene. *Hum Mutat* 1998; **11**: 62–68.
7 Tanner SM, Schneider V, Thomas NS, Clarke A, Lazarou L, Liechti-Gallati S: Characterization of 34 novel and six known MTM1 gene mutations in 47 unrelated X-linked myotubular myopathy patients. *Neuromuscul Disord* 1999; **9**: 41–49.
8 Tosch V, Vasli N, Kretz C *et al*: Novel molecular diagnostic approaches for X-linked centronuclear (myotubular) myopathy reveal intronic mutations. *Neuromuscul Disord* 2010; **20**: 375–381.
9 Desmet FO, Hamroun D, Lalande M, Collod-Beroud G, Claustres M, Beroud C: Human Splicing Finder: an online bioinformatics tool to predict splicing signals. *Nucleic Acids Res* 2009; **37**: e67.
10 Cartegni L, Chew SL, Krainer AR: Listening to silence and understanding nonsense: exonic mutations that affect splicing. *Nat Rev Genet* 2002; **3**: 285–298.
11 McEntagart M, Parsons G, Buj-Bello A *et al*: Genotype-phenotype correlations in X-linked myotubular myopathy. *Neuromuscul Disord* 2002; **12**: 939–946.
12 Laporte J, Kress W, Mandel JL: Diagnosis of X-linked myotubular myopathy by detection of myotubularin. *Ann Neurol* 2001; **50**: 42–46.
13 Komatsu A, Suzuki S, Inagaki T, Yamashita K, Hashizume K: A kindred with Cockayne syndrome caused by multiple splicing variants of the CSA gene. *Am J Med Genet A* 2004; **128A**: 67–71.
14 Nissim-Rafinia M, Kerem B: Splicing regulation as a potential genetic modifier. *Trends Genet* 2002; **18**: 123–127.
15 Targovnik HM, Edouard T, Varela V *et al*: Two novel mutations in the thyroglobulin gene as cause of congenital hypothyroidism: identification a cryptic donor splice site in the exon 19. *Mol Cell Endocrinol* 2012; **348**: 313–321.
16 Aminoff M, Carter JE, Chadwick RB *et al*: Mutations in CUBN, encoding the intrinsic factor-vitamin B12 receptor, cubilin, cause hereditary megaloblastic anaemia 1. *Nat Genet* 1999; **21**: 309–313.

Publication 3

Altered Splicing of the BIN1 Muscle-Specific Exon in Humans and Great Danes with Highly Progressive Centronuclear Myopathy

Johann Böhm, Nasim Vasli, Marie Maurer, Belinda Cowling, G. Diane Shelton, Wolfram Kress, Ivana Prokic, Ulrike Schara, Thomas James Anderson, Matthias Strittmatter, Joachim Weis, Laurent Tiret, Jocelyn Laporte

- Under revision

A- Introduction

Amphiphysin 2, encoded by *BIN1*, plays a central role in membrane sensing and remodeling and is involved in intracellular membrane trafficking in different cell types. The ubiquitously expressed *BIN1*, altered in centronuclear myopathy (CNM) and myotonic dystrophy (DM), possesses a muscle-specific exon coding for a phosphoinositide binding domain. There are at least 12 different isoforms, basically differing by the presence or absence of a phosphoinositide-binding domain (PI domain) and a clathrin-binding domain encoded by exon 11 and exons 13-16, respectively. The clathrin-binding domain is present in the brain isoforms, while the PI domain is found almost exclusively in skeletal muscle isoforms.

B- Aim of study

Detection and characterization of a novel mutation in the *BIN1* gene in a patient with CNM and in canine model.

C- Results

We identified and characterized the first human *BIN1* mutation affecting the splicing of the muscle-specific domain, resulting in a rapidly progressing myopathy in humans. We also identified a new canine model reproducing the human pathology with similar mutation in the *BIN1* gene.

D- Conclusion

Alternative splicing of *BIN1* exon 11 is mis-regulated in patients with myotonic dystrophy. In this study we reported the first mutation affecting the muscle-specific exon 11 of *BIN1* and having an impact on splicing. The major clinical and histological aspects of the patients and affected Great Danes include general muscle weakness, atrophy and nuclear centralization, consistent with the muscle phenotype in DM patients. Our data therefore strongly support the hypothesis that mis-splicing of *BIN1* exon 11 accounts for most muscle-specific signs in myotonic dystrophy.

E- My contribution

The molecular analysis for human was done by me.

Altered Splicing of the BIN1 Muscle-Specific Exon in Humans and Great Danes with Highly Progressive Centronuclear Myopathy

Johann Böhm[1,2,3,4,5], Nasim Vasli[1,2,3,4,5], Marie Maurer[6,7], Belinda Cowling[1,2,3,4,5], G. Diane Shelton[8], Wolfram Kress[9], Ivana Prokic[1,2,3,4,5], Ulrike Schara[10], Thomas James Anderson[11], Matthias Strittmatter[12], Joachim Weis[13], Laurent Tiret[6,7], Jocelyn Laporte[1,2,3,4,5*]

[1]IGBMC (Institut de Génétique et de Biologie Moléculaire et Cellulaire), Illkirch, France
[2]Inserm, U964, Illkirch, France
[3]CNRS, UMR7104, Illkirch, France
[4]Université de Strasbourg, Illkirch, France
[5]Collège de France, chaire de génétique humaine, Illkirch, France
[6]CNM Project, Université Paris-Est Créteil, Ecole nationale vétérinaire d'Alfort, Maisons-Alfort, France
[7]UMR955 de Génétique Fonctionnelle et Médicale, Institut National de la Recherche Agronomique, Maisons-Alfort, France
[8]Department of Pathology, University of California at San Diego, La Jolla, CA 92093;
[9]Department of Human Genetics, Julius-Maximilian University, Würzburg, Germany
[10]Department of Pediatrics, University of Essen, Essen, Germany
[11]Institute of Comparative Medicine, Division of Companion Animal Sciences, University of Glasgow Veterinary School, Glasgow, United Kingdom
[12]Neurology, SHG Klinikum, Merzig, Germany
[13]Institute of Neuropathology and JARA Brain Translational Medicine, RWTH Aachen University, Aachen, Germany

*Correspondence to: jocelyn@igbmc.fr

Short title: BIN1 muscle-specific defects in myopathies

Abstract

Amphiphysin 2, encoded by *BIN1*, is a key factor for membrane sensing and remodelling in different cell types. Homozygous *BIN1* mutations in ubiquitously expressed exons are associated with autosomal recessive centronuclear myopathy (CNM), a mildly progressive muscle disorder typically showing abnormal nuclear centralization on biopsies. In addition, misregulation of *BIN1* splicing partially accounts for the muscle defects in myotonic dystrophy (DM). However, the muscle-specific function of amphiphysin 2 and its pathogenicity in both muscle disorders are not well understood. In this study we identified and characterized the first mutation affecting the splicing of the muscle-specific *BIN1* exon 11 in a consanguineous family with rapidly progressive and ultimately fatal centronuclear myopathy. In parallel, we discovered a mutation in the same *BIN1* exon 11 acceptor splice site as the genetic cause of the Inherited Myopathy of Great Danes (IMGD). Analysis of RNA from patient muscle demonstrated complete skipping of exon 11 and *BIN1* constructs without exon 11 were unable to promote tubulation in differentiated myotubes. Comparative immunofluorescence and ultrastructural analyses of patient and canine biopsies revealed common structural defects, emphasizing the importance of amphiphysin 2 in membrane remodelling and maintenance of the skeletal muscle triad. Our data demonstrate that the alteration of the muscle-specific function of amphiphysin 2 is a common pathomechanism for centronuclear myopathy, myotonic dystrophy and IMGD. The IMGD dog is the first faithful model for human *BIN1*-related CNM and represents the only known mammalian model available for preclinical trials of potential therapies.

Key words: Centronuclear myopathy, CNM, BIN1, Myotonic dystrophy, DM, Inherited Myopathy of Great Danes, IMGD, muscle disorder, excitation-contraction coupling, ECC, T-tubule, splice mutation, dog model

Author Summary

The intracellular organization of muscle fibers relies on a complex membrane system important for muscle structural organization, maintenance, contraction and resistance to stress. Amphiphysin 2, encoded by *BIN1*, plays a central role in membrane sensing and remodelling and is involved in intracellular membrane trafficking in different cell types. The ubiquitously expressed *BIN1*, altered in centronuclear myopathy (CNM) and myotonic dystrophy (DM), possesses a muscle-specific exon coding for a phosphoinositide binding domain. We identified splice mutations affecting the muscle-specific *BIN1* isoform in humans and Great Danes presenting a clinically and histopathologically comparable highly progressive centronuclear myopathy. Our functional and ultrastructural data emphasize the importance of amphiphysin 2 in membrane remodeling and suggest that the defective maintenance of the triad structure is a primary cause for the muscle weakness. The Inherited Myopathy of Great Danes is the first faithful model for investigating other potential pathological mechanisms underlying centronuclear myopathy and for testing therapeutic approaches.

Introduction

Amphiphysin 2 is one of the key factors in muscular membrane remodeling. The gene, *BIN1*, has recently been associated with two different muscle disorders: centronuclear myopathy (CNM, MIM #255200) [1] and myotonic dystrophy (DM, MIM #160900 and #602668) [2]. However, the muscle-specific role of the ubiquitous protein amphiphysin 2 and the pathological mechanisms underlying the muscle disorders are not well understood. This is mainly due to the lack of faithful animal models.

Centronuclear myopathies are characterized by a generalized muscle weakness, atrophy, predominance of type I fibers, and aberrant positioning of nuclei and mitochondria [3]. All three forms are not or only moderately progressive. The X-linked and dominant CNM forms respectively result from mutations in the phosphoinositide phosphatase myotubularin (*MTM1*) and the large GTPase dynamin 2 (*DNM2*) [4,5]. The autosomal recessive form is caused by mutations in *BIN1*, probably involving a partial loss-of-function as the protein level was found to be normal in previously described patients [1]. Amphiphysin 2, encoded by *BIN1*, contains a N-terminal amphipathic helix, a BAR (Bin/Amphiphysin/Rvs) domain, able to sense and maintain membrane curvature, a Myc-binding domain and a SH3 domain, both implicated in protein-protein interactions [6,7,8]. There are at least 12 different isoforms, basically differing by the presence or absence of a phosphoinositide-binding domain and a clathrin-binding domain encoded by exon 11 and exons 13-16, respectively [9,10]. The clathrin-binding domain is present in the brain isoforms, while the phosphoinositide-binding (PI) domain is found almost exclusively in skeletal muscle isoforms [10,11,12]. Sequencing of cDNA demonstrated that all *BIN1* skeletal muscle isoforms contain exon 11 [12]. All ARCNM mutations described to date are in ubiquitously expressed exons [1,13,14,15], raising the question about the molecular basis of the muscle-specificity of the disease. The BAR domain mutations strongly decreased the amphiphysin 2 membrane tubulating properties when expressed in cultured cells, while SH3 truncating mutations were shown to impair the binding and recruitment of dynamin 2.

Mis-splicing of the *BIN1* muscle-specific exon 11 was reported in different forms of myotonic dystrophy (DM) [16]. It is one of the most common muscular dystrophies in neonates and adults, and results from the expression of mutant RNAs with expanded CUG or CCUG repeats leading to the sequestration of splicing factors and subsequent defects in RNA splicing. Splicing alterations of the muscle chloride channel *CLCN1* are suggested to be responsible for the myotonia, whereas aberrant splicing of the insulin receptor *INSR* gene is thought to cause insulin resistance in DM patients. Complete or partial skipping of *BIN1* exon 11 in congenital and adult DM was shown to involve structural T-tubule abnormalities and subsequently muscle weakness [16]. However, there are numerous splicing defects in DM. It is therefore challenging to assess the exact contribution of *BIN1* exon 11 skipping to the DM phenotype, even though severe hypotonia, respiratory failure and histopathological features such as fiber hypotrophy and centrally located nuclei in the congenital forms of DM show intriguing similarities to CNM.

Amphiphysins are key regulators of membrane curvature and trafficking [17]. They can sense membrane curvature and presumably promote the curvature and fission of membranes. They also bind and recruit other regulators of endocytosis to sites of plasma membrane inward budding. Amphiphysin 1 expression is restricted to neuronal tissues and the protein regulates synaptic vesicle recycling in the brain [18]. Amphiphysin 2 is highly expressed in adult striated muscle and its expression increases during muscle cell maturation [10,11,19,20]. The polybasic residues encoded by *BIN1* exon 11 are required for amphiphysin 2-induced membrane tubulation when exogenously expressed in cultured cells [1,21]. In skeletal muscle, amphiphysin 2 is localized at the T-tubules, which are deep sarcolemmal invaginations enabling excitation-contraction coupling [11]. This specific localization, together with the membrane tubulation properties of the muscle-specific isoform containing the PI domain, called iso8 or M-amphiphysin, have led to the suggestion that amphiphysin 2 is implicated in T-tubule biogenesis [21]. This is sustained by defects in the localization of nascent T-tubule markers as caveolin 3 following *BIN1* downregulation in cultured cells [22], and by the abnormal T-tubule structure seen in drosophila with null mutations in *amph*, the unique ortholog of mammalian amphiphysins 1 and 2 [23]. While faithful animal models were previously characterized for the *MTM1* and *DNM2* related CNM forms [24], the perinatal lethality of *Bin1*-null mice precludes the analysis of the role of amphiphysin 2 in skeletal muscle [25]. Therefore, critical questions concerning the muscle-specific function of amphiphysin 2 in mammals and the pathological mechanism of *BIN1*-related CNM remain unanswered. The lack of a faithful animal model for autosomal recessive centronuclear myopathy is a hurdle for a better comprehension of the pathological mechanisms and for the development of therapeutic approaches.

In this study, we identified and characterized the first human *BIN1* mutation affecting the muscle-specific domain. We also identified a novel spontaneous canine model reproducing the human pathology and allowing investigations on the physiological role of amphiphysin 2 in skeletal muscle after birth. Characterization of the dog model revealed an important role for amphiphysin 2 in triad structure and membrane trafficking, and we provide the evidence for a physiological function of the membrane-deforming properties of amphiphysin 2 and its alternative splicing-dependent activity. Our data support the hypothesis that the alteration of the muscle-specific function of amphiphysin 2 on membrane remodeling and trafficking is a common pathomechanism underlying several canine and human myopathies.

Results

BIN1 *exon 11 mutation in patients with rapidly progressive centronuclear myopathy*

To identify *BIN1* mutations affecting its function in skeletal muscle, we sequenced the muscle-specific exon 11 and the adjacent splice-relevant intronic regions in a cohort of 84 patients with various forms of centronuclear myopathy and without mutations in *MTM1*, *DNM2*, or in the other *BIN1* exons. We identified a homozygous *BIN1* exon 11 splice

acceptor mutation (IVS10-1G>A) in two affected members from a consanguineous family from Turkey (Figure 1A and 1B). Genotyping of patient 1 on an Affymetrix Genome-Wide Human SNP 6.0 Array revealed a homozygous region at 2q12.1-q34 region (chr2:104939155-211935990; 106 Mb) encompassing the *BIN1* locus on chromosome 2q14 (data not shown). DNA was not available for the third affected member. The healthy parents are first-degree cousins and were found to be heterozygous for the mutation, confirming autosomal recessive inheritance of the disease. The mutation was not found in unaffected individuals from different origins, including 37 DNAs from an ethnically matched control population, and is not listed in the SNP databases as dbSNP, 1000 genomes, or the NHLBI exome variant server.

Patients 1 and 2 are dizygotic twins. Pregnancy and birth, as well as motor and speech development were normal. General muscle weakness was diagnosed at 3.5 years. Hypotonia, respiratory distress and loss of motor skills were rapidly progressive and the twins died at age 5 and 7, respectively. Patient 3 is the younger brother, and as for his sisters, pregnancy, birth, motor and speech development were normal. Age of onset was 3.5 years and the myopathy is highly progressive, contrasting the slow progression of muscle weakness in the reported CNM cases with *BIN1* mutations in ubiquitous exons [1,13,14,15]. Patient 3 presented with predominant proximal muscle weakness, facial weakness, but no respiratory distress. Eye movement defects, as seen in the majority of the *MTM1*, *DNM2* and *BIN1* patients, were not noted. Deep tendon reflexes were absent and the patient had contractures. Electrophysiological evaluation was normal or showed only unspecific myopathic changes, with normal nerve conduction velocity. Cardiac defects were not noted.

Impact of the human BIN1 *mutation on splicing*

The *BIN1* IVS10-1G>A variation changes the AG acceptor splice site into AA, and is predicted to impair exon 11 splicing by various algorithms. The wild-type acceptor site was recognized by NNSPLICE (score 0.84) and Human Splice Finder (88.5), while the mutated sequence was not predicted to dispose splice sites. To confirm an impact on exon 11 splicing, we performed RT-PCR after RNA isolation from a muscle biopsy of patient 1, amplified a fragment encompassing exons 10 to 12, and obtained a shorter product compared to the control (Figure 1C). To analyze the transcript(s), we cloned the PCR-products and sequenced the resulting clones. As we and others previously reported, the skeletal muscle *BIN1* isoforms contain exon 11, but lack exons 7 and 13 to 16. Exon 17 can be either present or absent, corresponding to isoform 8 or M-amphiphysin [10,11]. Among the 30 analyzed clones, 29 did not contain exon 11 and directly combined exon 10 with exon 12, demonstrating a major skipping of exon 11 in the patient muscle (Figure 1D). The impact of the mutation on the amphiphysin 2 protein level in skeletal muscle was investigated by Western blot (Figure 1E). Using an anti-PI domain antibody, we detected multiple signals in the control as previously reported [1], most probably reflecting post-translational modifications of the different isoforms containing exon 11. In the patient muscle, we found a significant decrease of the level of the amphiphysin 2 isoform containing the PI domain, confirming exon 11 skipping in most *BIN1* muscle transcripts. The amphiphysin levels detected with the pan antibody were

similar in patient 1 and control. Together with the genetic data, we conclude that the rapidly progressive CNM form results from a splice mutation involving the skipping of the muscle-specific exon 11.

BIN1 *exon 11 is required for membrane tubulation in muscle cells*

Previous publications demonstrated the importance of the amphiphysin 2 PI domain in PtdIns(4,5)P2 binding and membrane tubulation [1,2,21]. We transfected C2C12 cells with *BIN1* constructs including or excluding exon 11, and we induced the differentiation of the murine myoblasts into myotubes. Myotubes transfected with the exon 11 containing isoform showed tubulation (arrows) [21,26], whereas the isoform without exon 11 did not have this effect (Figure 2). Immunolabelling of actin, caveolin-3 and RYR1 did not reveal significant differences between the differentially transfected myotubes, suggesting that the amphiphysin 2 PI domain is important for late muscle development or maintenance, rather than for early muscle development. This hypothesis is supported by the fact that the patients were unaffected at birth and during early childhood.

BIN1 *exon 11 mutation causes the Inherited Myopathy of Great Danes (IMGD)*

The perinatal lethality of *Bin1*-null mice precludes investigations on the role of amphiphysin 2 in skeletal muscle maintenance [25]. To identify and characterize an animal model for *BIN1*-related CNM, we analyzed canine pedigrees with molecularly unsolved myopathies. The Inherited Myopathy of Great Danes (IMGD) is characterized by rapidly progressive muscle atrophy and exercise intolerance with an age of onset of about 6 months. Histological examinations of muscle biopsies from autosomal recessive cases from the UK, Canada and Australia revealed increased nuclear internalization and centralization [27,28,29], consistent with centronuclear myopathy. We excluded mutations in *MTM1* [30] and *PTPLA* [31] before sequencing the coding regions and intron/exon boundaries of the canine *BIN1* gene (XM_540990.3). We identified a homozygous AG to GG substitution of the *BIN1* exon 11 acceptor splice site in five dogs from Canada and UK (IVS10-2A>G; Figures 3A and 3B). Pedigree reconstruction revealed a distant relationship between the Canadian and one UK dog (Figure 3C) and a previous publication reported a common ancestor for all IMGD dogs in the UK [28]. The *BIN1* IVS10-2A>G mutation was not found in 112 healthy Great Danes and in 35 dogs from 12 other breeds, strongly suggesting its pathogenicity.

Impact of the canine BIN1 mutation on exon 11 splicing

Like the human *BIN1* IVS10-1G>A mutation, the canine *BIN1* IVS10-2A>G variation affects the exon 11 acceptor splice site. To assess its impact on splicing, we performed RT-PCR on RNA isolated from skeletal muscle biopsies and found a strong reduction of the *BIN1* RNA level compared to healthy controls and compared to a control gene (*MTM1*, Figure 3D). We however detected a faint signal of expected size and cloned the amplicon. All clones contained exon 11 with 27 additional upstream nucleotides, encoding the amino acid sequence ASASRPFPQ (Figure 3E). This in-frame extension results from the disposition of a weak

cryptic 5' acceptor site. The intronic sequence upstream of exon 11 slightly differs between human and dog, possibly explaining the cryptic splicing in dogs versus exon skipping in human patients (Figure 3F). To confirm the impact of the splice mutation on the amphiphysin 2 protein level, canine muscle extracts were probed with an anti-PI domain antibody on Western blot. Compared to the healthy control, amphiphysin 2 was significantly reduced in the affected dog (Figure 3G). We conclude that the Inherited Myopathy of Great Danes results from a *BIN1* exon 11 splice mutation, provoking a strong reduction of the PI domain-containing RNA and protein. Consequently, IMGD represents a genetic model for autosomal recessive centronuclear myopathy, and more specifically for the rapidly progressive form described in this study.

Similar histopathology in affected humans and Great Danes

Medial gluteal muscle biopsies were performed for patient 1 as well as for patient 3 at the age of 3.5 years. H&E staining revealed prominent nuclear centralization, fiber atrophy and moderate endomysial fibrosis (Figure 4A), consistent with centronuclear myopathy. NADH-TR staining demonstrated predominant type I fiber atrophy (Figure 4C), and Gomori trichrome staining did not reveal any further abnormalities (Figure 4D). Similarly, H&E staining of biceps femoris muscle biopsies from affected dogs revealed nuclear internalization and fiber atrophy (Figure 4B). The central areas devoid of staining reflect perinuclear regions lacking myofibrils. Of note, the transverse canine muscle sections showed an unusual lobulated appearance with indentations of the sarcolemma (Figure 4B, arrowheads). To a lesser extent, this was also seen in the human muscle sections. Taken together, human and canine histopathologies were comparable.

Common ultrastructural and membrane defects in affected patients and dogs

To uncover the pathological defects underlying this highly progressive form of centronuclear myopathy and to validate the canine model, we analyzed human and dog muscle biopsies by electron microscopy. Ultrastructural analysis of the human muscle biopsy revealed centralized nuclei surrounded by an area devoid of myofibrils and containing glycogen granules and other organelles (Figure 5A), as commonly seen in *MTM1*, *DNM2* and *BIN1*-related CNM. Moderate Z-band deviations were seen in the adjacent sarcomeres. Triad structures were found to be aberrant and we observed frequent enlarged structures, most probably originating from the sarcoplasmic reticulum (Figure 5D). We also noted other membrane alterations, including sarcolemmal invaginations and subsarcolemmal proliferations of membranes and tubules (Figure 5C), as well as myelin-like membranous structures indicating autophagy (Figure 5B). Likewise, ultrastructural analysis of muscle biopsies from an affected Great Dane showed nuclear internalization, mitochondrial accumulations around the nuclei and myofibrillar disarray (Figure 5E). We furthermore found membranous whorls (Figure 5H) as reported for the X-linked CNM Labrador retriever model with *MTM1* mutation [30,32,33], lipofuscin granules (Figure 5F), and abnormal triads (Figure 5G, arrow). Taken together and considering the histological analysis described above, histopathology of IMGD dogs and

human patients appear strikingly similar, emphasizing common alterations of the membrane structures.

Amphiphysin 2 is present but altered in affected human and dog muscles

To further characterize the pathophysiology of the rapidly progressive human CNM and canine IMGD, we performed immunolocalization experiments on muscle biopsies. Using a pan antibody against all isoforms, amphiphysin 2 signals were detected as an intracellular network in transverse sections of human and canine controls (Figure 6). Signals were also detected in sections of muscles from patient and affected dog, reflecting the presence of different amphiphysin 2 isoforms without exon 11 as shown by Western blot. The amphiphysin 2 network appeared however abnormal in human and canine sections. In some fibers we noted central areas without any signal, while in other fibers accumulations around centralized nuclei were observed (arrows). To determine whether these anomalies were specific for the *BIN1* exon 11 splice mutation or rather a general *BIN1*-related CNM feature, we analyzed a muscle biopsy from a patient with the previously reported *BIN1* p.Asp151Asn mutation and a classical ARCNM phenotype [1]. We observed similar accumulations of amphiphysin 2, suggesting that different *BIN1* mutations in humans and dogs lead to similar amphiphysin 2 mis-localization in muscle (Figure 6A).

Alteration of triad and membrane trafficking regulators

Amphiphysin 2 has been proposed to be implicated in T-tubule biogenesis, but the exact link has barely been documented in mammalian skeletal muscle [21]. We therefore examined the skeletal muscle triad using antibodies against the junctional sarcoplasmic calcium channel RYR1 and the T-tubule marker DHPR in human and dog (Figure 7A and 7B). Both proteins were profoundly altered, showing focal accumulations or central areas without signal in the fibers. Compared to the control longitudinal sections, the transversal orientation of RYR1-labeled triads was lost in the patient muscle. However, these defects did not reflect a general disorganization of the sarcomere, as alpha-actinin labeling appeared normal (Figure S1).

We next wanted to know whether the aberrant triad structure was concurrent with more generalized membrane defects. Dysferlin and caveolin 3, key regulators of membrane repair and trafficking, were found to be mainly located at the sarcolemma in control muscle sections. In contrast, transverse and longitudinal sections of patient 1 and of an affected Great Dane revealed striking intracellular accumulations of both proteins, mainly around central nuclei (Figure 7C and 7D). Especially for the Great Dane, labeling of the sarcolemmal markers confirmed the presence of numerous fibers with unusual lobulated and indented sarcolemma, representing deep sarcolemmal invaginations pointing towards the center of the fibers (arrows, Figure 7D). Taken together, our data correlate the highly progressive human CNM and canine IMGD with general membrane alterations at the triad, the sarcolemma and within the fibers.

Altered myotubularin localization in BIN1-*mutated canine muscles*

As *MTM1* is mutated in X-linked human and canine CNM, we investigated the localization of myotubularin in muscle sections of IMGD dogs. Myotubularin formed an intracellular network in control sections and the signal was stronger in type II fibers labeled with the SERCA1 antibody (Figure 8A). In the IMGD muscle, myotubularin was mainly located as concentric strands pointing to the center in both type I and type II fibers (Figure 8B). We conclude that altered splicing of *BIN1* has a strong impact on myotubularin localization in muscle, revealing a potential link between IMGD and X-linked CNM.

Discussion

In this study, we identified and characterized *BIN1* mutations affecting the splicing of the muscle-specific exon 11, resulting in a rapidly progressing myopathy in humans and dogs. The IMGD dog is the first faithful mammalian model for *BIN1*-related centronuclear myopathy and its characterization unravelled muscle-specific functions of amphiphysin 2 in membrane structural organization, trafficking and remodelling. A schematic representation of the amphiphysin 2 protein domains and of the position of the mutations and splicing alterations causing classical autosomal recessive centronuclear myopathy, rapidly progressive human CNM and canine IMGD as well as myotonic dystrophy is shown in Figure 9.

BIN1 mutations in classical and highly progressive centronuclear myopathies

Classical *BIN1*-related ARCNM has been described with neonatal or childhood onset, hypotonia and ptosis and all mutations were found in ubiquitously expressed exons [1,13,14,15]. The muscle weakness was mildly to moderately progressive, the life expectancy of all previously reported ARCNM did not appear to be markedly reduced, and some patients could still walk at older age. In contrast, our patients with a splice mutation affecting the muscle-specific exon 11 showed a rapid disease progression involving strong care-dependence and leading to death within a few years, despite normal motor development and disease-onset not before 3.5 years. The histopathological findings of our patients and of the previously reported ARCNM cases partially overlap, including atrophy and prominent nuclear internalization in muscle fibers. However, there is no evidence for type I fiber predominance in the muscle biopsies of our patients. Previous RT-PCR experiments demonstrated a progressive integration of exon 11 in *BIN1* mRNA during human skeletal muscle development [2]. We therefore hypothesize that the muscle-specific exon 11 plays a major role in muscle maintenance, rather than in early muscle development. This is in accordance with the highly progressive phenotype of humans and dogs with a disease onset several months or years after birth. Consistently, we detected amphiphysin 2 in muscle tissue, but no *BIN1* transcripts containing exon 11. This suggests that the patients mainly express the embryonic *BIN1* isoform, which might not be able to assume the function of the adult *BIN1* isoform.

The Inherited Myopathy of Great Danes is a faithful model for BIN1-related centronuclear myopathy

The characterization of the pathological mechanisms leading to *BIN1*-related CNM and the development of potential therapeutic approaches is obviated by the lack of a faithful animal model. *BIN1*-null mice are perinatally lethal [25], so that a comprehensive analysis of skeletal muscle alterations during disease development is not possible. We sought for dog breeds with molecularly unsolved congenital myopathies and we identified the Inherited Myopathy of Great Danes as a disease model reproducing the molecular, histological and physiological defects observed in *BIN1*-related CNM patients. IMGD has been reported for cases in Canada, Australia and UK and is characterized by generalized muscle atrophy, exercise intolerance, exercise-induced tremor and muscle wasting [28]. The disease typically starts before 10 months of age and most of the affected dogs are euthanized before 18 months of age due to severe debilitating muscle weakness. Histological examinations revealed internalized or central nuclei without evidence of inflammation, disruption of the sarcomeric architecture with central fiber areas devoid of myofibrils, and central accumulations of mitochondria and glycogen granules [27,28,29]. In addition, type I fiber predominance in combination with an increased expression of genes implicated in the slow-oxidative metabolism was described [34]. In this study we demonstrate that IMGD and progressive CNM have a comparable etiopathology and both conditions result from mutations of the AG acceptor splice site of the *BIN1* muscle-specific exon 11. The histopathology and the cellular organization defects of the human and canine muscle disorders are almost identical, we therefore consider IMGD as the first faithful mammalian model for *BIN1*-related centronuclear myopathy.

Veterinary implications

Some Great Danes of our IMGD cohort were found to be negative for *BIN1* mutations, suggesting that IMGD encompasses several disorders with similar clinical and overlapping histopathological features. The proven relationship of two affected Great Danes demonstrates a common origin of the *BIN1* exon 11 splice mutation, and it is likely that all five affected dogs described here can be traced back to a common ancestor. As the muscle disorder is inherited as a recessive trait, and as canine pedigrees are generally highly inbred, it is likely that the mutation can be found in Great Danes populations from all over the world, as recently demonstrated for another autosomal recessive CNM form in Labrador retrievers (Maurer et al., PLoS ONE, in press). It is therefore of veterinary medical interest to sequence *BIN1* exon 11 in Great Danes. Also, veterinarians and veterinary pathologists should consider *BIN1* mutations as a possible cause of any unexplained progressive myopathy in dogs, especially when the biopsy displays central nuclei and lobulated or indented sarcolemma.

Insights into amphiphysin 2 muscle-specific functions and pathological mechanisms of centronuclear myopathy

Detailed immunological and ultrastructural analyses of muscles from patients and affected Great Danes revealed common membrane structure alterations and abnormal accumulations

of proteins regulating membrane trafficking. The aberrant skeletal muscle triad suggests that the amphiphysin 2 muscle-specific isoforms play an important role in triad buildup and/or maintenance. This is in accordance with the known biochemical function of amphiphysin 2 and other N-BAR domain proteins to sense membrane curvature and to potentially induce curvature through the insertion of an amphipathic helix into the membrane bilayer. *In vitro* and cell culture experiments have led to the suggestion that the exon 11 encoded PI-binding motif is essential for membrane tubulation in cultured muscle cells [21]. Indeed, Drosophila mutated for amphiphysin, the ortholog of both amphiphysin 1 and amphiphysin 2, display an abnormal T-tubule system [23]. The mammalian amphiphysin 2 has most probably retained the muscle function of the common amphiphysin ancestor while amphiphysin 1, implicated in synaptic vesicle recycling [18], may have gradually evolved a more specialized function of an upper neuronal control of motor [35] or cognitive functions [18]. T-tubule alterations and muscle weakness were reproduced in murine *Tibialis anterior* injected with a U7 small nuclear RNA construct harboring an antisense sequence promoting *BIN1* exon 11 skipping [2]. However, nuclear centralization and atrophy were not observed, contrasting with the IMGD model. This is possibly due to the low efficacy of the AAV-U7 method or alternatively to the examination time point 4 months post injection. As the triad is the membrane structure controlling excitation-contraction coupling, this suggests that impaired excitation-contraction coupling and subsequent calcium homeostasis defects are a primary cause of the myopathy. Of note, abnormal intracellular calcium release was observed in isolated murine muscle fibers after *BIN1* shRNA-mediated knock-down [36]. Together with the present characterization of the IMGD model, these data indicate that amphiphysin 2 has an important muscle-specific role in triad structural maintenance, and provide additional evidence that triad modifications are a common defect in myopathies.

Triads are not the only membrane compartment affected in patients and Great Danes harboring *BIN1* exon 11 mutations. We also noted central accumulations of caveolin 3 and dysferlin, two key regulators of membrane trafficking in skeletal muscle, numerous membranous whorls, and a peculiar remodeling of the sarcolemma, manifesting an indented fiber perimeter and invaginations towards the center of the fibers. These observations point to a general function of amphiphysin 2 in membrane trafficking and remodeling in skeletal muscle. Interestingly, caveolin 3 and dysferlin are mutated in limb girdle muscular dystrophies, defined by progressive muscle weakness and increased sarcolemma fragility. However, we did not observe a dystrophic phenotype in patients and affected Great Danes. Caveolin 3 regulates membrane tension at the sarcolemma and dysferlin controls membrane exocytosis in sarcolemmal membrane repair [37,38]. As both proteins are also present on regenerating T-tubules [39], their mislocalization resulting from a *BIN1* mutation would be in accordance with defective T-tubule regeneration. It also suggests that amphiphysin 2 could be implicated in membrane and protein trafficking in skeletal muscle. Indeed, Hong et al. have suggested that amphiphysin 2 participates in the transport of the L-type calcium channel DHPR to the T-tubule in a microtubule-dependent manner in cardiac muscle cells [40]. Moreover, data mainly obtained in cultured cells support a key role of amphiphysins in the formation of endocytic vesicles [17], and a study in *Caenorhabditis elegans* suggested a role of amphipysin in vesicle recycling [41]. Defects in intracellular signaling resulting from

calcium defects and impaired transport of ion channels and growth factor might explain the muscle weakness and atrophy in *BIN1*-related CNM.

Amphiphysin 2 links several forms of centronuclear myopathies and myotonic dystrophy

Our findings on the IMGD model uncovered possible links between *BIN1*-related and other forms of CNM. Altered triads and the presence of membranous whorls were reported for *MTM1* dog, mouse and zebrafish models as well as for patients with *MTM1* mutations involving protein loss [12,30,33,42,43]. Abnormal triad markers were also reported for *MTM1*-related and *DNM2*-related CNM [12,44]. Dysferlin localization was not extensively studied in *MTM1*-CNM but was increased in the cytoplasm of a mouse model and in patients with *DNM2*-CNM [45]. Moreover, we found myotubularin localization was strongly impaired in IMGD muscles. These findings suggest that myotubularin and amphiphysin 2 are in the same pathway regulating membrane remodeling in skeletal muscle and strengthen the hypothesis of a common pathological mechanism of the X-linked and the autosomal recessive CNM forms.

Alternative splicing of *BIN1* exon 11 is mis-regulated in patients with myotonic dystrophy [2]. Here we report the first mutation affecting the muscle-specific exon 11 of *BIN1* and having an impact on splicing. The major clinical and histological aspects of the patients and affected Great Danes include general muscle weakness, atrophy and nuclear centralization, consistent with the muscle phenotype in DM patients. Our data therefore strongly support the hypothesis that mis-splicing of *BIN1* exon 11 accounts for most muscle-specific signs in myotonic dystrophy.

Conclusion

We identified a mutation in *BIN1* as the genetic cause of the Inherited Myopathy in Great Danes. Comparison with the pathology of patients with an exon 11 acceptor site mutation demonstrates that the IMGD dog is a faithful model for *BIN1*-related centronuclear myopathy. The characterization of the IMGD model allows novel insights into the overlapping pathogenesis of centronuclear myopathy and myotonic dystrophy, resulting from *BIN1* mutations in coding regions or from mutations leading to altered splicing, respectively. Our data provide strong evidence for the physiological importance of the membrane-deforming properties of amphiphysin 2 in skeletal muscle. The affected Great Danes represent the only characterized mammalian model available for preclinical trials of potential therapies and are a precious resource to develop effective treatments for this severe human congenital myopathy.

Materials and methods

Ethics statement

Human sample collection was performed with informed consent from the patients according to the declaration of Helsinki and experimentation was performed following IGBMC institutional review-board-accepted protocols. Dog samples were collected under the ethical guidance of the Ethics committee of the University of Glasgow. Cryopreserved muscle specimens were processed and stored at the University of California, San Diego, under a tissue transfer approval from the institutional Animal Care and Use Committee. All dogs were examined with the consent of their owners.

Molecular genetics

Human Genomic DNA was prepared from peripheral blood by routine procedures and sequenced for all coding exons and intron/exon boundaries of *MTM1*, *DNM2*, and *BIN1* as described elsewhere [1,4,5]. Patient 1 had a normal CTG repeat length at the DMPK locus (7 and 13 repeats) and was therefore excluded for myotonic dystrophy. Control DNAs were from healthy individuals of Turkish origin.

Dog DNA samples were extracted from saliva, venous blood or muscle biopsy specimens (cryosections or paraffin embedded tissue) by routine procedures and sequenced for all coding exons and intron/exon boundaries of canine *MTM1* [30], *PTPLA* [31] and *BIN1* (primer sequences in TableS1). Control samples were from a world-wide collection of healthy Great Danes as well as from healthy individuals of 13 other breeds.

RNA studies

RNA was extracted from muscle biopsies by routine procedures and reverse transcribed using the SuperScript® III kit (Invitrogen, Carlsbad, USA). Human and dog amplicons were cloned into the pGEM®-T Easy vector (Promega, Madison, USA) and transfected into *E.coli* DH5α cells. Blue/white selection, repeated twice, resulted in 30 clones for the human cDNA and 3 clones for the canine cDNA. Control dog was an unaffected Drahthaar. Primer sequences are listed in Table S1.

Protein studies

Western blot and immunofluorescence were performed using routine protocols. Following antibodies were used for the study: R2406 (home-made rabbit anti-BIN1 PI binding domain), R2444 (home-made rabbit anti-BIN1 SH3 domain), R3062 (home-made rabbit anti-BIN1 exon 12 epitope), C99D (mouse anti-BIN1 exon 17 epitope, Upstate Biotechnology, Buffalo, USA), R2868 (home-made rabbit anti-MTM1), mouse anti-GAPDH (Merck Millipore, Darmstadt, Germany), mouse anti-α-actinin (Sigma-Aldrich, St. Louis, USA), mouse anti-ryanodine receptor 1 (Affinity BioReagents, Golden, USA), mouse anti-SERCA 1 (Affinity BioReagents, Golden, USA), rabbit anti-dysferlin (Euromedex, Souffelweyersheim, France), goat anti-caveolin-3 (Tebu-BIO, Le-Perray-en-Yvelines, France), rabbit anti-caveolin-3 (Affinity BioReagents, Golden, USA), mouse anti-DHPR (Affinity BioReagents, Golden, USA), and mouse anti-actin (Sigma-Aldrich, St. Louis, USA). For immunohistofluorescence,

transverse cryosections were prepared, fixed and stained by routine methods. Nuclei were stained with Hoechst or DAPI (Sigma-Aldrich, St. Louis, USA). Sections were mounted with slowfade antifade reagent (Invitrogen, Carlsbad, USA) and viewed using a laser scanning confocal microscope (TCS SP2; Leica Microsystems, Wetzlar, Germany) or a a Zeiss Axio Observer Z.1 microscope equipped with a 20X lens and Axioplan imaging with structured illumination (Carl Zeiss, Jena, Germany).

Muscle histology

For histochemical analyses, transverse sections of muscle cryosections (8 µm) of medial gluteal and biceps femoris muscle biopsies were stained with hematoxylin-eosin, modified Gomori trichrome, NADH-TR and myofibrillar ATPase then assessed for centralized nuclei, fiber morphology, fiber type distribution, cores, protein accumulation and cellular infiltrations.

Electron microscopy

Muscle biopsies were processed for electron microscopy as described previously [46]. Briefly, the tissue was fixed either in 6 % phosphate-buffered glutaraldehyde (human patient) or in 2.5% paraformaldehyde, 2.5% glutaraldehyde, and 50 mM $CaCl_2$ in 0.1 M cacodylate buffer at pH 7.4 (dog), and post-fixed with 2% OsO_4, 0.8% $K_3Fe(CN)_6$ in 0.1 M cacodylate buffer (pH 7.4) for 2 h at 4°C and incubated with 5% uranyl acetate for 2 h at 4°C. Samples were dehydrated in graded series of ethanol and embedded in Epoxy resin 812. Ultrathin sections (70 nm) were contrasted with uranyl acetate and lead citrate.

Membrane tubulation assay

Murine C2C12 myoblasts were seeded on coverslips and transfected at 50-60% confluency either with *BIN1* isoform 8 (including exon 11) or isoform 9 (excluding exon 11, both were a kind gift from Pietro de Camilli, Howard Hughes Medical Institute). Cells were differentiated after 24h by changing to medium containing 2% horse serum instead of FCS and fixed and stained after 5 days of differentiation by routine methods. Nuclei were stained with Hoechst/DAPI (Sigma-Aldrich, St. Louis, USA) and sections were mounted with slowfade antifade reagent and viewed using a laser scanning confocal microscope (TCS SP2; Leica Microsystems, Wetzlar, Germany).

Web resources

1000 genomes - A Deep Catalog of Human Genetic Variation (URL: http://www.1000genomes.org/)

Database of Single Nucleotide Polymorphisms (dbSNP). Bethesda (MD): National Center for Biotechnology Information, National Library of Medicine. (dbSNP Build ID: 134). (URL: http://www.ncbi.nlm.nih.gov/SNP/)

Exome Variant Server, NHLBI Exome Sequencing Project (ESP), Seattle, WA (URL: http://evs.gs.washington.edu/EVS/)

Online Mendelian Inheritance in Man (OMIM) (URL: http://www.omim.org/)

NNsplice: prediction of splice mutations (URL: http://www.fruitfly.org/seq_tools/splice.html)

Acknowledgements

We thank Valérie Biancalana for the myotonic dystrophy testing, Pietro de Camilli for the amphiphysin constructs, Anders Oldfors for the muscle biopsy of the p.Asp151Asn *BIN1* patient, Inès Barthélémy and Stéphane Blot from the UETM-ENVA for the control canine muscle biopsy, the Cani-DNA banking resource, Anne Thomas from Antagene and the owners for DNAs from control Great Danes.

Financial Disclosure

This study was supported by INSERM, CNRS, University of Strasbourg, and Collège de France and by grants from Association Française contre les Myopathies (AFM), Muscular dystrophy Association (MDA), Fondation Recherche Médicale (FRM), the E-rare program, Agence Nationale de la Recherche (ANR Centronucleus and CM-WES), and the CNM Project (www.labradorcnm.com). B.C. was supported by FRM; JL was supported by a contrat Hospitalier de Recherche Translationnelle from Assistance Publique - Hôpitaux de Paris. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author Contributions

JB, LT and JL conceived and designed the experiments. JB, NV, MM, BC, GDS and WK performed the experiments. JB, NV, WK, GDS, US and JW analyzed the data. GDS, IP, TJA, MS and JW contributed reagents/materials/analysis. JB and JL wrote the manuscript.

References

1. Nicot AS, Toussaint A, Tosch V, Kretz C, Wallgren-Pettersson C, et al. (2007) Mutations in amphiphysin 2 (BIN1) disrupt interaction with dynamin 2 and cause autosomal recessive centronuclear myopathy. Nat Genet 39: 1134-1139.
2. Fugier C, Klein AF, Hammer C, Vassilopoulos S, Ivarsson Y, et al. (2011) Misregulated alternative splicing of BIN1 is associated with T tubule alterations and muscle weakness in myotonic dystrophy. Nat Med 17: 720-725.
3. Jungbluth H, Wallgren-Pettersson C, Laporte J (2008) Centronuclear (myotubular) myopathy. Orphanet J Rare Dis 3: 26.

4. Bitoun M, Maugenre S, Jeannet PY, Lacene E, Ferrer X, et al. (2005) Mutations in dynamin 2 cause dominant centronuclear myopathy. Nat Genet 37: 1207-1209.
5. Laporte J, Hu LJ, Kretz C, Mandel JL, Kioschis P, et al. (1996) A gene mutated in X-linked myotubular myopathy defines a new putative tyrosine phosphatase family conserved in yeast. Nat Genet 13: 175-182.
6. Grabs D, Slepnev VI, Songyang Z, David C, Lynch M, et al. (1997) The SH3 domain of amphiphysin binds the proline-rich domain of dynamin at a single site that defines a new SH3 binding consensus sequence. J Biol Chem 272: 13419-13425.
7. Itoh T, De Camilli P (2006) BAR, F-BAR (EFC) and ENTH/ANTH domains in the regulation of membrane-cytosol interfaces and membrane curvature. Biochim Biophys Acta 1761: 897-912.
8. Sakamuro D, Elliott KJ, Wechsler-Reya R, Prendergast GC (1996) BIN1 is a novel MYC-interacting protein with features of a tumour suppressor. Nat Genet 14: 69-77.
9. Ren G, Vajjhala P, Lee JS, Winsor B, Munn AL (2006) The BAR domain proteins: molding membranes in fission, fusion, and phagy. Microbiol Mol Biol Rev 70: 37-120.
10. Wechsler-Reya R, Sakamuro D, Zhang J, Duhadaway J, Prendergast GC (1997) Structural analysis of the human BIN1 gene. Evidence for tissue-specific transcriptional regulation and alternate RNA splicing. J Biol Chem 272: 31453-31458.
11. Butler MH, David C, Ochoa GC, Freyberg Z, Daniell L, et al. (1997) Amphiphysin II (SH3P9; BIN1), a member of the amphiphysin/Rvs family, is concentrated in the cortical cytomatrix of axon initial segments and nodes of Ranvier in brain and around T tubules in skeletal muscle. J Cell Biol 137: 1355-1367.
12. Toussaint A, Cowling BS, Hnia K, Mohr M, Oldfors A, et al. (2011) Defects in amphiphysin 2 (BIN1) and triads in several forms of centronuclear myopathies. Acta Neuropathol 121: 253-266.
13. Bohm J, Yis U, Ortac R, Cakmakci H, Kurul SH, et al. (2010) Case report of intrafamilial variability in autosomal recessive centronuclear myopathy associated to a novel BIN1 stop mutation. Orphanet J Rare Dis 5: 35.
14. Claeys KG, Maisonobe T, Bohm J, Laporte J, Hezode M, et al. (2010) Phenotype of a patient with recessive centronuclear myopathy and a novel BIN1 mutation. Neurology 74: 519-521.
15. Mejaddam AY, Nennesmo I, Sejersen T (2009) Severe phenotype of a patient with autosomal recessive centronuclear myopathy due to a BIN1 mutation. Acta Myol 28: 91-93.
16. Fugier C, Klein AF, Hammer C, Vassilopoulos S, Ivarsson Y, et al. (2011) Misregulated alternative splicing of BIN1 is associated with T tubule alterations and muscle weakness in myotonic dystrophy. Nat Med 17: 720-725.
17. Qualmann B, Koch D, Kessels MM (2011) Let's go bananas: revisiting the endocytic BAR code. EMBO J 30: 3501-3515.
18. Di Paolo G, Sankaranarayanan S, Wenk MR, Daniell L, Perucco E, et al. (2002) Decreased synaptic vesicle recycling efficiency and cognitive deficits in amphiphysin 1 knockout mice. Neuron 33: 789-804.
19. Mao NC, Steingrimsson E, DuHadaway J, Wasserman W, Ruiz JC, et al. (1999) The murine Bin1 gene functions early in myogenesis and defines a new region of synteny between mouse chromosome 18 and human chromosome 2. Genomics 56: 51-58.
20. Wechsler-Reya RJ, Elliott KJ, Prendergast GC (1998) A role for the putative tumor suppressor Bin1 in muscle cell differentiation. Mol Cell Biol 18: 566-575.
21. Lee E, Marcucci M, Daniell L, Pypaert M, Weisz OA, et al. (2002) Amphiphysin 2 (Bin1) and T-tubule biogenesis in muscle. Science 297: 1193-1196.
22. Kojima C, Hashimoto A, Yabuta I, Hirose M, Hashimoto S, et al. (2004) Regulation of Bin1 SH3 domain binding by phosphoinositides. Embo J 23: 4413-4422.
23. Razzaq A, Robinson IM, McMahon HT, Skepper JN, Su Y, et al. (2001) Amphiphysin is necessary for organization of the excitation-contraction coupling machinery of muscles, but not for synaptic vesicle endocytosis in Drosophila. Genes Dev 15: 2967-2979.

24. Cowling BS, Toussaint A, Muller J, Laporte J (2012) Defective membrane remodeling in neuromuscular diseases: insights from animal models. PLoS Genet 8: e1002595.
25. Muller AJ, Baker JF, DuHadaway JB, Ge K, Farmer G, et al. (2003) Targeted disruption of the murine Bin1/Amphiphysin II gene does not disable endocytosis but results in embryonic cardiomyopathy with aberrant myofibril formation. Mol Cell Biol 23: 4295-4306.
26. Spiegelhalter C, Tosch V, Hentsch D, Koch M, Kessler P, et al. (2010) From dynamic live cell imaging to 3D ultrastructure: novel integrated methods for high pressure freezing and correlative light-electron microscopy. PLoS One 5: e9014.
27. Davies SE, Davies DR, Richards RB, Bruce WJ (2008) Inherited myopathy in a Great Dane. Aust Vet J 86: 43-45.
28. Lujan Feliu-Pascual A, Shelton GD, Targett MP, Long SN, Comerford EJ, et al. (2006) Inherited myopathy of great Danes. J Small Anim Pract 47: 249-254.
29. McMillan CJ, Taylor SM, Shelton GD (2006) Inherited myopathy in a young Great Dane. Can Vet J 47: 891-893.
30. Beggs AH, Bohm J, Snead E, Kozlowski M, Maurer M, et al. (2010) MTM1 mutation associated with X-linked myotubular myopathy in Labrador Retrievers. Proc Natl Acad Sci U S A 107: 14697-14702.
31. Pele M, Tiret L, Kessler JL, Blot S, Panthier JJ (2005) SINE exonic insertion in the PTPLA gene leads to multiple splicing defects and segregates with the autosomal recessive centronuclear myopathy in dogs. Hum Mol Genet 14: 1417-1427.
32. Buj-Bello A, Laugel V, Messaddeq N, Zahreddine H, Laporte J, et al. (2002) The lipid phosphatase myotubularin is essential for skeletal muscle maintenance but not for myogenesis in mice. Proc Natl Acad Sci U S A 99: 15060-15065.
33. Dowling JJ, Vreede AP, Low SE, Gibbs EM, Kuwada JY, et al. (2009) Loss of myotubularin function results in T-tubule disorganization in zebrafish and human myotubular myopathy. PLoS Genet 5: e1000372.
34. Chang KC, McCulloch ML, Anderson TJ (2010) Molecular and cellular insights into a distinct myopathy of Great Dane dogs. Vet J 183: 322-327.
35. Patterson EE, Minor KM, Tchernatynskaia AV, Taylor SM, Shelton GD, et al. (2008) A canine DNM1 mutation is highly associated with the syndrome of exercise-induced collapse. Nat Genet 40: 1235-1239.
36. Tjondrokoesoemo A, Park KH, Ferrante C, Komazaki S, Lesniak S, et al. (2011) Disrupted membrane structure and intracellular Ca(2)(+) signaling in adult skeletal muscle with acute knockdown of Bin1. PLoS One 6: e25740.
37. Bansal D, Miyake K, Vogel SS, Groh S, Chen CC, et al. (2003) Defective membrane repair in dysferlin-deficient muscular dystrophy. Nature 423: 168-172.
38. Sinha B, Koster D, Ruez R, Gonnord P, Bastiani M, et al. (2011) Cells respond to mechanical stress by rapid disassembly of caveolae. Cell 144: 402-413.
39. Klinge L, Harris J, Sewry C, Charlton R, Anderson L, et al. (2010) Dysferlin associates with the developing T-tubule system in rodent and human skeletal muscle. Muscle Nerve 41: 166-173.
40. Hong TT, Smyth JW, Gao D, Chu KY, Vogan JM, et al. (2010) BIN1 localizes the L-type calcium channel to cardiac T-tubules. PLoS Biol 8: e1000312.
41. Pant S, Sharma M, Patel K, Caplan S, Carr CM, et al. (2009) AMPH-1/Amphiphysin/Bin1 functions with RME-1/Ehd1 in endocytic recycling. Nat Cell Biol 11: 1399-1410.
42. Buj-Bello A, Laugel V, Messaddeq N, Zahreddine H, Laporte J, et al. (2002) The lipid phosphatase myotubularin is essential for skeletal muscle maintenance but not for myogenesis in mice. Proc Natl Acad Sci U S A 99: 15060-15065.
43. Pierson CR, Dulin-Smith AN, Durban AN, Marshall ML, Marshall JT, et al. Modeling the human MTM1 p.R69C mutation in murine Mtm1 results in exon 4 skipping and a less severe myotubular myopathy phenotype. Hum Mol Genet 21: 811-825.
44. Romero NB, Bitoun M (2011) Centronuclear myopathies. Semin Pediatr Neurol 18: 250-256.

45. Durieux AC, Vignaud A, Prudhon B, Viou MT, Beuvin M, et al. (2010) A centronuclear myopathy-dynamin 2 mutation impairs skeletal muscle structure and function in mice. Hum Mol Genet 19: 4820-4836.
46. Weis J, Schroder JM (1988) Adult polyglucosan body myopathy with subclinical peripheral neuropathy: case report and review of diseases associated with polyglucosan body accumulation. Clin Neuropathol 7: 271-279.

Tables

Table S1. Primer sequences

Figures legends

Figure 1. Human *BIN1* mutation of the exon 11 acceptor splice site and impact on splicing. (A) Pedigree and (B) Chromatopherogram. Patients 1 and 3 are homozygous for the IVS10-1G>A mutation, while both parents are heterozygous carriers. DNA from patient 2 was not available. (C) RT-PCR on mRNA isolated from muscle using primers encompassing *BIN1* exons 10-12 demonstrated amplification of a shorter product in patient 1 compared to a healthy control. For the negative control (Ctrl-) PCR was performed without RNA. (D) Sequencing of the *BIN1* cDNA from muscle demonstrated skipping of *BIN1* exon 11 in patient 1. (E) Western blot analysis of patient muscle extracts detected a strong reduction of the amphiphysin 2 isoforms containing the exon 11 encoded PI-binding domain. The level of amphiphysin 2 detected with a pan antibody was comparable between patient 1 and control.

Figure 2. Essential role of *BIN1* exon 11 in membrane tubulation in myotubes. C2C12 myotubes overexpressing *BIN1* isoform 8 (including exon 11) showed strong tubulation, whereas *BIN1* isoform 9 (without exon 11) does not induce tubulation. The intracellular localization of actin, caveolin 3, and RYR1 was not affected by tubulation in myotubes 5 days post differentiation.

Figure 3. The inherited myopathy of Great Danes results from a *BIN1* mutation in the exon 11 acceptor splice site. (A) Picture of an affected 3-year-old Great Dane. (B) Chromatopherograms and sequence alignment showing the *BIN1* IVS10-2A>G mutation in 5 affected dogs. (C) Pedigree showing the distant relationship of two affected Great Danes from the UK and Canada. (D) RT-PCR on skeletal muscle RNA showed a strong reduction of the *BIN1* RNA level in the affected Great Dane compared to the healthy canine control. Amplification of a control gene (*MTM1*) was normal. M = Marker (E) Sequencing of the residual cDNA revealed the presence of 27 additional nucleotides due to the use of a weak cryptic 5' splice acceptor site. (F) Sequence alignment of human and canine *BIN1* intron/exon boundary of exon 11. (G) Western blot using an anti-PI domain antibody showed a strong decrease of the amphiphysin 2 protein level.

Figure 4. Histopathological comparison of muscles from human patient and affected Great Dane. (A) Human and (B) canine muscle biopsy sections revealed nuclear centralization (arrows), fiber atrophy and lobulation as well as sarcolemmal invaginations (arrowheads) on H&E staining. (C) NADH-TR staining of human biopsy sections demonstrated predominant type I fiber atrophy. (D) Gomori trichrome staining of patient sections did not reveal any further abnormalities.

Figure 5. Common ultrastructural and membrane defects in affected human and dog. (A-D) Electron microscopic analysis of a patient biopsy showing a centralized nucleus surrounded by glycogen granules and mild sarcomeric disarray (A), membranous cytoplasmic bodies (B), proliferation of subsarcolemmal membranes and tubules, possibly representing extensions of the sarcoplasmic reticulum (C), and widened tubules at the triads (arrow, D). (E-H) Ultrastructural analysis of a Great Dane biopsy revealed central nuclei surrounded by mitochondrial accumulations (E), lipofuscin granules (F), abnormal triads (arrow) and myofibrillar disarray (G), as well as membranous whorls (H).

Figure 6. Amphiphysin 2 is present but altered in muscles from affected humans and dogs. (A) Amphiphysin 2 localization in control (left), patient 1 (middle) and a CNM patient with the p.Asp151Asn mutation (right). Arrows indicate abnormal accumulations of amphiphysin 2 around centralized nuclei in both patient muscles. (B) Abnormal localization of amphiphysin 2 in an affected Great Dane muscle compared to a control.

Figure 7. Alteration of triad components and proteins regulating membrane trafficking. (A) Immunolocalization on longitudinal and transversal sections revealed an abnormal RYR1 pattern and intracellular RYR1 accumulations in the patient, (C) as well as a strong dysferlin signal around large unstained areas including centralized nuclei. (B) The muscle of an affected Great Dane showed RYR1 and DHPR accumulations, and (D) an abnormal intracellular localization of dysferlin, caveolin 3 and SERCA1

Figure 8. Myotubularin is mis-localized in IMGD muscle. (A) In muscle sections from control dogs, myotubularin is predominantly expressed in type II fibers expressing SERCA1. (B) In affected Great Danes, massive myotubularin accumulations formed a concentric network around the fiber center.

Figure 9. *BIN1* alterations in different myopathies. Schematic representation of the amphiphysin 2 protein domains and position of the known mutations causing autosomal recessive centronuclear myopathy, the new splice mutations resulting in rapidly progressive centronuclear myopathy and Inherited Myopathy of Great Danes. Myotonic dystrophy induces mis-splicing of *BIN1* exon 11. Nomenclature is based on isoform 1 (NM_139343), containing all exons except exon 11.

Figure S1. Normal alpha-actinin labeling in patient muscle. Labeling of α-actinin did not reveal major structural differences between the patient and the control.

Figure 1. Human *BIN1* mutation of the exon 11 acceptor splice site and impact on splicing

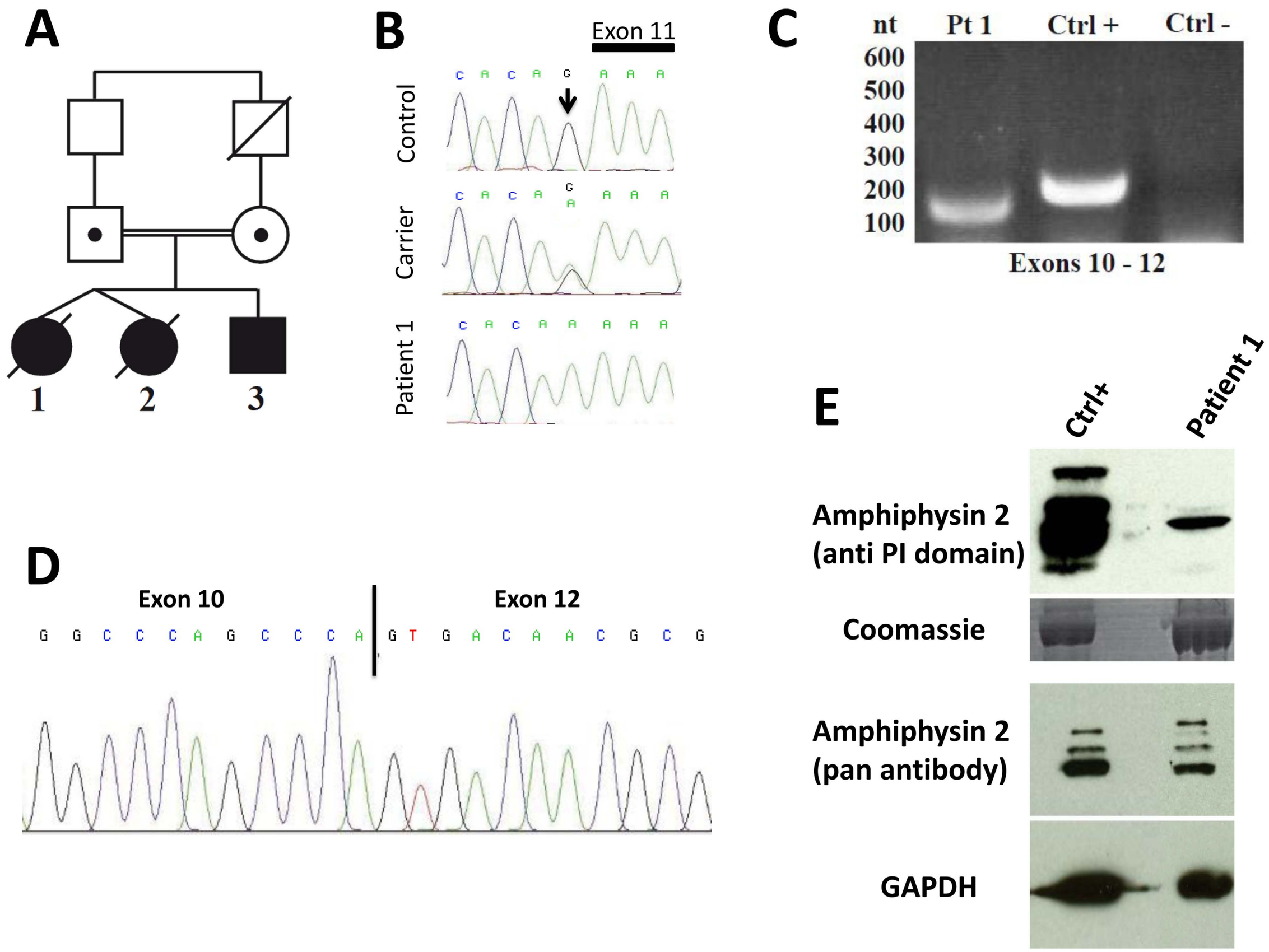


Figure 2. Essential role of *BIN1* exon 11 in membrane tubulation in myotubes

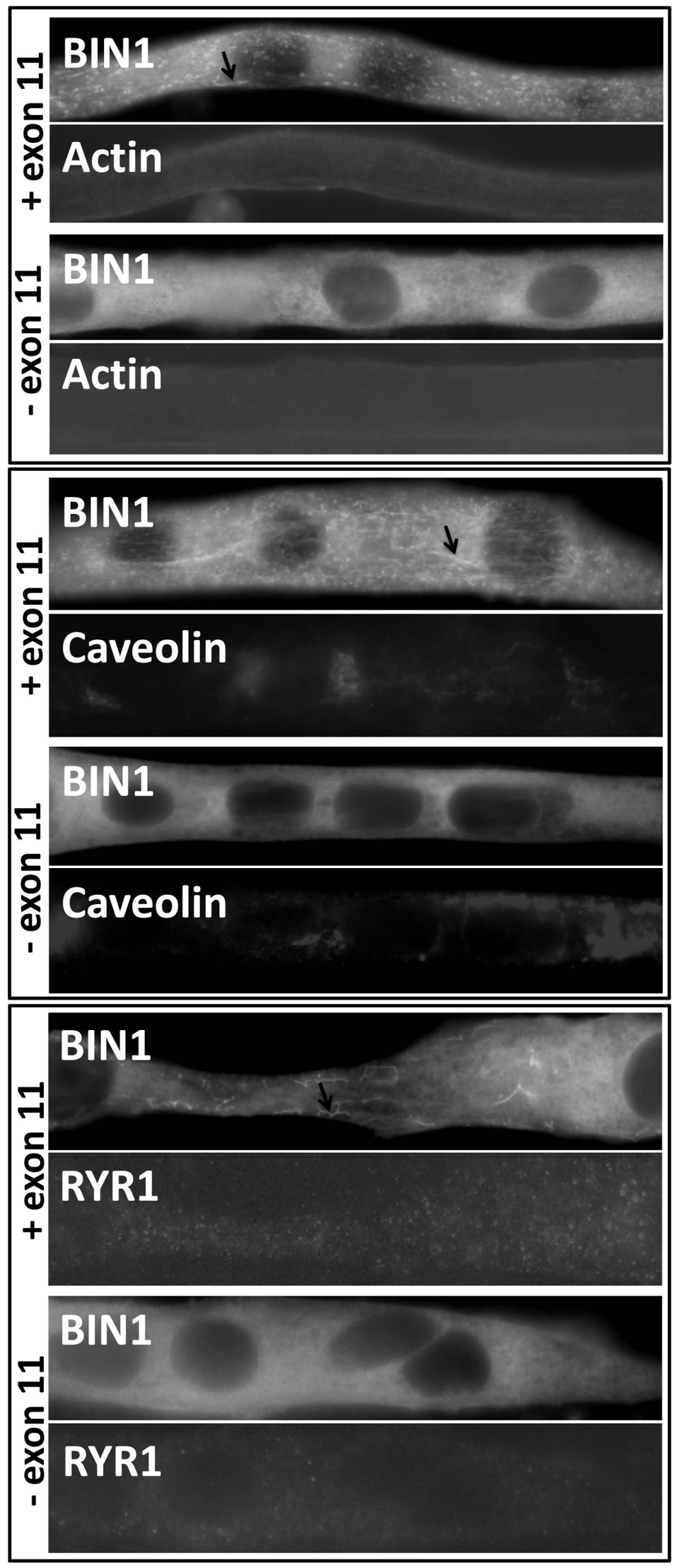
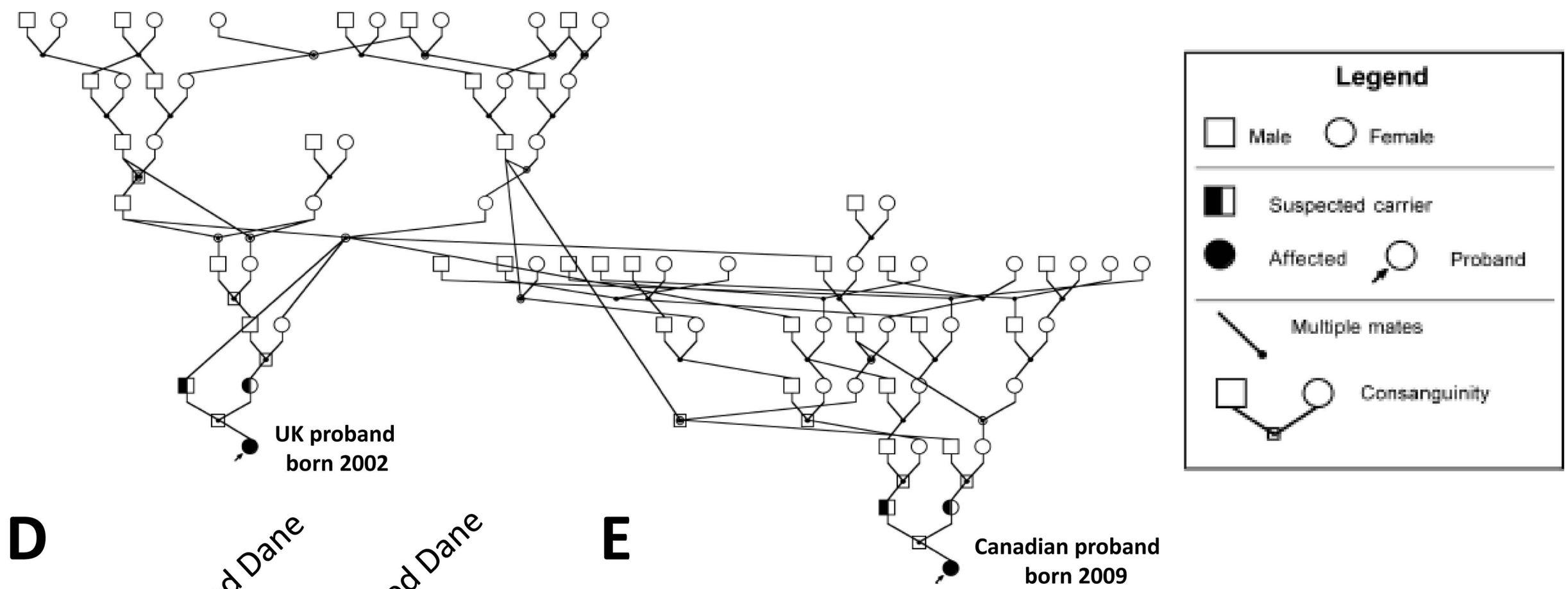
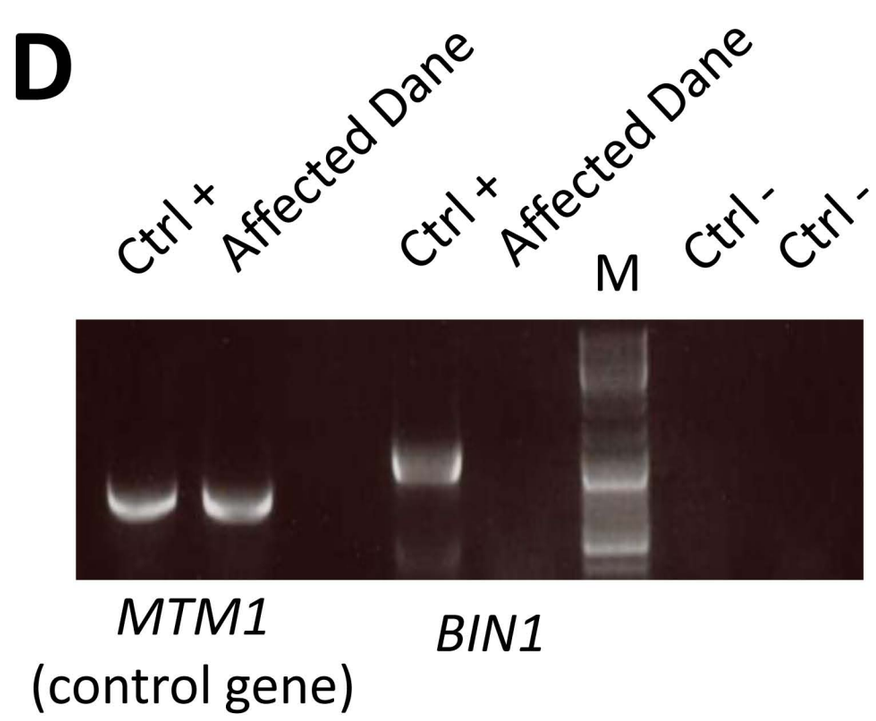
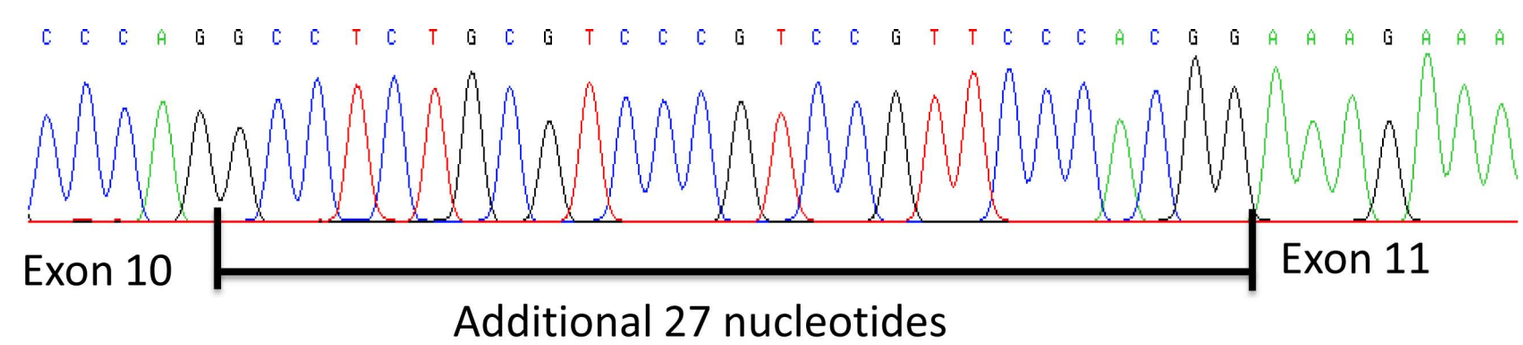
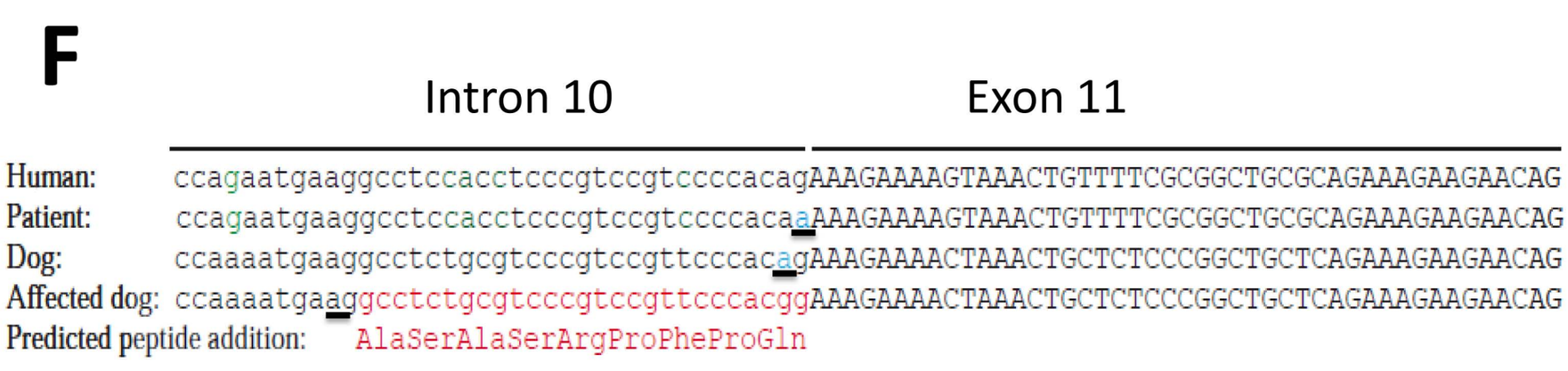


Figure 3. The inherited myopathy of Great Danes results from a *BIN1* mutation in the exon 11 acceptor splice site









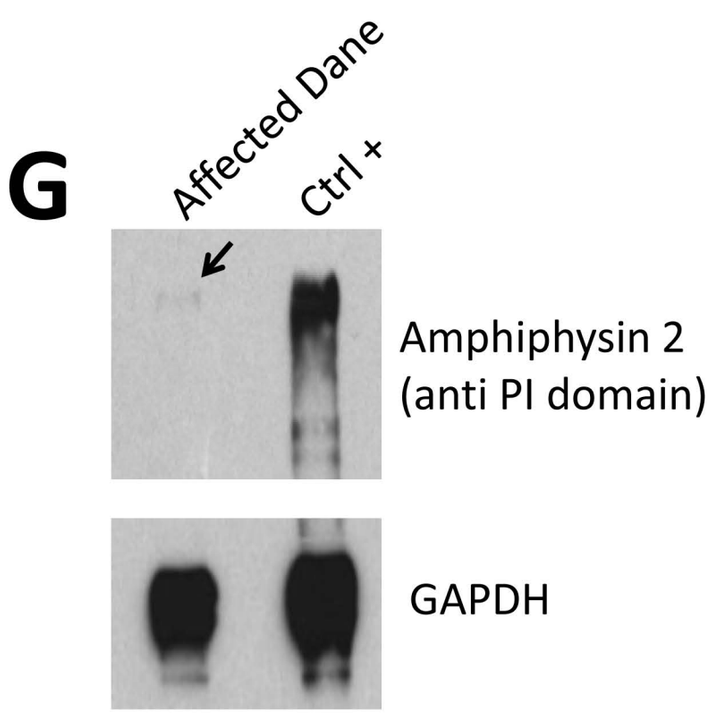


Figure 4. Histopathological comparison of muscles from human patient and affected Great Dane

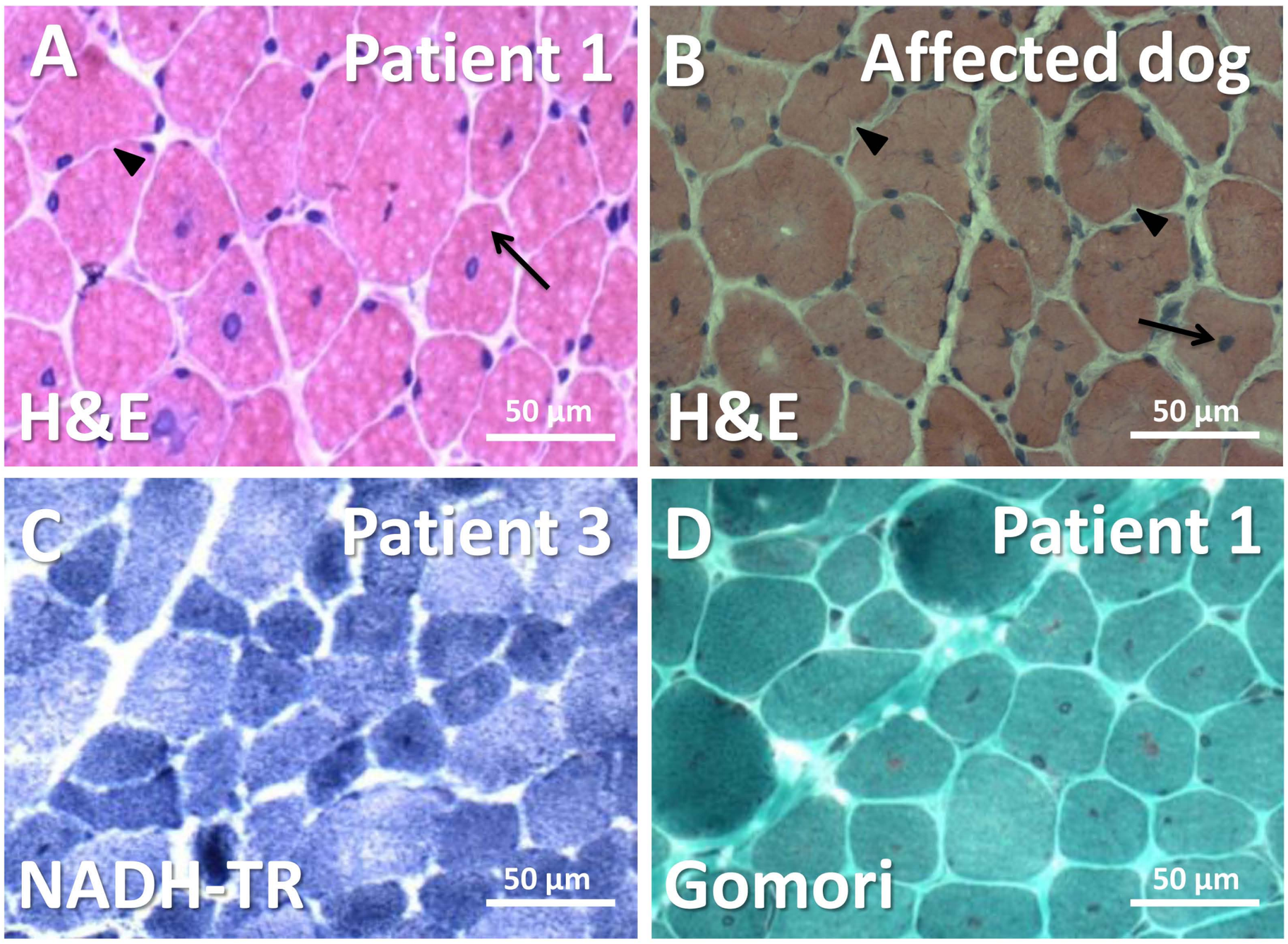


Figure 5. Common ultrastructural and membrane defects in affected human and dog

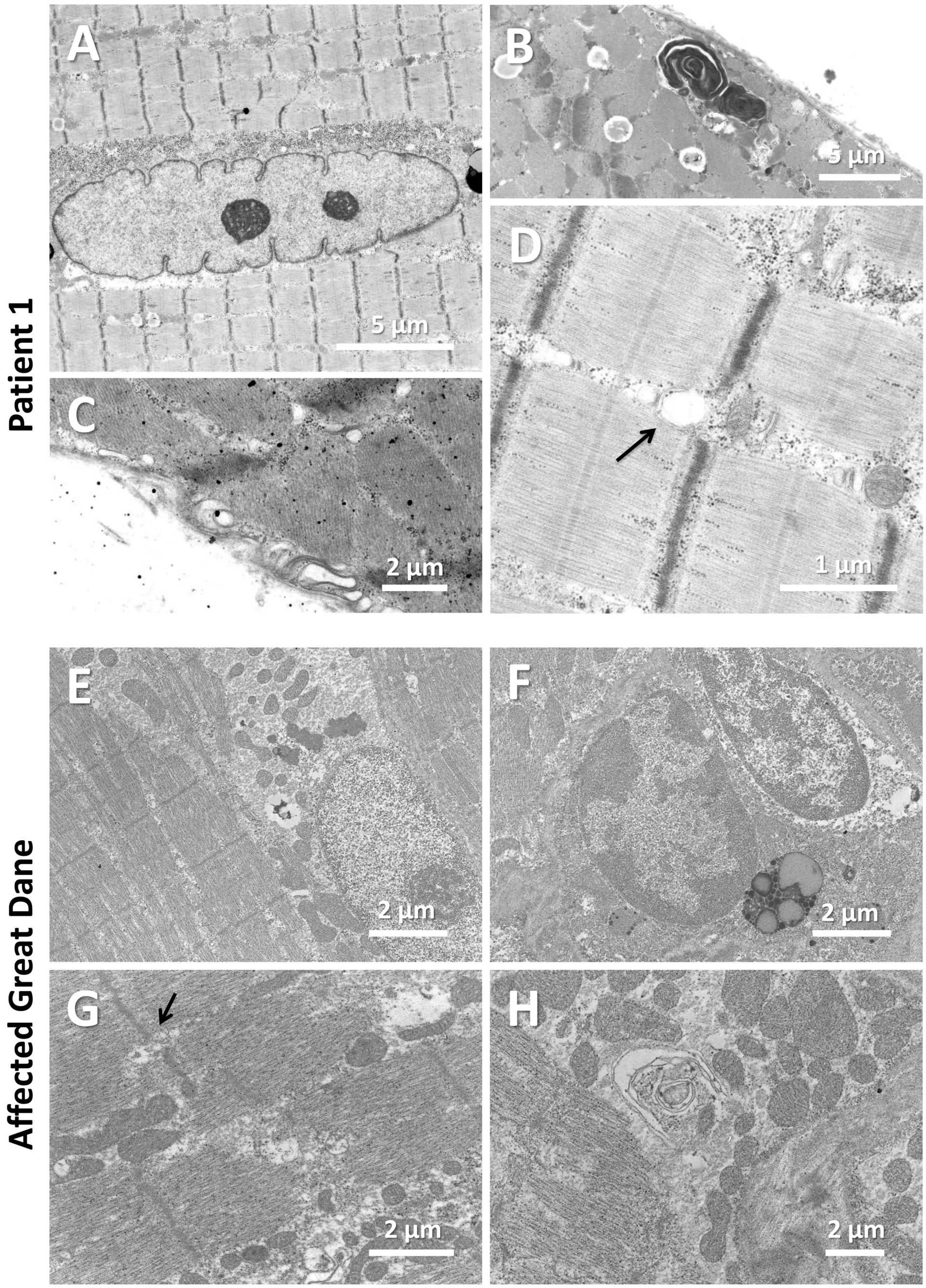
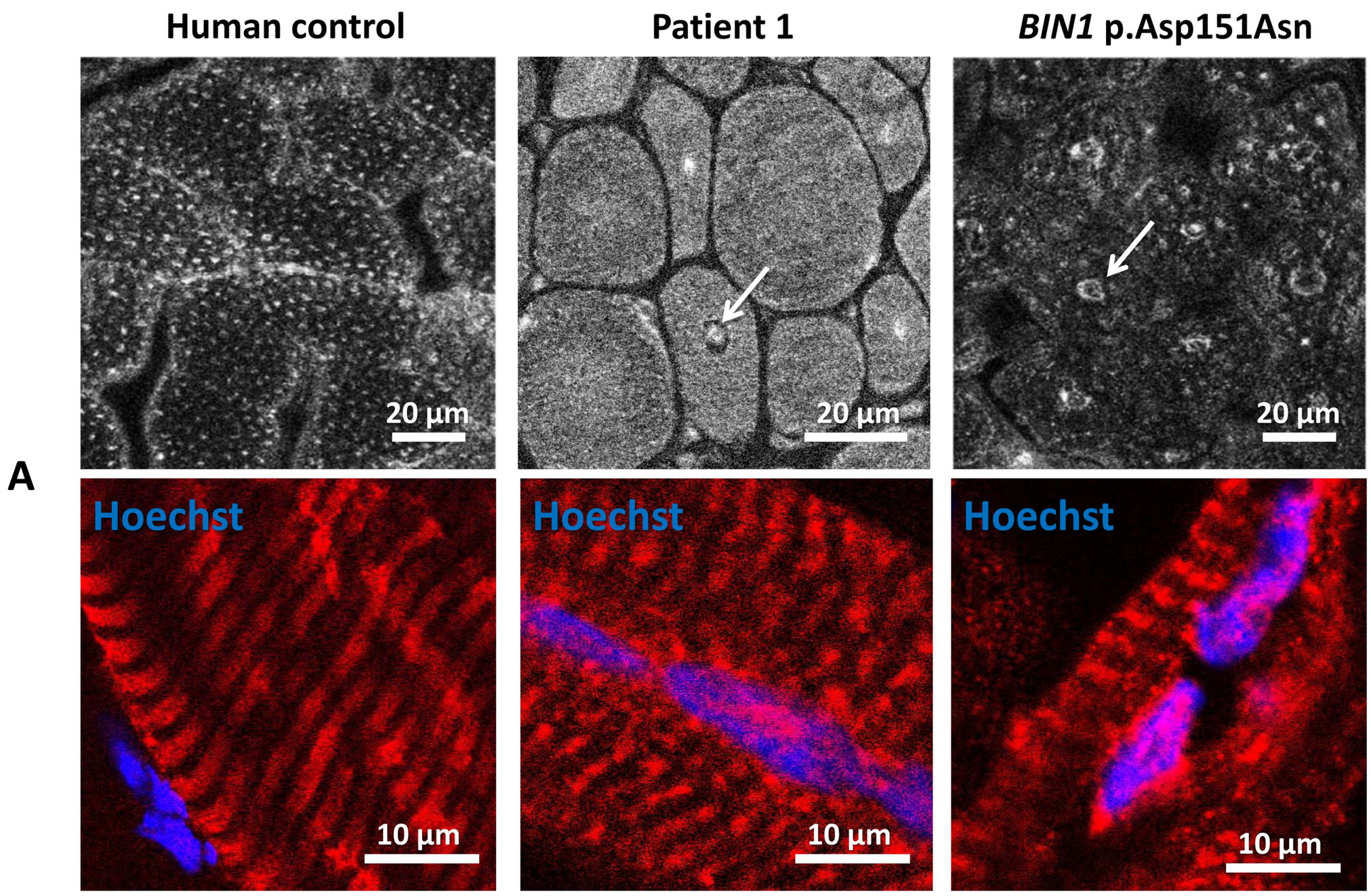


Figure 6. Amphiphysin 2 is present but altered in muscles from affected humans and dogs.



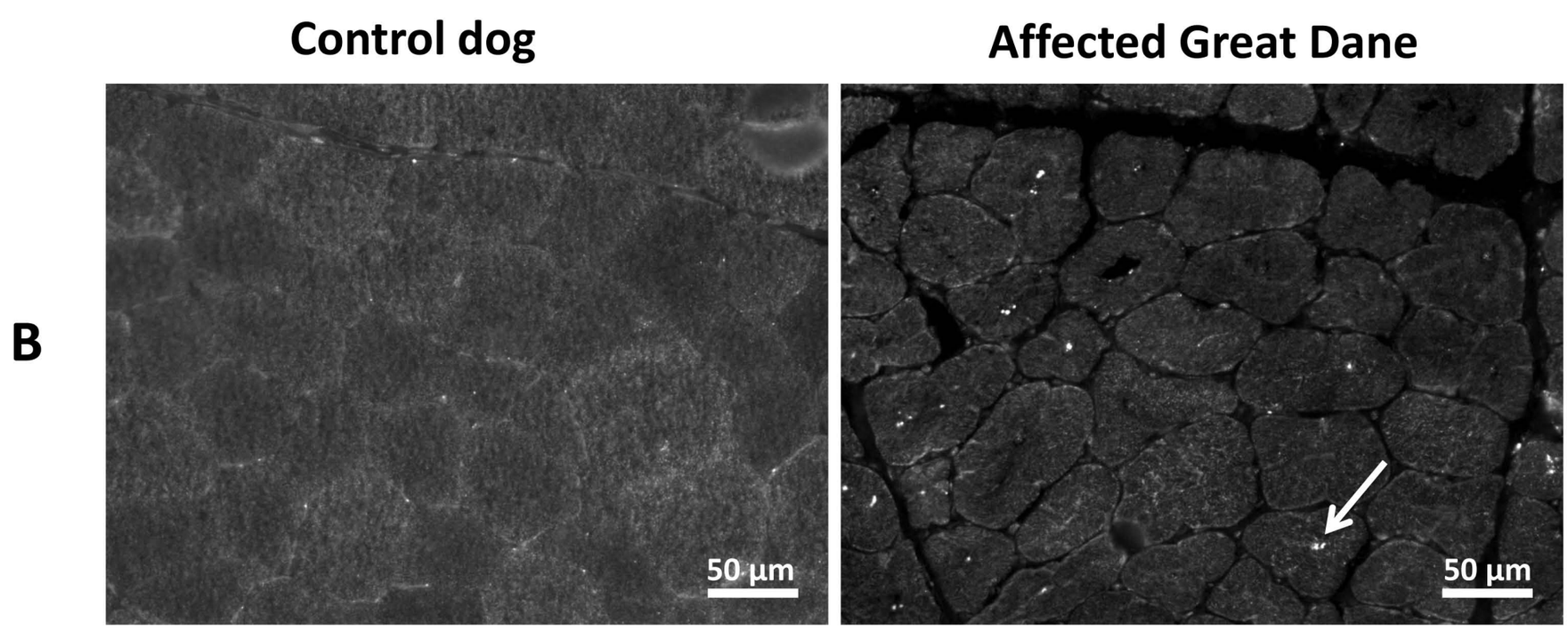
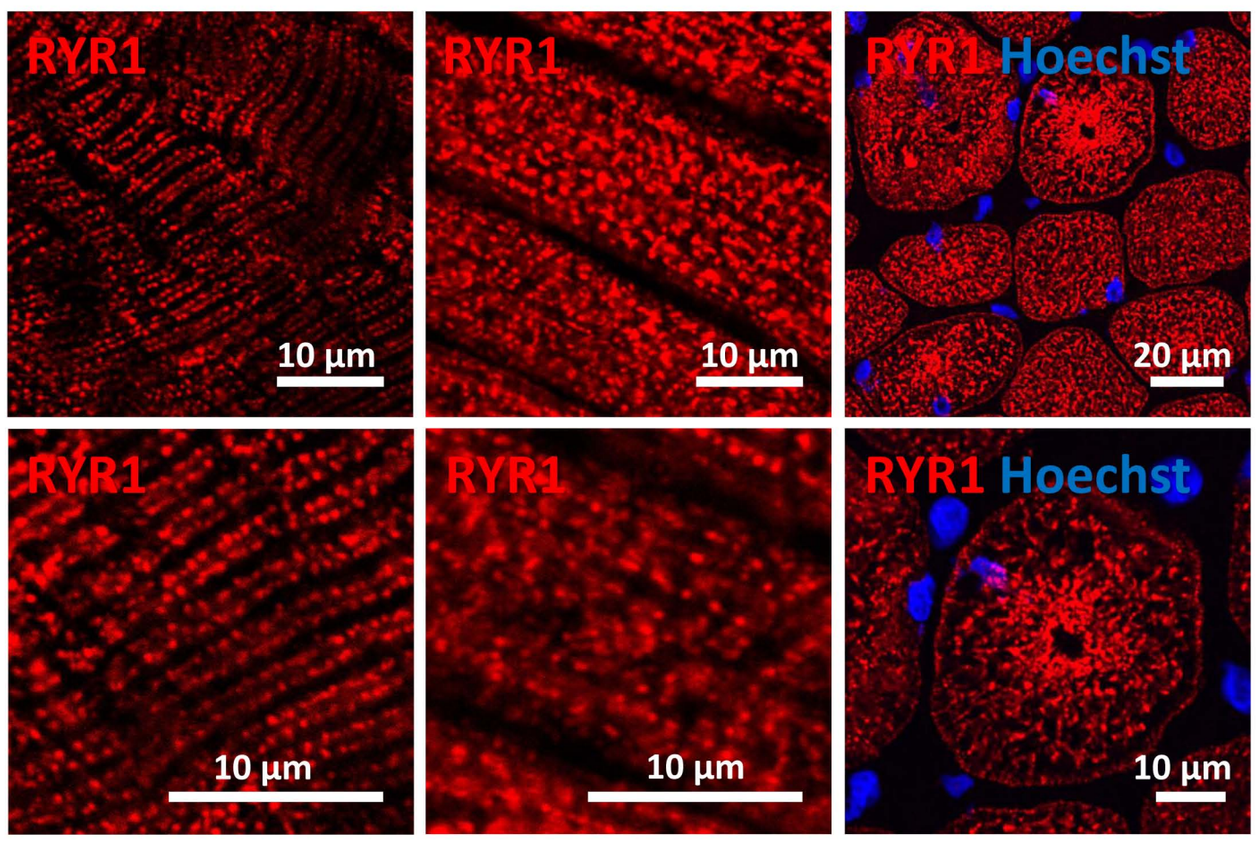
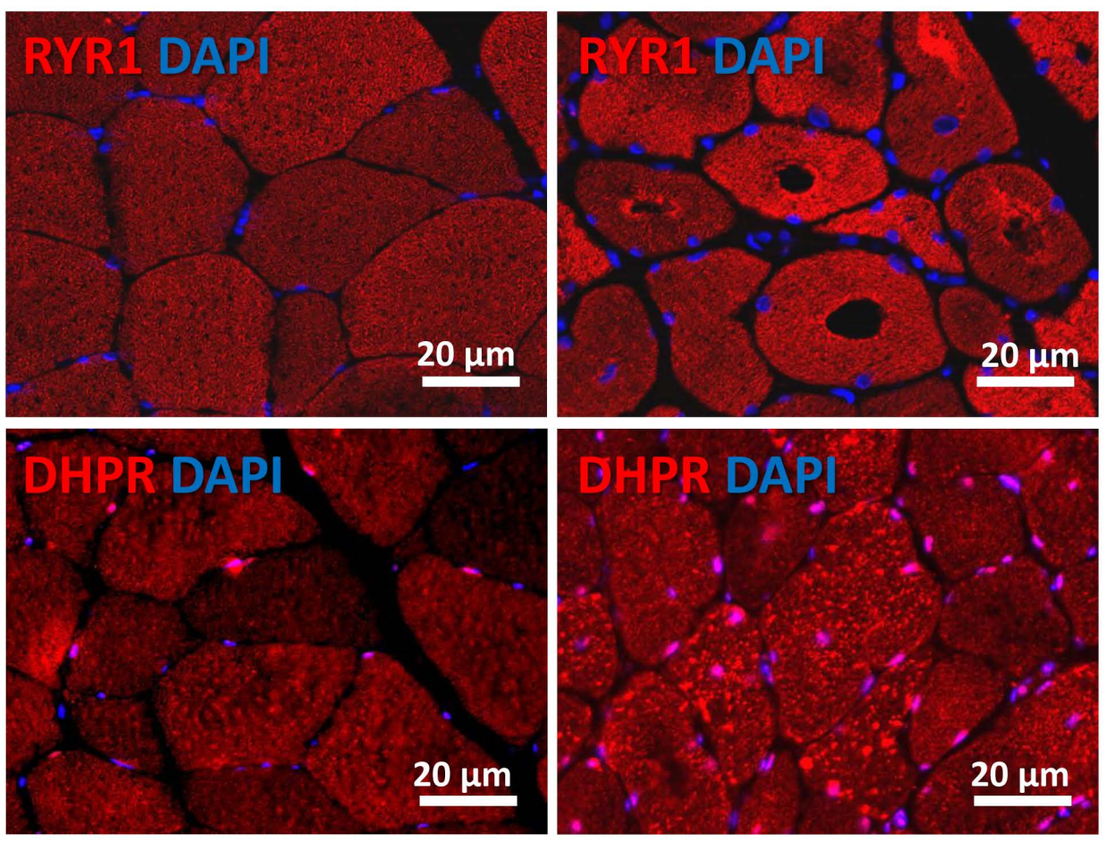
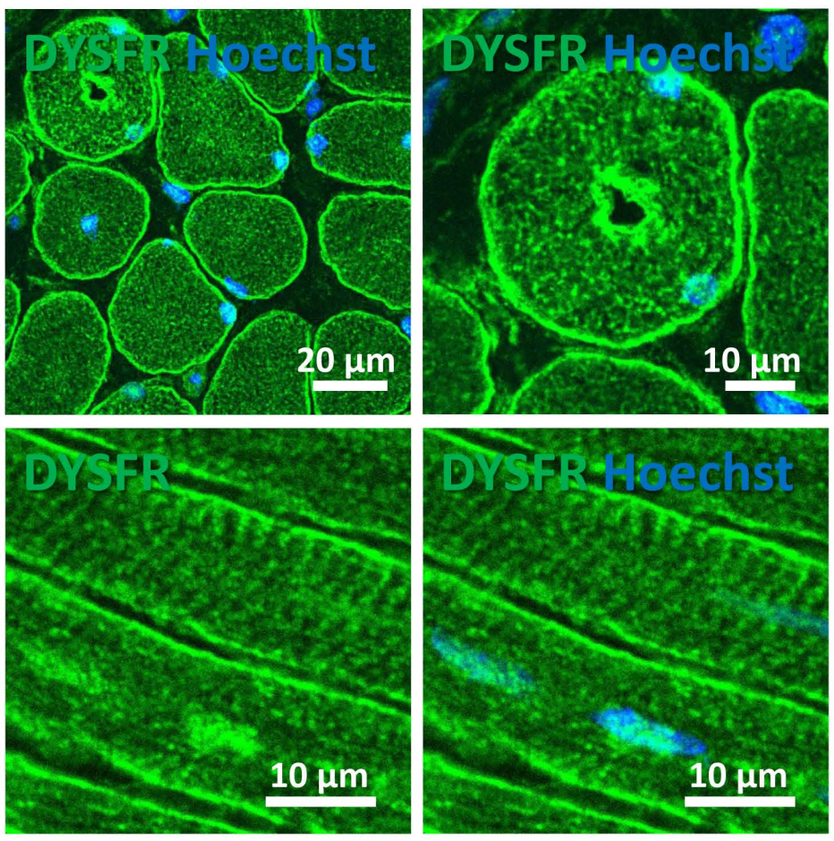


Figure 7. Alteration of triad components and proteins regulating membrane trafficking







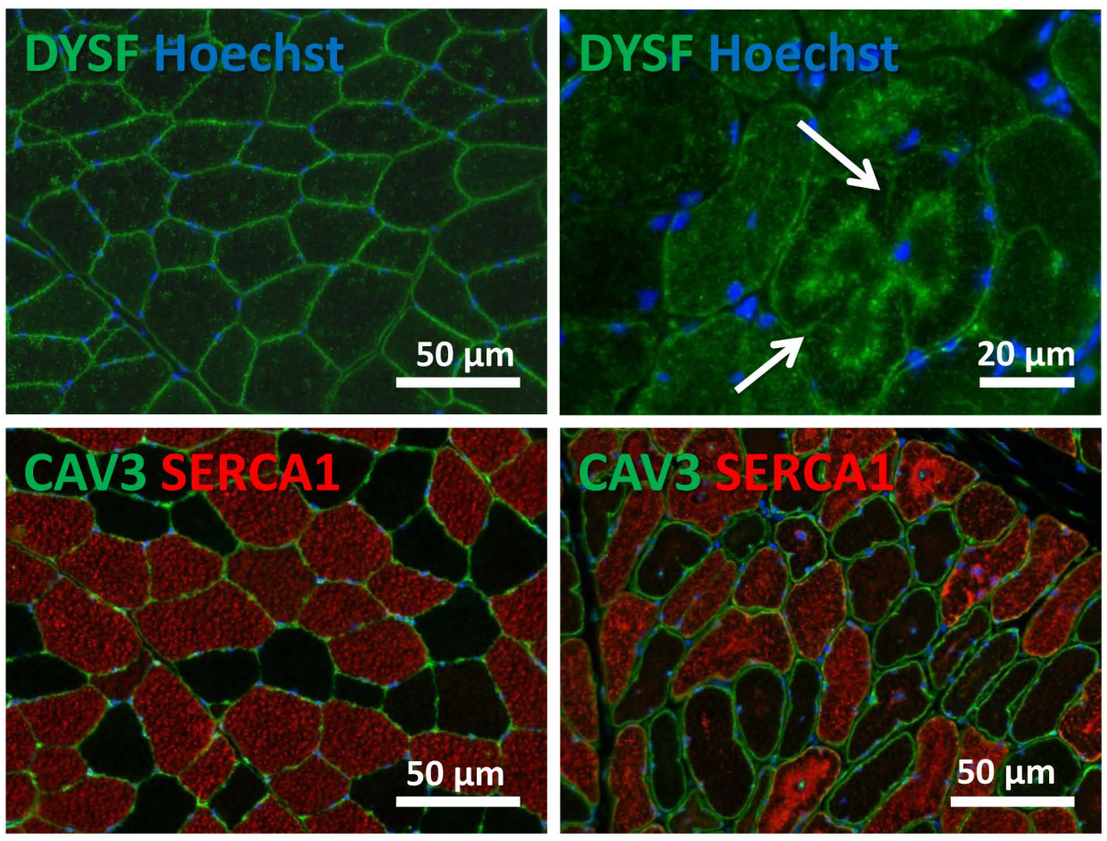
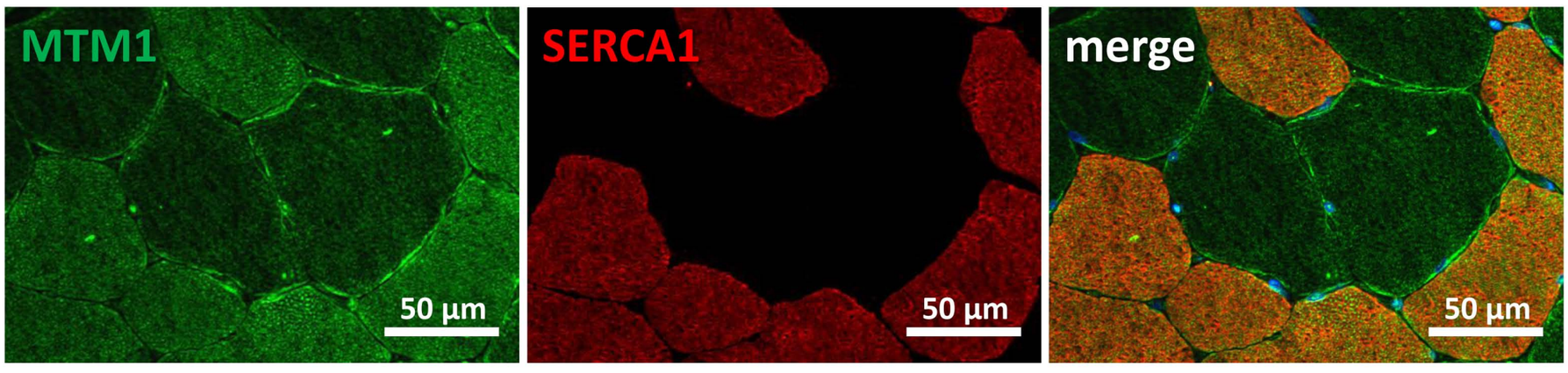


Figure 8. Myotubularin is mis-localized in IMGD muscle.

A

Control dog



B

Affected Great Dane

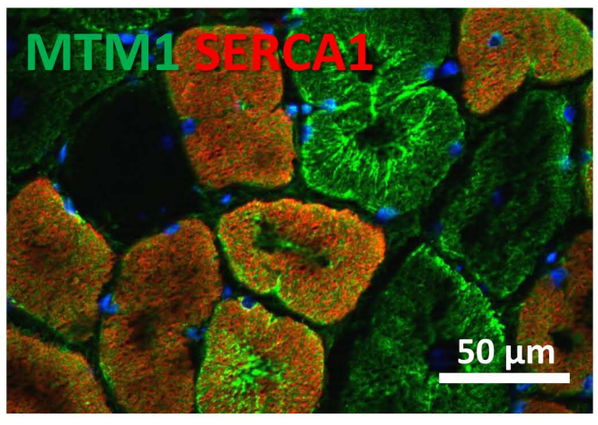


Figure 9. *BIN1* alterations in different myopathies

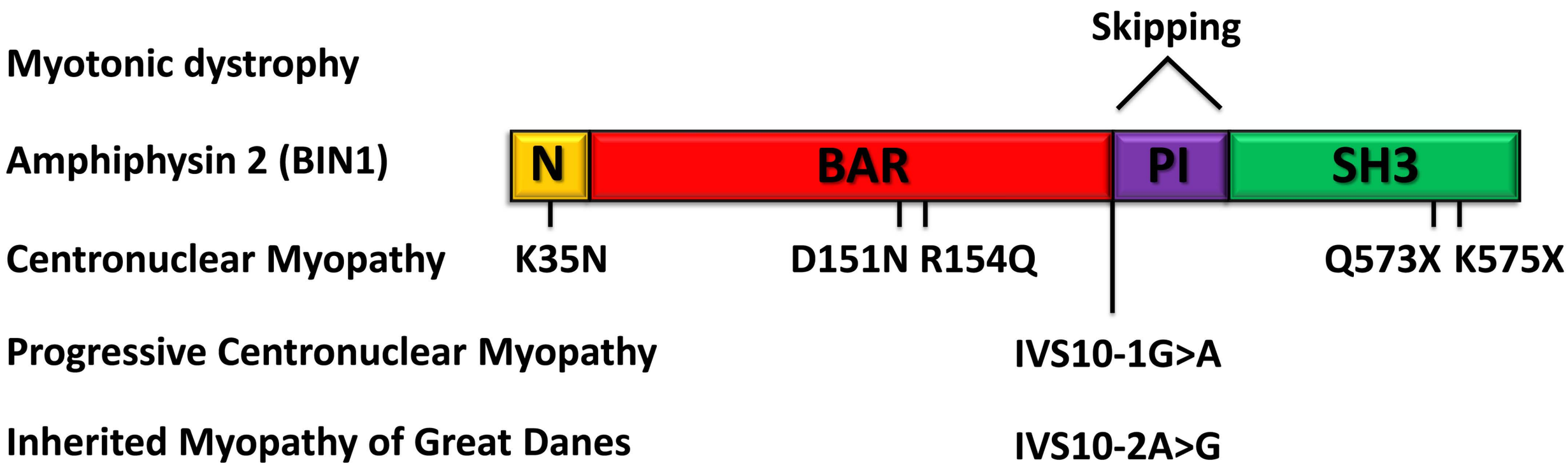


Figure S1. Normal alpha-actinin labeling in patient muscle

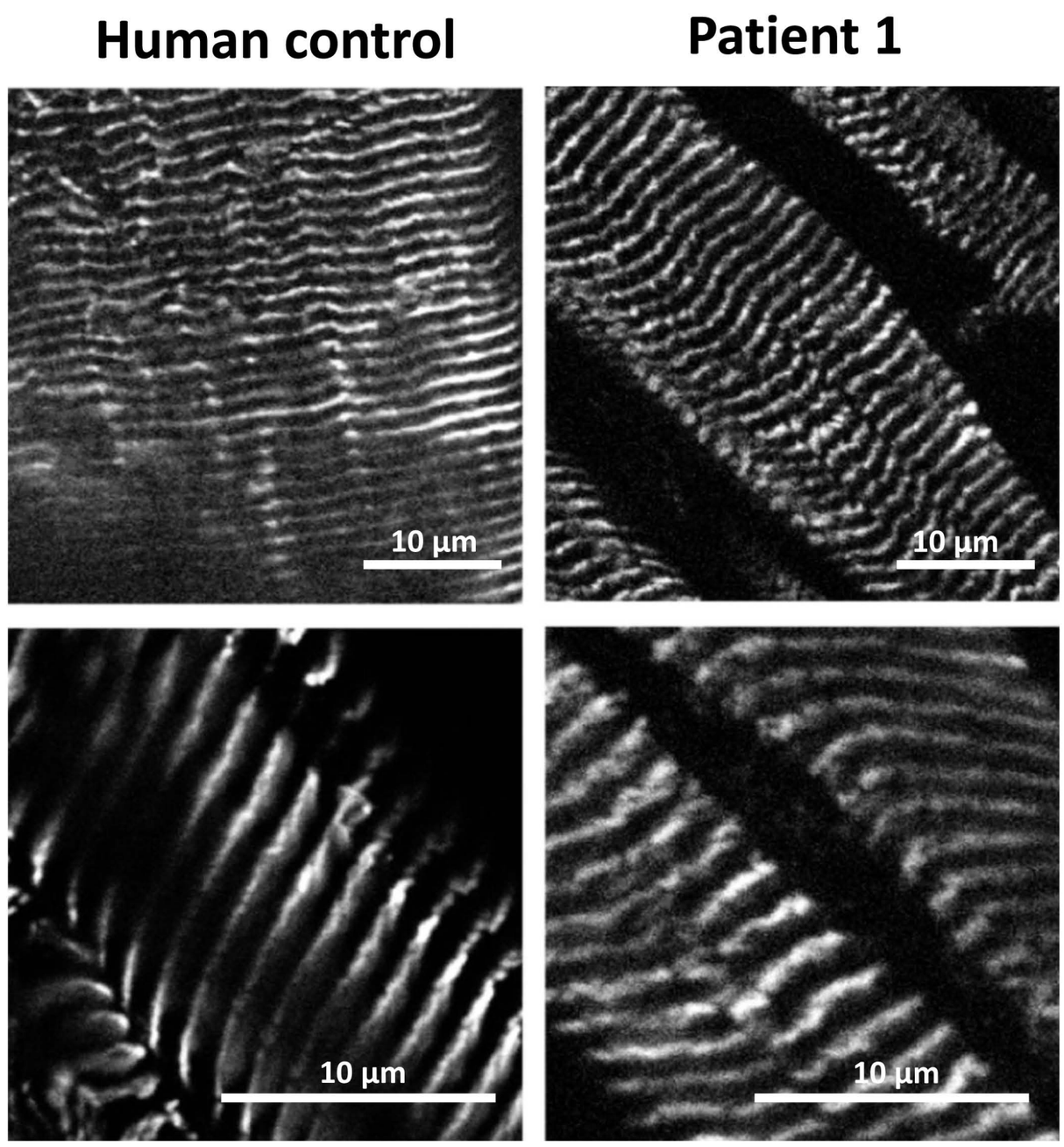


Table S1. Primer sequences

Amplification	Starting material	Species	5' -3'	5' -3'	Size (nt)
BIN1 exon 11	genomic DNA	human	TCCTCTGAGCAGAAGGGTTG	CACTGCACACAGAGCCAGAT	
BIN1 exons 6 to 18	cDNA	human	GCAAGCTGGTGGACTACGACAG	ACAGTTGCTGGGAAGGTCTC	1106
BIN1 exons 10 to 12	cDNA	human	AGAACCTCAATGATGTGCTGG	GGCTCGTGGTTGACTCTGAT	209
BIN1 exons 6 to 12	cDNA	human	GCAAGCTGGTGGACTACGACAG	GGCTCGTGGTTGACTCTGAT	547
BIN1 exons 10 to 18	cDNA	human	AGAACCTCAATGATGTGCTGG	ACAGTTGCTGGGAAGGTCTC	768
BIN1 exon 1	genomic DNA	dog	GTCGGGGCTGCAAGATCG	TCGGGGGTGCGCGAGGTG	
BIN1 exon 2	genomic DNA	dog	GAGCCATCTGTGGTATTGG	CTTCCAGCTCGCTTACTCC	
BIN1 exons 3 + 4	genomic DNA	dog	GCGTTTGTCCACCTGAGAG	GTCCAAGAAGGTGAGGCAG	
BIN1 exon 5	genomic DNA	dog	GGAAGCAGTGGGGCTCAAG	CTTCACCTCCAGTGCACAG	
BIN1 exon 6	genomic DNA	dog	CTGAACATCTCGACATCTCG	CAGTGGGCACAGCACAAGAG	
BIN1 exon 7	genomic DNA	dog	CCACTCTCCTTTGCCGTGAC	CAGGAAAGAGACTGAGCCCTC	
BIN1 exons 8 + 9	genomic DNA	dog	GACTTGGCTAGGCCTAAGC	GTGGCCACTAGTGTGTACC	
BIN1 exon 10	genomic DNA	dog	GTGCAAGGAAGGGGCTAAGATG	CTACAGCCAGGGCAGTAG	
BIN1 exon 11	genomic DNA	dog	GCTGCTGATGAGGGAACAGG	GCACCAGGCGCATGCACAG	
BIN1 exon 12	genomic DNA	dog	CTGTGGATGCACATGGTCC	CCATGAGCCCAGTGTTGC	
BIN1 exon 13	genomic DNA	dog	GTGACTAACTGTGGCTTTGTC	GGAAGAAGGCCATGCACAC	
BIN1 exons 14 + 15	genomic DNA	dog	GCACCCTGCCGAATGTTCC	GTGCAAGTGCATGCACCTG	
BIN1 exon 16	genomic DNA	dog	CTTGTCTCGTGCCCTTGCC	GCAAATGTCTAGGTTAGAGTG	
BIN1 exons 17 + 18	genomic DNA	dog	CCATTGCAGGGACCTGAC	CAACGTCGGCTCAGATAGC	
BIN1 exon 19	genomic DNA	dog	CCTGGTGCACCATGCTTG	GCAGGGTACAGAGACAG	
BIN1 exon 20	genomic DNA	dog	GGTTCCAGTGCCCTCTG	GGTGTTCTTCACACGCCCAG	
BIN1	cDNA	dog	CAAGCAGCTGACTGAGGGCAC	GGTGTTCTTCACACGCCCAG	
MTM1	cDNA	dog	CCGAGTTTCCAGTATGGCTTC	GTCTCCCTGATAACATCGAG	

3-2- Targeted sequencing

3-2-1- Sequencing of 76 selected candidate genes

In a study, 76 genes were selected based on their functions (Table 5). DNA from 20 patients with CNM was selected and pooled without tagging. The pool of samples was sequenced, using one lane of Illumina GAIIx sequencer, single-read with 72 bp length.

The aim of the study was to detect any potential disease-causing mutations in any of these genes, using the pool of 20 patients without barcoding the samples. One DNA (ACJ7) with hemizygous c.529-1G>T mutation in *MTM1* gene was used as positive control.

Table 5- List of genes selected for targeted sequencing of 76 genes.

ANK2	CLIP1	EHD3	MTM1	RANBP9	TOR1A
ANK3	DES	EHD4	MTMR10	RUFY3	TRDN
APPL1	DLG1	EXOC3	MTMR11	RYR1	TRIM72
BIN1	DLG2	FGD2	MTMR12	SH3GL2	TRIP10
C14ORF49	DLG3	FGD3	MTMR14	SH3GLB1	UNC84A
C19orf46	DLG4	FGD4	OBSCN	SNX18	UNC84B
C9ORF100	DLG5	FIG4	PIK3C2A	SNX33	
CAPZA1	DNM2	FNBP1	PIK3C2B	SRPK3	
CAPZA2	DNM3	FNBP1L	PIK3C2G	STIM1	
CAPZB	DNMBP	JPH1	PIK3C3	SYNE1	
CAV3	DST	JPH4	PIK3R4	SYNE2	
CCDC47	DYSF	KCNN3	PIP5K3	SYPL2	
CDC42BPA	EHD1	LDB3	PLEKHO1	TIAM1	
CDK5	EHD2	MAP4K3	PTK2	TIAM2	

The capture was done for 1854 non-overlapping regions, covering 472657 target nucleotides. Raw sequencing data was analyzed using Illumina Pipeline RTA (Real-Time Analysis) version 1.9 and the alignment was done, using BWA version 0.5.9. Indels of 50nt (nucleotides) were allowed and aligned reads can contain up to 3 mismatches. Number and percentage of input, mapped, duplicate and multi-mapped reads are shown in table 6.

Table 6- Statistics regarding NGS reads for targeted sequencing of 76 genes.

No. of input reads	27718017
No. of Mapped reads	23723262
Percentage of mapped reads	85,5 %
No. of multi-mapped reads	1261042
Percentage of multi-mapped reads	5,3 %
Number of duplicate reads	14689822
Percentage of duplicate reads	61,9 %
Number of remaining reads after filtration	7772398

All 1854 target regions were covered. Achieved nucleotide coverage is shown in table 7 and figure 21.

Table 7- Statistics regarding nucleotide coverage for targeted sequencing of 76 genes.

Mean of coverage	86,25
Median of coverage	89
Percentage of reads with ≥ 1x	99,99 %
Percentage of reads with ≥ 5x	99,93 %
Percentage of reads with ≥ 10x	99,87 %
Percentage of reads with ≥ 20x	99,79 %
Percentage of reads with ≥ 40x	99,12 %
Percentage of reads with ≥ 60x	94,94 %
Percentage of reads with ≥ 80x	73,11 %

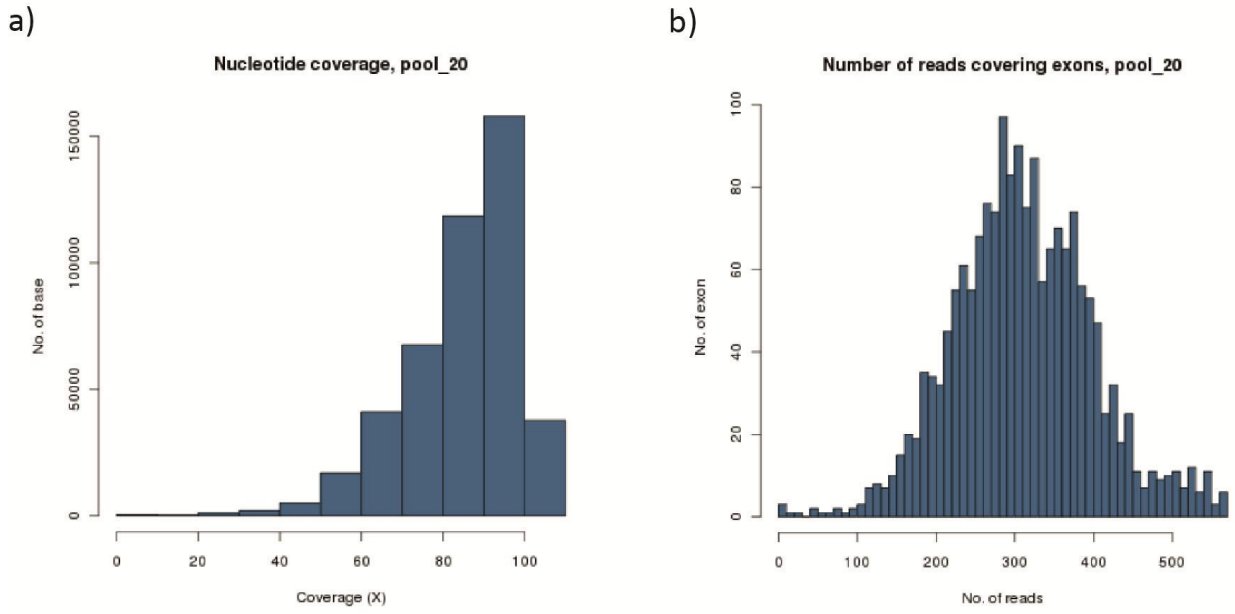


Figure 21: coverage for targeted nucleotides and exons. Most of the nucleotides have depth of coverage more than 60 times and most of the targeted exons have more than 200 reads.

After variant calling and functional annotation, using samtools, Sequence Variant Analyzer (SVA) (http://www.svaproject.org/), Ensembl 60 and dbSNP131, 64 indels and 13104 SNVs were detected. 941 SNVs were known and 12163 were new.

After filtering out the variants in introns and UTR regions, synonymous variants and variants with Rs number (variants which could be potentially polymorphisms), 5775 SNV and 1 indel remained.

The number of detected variants was too high and we believe that a substantial number of them were sequencing/PCR errors. As the 20 DNAs were pooled and sequenced without tagging, the detected variations in any positions should be checked for all 20 samples by direct sequencing to distinguish between the false positives and the real variants however it was not cost-effective to

carry out. For the hemizygous c.529-1 G>T mutation in the *MTM1* gene, the positive control, 2 reads out of 57, i.e. 3,5% of reads showed the change. If the criterion of "keeping variants with 3,5% of reads showing the change" was used as threshold, almost all of the variants would remain leading to have a lot of false positives among real variants. More stringent filtering would remove the change in the positive control. Therefore pooling 20 DNAs without tagging followed by NGS of 76 genes in one lane of Illumina GAIIx sequencer, single-read with 72 bp length, did not lead to a reliable data.

3-2-2- Sequencing of 2500 selected candidate genes

In a study, 2500 genes were selected based on their functions, chromosomal positions, genes implicated in different neuromuscular disorders and genes selected from GO (Gene Ontology website) (http://www.geneontology.org), using different keywords.

DNA of 7 patients with CNM was selected and sent to Genoscope sequencing platform (http://www.genoscope.cns.fr/spip/Sequencing.html) to perform NGS (in 2009), using Nimblegen array for capturing 2500 candidate genes followed by sequencing in one lane of Roche 454 pyrosequencer machine. Number and percentage of input, mapped and unique reads, as well as depth of coverage for each patient are shown in table 8.

Table 8- Statistics regarding NGS reads for targeted sequencing of 2500 genes.

Ind.	No. of reads	Average length (bp)	% of mapped reads	% of unique reads	% of reads in target regions	coverage
A	159804	233	69 %	44 %	25 %	1 X
B	122588	218	91 %	46 %	27 %	1 X
C	875398	315	97 %	66 %	47 %	10 X
D	1339069	209	92 %	43 %	26 %	7 X
E	882238	233	84 %	20 %	13 %	2 X
F	1235954	368	99 %	69 %	45 %	15 X
G	995617	234	82 %	40 %	24 %	5 X

The coverage for patients C, D and F was more than 7x, thus further analysis was done for patients C, D and F. Conversely, the coverage for other samples was not enough due to problems in Genoscope sequencing platform and therefore we did not analyze those samples further.

After mapping, variant calling and functional annotation, using gsMapper (ROCHE) and Genoscope's pipeline, different types of homozygous and heterozygous exonic variations were detected (Table 9).

Table 9- Number of different types of exonic variants in patients C, D and F for targeted sequencing of 2500 genes.

		patient C	patient D	patient F
Homozygous variations	Number of Non-synonymous variations	13	14	13
	Number of Stop variations	7	7	8
	Number of synonymous variations	13	13	6
	Number of variations in total	33	34	27
Heterozygous variations	Number of Non-synonymous variations	66	38	50
	Number of Stop variations	41	20	18
	Number of synonymous variations	41	25	36
	Number of variations in total	148	83	104
	Number of all variations	181	117	131

Variations presenting in dbSNP131, HapMap project and 1000 genomes as well as synonymous changes were filtered out. Numbers of remaining variants are shown in table 10.

Table 10- Number of exonic variants after filtration in patients C, D and F for targeted sequencing of 2500 genes.

		C	D	F
Homozygous variations	Number of Non-synonymous variations	5	9	8
	Number of Stop variations	6	6	6
	Number of variations in total	11	15	14
Heterozygous variations	Number of Non-synonymous variations	48	33	34
	Number of Stop variations	40	17	13
	Number of variations in total	88	50	47
	Number of all variations	99	65	61

3-2-2-1- Results for patient C (AHJ97)

Patient C (AHJ97) is a French male, born in 1987 with centronuclear myopathy from a consanguineous family. The age of onset was 14 month-old. He can walk but has difficulty in climbing the stairs and he cannot run. He has diffuse amyotrophy without any facial, ocular or respiratory symptoms. Histological analysis showed type I fiber predominance, the central nuclei in 50% of cells

and some cells with 3-4 nuclei. Homozygosity mapping analysis revealed the homozygous regions in chromosomes 2, 3, 4, 12, 14, 15 and 20. The biggest homozygous region was in chromosome 2.

Looking to these target regions, a homozygous deletion of two nucleotides leading to frameshift (NM_016653.2 (ZAK):c.490_491del, p.Met164ValfsX24) was detected in exon 7 of the *ZAK* gene.

The NGS data, direct sequencing result and implicated domains and amino acid conservation through evolution are shown in figure 22.

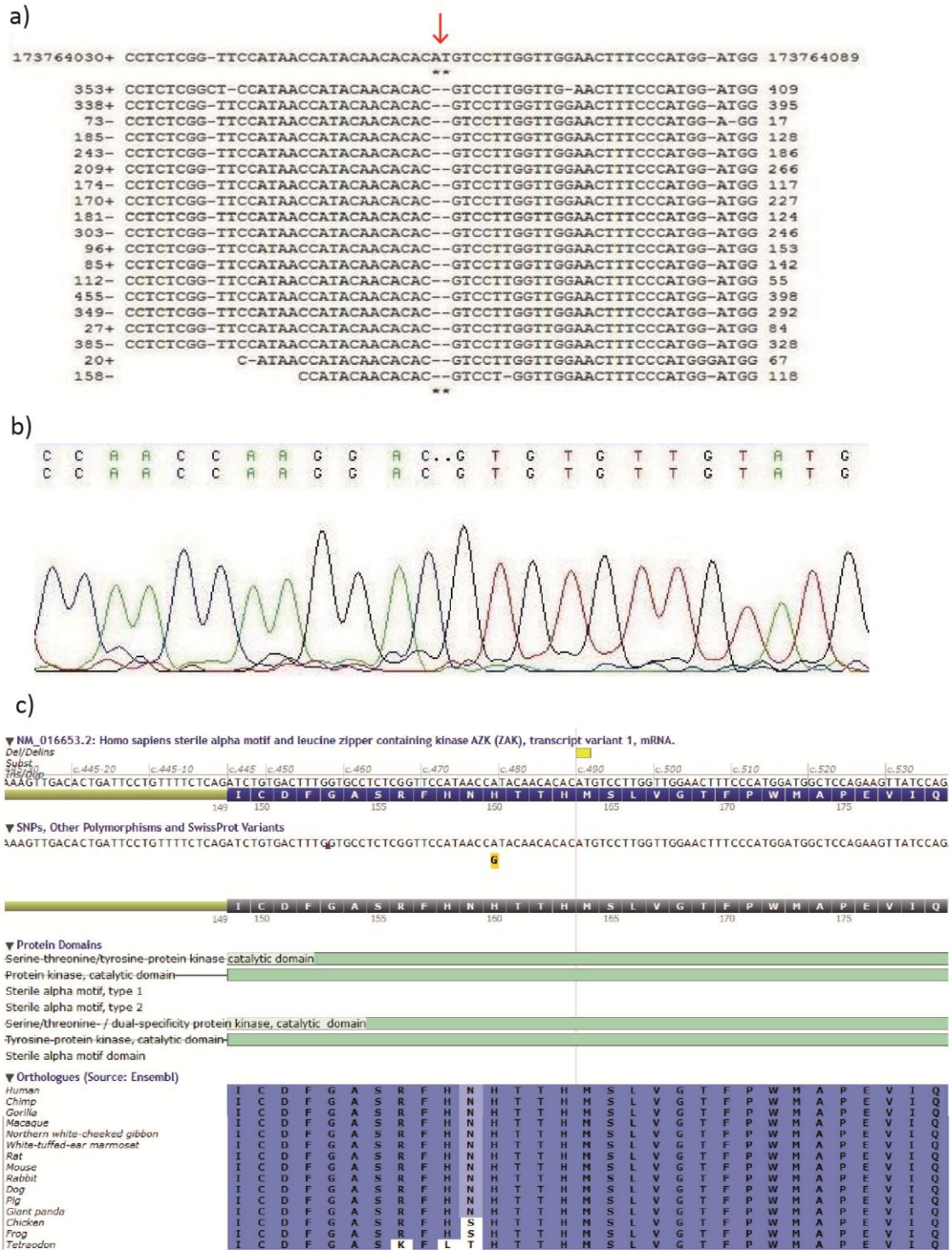


Figure 22: The NGS reads, direct sequencing result and implicated domains and amino acid conservation through evolution for patient C (AHJ97) with c.490_491del, p.Met164ValfsX24 variant in *ZAK*. a) NGS reads in + and – directions showing the homozygous deletion of 2 nucleotides (AT) in exon 7 of *ZAK*. b) Direct sequencing of exon 7 of *ZAK* in patient C (AHJ97) showing the homozygous deletion of 2 nucleotides (AT). c) The variation is c.490_491del, p.Met164ValfsX24. The conservation of M164 is shown in different species by red line. This amino acid is in two catalytic domains: 1-Serine/Threonine dual specificity protein kinase and Tyrosine protein kinase domains. (Part C is depicted, using ALAMUT software)

ZAK is a serine-threonine kinase, belonging to the MAPKKK (MAP kinase kinase kinase) family of signal transduction molecules and encodes a protein with an N-terminal kinase catalytic domain, a leucine zipper motif and a sterile-alpha motif (SAM). This magnesium-binding protein forms homo-dimers and is located in the cytoplasm (Gross et al, 2002). The gene is located in chromosome 2q24.2 and has two isoforms. The longest isoform contains 21 exons, 20 coding, with 3741 base pairs coding a protein with 800 amino acids and it is highly expressed in skeletal muscle (Figure 23). The homozygous deletion of 2 nucleotides deletes the dimerization domain and truncates the kinase domain.

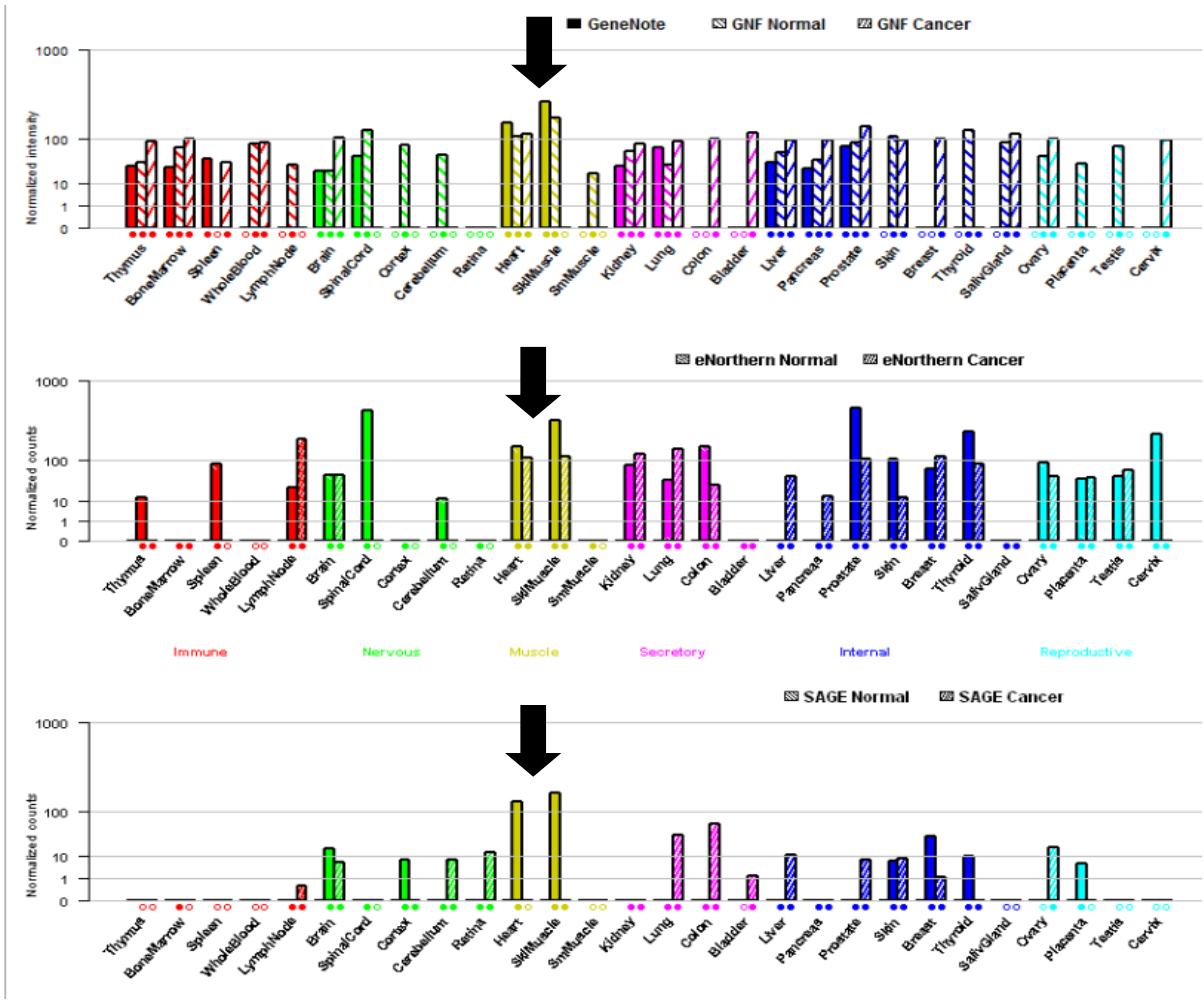


Figure 23: Expression level of ZAK in different tissues. As shown in 3 panels, the highest expression level of ZAK is in skeletal muscle (yellow bars), using three different methods: Experimental tissue vector (upper panel), Electronic Northern (middle panel) and serial analysis of gene expression (lower panel).

Reference: http://www.genecards.org/cgi-bin/carddisp.pl?gene=ZAK&search=zak

In an experiment the RNA extracts from lymphoblasts of two CNM patients with mutations in *MTM1* (deletions of exons 1 to 13 in patient one and exons 2 to 8 in patient two), three healthy controls and patient C (AHJ97) were used to detect the most down-regulated genes, using Affymetrix GeneChip® Human Gene 1.0 ST Array. The results showed that the expression level of ZAK was decreased more than 50% in AHJ97 as well as in patient one with hemyzygous deletion of *MTM1* compare to other samples. This result can highlight two points: Firstly, the homozygous deletion of two nucleotides in *ZAK* leads to non-sense mRNA decay in patient C (AHJ97). Secondly, the decreased level of expression in patient one with hemyzygous deletion of exons 1 to 13 in *MTM1* is as expected assuming that ZAK and MTM1 are in the same cascade pathway and a decrease in MTM1 expression leads to decrease

in expression of ZAK. Whereas, decrease in ZAK expression does not lead to decrease in expression of MTM1, as the expression level of MTM1 in AHJ97 was normal.

RT-PCR analysis of this patient and two samples without any variations in ZAK showed the non-sense mRNA decay in patient C (AHJ97) in comparison with controls (Figure24).

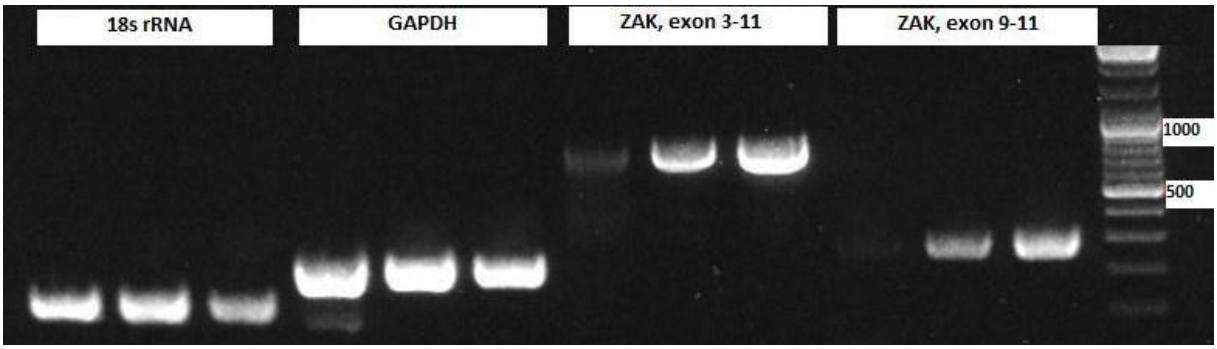


Figure 24: RT-PCR results for AHJ97 (first lane for each couple of primers) and two negative controls without any variations in ZAK (second and third lanes for each couple of primers). The cDNA amount of AHJ97 is similar to two controls when it amplifies with human 18s rRNA and GAPDH primers whereas it decreases when it amplifies using primers designed for ZAK exon 3 to 11 and exon 9 to 11. This shows the non-sense mRNA decay in patient C (AHJ97).

The c.490_491del, p.Met164ValfsX24 variation is segregated in the family as both parents have the same variation in heterozygous status. This variation is not listed in the SNP (Single nucleotide Polymorphism) databases such as dbSNP or 1000 genomes. This gene is located in the candidate region based on homozygosity mapping results, and is highly expressed in skeletal muscle. It is a serine-threonine kinase which can potentially be in the same pathway as MTM1 and there is no study reporting a disease-causing mutation in this gene, so far. All of this data together shows that this gene could be a potential candidate for CNM. As 2500 selected genes were sequenced in this study and other potential variations could be missed, we decided to do exome sequencing for this patient to see whether this variant is the highest-ranked variant in the exome data, as well. Results will be shown in exome section (3-3-1- Trio sequencing for AHJ97).

3-2-2-2- Results for patient D (34263)

Patient D (34263) is an Italian male, born in 1984 with centronuclear myopathy from healthy parents without consanguinity. The newborn was hypotonic with severe ophthalmoplegia, facial weakness, delayed motor milestones with respiratory distress and feeding difficulties. Histological analysis showed type I fiber predominance and central nuclei in 25% of cells. As this patient is a sporadic case, there were a lot of variations after NGS of 2500 candidate genes in recessive as well as dominant

scenarios, thus distinguishing between private rare polymorphisms and disease-causing mutation was impossible and we could not detect any potential CNM-causing mutations in this patient.

3-2-2-3- Results for patient F (AHH42)

Patient F (AHH42) is a German/British male, born in 2007 with centronuclear myopathy from healthy parents without consanguinity. The newborn was hypotonic with facial weakness, delayed motor milestones and respiratory distress. Histological analysis showed round fibers with central nuclei and abnormal mitochondrial distribution. Like patient D, this patient is a sporadic case also and there were a lot of variations after NGS of 2500 candidate genes in recessive as well as dominant scenarios, thus distinguishing between private rare polymorphisms and disease-causing mutation was impossible and we could not detect any potential CNM-causing mutations in this patient.

3-3- Exome sequencing

For finding new gene(s) implicated in CNM, we did exome sequencing for several patients with CNM without mutations in the three classical genes. In some of the cases, we detected disease-causing mutations in *RYR1*. This data was submitted as a paper.

Publication 4

An integrated diagnosis strategy for congenital myopathies

Johann Böhm*, Nasim Vasli*, Edoardo Malfatti, Stéphanie Le Gras, Claire Feger, Bernard Jost, Nicole Monnier, Hatice Karasoy, Marion Gérard, Maggie C. Walter, Peter Reilich, Valérie Biancalana, Christine Kretz, Nadia Messaddeq, Joël Lunardi, Norma B. Romero, Jocelyn Laporte

* These authors are contributed equally to this study

- Under revision

A- Introduction

Congenital myopathies (CM) are rare disorders characterized by early-onset muscle weakness and classified based on the predominance of particular histological anomalies on muscle biopsies with the prevalence of about 1:25,000 and are usually associated with neonatal or childhood onset, progressive or non-progressive muscle weakness, breathing difficulties and delayed motor milestones.

B- Aim of study

Proposing and validation of an integrated approach including exome sequencing for the diagnosis of congenital myopathies with neonatal and adult onset.

C- Results

In this study we used an integrated exome sequencing strategy to identify the causative mutations in eight patients from six families with clinically different neonatal or adult-onset congenital myopathies. We found pathogenic mutations in the large *RYR1* and *NEB* genes, and histopathological and ultrastructural analysis of the muscle biopsies of the patients confirmed and validated the exome sequencing results.

D- Conclusion

This study provides the evidence that exome sequencing, in combination with clinical and histological analyses, is a fast, efficient and reliable molecular diagnosis tool for congenital myopathies, and can accelerate the patient's access to a better healthcare and disease management.

An integrated diagnosis strategy for congenital myopathies

Johann Böhm, PhD[1,2,3,4,5*], Nasim Vasli[1,2,3,4,5*], Edoardo Malfatti, MD[6,7,8,9], Stéphanie Le Gras[1,2,3,4,10], Claire Feger[1,2,3,4,5], Bernard Jost, PhD[1,2,3,4,10], Nicole Monnier, PhD[11], Hatice Karasoy, MD[12], Marion Gérard, MD[13], Maggie C. Walter, MD, MA[14], Peter Reilich, MD, MA[14], Valérie Biancalana, PhD,[1,2,3,4,5,15], Christine Kretz[1,2,3,4,5], Nadia Messaddeq, PhD[1,2,3,4,16], Joël Lunardi, PhD[11], Norma B. Romero, MD, PhD[6,8,9], Jocelyn Laporte, PhD[1,2,3,4,5]

[1]Department of Translational Medicine and Neurogenetics, IGBMC, Illkirch, France

[2]Inserm, U964, Illkirch, France

[3]CNRS, UMR7104, Illkirch, France

[4]Université de Strasbourg, Illkirch, France

[5]Collège de France, chaire de génétique humaine, Illkirch, France

[6]Institut de Myologie, Unité de Morphologie Neuromusculaire, GHU La Pitié-Salpêtrère, Paris, France

[7]Department of Neurological, Neurosurgical, and Behavioral Sciences, University of Siena, Italy

[8]Université Paris 6 UR76, INSERM UMR 974, CNRS UMR 7215, Institut de Myologie, GHU La Pitié-Salpêtrière, Paris, France

[9]Centre de référence de pathologie neuromusculaire Paris-Est, Institut de Myologie, Groupe Hospitalier La Pitié-Salpêtrière, Assistance Publique-Hôpitaux de Paris, Paris, France

[10]DNA microarrays and Sequencing platform, IGBMC, Illkirch, France

[11]Laboratoire de Biochimie Génétique et Moléculaire, CHU de Grenoble, Grenoble, France

[12]Department of Neurology, Ege University School of Medicine, Izmir, Turkey

[13]Service de Génétique, CHU de Caen, Caen, France

[14]Friedrich-Baur-Institute, Department of Neurology, Ludwig-Maximilians University, Munich, Germany

[15]Faculté de Médecine, Laboratoire de Diagnostic Génétique, Nouvel Hopital Civil, Strasbourg, France

[16]Imaging Center, Illkirch, France

[*]These authors contributed equally to the manuscript

Correspondence to: Jocelyn Laporte

1, Rue Laurent Fries

67404 Illkirch, France

Tel.: +33 3 88 65 34 12

Fax: +33 3 88 65 32 01

jocelyn@igbmc.fr

Study funding: This work was supported by INSERM, CNRS, University of Strasbourg, Collège de France and grants from ANR, GIS Institute for rare diseases and IBiSA, Association Francaise contre les myopathies, Muscular Dystrophy Association (USA) and the Myotubular Trust. This work was supported by the Institut National de la Santé et de la Recherche Médicale (INSERM), the Centre National de la Recherche Scientifique (CNRS), University of Strasbourg (UdS), Collège de France and grants from the Agence Nationale de la Recherche (ANR, grant CM-WES), Muscular Dystrophy Association (MDA, grant 2010-52655) and Myotubular Trust.

Search terms: [176] All Neuromuscular Disease

[185] Muscle disease

[111] Diagnostic test assessment

Exome sequencing

Integrated diagnosis

Characters in the title (including spaces):	58
Words in the abstract:	224
Word in the manuscript:	2658
Figures:	4
Tables:	1
Supplementary Data:	Patients and Methods, 1 table

Disclosures:

None of the authors reports competing interests.

Author Contributions:

JB, NBR and JL conceived and designed the experiments. JB, NV, CF, NM, VB, CK, and NM performed the experiments. JB, NV, EM, SLG and NM analyzed the data. BJ, HK, MG, MCW, PR and JL contributed reagents/materials/analysis. JB and JL wrote the manuscript.

Abstract

Objective: Congenital myopathies are severe muscle disorders affecting adults as well as children in all populations. The diagnosis of congenital myopathies is constrained by strong clinical and genetic heterogeneity. Moreover, the majority of patients present with unspecific histological features, precluding purposive molecular diagnosis and demonstrating the need for an alternative and more efficient diagnostic approach.

Methods: We used exome sequencing complemented by histological and ultrastructural analysis of muscle biopsies to identify the causative mutations in eight patients with clinically different skeletal muscle pathologies, ranging from a fatal neonatal myopathy to a mild and slowly progressive myopathy with adult onset.

Results: We identified *RYR1* (ryanodine receptor) mutations in six patients and *NEB* (nebulin) mutations in two patients. We found novel missense and nonsense mutations, unraveled small insertions/deletions and confirmed the impact of splice mutations by RNA analysis. Histological and ultrastructural findings of the muscle biopsies of the patients were in agreement with the exome sequencing results.

Conclusions: We provide the evidence that an integrated strategy combining exome sequencing with clinical and histopathological investigations overcomes the limitations of the individual approaches to allow a fast and efficient diagnosis, accelerating the patient's access to a better healthcare and disease management. This is of particular interest for the diagnosis of congenital myopathies, which involve very large genes like *RYR1* and *NEB* as well as genetic and phenotypic heterogeneity.

Introduction

Congenital myopathies (CM) are rare disorders characterized by early-onset muscle weakness and classified based on the predominance of particular histological anomalies on muscle biopsies. They have an estimated prevalence of about 1:25 000 and are usually associated with neonatal or childhood onset, progressive or non-progressive muscle weakness, breathing difficulties and delayed motor milestones(1, 2). The main congenital myopathy subgroups are protein aggregate myopathies (primarily nemaline myopathy), core myopathies and centronuclear myopathies (CNM), respectively characterized by rod-like protein accumulations, focal myofibrillar disorganization, and nuclear centralization on muscle biopsies(3). Other congenital myopathy subgroups have been reported with different structural hallmarks(4). Although many genes have been associated with congenital myopathies in the past years, a recent study reported that only 16 out of 46 US patients were molecularly diagnosed(5). This is due to the fact that despite clinical and histological examinations, the majority of the patients presented with unspecific features. Especially for the neonatal cases, a reliable diagnosis is often challenging. Another reason is the genetic heterogeneity in congenital myopathies with the implication of more than 20 known genes(4), opposing efficient molecular diagnosis. In addition, some of the genes implicated in congenital myopathies belong to the largest genes of the human genome, as *TTN* (363 exons; MIM#611705) mutated in congenital myopathy with fatal cardiomyopathy, *NEB* (183 exons; MIM#256030) mutated in nemaline myopathy, or *RYR1* (106 exons; MIM#180901) mutated in different pathologies. As congenital myopathies are usually severe with a high recurrence risk in affected families, molecular diagnosis is important to provide an adequate healthcare and genetic counseling.

The aim of this study was to propose and validate an integrated approach including exome sequencing for the diagnosis of congenital myopathies with neonatal and adult onset. The next

generation sequencing technology has become an effective strategy for massively parallel analysis of a large number of genes and has led to the successful identification of several Mendelian disease genes(6). This approach is however not yet routinely used in molecular diagnosis despite its potential synergy with clinical and histological investigations. Sanger sequencing of single genes remains the major technique for monogenetic pathologies with characteristic clinical manifestations. Sanger sequencing, i.e. exon by exon sequencing of the most common gene or the best candidate gene is time-consuming and not centralized, demonstrating the need for a more efficient diagnostic approach. In this study we used an integrated exome sequencing strategy to identify the causative mutations in eight patients from six families with clinically different neonatal or adult-onset congenital myopathies. We found pathogenic mutations in the large *RYR1* and *NEB* genes, and histopathological and ultrastructural analysis of the muscle biopsies of the patients confirmed and validated the exome sequencing results. In conclusion, we provide the evidence that exome sequencing is a fast, efficient and reliable method to identify disease-causing mutations in unsolved myopathy cases. Our integrated approach is particularly relevant for disease groups with genetic and phenotypic heterogeneity.

Results

An integrated diagnosis approach for congenital myopathies

We studied eight patients from six families with different clinical and histological features suggestive of congenital myopathies. The neonatal forms ranged from fatal shortly after birth to moderately progressive, and the adult form was mild and slowly progressive (Table 1). Using Sanger sequencing, the patients were previously excluded for several genes implicated in nemaline or centronuclear myopathies, including *ACTA1*, *TPM2*, *TPM3*, *TNNT1* for

families 1 and 2, *MTM1* and *BIN1* for families 3 to 6, and *DNM2* for families 3, 4 and 6. These inconclusive preliminary analyses underscore the basic necessity of an alternative and more efficient molecular diagnosis. We therefore used an integrated approach, combining exome sequencing and histological investigations. Exome sequencing was performed for all patients and for the parents of families 1 and 2 (trio sequencing). A statistical overview of the sequencing results is shown in table 2. The inheritance pattern was taken into account for the selection of candidate genes. For the sporadic cases in families 1 and 2, genes with two heterozygous variants segregating from each parent were selected as the analysis for de novo mutations was not conclusive. For the non-consanguineous families 3 and 5 with two affected members each, genes with two common heterozygous variations were selected. For family 4, we verified dominant and recessive scenarios. For patient AHE6 from the consanguineous family 6, we focused on homozygous variants mapped in large homozygous regions, determined by homozygosity-by-descent (Table e-1). The impact of potential mutations was predicted with SIFT and PolyPhen for amino acid changes and with NNSPLICE and Human Splicing Finder for changes potentially affecting splicing. All identified mutations were verified by Sanger sequencing in the starting genomic DNA and also in the cDNA for families 1, 2, 3 and 6. Histological analysis of muscle biopsies was performed for all patients. For five patients (ARX30, AKY21, IM26, AHY58 and AHE6), we additionally analyzed the muscle biopsy by electron microscopy.

Clinical and histological findings and mutation detection by exome sequencing

For patient ARX30 from family 1, polyhydramnios and fetal akinesia were diagnosed during pregnancy and the patient presented with severe neonatal hypotonia, respiratory distress, arthrogryposis, hip hyperlaxity, club feet and dysmorphic features. Through exome sequencing, we identified two heterozygous mutations in *NEB*: the c.5574C>A (exon 46;

p.Tyr1858Stop) nonsense mutation on the maternal allele and the IVS124+5G mutation on the paternal allele, confirming autosomal recessive inheritance (Figure 1). A deltoid muscle biopsy revealed nemaline rods, marked fiber size variability and type I fiber predominance (Figure 2). Ultrastructural analysis confirmed the presence of numerous nemaline bodies, and revealed mild disorganization of the myofibrillar structure and Z-band streaming (Figure 3). These findings were consistent with the mutations in *NEB* found by exome sequencing. We performed additional molecular analysis to confirm the impact of the mutations. The paternal mutation was predicted to impair splicing of exon 124 and RNA reverse transcription and cDNA sequencing confirmed major skipping of this in-frame exon (Figure 4). The nonsense mutation was not seen on the cDNA sequence, suggesting degradation of the maternal allele by nonsense-mediated mRNA decay (NMD).

Patient ARX33 from family 2 presented with a severe muscle weakness at birth and deceased in the following days due to cardio-respiratory arrest. We identified one *NEB* mutation on the paternal allele (c.5783-5784delTA) in exon 47, involving a frameshift and a premature stop codon (p.Tyr1928fsX2), and one *NEB* donor splice site mutation on the maternal allele (IVS59+1G>A) (Figure 1). Gomori trichrome staining of a deltoid muscle biopsy revealed nemaline rods, electron microscopy data were not available. The impact of the splice mutation was verified by muscle mRNA analysis and demonstrated complete skipping of the in-frame exon 59. The 2 nucleotides deletion was not seen on the cDNA sequence, suggesting nonsense-mediated mRNA decay of the paternal allele (Figure 4).

AKY21 and IM26 from family 3 deceased from a severe muscle disorder shortly after birth. Pregnancy was normal and both had low Apgar scores, generalized hypotonia and respiratory distress at birth. Cardiac examinations were normal. We detected three heterozygous *RYR1* mutations in exons 25 (c.3223C>T; p.Arg1075Trp), 43 (c.7025A>G; p.Asn2342Ser), and 48

(c.7645-7650dup; p.Ala2549-Leu2550dup). A DNA sample of the father was not available and sequencing of the maternal DNA revealed the presence of the c.3223C>T mutation in the absence of the other sequence aberrations, suggesting autosomal recessive inheritance with compound heterozygosity (Figure 1). The c.7025A>G (p.Asn2342Ser) missense on the paternal allele has been associated with malignant hyperthermia susceptibility(7) (rs147213895), known to result from heterozygous *RYR1* mutations. The deltoid muscle biopsies of both patients revealed nuclear internalization, atrophy, fiber size variability, and areas devoid of oxidative enzyme activity (Figure 2). Ultrastructural analysis of the biopsy of the patients showed that the disorganized myofibrillar areas around the internalized nuclei cover a large part of the fiber diameter. The longitudinal muscle section revealed prominent Z-band streaming (Figure 3). These findings were suggestive of a myopathy with cores. As *RYR1* mutations mostly involve cores, the histopathological findings and the exome sequencing results are in agreement.

AHY58 from family 4 was born with general amyotrophy, axial and peripheral hypotonia and arthrogryposis after normal pregnancy. Although the health status slightly improved, the patient had temporal respiratory distress and deglutition problems. We identified one heterozygous *RYR1* mutation on each parental allele, confirming autosomal recessive inheritance (Figure 1). The paternal c.8953C>T (p.Arg2985Stop) mutation in exon 59 creates a premature stop codon and the maternal c.9758T>C (p.Ile3253Thr) involves a missense substitution in exon 66, predicted to be deleterious. None of these mutations were listed in the SNP databases. The deltoid muscle biopsy revealed nuclear internalization, atrophy, fiber size variability, and areas devoid of oxidative enzyme activity corresponding to cores (Figure 2). Ultrastructural analysis revealed atrophy, myofibrillar disarray and morphological alterations of the Z-line (Figure 3). Previous studies reported *RYR1* mutations associated with prominent

nuclear internalization and areas of myofibrillar disorganization(8, 9) as for AHY58. The *RYR1* mutations identified by exome sequencing are therefore likely to be disease-causing.

AGT66 and AGT67 from family 5 are now 45 and 31 years old and presented with neonatal hypotonia without need for respiratory support. Motor milestones were delayed. Currently, AGT66 is wheelchair-bound and AGT67 is able to walk short distances. For both patients, diffuse muscle weakness, facial weakness and ptosis were diagnosed. They have contractures and absent reflexes. By comparing the exome sequencing data of both patients, we identified common compound heterozygous *RYR1* mutations, confirming autosomal recessive inheritance (Figure 1). The c.325C>T (p.Arg109Trp) missense mutation in exon 4 on the maternal allele has been reported to be associated (in combination with a second *RYR1* mutation) with multi-minicore and central core disease(10, 11). The c.8140-8141delTA deletion in exon 51 on the paternal allele has never been described and is predicted to induce a frameshift and a premature TAA stop codon (p.Tyr2714fsX7). The biopsy of patients AGT66 and AGT67 revealed nuclear internalization, fiber size variability, type I fiber predominance, and multiple dot-like areas devoid of oxidative enzyme activity, suggestive of multiminicore disease (MmD) (Figure 2). MmD is a recessive disorder linked to mutations in *RYR1*, the exome sequencing results are therefore in agreement with the histological data.

Patient AHE6 from family 6 is a 35 year old male from a consanguineous family. Pregnancy and motor development were normal. At the age of 30 he started to feel increased fatigability, had walking difficulties and impaired manual capacities. Calf muscles were hypertrophic and mild muscle weakness was only present in the upper limbs. Except for the ankles, deep tendon reflexes were absent. CPK levels were elevated. We identified a homozygous *RYR1* missense mutation in exon 58 (c.8888T>C; p.Leu2963Pro) in a homozygous 21.1 Mb region on chromosome 19 encompassing the entire *RYR1* gene (Table e-1) and predicted to be

deleterious. Both parents were found to be heterozygous for the mutation, confirming autosomal recessive inheritance (Figure 1). The mutation was not listed in the SNP databases and was not found in 80 Turkish control chromosomes. The deltoid muscle biopsy of AHE6 revealed type I fiber predominance and core-like structures, suggestive of a core myopathy in agreement with the exome sequencing results (Figure 2). In addition, aberrant nuclear positioning, marked fiber size variation fibrosis and fatty infiltrations were observed. The radial arrangements of sarcoplasmic strands ("spoke of a wheel") and necklace fibers are however untypical for core myopathies and are rather seen in centronuclear myopathy. However, mutations in *MTM1*, *BIN1* and *DNM2* were excluded by Sanger sequencing. Major findings by electron microscopy were nuclear internalization, fibrosis, massive fatty infiltrations, myofibrillar disorganization, myofiber degeneration, and lipofuscin granules (Figure 3). Summing up the histological and ultrastructural findings as well as the linkage analysis and exome sequencing results, the *RYR1* p.Leu2963Pro missense ist most probably the disease-causing mutation.

Discussion

This study provides the evidence that exome sequencing – in combination with clinical and histological analyses - is a fast, efficient, and reliable molecular diagnosis tool for congenital myopathies. Using this integrated diagnosis strategy, we identified the pathogenic mutations in eight patients from six families with different muscle phenotypes, including a fatal muscle disorder with neonatal onset, a severe myopathy with neonatal onset, a slowly progressive form with neonatal onset and a mild and slowly progressive myopathy with adult onset.

Patients from families 1 and 2 died from a severe myopathy shortly after birth, and both were found to harbour *NEB* mutations. *NEB* codes for the contractile unit scaffold protein nebulin and mutations in *NEB* usually give rise to a mild childhood or adult onset myopathy, but also rare fatal cases as for our patients have been reported(12-14). The patients from families 3, 4, 5 and 6 were all found to carry mutations in *RYR1*, although they were clinically different. The patients from family 3 had a severe neonatal myopathy and deceased within a few days after birth. The patient from family 4 had a severe, but not fatal neonatal myopathy. Both patients from family 5 presented with a slowly progressive diffuse myopathy at birth and the patient from family 6 had a mild and adult-onset muscle weakness. These findings emphasize the phenotypic variability of *RYR1*–related disorders. Mutations in *RYR1* have been associated with different neuromuscular phenotypes including central core disease (CCD, MIM# 117000)(15, 16), multiminicore disease (MmD, MIM# 255320)(17), congenital myopathy with central or internalized nuclei(8, 9), congenital fiber-type disproportion (CFTD, MIM# 255310)(18), foetal akinesia(19), benign Samaritan congenital myopathy(20), and malignant hyperthermia susceptibility (MH, MIM# 145600)(21). *RYR1* codes for the skeletal muscle ryanodine receptor, a key player in skeletal muscle excitation-contraction coupling.

The integrated diagnosis strategy combining exome sequencing with clinical and histological investigations is suitable for congenital myopathies for several reasons. First, congenital myopathies are clinically heterogeneous and our and other studies have demonstrated that especially the neonatal forms are often not clinically consistent, so that several candidate genes can be considered. Second, congenital myopathies are also genetically heterogeneous and mutations in several large genes account for a large number of cases. Protein aggregate myopathy for instance is linked to mutations in 7 different genes to date (*ACTA1*, *TPM2*, *TPM3*, *TNN1*, *NEB*, *CFL2*, *KBTBD13*). Third, congenital myopathies are classified based on histological hallmarks, but the majority of patients present non-specific features(5). Some

patient biopsies display a mix of several histological hallmarks as it is the case for our patient from family 6, increasing the number of candidate genes. Massively parallel sequencing therefore represents the most suitable molecular diagnosis approach, as it allows a fast testing of all genes previously linked to a given disease, including the large genes. Importantly, exome sequencing also covers new myopathy genes that will be discovered in the future. Another advantage is the possibility to perform the analysis in a unique laboratory, while Sanger sequencing is mostly performed in different diagnostic centers specialized for single or a small number of genes. The dropping costs of exome sequencing pave the way for a routine use in molecular diagnosis and are already far below the estimated expenses for classical Sanger sequencing of all exons of large genes as *RYR1* or *NEB*. However, it does not detect intronic mutations and may generate a large list of variants of uncertain significance. The validation of the disease-causing mutations therefore requires the synergistic combination of the exome sequencing data with clinical and histological analyses. The results need to be evaluated by specialized diagnostic centers and associated research laboratories with an expertise on the underlying pathophysiological mechanisms.

In conclusion, exome sequencing or targeted massively parallel sequencing improves molecular diagnosis of myopathies when combined with histopathology and molecular validation, and can accelerate the patient's access to a better healthcare and disease management.

Web resources

1000 genomes (URL: http://www.1000genomes.org/)

Database of Single Nucleotide Polymorphisms (dbSNP). Bethesda (MD): National Center for Biotechnology Information, National Library of Medicine. (dbSNP Build ID: 13(22)4). (URL: http://www.ncbi.nlm.nih.gov/SNP/)

Exome Variant Server, NHLBI Exome Sequencing Project (ESP), Seattle, WA (URL: http://evs.gs.washington.edu/EVS/) [March, 2012])

Human Splicing finder (URL: http://www.umd.be/HSF/)

Online Mendelian Inheritance in Man (OMIM) (URL: http://www.omim.org/)

NNsplice (URL: http://www.fruitfly.org/seq_tools/splice.html)

PolyPhen (URL: http://genetics.bwh.harvard.edu/pph/)

SIFT (URL: http://sift.jcvi.org/)

Acknowledgements

We thank the families for their cooperation and interest in this study, and Muriel Philipps, Serge Vicaire, Christelle Thibault-Carpentier, Jean-Luc Weickert and Linda Manère for technical assistance.

References

1. North K. What's new in congenital myopathies? Neuromuscul Disord 2008;18:433-442.

2. Wang CH, Dowling JJ, North K, et al. Consensus statement on standard of care for congenital myopathies. J Child Neurol 2012;27:363-382.

3. Nance JR, Dowling JJ, Gibbs EM, Bonnemann CG. Congenital myopathies: an update. Curr Neurol Neurosci Rep 2012;12:165-174.

4. Sewry CA. Pathological defects in congenital myopathies. J Muscle Res Cell Motil 2008;29:231-238.

5. Amburgey K, McNamara N, Bennett LR, McCormick ME, Acsadi G, Dowling JJ. Prevalence of congenital myopathies in a representative pediatric united states population. Ann Neurol 2011;70:662-665.

6. Ng SB, Nickerson DA, Bamshad MJ, Shendure J. Massively parallel sequencing and rare disease. Hum Mol Genet 2010;19:R119-124.

7. Robinson R, Carpenter D, Shaw MA, Halsall J, Hopkins P. Mutations in RYR1 in malignant hyperthermia and central core disease. Hum Mutat 2006;27:977-989.

8. Bevilacqua JA, Monnier N, Bitoun M, et al. Recessive RYR1 mutations cause unusual congenital myopathy with prominent nuclear internalization and large areas of myofibrillar disorganization. Neuropathol Appl Neurobiol 2011;37:271-284.

9. Wilmshurst JM, Lillis S, Zhou H, et al. RYR1 mutations are a common cause of congenital myopathies with central nuclei. Ann Neurol 2010;68:717-726.

10. Jungbluth H, Zhou H, Hartley L, et al. Minicore myopathy with ophthalmoplegia caused by mutations in the ryanodine receptor type 1 gene. Neurology 2005;65:1930-1935.

11. Zhou H, Yamaguchi N, Xu L, et al. Characterization of recessive RYR1 mutations in core myopathies. Hum Mol Genet 2006;15:2791-2803.

12. Wallgren-Pettersson C, Donner K, Sewry C, et al. Mutations in the nebulin gene can cause severe congenital nemaline myopathy. Neuromuscul Disord 2002;12:674-679.

13. Pelin K, Hilpela P, Donner K, et al. Mutations in the nebulin gene associated with autosomal recessive nemaline myopathy. Proc Natl Acad Sci U S A 1999;96:2305-2310.

14. Lawlor MW, Ottenheijm CA, Lehtokari VL, et al. Novel mutations in NEB cause abnormal nebulin expression and markedly impaired muscle force generation in severe nemaline myopathy. Skelet Muscle 2011;1:23.

15. Quane KA, Healy JM, Keating KE, et al. Mutations in the ryanodine receptor gene in central core disease and malignant hyperthermia. Nat Genet 1993;5:51-55.

16. Zhang Y, Chen HS, Khanna VK, et al. A mutation in the human ryanodine receptor gene associated with central core disease. Nat Genet 1993;5:46-50.

17. Monnier N, Ferreiro A, Marty I, Labarre-Vila A, Mezin P, Lunardi J. A homozygous splicing mutation causing a depletion of skeletal muscle RYR1 is associated with multi-minicore disease congenital myopathy with ophthalmoplegia. Hum Mol Genet 2003;12:1171-1178.

18. Clarke NF, Waddell LB, Cooper ST, et al. Recessive mutations in RYR1 are a common cause of congenital fiber type disproportion. Hum Mutat 2010;31:E1544-1550.

19. Romero NB, Monnier N, Viollet L, et al. Dominant and recessive central core disease associated with RYR1 mutations and fetal akinesia. Brain 2003;126:2341-2349.

20. Bohm J, Leshinsky-Silver E, Vassilopoulos S, et al. Samaritan myopathy, an ultimately benign congenital myopathy, is caused by a RYR1 mutation. Acta Neuropathol 2012.

21. Gillard EF, Otsu K, Fujii J, et al. A substitution of cysteine for arginine 614 in the ryanodine receptor is potentially causative of human malignant hyperthermia. Genomics 1991;11:751-755.

Tables

Table 1: Phenotypic and molecular data

Table 2: Statistical overview of the exome sequencing results

A) Sporadic cases (trio sequencing): ARX 30, ARX33

B) Familial cases (non-consanguineous): AKY21/IM26, AGT66/AGT67

C) Sporadic case: AHY58

D) Sporadic case (consanguineous): AHE6

Table e-1: Homozygosity mapping for AHE6

Figure Legends

Figure 1. Pedigrees and *RYR1/NEB* mutations in six families with different muscle disorders. Patient ARX30 harbors the *NEB* c.5574C>G mutation on the maternal and the *NEB* IVS124+5G mutation on the paternal allele. Patient ARX33 harbors the *NEB* IVS55+1G>A mutation on the maternal and the *NEB* c.5783-5784delTA mutation on the paternal allele. Patients AKY21 and IM26 carry the heterozygous c.3223C>T and c.7025A>G mutations in *RYR1*. The c.3223C>T mutation was found on the maternal allele, the father's DNA was not available. AHY58 harbors two heterozygous *RYR1* mutations: c.8953C>T on the paternal allele and c.9758T>C on the maternal allele. In patients AGT66 and AGT67 we identified the heterozygous *RYR1* mutations c.325C>T on the maternal and c.8140-8141delTA on the paternal allele. Patient AHE6 from a consanguineous family was found to harbor the homozygous *RYR1* c.8888T>C mutation, both parents were heterozygous.

Figure 2. Histological analysis of muscle biopsies from patients ARX30 and ARX33, IM26, AHY58, AGT66/AGT67, and AHE6. The deltoid muscle biopsy of patient ARX30 was performed 2 days after birth and revealed nemaline bodies, fiber size variability and type I fiber predominance. Nemaline bodies were also found on the muscle biopsy of patient ARX33. On the deltoid muscle biopsies from IM26 (shortly after birth), nuclear centralization, atrophy, fiber size variability, and areas devoid of oxidative enzyme activity became apparent. Analysis of the deltoid muscle biopsy of patient AHY58 (20 days) demonstrated fiber size variability, atrophy, internal nuclei, and discrete areas of reduced oxidative enzyme activity. Biceps brachii biopsy from AGT66 (8 years) and AGT67 (shortly after birth) revealed nuclear internalization, fiber size variability, multiple minicores devoid of oxidative enzyme activity and type I fiber predominance. Left deltoid muscle biopsy from

patient AHE6 (performed at 30 years) revealed internalized nuclei, fiber size variation, radial arrangements of sarcoplasmic strands, necklace fibers, type I fiber predominance, core-like structures, fibrosis and fatty infiltrations.

Figure 3. Ultrastructural analysis of muscle biopsies. Ultrastructural analysis of the biopsy from patient ARX30 revealed myofibrillar disorganization, Z-line streaming of adjacent sarcomeres (arrows), and prominent nemaline rods. The biopsy of patient IM26 showed large disorganized areas around internalized nuclei, the longitudinal muscle section of AKY21 revealed prominent Z-band streaming. Analysis of the biopsy of AHY58 demonstrated marked myofibrillar disorganization, fragmented Z-bands and internalized nuclei. The biopsy of patient AHE6 displayed nuclear centralization, myofibrillar disorganization, fibrosis, lipofuscin granules and myofiber degeneration. R = nemaline rods, N = nuclei, L = lipids.

Figure 4. Analysis of the *NEB* splice mutations. (A) The c.5574C>A nonsense mutation (exon 46, ARX30) and the c.5783-5784delTA deletion (exon 47, ARX33) most probably involved NMD and did not have an effect on the cDNA amplicon size of exons 45-48 of the allele not harboring these mutations. The splice mutation IVS59+1G>A (ARX33) resulted in a shorter *NEB* cDNA amplicon (exons 58-61) compared to the control. The splice mutation IVS124+5G (ARX30) involved a weak cDNA amplicon (exons 122-126) of normal size and a strong amplicon of smaller size. (B) The *NEB* IVS59+1G>A splice mutation (ARX33) causes a complete skipping of the in-frame exon 59. The c.5783-5784delTA was not seen in the cDNA, indicating mRNA degradation by NMD of the allele containing the deletion. (C) The *NEB* cDNA amplicon of patient ARX30 (exons 122-126) did not contain the in-frame exon 124. The c.5574C>A mutation in exon 46 was not seen by cDNA sequencing, suggesting nonsense-mediated mRNA decay (NMD) of the allele harboring the nonsense mutation.

Table 1: Phenotypic and molecular data

	Family 1	Family 2	Family 3		Family 4	Family 5		Family 6
Patient	ARX30	ARX33	AKY21	IM26	AHY58	AGT66	AGT67	AHE6
Disease occurence	Sporadic	Sporadic	2 affected children		Sporadic	2 affected children		Sporadic (consanuineous)
Origin	France	France	Greece/Morocco		Antilles	Germany		Turkey
Myopathy	Severe neonatal with arthrogryposis	Severe neonatal	Severe neonatal		Severe neonatal	Moderately progressive neonatal		Slowly progressive
Onset	Neonatal	Neonatal	Neonatal	Neonatal	Neonatal	Neonatal	Neonatal	Adult onset
Age at last examination	Deceased at 10 days	Deceased at 2	Deceased at 45 days	Deceased at 7 days	1	45	31	35
Motor function	Generalized hypotonia	Generalized hypotonia	Generalized hypotonia	Generalized hypotonia	Mild muscle weakness of upper limbs, walking difficulties	Diffuse muscle weakness, wheelchair-bound	Diffuse muscle weakness, walks short distances	Mild muscle weakness of upper limbs, walking difficulties
Respiration	Abnormal	Abnormal	Abnormal	Abnormal	Abnormal	Normal	Normal	Normal
Facial involvement	n.d.	n.d.	n.d	n.d.	Amimic face	Facial weakness, ptosis	Facial weakness, ptosis	No
Histopathologic al hallmarks	Nemaline bodies, fiber size variability, type I fiber predominance	Nemaline bodies	Internal nuclei, fiber size variability, atrophy, central cores	Internal nuclei, fiber size variability, atrophy, central cores	Internal nuclei, fiber size variability, atrophy, cores	Internal nuclei, type I fiber predominance, fiber size variability, multi minicores	Internal nuclei, type I fiber predominance, fiber size variability, multi minicores	Internal nuclei, type I fiber predominance, fiber size variability, radial arrangements of sarcoplasmic strands, necklace fibers, central cores
Gene	NEB	NEB	RYR1		RYR1	RYR1		RYR1
Protein	Nebulin	Nebulin	Ryanodine receptor 1		Ryanodine receptor 1	Ryanodine receptor 1		Ryanodine receptor 1
Mutation	c.5574C>G; IVS124+5G>A	c.5783-5784delTA; IVS59+1G>A	c.3223C>T; c.7025A>G; (c.7645-7650dup)		c.8953C>T; c.9758T>C	c.325C>T; c.8140-8141delTA		c.8888T>C, homozygous
Predicted protein impact	p.Tyr1858Stop; Del exon 124	p.Tyr1928fsX2; Del exon 59	p.Arg1075Trp; p.Asn2342Ser; (p.Ala2549-Leu2550dup)		p.Arg2985Stop; p.Ile3253Thr	p.Arg109Trp; p.Tyr2714fsX7		p.Leu2963Pro

n.d. = not determined

For family 3, the (c.7645-7650dup) change is a potential modifier of the phenotype

Table 2: Statistical overview of the exome sequencing results

A) Sporadic cases (trio sequencing); paired-end 72nt	ARX30	ARX33
Mean coverage	56	55
Coverage nt ≥ 10x	86 %	87 %
Total SNVs	43 062	43 733
SNVs spice sites	87	41
SNV nonsense	10	12
SNV missense	563	445
Total indels	6245	5838
Indels frameshift	595	329
Heterozygous SNVs splice shared with one parent	12	11
Heterozygous SNVs nonsense shared with one parent	3	2
Heterozygous SNVs missense shared with one parent	75	89
Heterozygous indels frameshift shared with one parent	17	16
Two het. SNVs/indels in same gene from distinct parents	**2**	**1**
Myopathy gene	**1 (NEB)**	**1 (NEB)**

B) Familial cases (non-consanguineous); paired-end 72 nt	AKY21	IM26	AGT66	AGT67
Mean coverage	59	73	63	63
Coverage nt ≥ 10x	90.0 %	90.7 %	90.2%	90.1 %
Total SNVs	26 897	29 011	27 273	26 815
SNVs spice sites	164	174	138	110
SNV nonsense	24	16	15	9
SNV missense	3 573	4 083	3 685	2 623
Total indels	1 838	2 048	1 864	1 864
Indels frameshift	51	69	50	51
Common heterozygous SNVs splice	3		2	
Common heterozygous SNVs nonsense	8		5	
Common heterozygous SNVs missense	385		306	
Common heterozygous indels frameshift	15		12	
Two Common het. SNVs/indels in same gene	**39**		**25**	
Myopathy gene	**1 (RYR1)**		**1 (RYR1)**	

C) Sporadic case; paired-end 72nt	AHY58
Mean coverage	59
Coverage nt ≥ 10x	94%
Total SNVs	29182
SNVs spice sites	103
SNV nonsense	167
SNV missense	9480
Total indels	2381
Indels frameshift	155
Two heterozygous SNVs/indels in same gene	20
Myopathy gene	**1 (RYR1)**

D) Sporadic case (consanguineous); single read 72nt	AHE6
Mean coverage	23
Coverage nt ≥ 10x	75%
Total SNVs	35755
SNVs spice sites	238
SNV nonsense	31
SNV missense	4756
Total indels	3393
Indels frameshift	256
Linked homozygous SNVs splice	0
Linked homozygous SNVs nonsense	0
Linked homozygous SNVs missense	18
Linked homozygous indels frameshift	0
Total linked homozygous SNVs/indels	**18**
Myopathy gene	**1 (RYR1)**

Figure 1. Pedigrees and *RYR1/NEB* mutations in six families with different muscle disorders

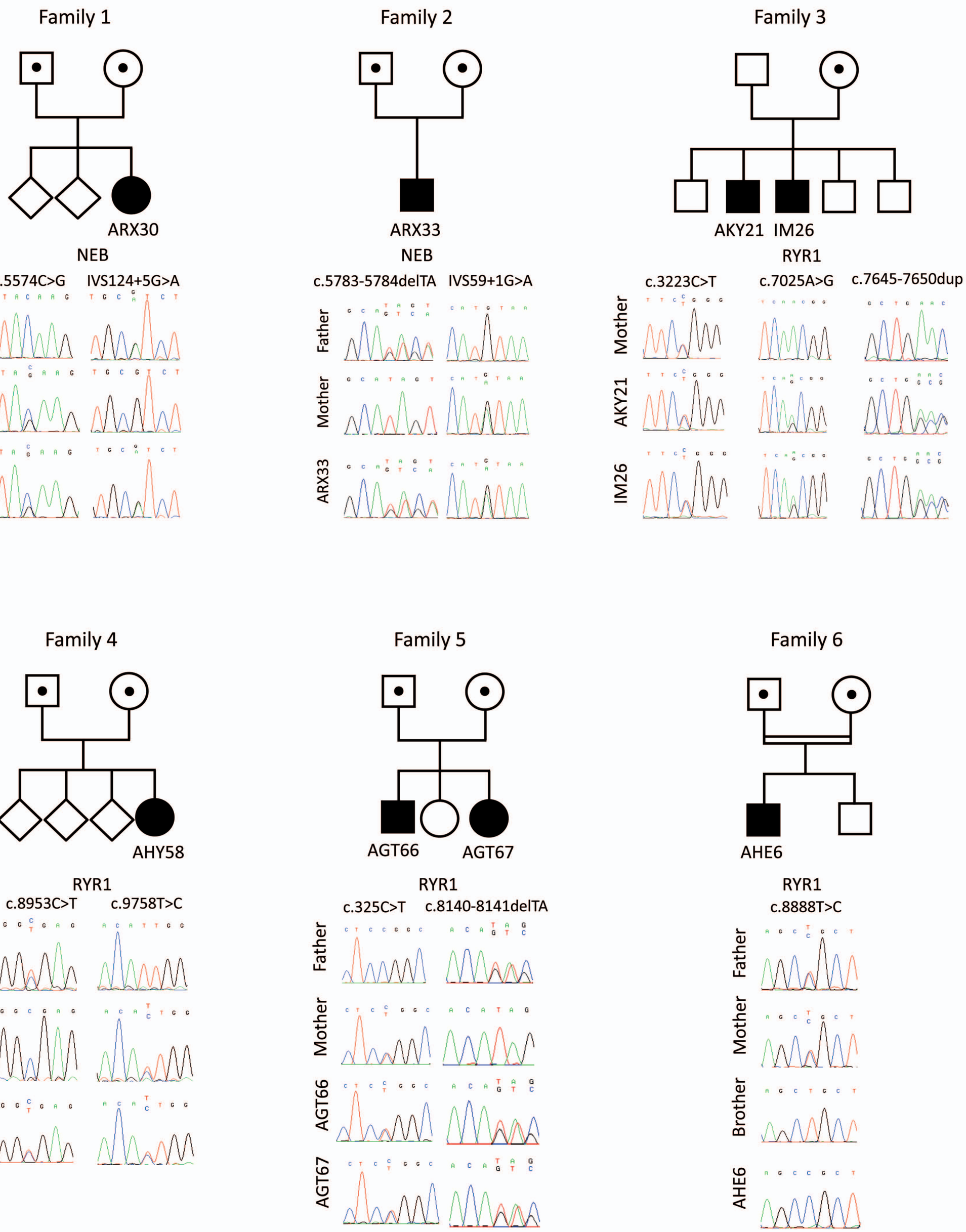


Fig. 2. Histological muscle biopsy analysis of the patients

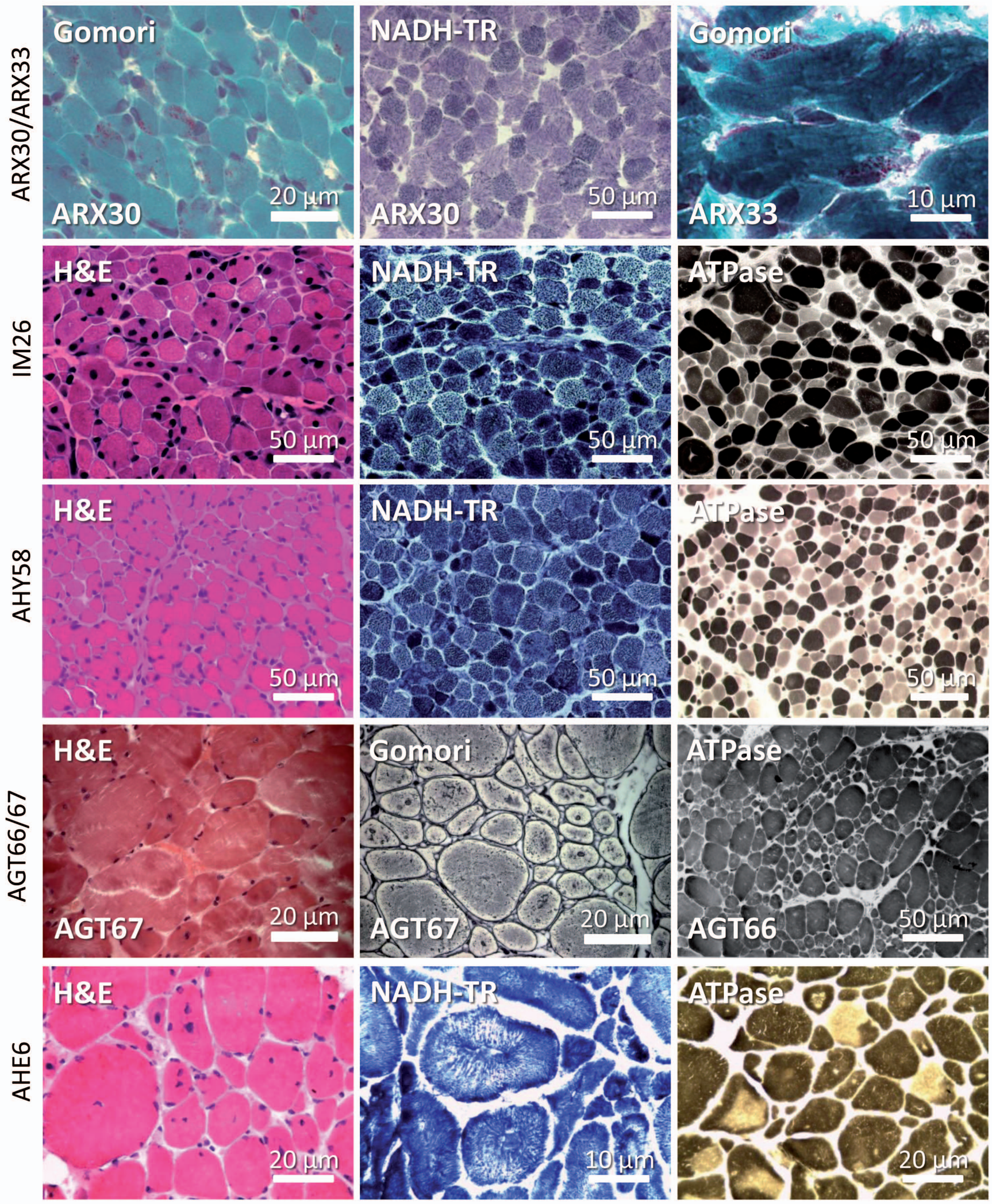


Fig. 3. Ultrastructural analysis of muscle biopsies

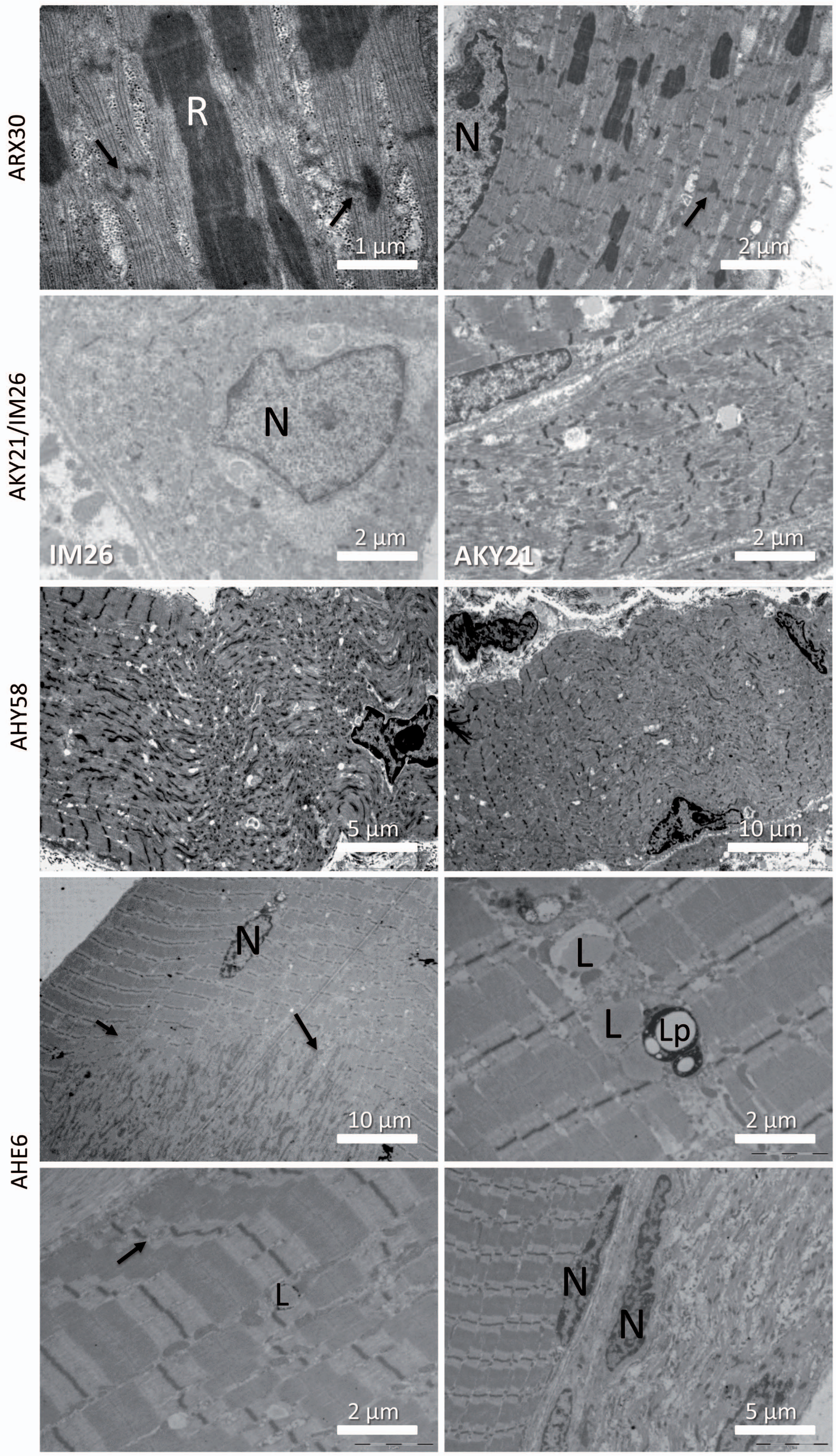
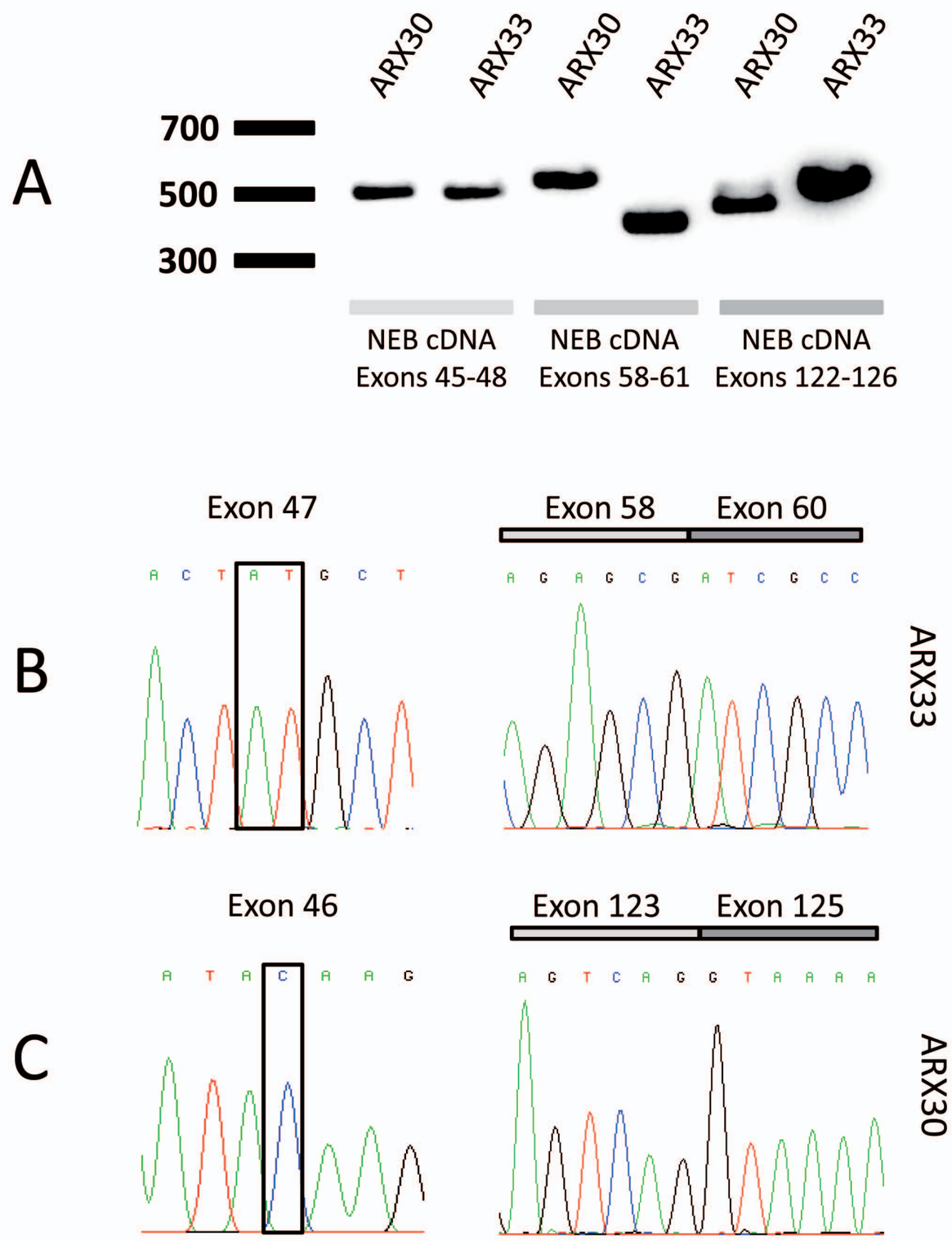


Fig. 4. Impact of the *NEB* splice mutations



SUPPLEMENTARY MATERIAL

Patients and Methods

Patients

Patients originated from France (ARX30 and ARX33), Greece/Morocco (IM26 and AKY21), French West Indies (AHY58), Germany (AGT66, AGT67), and Turkey (AHE6). Sample collection was performed with informed consent from the patients according to the declaration of Helsinki and experimentation was performed following institutional IRB-accepted protocols.

Linkage analysis

For whole-genome analysis, the genomic DNA of patient AHE6 was hybridized on Affymetrix SNP array 6.0 according to the manufacturer's instructions. Loss of heterozygosity was analyzed with GeneChip DNA Analysis and Chromosome Copy Number Analysis softwares (Affymetrix, Santa Clara, CA, USA).

Exome sequencing

Genomic DNA was prepared from peripheral blood by routine procedures and quality-controlled. DNA was sheared using the Covaris E210 (KBioscience, Herts, UK) followed by automatic library preparation with the SPRI-TE machine (Beckman Coulter Inc., Brea, CA, USA). Exon capture was performed with the Agilent SureSelect Human all Exon 50 Mb Kit (Agilent Technologies, Santa Clara, CA, USA). Enriched DNA fragments were sequenced on an Illumina Genome Analyzer IIx to generate 72nt single reads for AHE6 and paired-end

reads for AGT66, AGT67, AHY58, AKY21 and IM26. For patients ARX30 and ARX33 we performed “trio sequencing”, i.e. exome sequencing of the patient and both healthy parents.

Bioinformatic analysis

Sequence data were analyzed using Illumina Pipeline RTA (Real-Time Analysis) version 1.7 and aligned to the reference genome GRCh37/hg19 using BWA(1). Variant calling and filtering of reads sharing the same start position and strand was done with Samtools(2). Variants were considered as heterozygous when present in 20 - 80 % of the reads and as homozygous when present in ≥ 80 % of the reads. For SNP/indel annotation and filtering SVA, Ensembl60 and dbSNP131, 1000 genomes, and NHLBI exome variant server were used. Impact of variations were predicted using SIFT(3), PolyPhen V2(4), NNSPLICE(5) and Human Splicing Finder(6).

Mutation characterization

Mutation confirmation and segregation analysis were performed by PCR and Sanger sequencing of the *RYR1*/*NEB* exons harboring the mutations and the adjacent exon-intron boundaries. The mutations were numbered according to GenBank NM_000540.2 and NP_000531.2 (*RYR1*) and NM_001164507.1 and NP_001157979.1 (*NEB*). Nucleotide position reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon.

Muscle histology

For histochemical analyses, transverse sections (10 µm) of the muscle biopsies were stained with hematoxilin-eosin, Gomori trichrome, NADH tetrazolium reductase (NADH-TR) and ATPase and assessed for nuclei position, fiber morphology, fiber type distribution, cores and accumulation/infiltrations.

Electron microscopy

Muscle sections were fixed in 2.5% paraformaldehyde, 2.5% glutaraldehyde, and 50 mM $CaCl_2$ in 0.1 M cacodylate buffer (pH 7.4), and post-fixed with 2% OsO_4, 0.8% $K_3Fe(CN)_6$ in 0.1 M cacodylate buffer (pH 7.4) for 2 h at 4°C and incubated with 5% uranyl acetate for 2 h at 4°C. Samples were dehydrated in graded series of ethanol and embedded in Epon resin 812. Ultrathin sections (70nm) were contrasted with uranyl and lead citrate and viewed at 70kv with a Morgagni 268D electron microscope and a Mega View III camera (Soft Imaging System, Münster, Germany).

RNA

RNA was extracted from the deltoid muscle biopsy of patients ARX30, ARX33, IM26 and AHE6 using Tri reagent (Molecular Research Center Inc., Cincinnati, OH, USA), reverse transcribed using the SuperScript® III kit (Invitrogen, Carlsbad, CA, USA), and amplified using *RYR1* and *NEB* specific primers.

Table e-1: Homozygosity mapping for AHE6

Chromosome	Homozygous regions (Positions in Mb)	Number of genes
1	33.9 - 43.5	238
	60.4 - 86.4	325
	95.9 - 120.1	423
	193.6 - 196.0	12
	236.6 - 240.5	54
2	97.0 - 99.7	59
	207.8 - 227.4	316
	239.3 - 244.0	87
3	45.9 - 73.4	479
4	0.7 - 30.6	439
	182.5 - 190.0	128
5	10.3 - 33.7	259
	54.0 - 67.4	186
	163.0 - 169.8	63
6	38.8 - 58.0	339
	62.0 - 71.0	56
	153.8 - 166.0	146
7	7.5 - 25.4	210
	61.0 - 67.0	190
	105.8 - 111.9	63
9	129.8 - 135.0	171
11	74.0 - 174.0	526
	126.8 - 132.2	74
12	4.2 - 18.1	434
	39.7 - 44.2	66
13	40.8 - 44.7	64
	54.0 - 64.1	49
	80.2 - 91.0	57
14	42.3 - 89.7	790
	98.2 - 107.0	450
16	48.5 - 53.5	556
	72.5 - 77.5	57
17	3.6 - 12.6	247
	68.0 - 73.0	64
19	3.0 - 24.0	664
	32.5 - 53.6	803
20	1.9 - 15.5	212

References

1. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 2009;25:1754-1760.

2. Li H, Handsaker B, Wysoker A, et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics 2009;25:2078-2079.

3. Ng PC, Henikoff S. SIFT: Predicting amino acid changes that affect protein function. Nucleic Acids Res 2003;31:3812-3814.

4. Ramensky V, Bork P, Sunyaev S. Human non-synonymous SNPs: server and survey. Nucleic Acids Res 2002;30:3894-3900.

5. Reese MG, Eeckman FH, Kulp D, Haussler D. Improved splice site detection in Genie. J Comput Biol 1997;4:311-323.

6. Desmet FO, Hamroun D, Lalande M, Collod-Beroud G, Claustres M, Beroud C. Human Splicing Finder: an online bioinformatics tool to predict splicing signals. Nucleic Acids Res 2009;37:e67.

3-3-1- Trio sequencing for AHJ97

This patient was sequenced in the project of NGS for 2500 candidate genes and one interesting gene, *ZAK*, was detected as a potential candidate for CNM. As all the genes were not sequenced in that project, we decided to carry out exome sequencing for the affected person and his parents (Trio sequencing). The NGS for parents was done in the BGI sequencing platform in China, using one lane of Illumina HiSeq2000 sequencer, paired-end with 90bp length. NGS for the affected child was done in IGBMC sequencing platform, using one lane of Illumina HiSeq2000 sequencer, paired-end with 100bp length. For DNA enrichment and capture Agilent SureSelect Human All Exon 50 Mb kit was used in BGI whereas in IGBMC we tested three different capture kits: TruSeq Exome Enrichment kit (v1.3.0) from Illumina, SeqCap EZ Human Exome Library (v2.0) from Nimblegen and Agilent SureSelect Human All Exon 50 Mb kit (data was shown in materials and methods section). For trio analysis, the NGS data for the affected child (AHJ97) after capturing by Agilent SureSelect Human All Exon 50 Mb kit was compared to parents' data.

The capture was done for covering 51756122 target nucleotides in AHJ97 and 51543125 and 51347872 target nucleotides in father and mother, respectively. Target regions herein refer to the regions that are covered by the designed probes. Raw sequencing data was analyzed, using Illumina Pipeline RTA (Real-Time Analysis) version 1.9 for AHJ97 and Illumina base-calling Software version 1.7 with default parameters for the parents. The alignment was done, using BWA version 0.5.9 and aligned reads can contain up to 3 mismatches for AHJ97. For parents, SOAP aligner/SOAP2 was used and reads can contain up to 3 mismatches. Number and percentage of input, mapped and duplicate reads are shown in table 11.

Table 11- Statistics regarding NGS reads for trio sequencing.

	AHJ97	13560(Father)	13561(Mother)
No. of input reads	470149014	81065438	78223218
No. of Mapped reads	447567387	69338784	69489550
Percentage of mapped reads	95,2 %	85,5 %	85,8 %
Percentage of duplicate reads	50,5 %	12,25 %	8,82 %

Achieved nucleotide coverage is shown in table 12 and figure 25.

Table 12- Statistics regarding nucleotide sequencing coverage for trio sequencing.

	AHJ97	13560(Father)	13561(Mother)
Mean of coverage	253	72,6	72,8
Percentage of reads with ≥ 10x	95,5 %	89,3 %	89,2 %
Percentage of reads with ≥ 20x	93,5 %	80,91 %	81 %

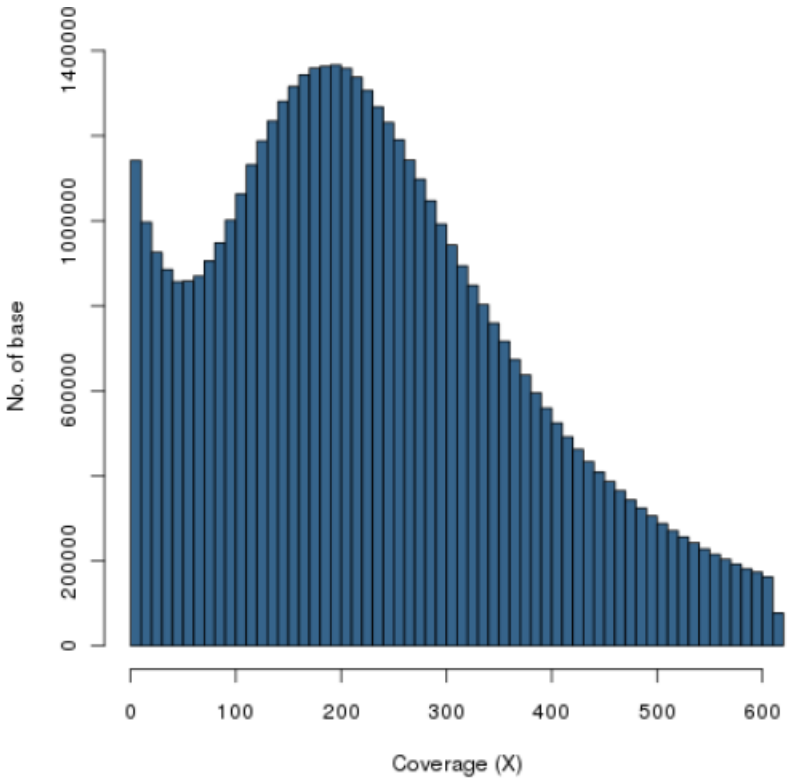


Figure 25: Sequence coverage for targeted nucleotides for AHJ97. As shown in graph the sequence coverage is more than 200 for most of the nucleotides.

Variant calling and functional annotation were performed, using samtools, Sequence Variant Analyzer (SVA) (http://www.svaproject.org/), Ensembl 60 and dbSNP135 for SNPs and 132 for Indels for AHJ97, and SOAPsnp and GATK program for calling SNVs and Indels, respectively for parents. Numbers of detected SNVs and Indels in three samples are shown in table 13.

Table 13- Statistics regarding detected SNVs and Indels.

	AHJ97	13560(Father)	13561(Mother)
Number of detected SNVs	46739	99919	97186
Number of detected Indels	6802	7107	7123

After filtering out the variants in introns and UTR regions, synonymous variants, and variants present in dbSNP, three different scenarios were checked in this family. As the affected child is from a consanguineous family with healthy parents, three scenarios could be possible:

1- Recessive scenario, where the affected child has one homozygous variation and the same variation in heterozygous status presents in each of parents. This is the most probable scenario as the affected person is from a consanguineous marriage.

2- Recessive scenario, where the affected child has two heterozygous variations in a gene (compound heterozygous) and each of the parents has one of the two variations.

3- *De novo* scenario, where the child has either homozygous or heterozygous variations that are not present in any of the parents.

3-3-1-1- Recessive scenario due to homozygous change

In this scenario, homozygous changes in the affected child would be listed if the same variation in heterozygous status presented in each of parents. After filtering out the variants in introns and UTR regions, synonymous variants, and variants present in dbSNP, two variants remained that are shown in table14.

Table 14- Two homozygous variants detected in AHJ97.

PP2: PolyPhen2

EVS: NHLBI exome variant server

coordinate	Gene	Chr	Variation type	Variation	expression	EVS	PP2	SIFT
14533105	POTEC	18	NON_SYNONYMOUS_CODING	NM_001137671.1(POTEC):c.1010C>G, p.Ser337Cys	skeletal muscle and testis	No	probably damaging	tolerated
174055813	ZAK	2	CODING_DISRUPTED_FRAMESHIFT	NM_016653.2 (ZAK):c.490_491del, p.Met164ValfsX24	High in skeletal muscle	No	-	-

Homozygosity mapping analysis revealed homozygous regions in chromosomes 2, 3, 4, 12, 14, 15 and 20 and the biggest homozygous region was in chromosome 2. Thus, the first variant in “POTE ankyrin domain family, member C” gene which is located in chromosome 18 is not in a homozygous region.

The pathogenicity predictions by polyphen2 and SIFT for the NM_001137671.1 (POTEC): c.1010C>G, p.Ser337Cys variation are contradictory, as polyphen2 predicts the effect of change is probably damaging whereas SIFT prediction shows that the effect of change is benign.

The NM_016653.2 (ZAK): c.490_491del, p.Met164ValfsX24 variation is a deletion of two nucleotides leading to a frameshift whereas the NM_001137671.1 (POTEC): c.1010C>G, p.Ser337Cys variation is a missense change. In general, loss of function variations such as Indels leading to frameshifts, are considered more damaging than missense changes. However, both genes are expressed highly in skeletal muscle. All these data together suggest that the NM_016653.2 (ZAK): c.490_491del, p.Met164ValfsX24 variation is ranked higher than NM_001137671.1 (POTEC): c.1010C>G, p.Ser337Cys variation.

3-3-1-2- Recessive scenario due to compound heterozygous changes

In this scenario, genes with at least two heterozygous variations in the affected child would be listed if each of the parents had one of the two variations in heterozygous status. After filtering out the variants in introns and UTR regions, synonymous variants, variants in “black genes”, and variants present in dbSNP, no genes remained.

3-3-1-3- *De novo* scenario

In this scenario, homozygous or heterozygous variations that did not present in any of the parents were listed. 2263 such variations were detected. We believed that most of these variations could be false positives or were detected as *de novo* in the affected child because the same regions/genes were not covered in any of parents.

In one hand, Sanger sequencing of the affected child and segregation analysis for this high number of variations were not cost-effective for removing false positives or non-segregated variations. On the other hand, this scenario is less likely to cause the disease in the affected child. Therefore the 2263 *de novo* variations were not verified by Sanger sequencing.

As a result, the best candidate in this patient was NM_016653.2 (ZAK): c.490_491del, p.Met164ValfsX24 variation in *ZAK*, detected in “recessive scenario due to homozygous change” as a potential CNM-causing mutation. Therefore, the panel of 100 patients with CNM was analyzed for all of the 20 exons of *ZAK* by Sanger sequencing, in order to find at least another CNM patient with the mutation in the same gene. Apart from one heterozygous in frame deletion of three nucleotides in exon 19 that was detected in one patient, no other unknown variations were detected in other patients.

Re-verifying the clinical and histological data of AHJ97 showed the atypical features for CNM in this patient such as predominant affection of shoulder girdle with detachment of two scapulas, diffuse amyotrophy (progressive wasting of muscle tissues) and high CK level (1334 U/l).We tried to find CNM patients with similar features and we identified three other patients. Sanger sequencing of all 20 exons of *ZAK* in these three patients did not reveal any mutations in *ZAK*.

3-3-2- Exome sequencing for centronuclear myopathy sporadic cases

Apart from samples where we found disease-causing mutations in *RYR1*, DNA from seven sporadic CNM cases in different families was used for exome sequencing in different sequencing platforms. The age of onset and severity of disorder as well as histological findings were not similar in all these sporadic cases. In these samples different enrichment kits with different versions, different sequencing machines and different length of reads were used. Although for the mapping and variant

calling, several programs had been used in different sequencing platforms, as our final aim was comparing all NGS data of these sporadic cases together, assuming that most of them have mutations in the same gene, for having less bias, we repeated mapping and variant calling analyses using BWA and samtools for all of these sporadic cases. The list of these DNAs is shown in table 15.

Table 15- Sporadic cases sequenced on different sequencing platforms.

[1]Otogenetics: http://www.otogenetics.com/

[2]DNA vision: http://www.dnavision.com/

[3]IGBMC: http://www-microarrays.u-strasbg.fr/

Patient	Sequencing platform	Enrichment kit	Read length	Sequencing machine
Li65	[1]Otogenetics	Nimblegen SeqCap exome library v.2.0	2x100	Illumina HiSeq2000
EO33	Otogenetics	Nimblegen SeqCap exome library v.2.0	2x100	Illumina HiSeq2000
G95-320	Otogenetics	Nimblegen SeqCap exome library v.2.0	2x100	Illumina HiSeq2000
AHY63	Otogenetics	Nimblegen SeqCap exome library v.2.0	2x100	Illumina HiSeq2000
ADE53	[3]IGBMC	Agilent SureSelect Human All Exon	2x72	Illumina GAIIx
AFZ87	IGBMC	Agilent SureSelect Human All Exon	2x72	Illumina GAIIx
AIZ36	[2]DNA vision	Agilent SureSelect Human All Exon	2x72	Illumina GAIIx

The statistics regarding NGS reads, depth of coverage and called SNVs and Indels are shown in table 16.

Table 16- Statistics regarding NGS reads, depth of coverage and detected SNVs and Indels in CNM sporadic cases.

	Li65	EO33	G95-320	AHY63	ADE53	AFZ87	AIZ36
No. of input reads	49495670	44722808	58017626	50480008	68106720	46754200	124561494
No. of Mapped reads	41996704	38294338	51424984	42946999	67106411	45991794	123278510
Percentage of mapped reads	84,8%	85,6%	88,6%	85%	98,5%	98,3%	98,9%
No. of multi-mapped reads	1926005	1706820	2120558	1860268	5101655	3677180	4668152
Percentage of multi-mapped reads	4,5%	4,4%	4,1%	4,3%	7,6%	7,9%	3,7%
Number of duplicate reads	7561036	8296650	12156336	8566608	4732346	3726020	66074382
Percentage of duplicate reads	18%	21,6%	23,6%	19,9%	7%	8,1%	53,5%
Number of remained reads	32509663	28290868	37148090	32520123	57272410	38588594	52535976
Number of targeted regions	195031	195031	195031	195031	213240	213240	171599
Number of non-covered regions	2232	2275	2181	2389	5984	6390	3507
Number of covered regions	189397	188854	189540	189090	197997	195373	164767
Mean of coverage	32,6	27,2	35,6	31	47,3	31	58,2
Percentage of reads with ≥ 1x	97,8%	97,7%	97,9%	97,7%	96,3%	95,9%	97,6%

Percentage of reads with ≥ 10x	87,8%	84,8%	89,2%	87,3%	86,8%	82,7%	93%
Percentage of reads with ≥ 20x	67,3%	58,5%	72,4%	65,6%	76,6%	64,44%	86,4%
Number of detected SNVs	37614	37892	36275	37058	43276	42652	43060
Number of detected Indels	3740	3567	3919	3712	5934	5552	3632

Each of these sporadic cases was analyzed separately for different scenarios based on mode of inheritance and a lot of candidates were detected in each of those scenarios. As distinguishing between the disease-causing mutation and individual rare variations was difficult by sequencing a single sporadic case in each family, we decided to overlap the NGS data from multiple unrelated individuals with similar disorder and to prioritize the common genes, assuming that the genetic heterogeneity is absent in this disorder.

The depth of coverage was not similar for all sporadic cases. The number and location of non-covered regions were different as well, which may lead to miss real variations. The heterogeneity in clinical and histological data in all these sporadic cases was also an important issue to be consider. However, we decided to overlap the NGS data of these seven samples in order to detect the common genes with loss of function variations (nonsense, splice sites or Indels leading to frameshift). Data are shown in table 17.

Table 17- Common genes with different variations in at least four CNM sporadic samples.

No. of patients with different variations	Gene	Complete name of the gene	chr	Expressed tissues	Variations	patient showing the variation
4	*RP1L1*	retinitis pigmentosa 1-like 1	8	Visual system	8_10467467_10467470_DEL_GCCC	EO33
					8_10467472_10467473_DEL_TC	AIZ36
					8_10467517_10467518_DEL_CC	AIZ36
					8_10467527_10467527_DEL_C	EO33
					8_10467546_10467547_INS_A	G95
					8_10467673_10467682_DEL_*	Li65
4	KRTAP 21-1	keratin associated protein 21-1	21	Thyroid	21_32127550_32127551_INS_C	Ahy63-Li65
					21_32127573_32127574_INS_C	EO33
					21_32127604_32127605_INS_C	G95
					21_32127606_32127607_INS_A	Ahy63-Li65

As shown in table 17, the two genes with different loss of function variations in at least four out of seven patients do not express in muscle so it is less likely that the variations detected in these two genes are CNM-causing mutations.

TTN gene was present in almost all of the patients with several variations. This gene has important roles in skeletal muscle but it is a large gene that accumulates numerous variations listed in different

databases such as 1000 genomes and known as “black gene” in literature. The verification of detected *TTN* variations in our CNM patients is ongoing to see whether these changes are sequencing errors or real variations.

As mentioned above, due to different depth of coverage, different covered and non-covered regions, different number of detected SNVs and Indels and clinical and histological heterogeneities in seven patients, overlapping NGS data did not lead to promising result.

3-3-3- Exome sequencing for ABJ family

A CNM male patient (ABJ79) with reduced muscle tone at birth, delayed motor milestones, general muscle weakness and difficulties in climbing the stairs and his sister (ABJ75) from a big, non-consanguineous family were selected for exome sequencing in Integragen sequencing platform, using Agilent Sureselect liquid capture-whole exome kit version 1 for enrichment followed by NGS, using one lane of Illumina GAIIx sequencer, paired-end with 75bp length. The pedigree of this family is shown in figure 26.

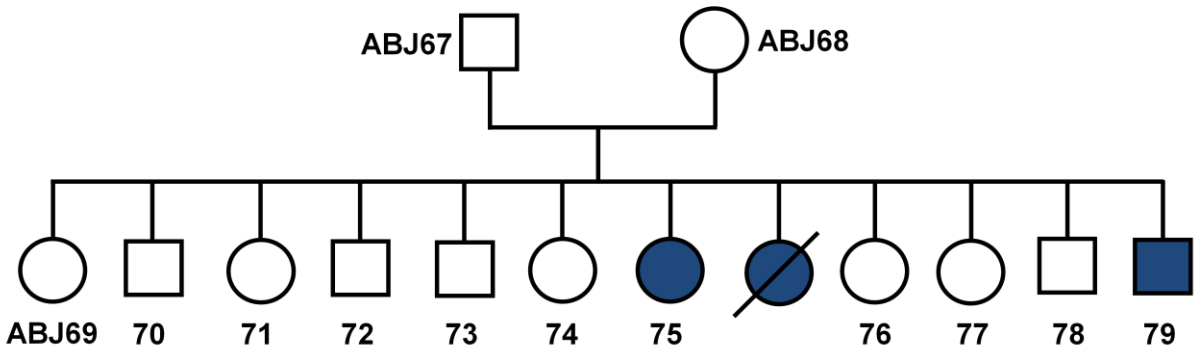


Figure 26: Pedigree of ABJ family. The affected patients are shown in bleu.

The capture was performed for 171599 non-overlapping regions, covering 38925539 target nucleotides. Raw sequencing data was analyzed, using Illumina Pipeline RTA (Real-Time Analysis) version 1.9, and the alignment was carried out, using BWA version 0.5.9. Indels of 50nt were allowed and aligned reads can contain up to 3 mismatches. Number and percentage of input, mapped, duplicate and multi-mapped reads are shown in table 18.

Table 18- Statistics regarding NGS reads for ABJ79 and ABJ75.

	ABJ75	ABJ79
No. of input reads	74369792	83009418
No. of Mapped reads	73298474	80873292

Percentage of mapped reads	98,5%	97,4%
No. of multi-mapped reads	4212233	4630358
Percentage of multi-mapped reads	5,7%	5,7%
Number of duplicate reads	6235286	6080746
Percentage of duplicate reads	8,5%	7,5%
Number of remained reads after filtration	62850955	70162188

Achieved nucleotide coverage is shown in table 19 and figure 27.

Table 19- Statistics regarding nucleotide coverage for ABJ79 and ABJ75.

	ABJ75	AB79
Mean of coverage	63,1%	69,2%
Median of coverage	51	54
Percentage of reads with ≥ 1x	96,6%	96,7%
Percentage of reads with ≥ 10x	86%	85,3%
Percentage of reads with ≥ 20x	75,7%	75%
Percentage of reads with ≥ 40x	58,7%	59,2%
Number of targeted region	171599	171599
Number of non-covered regions	4573	4296
Number of totally covered regions	159976	159814

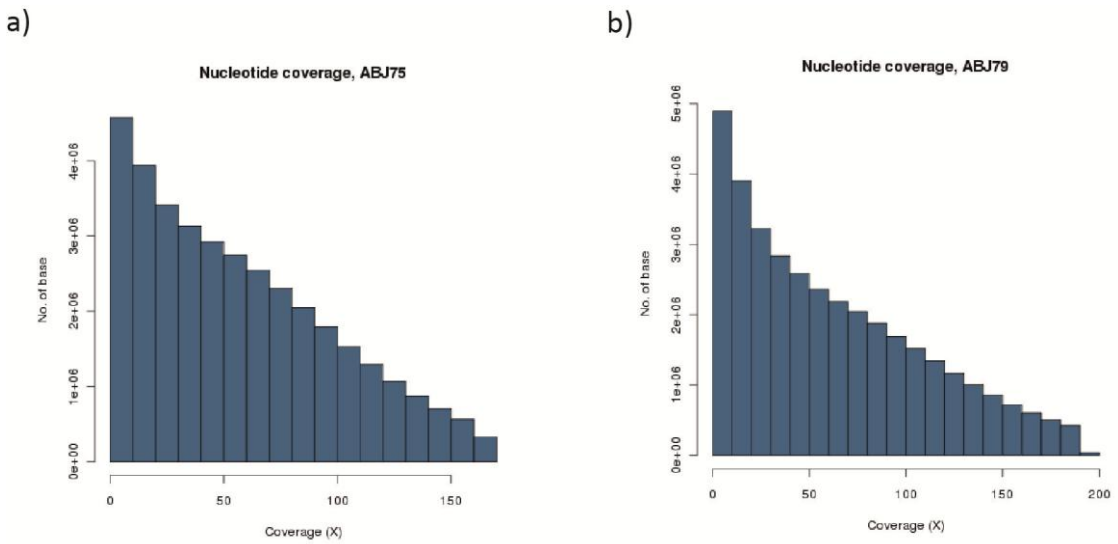


Figure 27: Sequence coverage for targeted nucleotides in ABJ75 and ABJ79. Most of the nucleotides have depth of coverage more than 60 times in both samples.

After variant calling and functional annotation, using samtools, Sequence Variant Analyzer (SVA) (http://www.svaproject.org/), Ensembl 60 and dbSNP134 for SNVs and dbSNP132 for Indels, SNVs and Indels were detected. Number of called SNVs and Indels are shown in table 20.

Table 20- Statistics regarding detected SNVs and Indels.

	ABJ75	ABJ79
Number of detected SNVs	45870	53715
Number of detected Indels	5336	5559

After filtering out the variants in introns and UTR regions, synonymous variants, and variants with Rs number (variants which could be potentially polymorphisms), variants that were present in both samples were detected and were analyzed in different scenarios.

The two affected patients are from a non-consanguineous family, thus the most probable scenario in this family was compound heterozygous variations in recessive mode of inheritance. The common genes in both affected patients with more than one variation were detected.

The common homozygous variations in both affected as "recessive scenario due to homozygous variations" was also checked. The linkage analysis in this family had revealed the linked regions in chromosome 2 and 9 but the Lod scores were about 2 which is not considered high enough. Thus, variations in these two chromosomes as well as variations in other chromosomes were checked in both scenarios.

3-3-3-1- Recessive scenario due to compound heterozygous changes

The most probable scenario in this family was compound heterozygous changes in a gene in both affected patients and each parent with one of the two variations in heterozygous status.

After filtering out the variants in introns and UTR regions, synonymous variants, and variants with Rs number, common genes with more than one variation in both samples were extracted and were Sanger sequenced to remove false positive changes due to sequencing or mapping errors. After removing false positives, for other variations, direct sequencing of parents and other siblings were performed to verify the segregation of changes in the family. These data are shown in table 21.

Table 21- Compound heterozygous variations in ABJ75 and ABJ79. Each gene with two or three changes is shown with different colors. After Sanger sequencing of variants for ABJ75 and AB79, if the variants were not false positives, they would check in parents as well as other siblings by direct sequencing. The reasons of filtering out the variations are listed in the last column.

Gene	Chr	Exon with change	Variation; codon; amino acid	Sanger result	Reason of filtering
NBPF14	1	22	Missense; TCA>TTA; S>L	Not detected	False+
NBPF14	1	22	Missense; GTC>ATC; V>I	Not detected	False+
CFTR	**7**	**10**	**Deletion AAAGC- frameshift**	**Not detected**	**False+**
CFTR	**7**	**10**	**Deletion ATTA- frameshift**	**Not detected**	**False+**
PNPLA8	7	7	Missense; CAT>ATT; H>N	Detected	Seen in several unaffected in family
PNPLA8	7	7	Missense; ATA>GTA; I>V	Detected	Seen in several unaffected in family
PPIF	**10**	**4**	**Insertion C- frameshift**	**Detected**	**Seen in several unaffected in family**
PPIF	**10**	**6**	**Deletion TG- frameshift**	**Detected**	**Seen in several unaffected in family**
XIRP2	2	9	Missense; CGG>CAG; R>Q	Detected	All three changes from unaffected father
XIRP2	2	9	Missense; ATG>ATT; M>I	Detected	All three changes from unaffected father
XIRP2	2	11	Missense; AGA>ACA ; R>T	Detected	All three changes from unaffected father
NCKAP1	**2**	**25**	**Deletion A- frameshift**	**Detected**	**Seen in several unaffected in family**
NCKAP1	**2**	**6**	**Insertion A- frameshift**	**Detected**	**Seen in several unaffected in family**
AGAP4	10	7	Missense; CGC>TGC; R>C	Detected	Parents have same variation & No more in compound heterozygous scenario
AGAP4	10	7	Missense; CGC>TGC; R>C	Not detected	False+
LGALS9C	**17**	**6**	**Missense; GTG>ATG; V>M**	**Detected**	**Two changes from unaffected father**
LGALS9C	**17**	**11**	**Missense; TTG>TTC; L>F**	**Detected**	**Two changes from unaffected father**
NCOR1	17	15	Missense; TCA>TTA; S>L	Not detected	False+
NCOR1	17	21	Missense; GAC>AAC ; D/N	Detected	No more in compound heterozygous scenario
PDE4DIP	**1**	**25**	**Missense; GCT>ACT; A>T**	**Detected**	**Seen in one unaffected in family**
PDE4DIP	**1**	**40**	**Missense; GGC>AGC; G>S**	**Detected**	**Seen in one unaffected in family**
CTBP2	10	3	Deletion T- frameshift	Not detected	False+
CTBP2	10	11	Deletion A- frameshift	Not detected	False+
GFI1B	**9**	**4**	**Missense; TCC>CCC; S>P**	**Not detected**	**False+**
GFI1B	**9**	**4**	**Missense; ACC>CCC ; T>P**	**Not detected**	**False+**

As it is shown in table 21, all candidates were filtered out due to different reasons such as being false positive or one change was false positive and the other one was detected by Sanger sequencing therefore, the gene could not be in compound heterozygous scenario anymore. The other reason could be that both variations in a gene were detected in several non-affected sisters/brothers or both variations were detected in one of the non-affected parent. Among these genes, variations in the *PDE4DIP* gene were detected only in one non-affected sister (ABJ74). Thus, we were wondering whether the DNA that we had for this sister was not contaminated or mixed with the other affected

sister who died after birth and we did not receive her DNA. Unfortunately, we could not have a new batch of DNA or blood or saliva samples from ABJ74 to re-sequence it.

The variations in *PDE4DIP* gene detected by exome sequencing, Sanger sequencing and segregation in family are shown in figure 28 and figure 29.

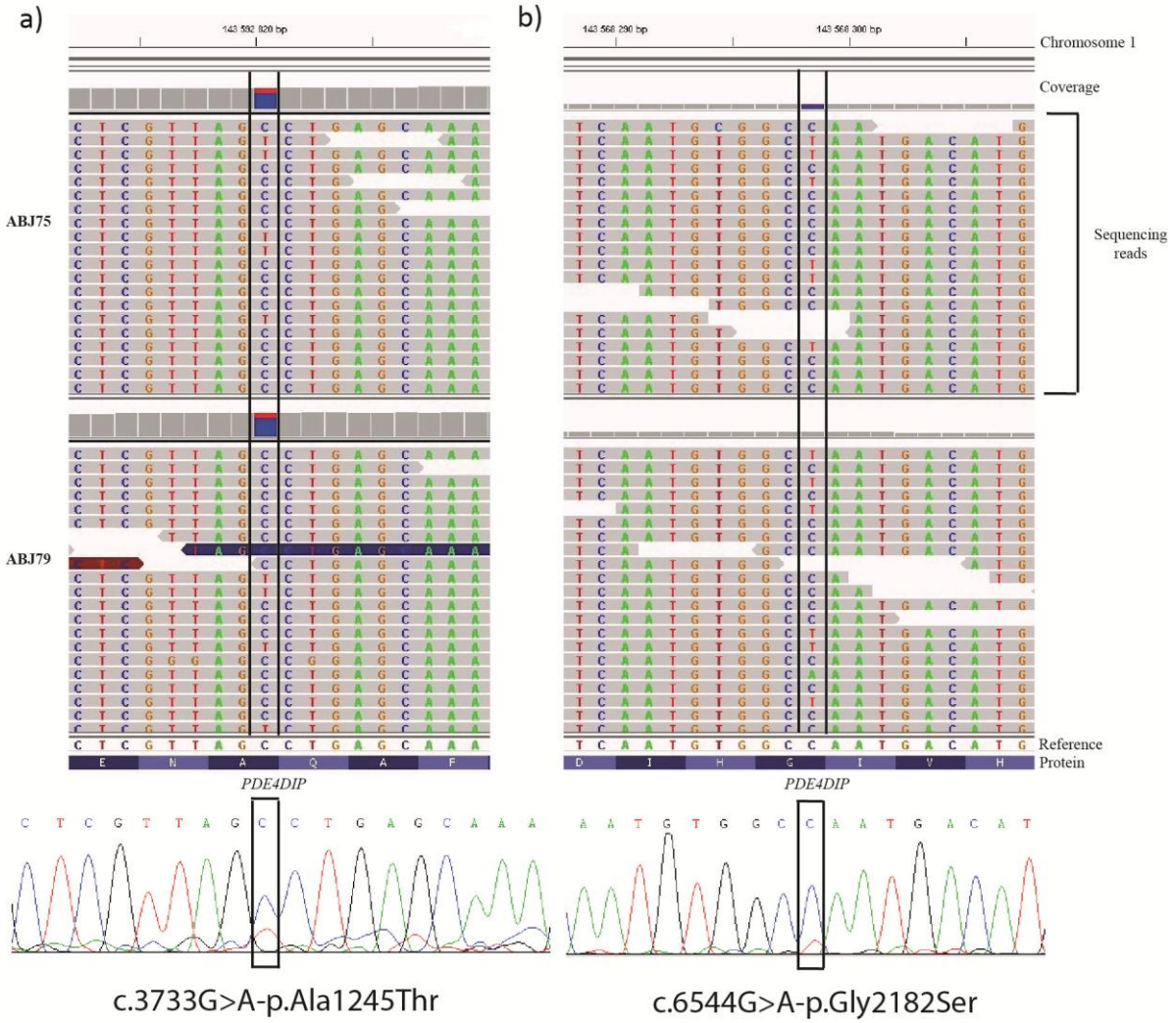


Figure 28: The variations in *PDE4DIP* gene detected by exome sequencing. The c.3733G>A-p.Ala1245Thr variation in exon 25 of *PDE4DIP* (panel a) and c.6544G>A-p.Gly2182Ser variation in exon 40 of *PDE4DIP* (panel b) in AB75 (upper panel) and ABJ79 (lower panel), detected by exome sequencing and depicted by IGV tool. Sanger sequencing confirmed the detected variations in both patients which are shown at the bottom of the picture, using Chromas software. The gene is in the reverse strand so the nucleotides shown by IGV and Chromas are reverse, complement.

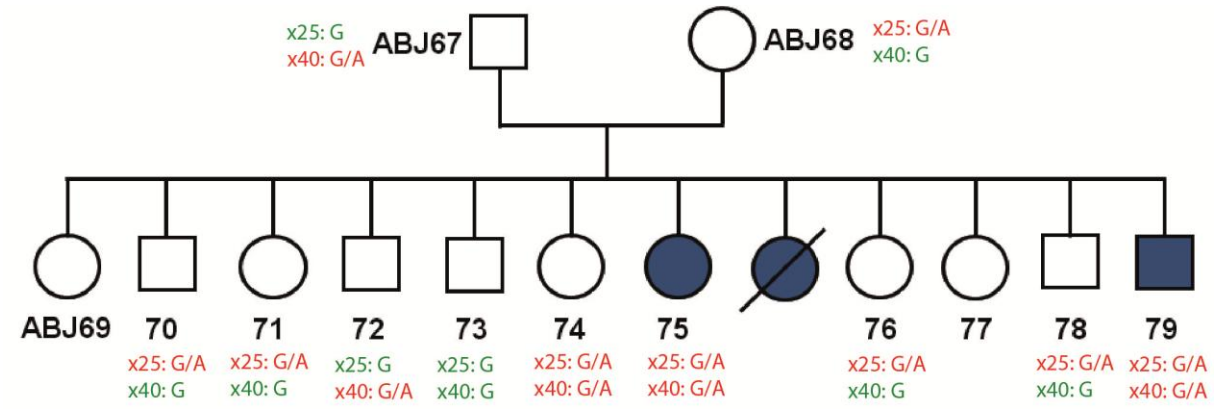


Figure 29: *PDE4DIP* variations segregation in ABJ family. The c.3733G>A-p.Ala1245Thr variation in exon 25 and c.6544G>A-p.Gly2182Ser variation in exon 40 of *PDE4DIP* were verified in parents and all sisters and brothers of affected patients with available DNA. The wild type allele is shown in green and the changed allele is shown in red. ABJ74 who does not show any sign of the disease until now has both changes, similar to ABJ75 and ABJ79, the two affected patients in this family. Each parent has one of the two changes. For AB69 and ABJ77, enough DNAs were not available.

The c.3733G>A-p.Ala1245Thr variation in exon 25 of *PDE4DIP* and c.6544G>A-p.Gly2182Ser variation in exon 40 of *PDE4DIP* were checked in Exome Variant Server, NHLBI Exome Sequencing (http://evs.gs.washington.edu/EVS/), to see whether these changes were detected in other exome datasets and with which frequency. As shown in table 22 both changes have Rs number and were detected in several controls in new version of the Exome Variant Server database. Thus, these changes cannot be CNM-causing mutations in this family.

Table 22- Variations in *PDE4DIP* detected and shown in new version of Exome variant Server. As shown in the table both changes have Rs number and were detected in several controls. "EA" in column four means "European American" and "AA" in column five means "African American".

SNP Pos	rs ID	Alleles	EA Allele #	AA Allele #	All Allele #	Avg. Sample Read Depth	Genes	GVS Function	Amino Acid
1:144881463	rs147815016	T/C	T=43/C=8549	T=6/C=4400	T=49/C=12949	181	PDE4DIP	missense	THR,ALA
1:144856941	rs150294397	T/C	T=385/C=8197	T=41/C=4365	T=426/C=12562	45	PDE4DIP	missense	SER,GLY

3-3-3-2- Recessive scenario due to homozygous change

Another less probable scenario was a common homozygous change in both affected patients (ABJ75 and ABJ79) which was not detected in non-affected parents and siblings in homozygous status. After

filtering out the variants in introns and UTR regions, synonymous variants, and variants with Rs number, common homozygous variations in both samples were extracted and were Sanger sequenced to remove false positives due to sequencing or mapping errors. After these filtration steps, no variation remained.

Since no promising data was achieved by exome sequencing of the two affected patients in ABJ family, we decided to carry out whole genome sequencing of one affected (ABJ79) and mother (ABJ68), as we did not have enough DNA from the other affected patient (ABJ75).

3-4-Whole genome sequencing

Two members of the ABJ family, affected child (ABJ79) and his mother (ABJ68), and three members of the AIZ family, affected child (AIZ36) and his healthy parents (APX27/mother & APX28/father) were selected for whole genome sequencing at the Complete genomics sequencing platform.

3-4-1- Whole genome sequencing for ABJ family

In this family, the affected child (ABJ79) and his mother (ABJ68) were sequenced. The statistics regarding genome and exome coverages are shown in table 23 and statistics regarding numbers of detected variations are shown in table 24.

Table 23- Statistics regarding genome and exome coverages for ABJ79 and ABJ68.

	ABJ79	ABJ68
Fully called genome	96%	95,9%
Partially called genome	0%	0%
No-called genome	4%	4%
Fully called exome	98,2%	98,1%
Partially called exome	0%	0%
No-called exome	1,8%	1,9%
Genome Coverage >= 5x	98,7%	98,9%
Genome Coverage >= 10x	96,5%	97,1%
Genome Coverage >= 20x	88,1%	90,5%
Genome Coverage >= 40x	59,5%	63,3%
Exome Coverage >= 5x	99,6%	99,6%
Exome Coverage >= 10x	98,8%	98,8%
Exome Coverage >= 20x	94,7%	94,7%
Exome Coverage >= 40x	74,1%	72,6%

Table 24- Statistics regarding numbers of detected variations in ABJ79 and ABJ68.

Genome/Exome		AB75	AB79
Genome	SNP total count	3424591	3452421
Genome	Homozygous SNP count	1225958	1267124
Genome	Heterozygous SNP count	2022384	2078676
Genome	SNP novel	5,6%	5,7%
Genome	Homozygous SNP novel	0,5%	0,5%
Genome	Heterozygous SNP novel	8,4%	8,6%
Genome	SNP heterozygous/homozygous ratio	1,65	1,64
Genome	SNP transitions/transversions ratio	2,121	2,118
Genome	INS total count	272294	280097
Genome	INS novel	29,7%	30,2%
Genome	INS heterozygous/homozygous ratio	2,194	2,216
Genome	DEL total count	266907	273614
Genome	DEL novel	26,3%	26,7%
Genome	DEL heterozygous/homozygous ratio	2,033	2,035
Genome	SUB total count	94223	94959
Genome	SUB novel	42,7%	42,9%
Genome	SUB heterozygous/homozygous ratio	2,81	2,803
Exome	SNP total count	22191	22602
Exome	Homozygous SNP count	7954	8028
Exome	Heterozygous SNP count	13441	14021
Exome	SNP novel	7,2%	7,6%
Exome	Homozygous SNP novel	0,2%	0,3%
Exome	Heterozygous SNP novel	11,3%	11,6%
Exome	SNP heterozygous/homozygous ratio	1,69	1,747
Exome	SNP transitions/transversions ratio	2,975	2,934
Exome	INS total count	349	350
Exome	INS novel	44,4%	45,8%
Exome	INS heterozygous/homozygous ratio	1,833	1,714
Exome	DEL total count	345	343
Exome	DEL novel	48,7%	51%
Exome	DEL heterozygous/homozygous ratio	2,987	3,051
Exome	SUB total count	494	494
Exome	SUB novel	50,9%	51,9%
Exome	SUB heterozygous/homozygous ratio	4,069	5,394
Functional impact	Synonymous SNP loci	10701	10802
Functional impact	Non-synonymous SNP loci	10130	10426
Functional impact	Missense SNP loci	9994	10294
Functional impact	Nonsense SNP loci	103	95
Functional impact	Nonstop SNP loci	11	12
Functional impact	Misstart SNP loci	22	25
Functional impact	Disrupt SNP loci	90	91

Functional impact	Frame-shifting INS loci	166	160
Functional impact	Frame-shifting DEL loci	144	167
Functional impact	Frame-shifting SUB loci	34	32
Functional impact	Frame-preserving INS loci	184	191
Functional impact	Frame-preserving DEL loci	204	179
Functional impact	Frame-preserving SUB loci	457	462

In this family the NGS data were analyzed for two possible scenarios: Firstly the recessive scenario due to compound heterozygous changes where the affected child had more than one heterozygous change in a gene and mother had one of the two or more changes. Secondly the recessive scenario due to homozygous change where the affected child had a homozygous change and mother had similar change in heterozygous status.

3-4-1-1- Recessive scenario due to compound heterozygous changes

After filtering out the variants in introns and UTR regions, synonymous variants, and variants with Rs number in ABJ79 and ABJ68 data, genes with more than one heterozygous variation in affected child (ABJ79) were selected and were crossed to mother's data (ABJ68). Therefore, genes with more than one heterozygous variation in ABJ79 and only one of the two or more heterozygous changes in AB68 were extracted. The selected variations were checked by Sanger sequencing to remove false positives due to sequencing errors. If the variations were detected by Sanger sequencing, segregation of the variations in another affected member (AB75) and the other members of family would check. The data is shown in table 25.

Table 25- Compound heterozygous variations in ABJ75. Each gene with two or three variations is shown in different colors. Following Sanger sequencing of variants in AB79, if the variants were not false positives, they would check in ABJ75 as well as parents and other sisters and brothers by direct sequencing. The reasons of filtering out the variations are listed in the last column.

Gene	Chr	Exon with change	Variation; codon; amino acid	Sanger result in ABJ79	Reason of filtering
EPPK1	8	1	Substitution; GTC>CAT; D>M	Not detected	False+
EPPK1	8	1	Substitution; GCG> CGA; R>S	Not detected	False+
LOC389333	**5**	**1**	**Missense ; CCC>CTC; P>L**	**Not detected**	**False+**
LOC389333	**5**	**1**	**Insertion T- frameshift**	**Not detected**	**False+**
MTIF2	2	5	Missense; GCA>CCA; A>P	Detected	Not detected in ABJ75 (another affected member)
MTIF2	2	12	Donor splice site mutation; GT>CT	Detected	Not detected in ABJ75 (another affected member)
WNT6	**2**	**2**	**Missense ; GAC>GAA; D>E**	**Detected**	**Not detected in ABJ75 (another affected member)**
WNT6	**2**	**4**	**Substitution ; GAC>CCA; D>P**	**Not detected**	**False+**
ARFGEF1	8	29	Missense; GAT>GTT; D>V	Not detected	False+
ARFGEF1	8	34	Missense; AGG>AAG; R>K	Was not checked	No more in compound heterozygous scenario

ARFGEF1	8	36	Missense; TCT>CCT ; S>P	Not detected	False+
CLSTN3	**12**	**5**	**Missense ; TAT>CAT; Y>H**	**Not detected**	**False+**
CLSTN3	**12**	**11**	**Missense ; GAC>GTC; D>V**	**Detected**	**No more in compound heterozygous scenario**
CPNE2	16	2	Deletion T- frameshift	Not detected	False+
CPNE2	16	7	Change in +3; G>C	Was not checked	No more in compound heterozygous scenario
IL17RD	**3**	**1**	**Substitution ;TAC>TGT; Y>C**	**Was not checked**	**No more in compound heterozygous scenario in ABJ75**
IL17RD	**3**	**11**	**Missense; GCT>CCT; A>P**	**Detected**	**Not detected in ABJ75 (another affected member)**
PYROXD1	12	5	Substitution; ATT>AAA; I>X	Not detected	False+
PYROXD1	12	6	Donor splice site mutation; GT>AT	Detected	No more in compound heterozygous scenario

As shown in table 25, all candidates were filtered out due to different reasons such as being false positive. One change was false positive and the other one was detected by Sanger sequencing, therefore the gene could not be in compound heterozygous scenario anymore, or both variations in a gene were not detected in another affected member (ABJ75).

3-4-1-2- Recessive scenario due to homozygous change

Another possible scenario with low probability was a common homozygous change in affected child (ABJ79) and similar change in heterozygous status in mother. After filtering out the variants in introns and UTR regions, synonymous variants, and variants with Rs number, homozygous variations in ABJ79 were extracted and were crossed to ABJ68 data to extract genes with homozygous variations in ABJ79 and same variations in ABJ68 in heterozygous status. Only one homozygous deletion was detected in the *EIF3CL* gene, and was confirmed by Sanger sequencing in ABJ79 and the other affected member (ABJ75). Sanger sequencing of parents detected similar change in homozygous status in both non-affected parents showing the non-pathogenicity of this variation.

Copy number variations (CNV) and structural variations (SV) listed by complete genomics were also analyzed in this family. For being sure that these detected variations were correct and not due to sequencing or mapping errors, these data were compared to similar data from a different family (AIZ, described below). The comparison showed that the same structural or copy number variations were detected in affected and non-affected members in both families suggesting that these variations were detected due to sequencing or mapping errors. Thus, no candidates were detected by CNV or SV analysis.

Therefore, no promising data was achieved by whole genome sequencing of one affected patient and his mother in ABJ family.

3-4-2- Whole genome sequencing for AIZ family

In this family, the affected child (AIZ36) had sequenced in the project of exome sequencing of CNM sporadic cases. Several candidates were detected in different scenarios and it was impossible to distinguish between private, rare polymorphisms and disease-causing mutation. Therefore, we decided to perform whole genome trio-sequencing for the affected child and his healthy parents (APX27/mother & APX28/father).

This patient (AIZ36) is an American male, born in 2001 with centronuclear myopathy from a non-consanguineous family. The age of onset was 15 months. He can walk and almost run. Histological analysis showed type I fiber predominance, several small-rounded type I fibers with central nuclei and very large type II fibers with spiral shaped appearances which is an atypical feature in patients with CNM.

The statistics regarding genome and exome coverages are shown in table 26 and statistics regarding numbers of detected variations in three individuals are shown in table 27.

Table 26- Statistics regarding genome and exome coverages for AIZ family.

	AIZ36	APX27	APX28
Fully called genome	96,4%	96,4%	95,9%
Partially called genome	0%	0%	0%
No-called genome	3.6%	3.6%	4%
Fully called exome	98,3%	98,3%	98,1%
Partially called exome	0%	0%	0%
No-called exome	1,7%	1,7%	1,9%
Genome Coverage >= 5x	98,9%	99,1%	98,7%
Genome Coverage >= 10x	97,1%	97,1%	96,3%
Genome Coverage >= 20x	89,8%	92%	87,5%
Genome Coverage >= 40x	61,7%	65,4%	58,7%
Exome Coverage >= 5x	99,7%	99,7%	99,6%
Exome Coverage >= 10x	99%	99%	98,7%
Exome Coverage >= 20x	95,3%	95,3%	94%
Exome Coverage >= 40x	74,1%	73,2%	72%

Table 27- Statistics regarding numbers of detected variations in AIZ family.

Genome/Exome		AIZ36	APX27	APX28
Genome	SNP total count	3435663	3474950	3436291
Genome	Homozygous SNP count	1228685	1271940	1208863
Genome	Heterozygous SNP count	2037803	2104029	2045757
Genome	SNP novel	3,3%	5,6%	5,8%
Genome	Homozygous SNP novel	0,5%	0,6%	0,5%
Genome	Heterozygous SNP novel	8,4%	8,3%	8,6%

Genome	SNP heterozygous/homozygous ratio	1,65	1,65	1,69
Genome	SNP transitions/transversions ratio	2,119	2,119	2,115
Genome	INS total count	285261	290376	279417
Genome	INS novel	30,4%	31%	30,3%
Genome	INS heterozygous/homozygous ratio	2,302	2,302	2,328
Genome	DEL total count	276553	279315	272658
Genome	DEL novel	26,5%	26,7%	26,6%
Genome	DEL heterozygous/homozygous ratio	2,070	2,058	2,117
Genome	SUB total count	94439	95495	95559
Genome	SUB novel	42,2%	42,2%	43,5%
Genome	SUB heterozygous/homozygous ratio	2,78	2,763	2,955
Exome	SNP total count	22235	22506	22249
Exome	Homozygous SNP count	7588	8117	7675
Exome	Heterozygous SNP count	13917	13869	13721
Exome	SNP novel	7 %	7,3%	7,6%
Exome	Homozygous SNP novel	0,2%	0,4%	0,2%
Exome	Heterozygous SNP novel	10,6%	11,2%	11,6%
Exome	SNP heterozygous/homozygous ratio	1,83	1,709	1,788
Exome	SNP transitions/transversions ratio	2,958	2,944	2,908
Exome	INS total count	342	366	369
Exome	INS novel	43,3%	43%	47,7%
Exome	INS heterozygous/homozygous ratio	1,897	2,009	1,991
Exome	DEL total count	334	346	312
Exome	DEL novel	47,6%	48,6%	48,1%
Exome	DEL heterozygous/homozygous ratio	2,829	2,916	2,720
Exome	SUB total count	465	445	513
Exome	SUB novel	53,3%	53,1%	56%
Exome	SUB heterozygous/homozygous ratio	6,863	4,091	6,793
Functional impact	Synonymous SNP loci	10626	10818	10616
Functional impact	Non-synonymous SNP loci	10264	10348	10282
Functional impact	Missense SNP loci	10153	10214	10158
Functional impact	Nonsense SNP loci	80	9104	95
Functional impact	Nonstop SNP loci	13	11	12
Functional impact	Misstart SNP loci	18	19	17
Functional impact	Disrupt SNP loci	77	91	89
Functional impact	Frame-shifting INS loci	162	165	187
Functional impact	Frame-shifting DEL loci	151	152	129
Functional impact	Frame-shifting SUB loci	35	29	45
Functional impact	Frame-preserving INS loci	181	202	184
Functional impact	Frame-preserving DEL loci	183	192	163
Functional impact	Frame-preserving SUB loci	431	416	468

In this family three scenarios were checked: Firstly the recessive scenario due to compound heterozygous changes where the affected child had more than one heterozygous change in a gene and each of the parents had one of the changes. Secondly the recessive scenario due to homozygous changes where the affected child had a homozygous change and both of the parents had similar change in heterozygous status. The last scenario was *de novo* scenario where the affected child had variation which was absent in both parents.

3-4-2-1- Recessive scenario due to compound heterozygous changes

After filtering out the variants in introns and UTR regions, synonymous variants, and variants with Rs number in three individuals, genes with more than one heterozygous variation in affected child (AIZ36) were selected and were crossed to parents data (APX27 & APX28), in order to extract genes with more than one heterozygous variation in AIZ36 and only one of the two or more heterozygous changes in each parent. The selected variations were checked by Sanger sequencing to remove false positives due to sequencing errors. The data is shown in table 28.

Table 28- Compound heterozygous variations in AIZ36. Each gene with two or three changes is shown with different colors. Sanger sequencing was performed for affected child as well as parents for removing false positives.

Gene	Chr	Exon with change	Variation; codon; amino acid	Sanger result in AIZ36	Sanger result in APX27 & APX28
MYO5B	18	4	Missense; GCC>GGC; A>G	Detected	Detected in APX27
MYO5B	18	20	Missense; AAA>GAA; K>E	Detected	Detected in APX28
TTN	**2**	**42**	**Missense ; AAA>AGA; K>R**	**Detected**	**Detected in APX27**
TTN	**2**	**168**	**Missense ; GTT>CTT; V>L**	**Detected**	**Detected in APX28**
TTN	**2**	**192**	**Acceptor splice site mutation; AG>AA**	**Detected**	**Detected in APX27**

As shown in table 28, the variations in *MYO5B* as well as variations in *TTN* were not false positives in AIZ36 and they segregated in parents as well. It is important to note that the mother (APX27) had two *TTN* variations out of three, thus one of these two variations is not pathogenic, since the mother is not affected. Sanger sequencing data for the affected child as well as parents are shown in figure 30.

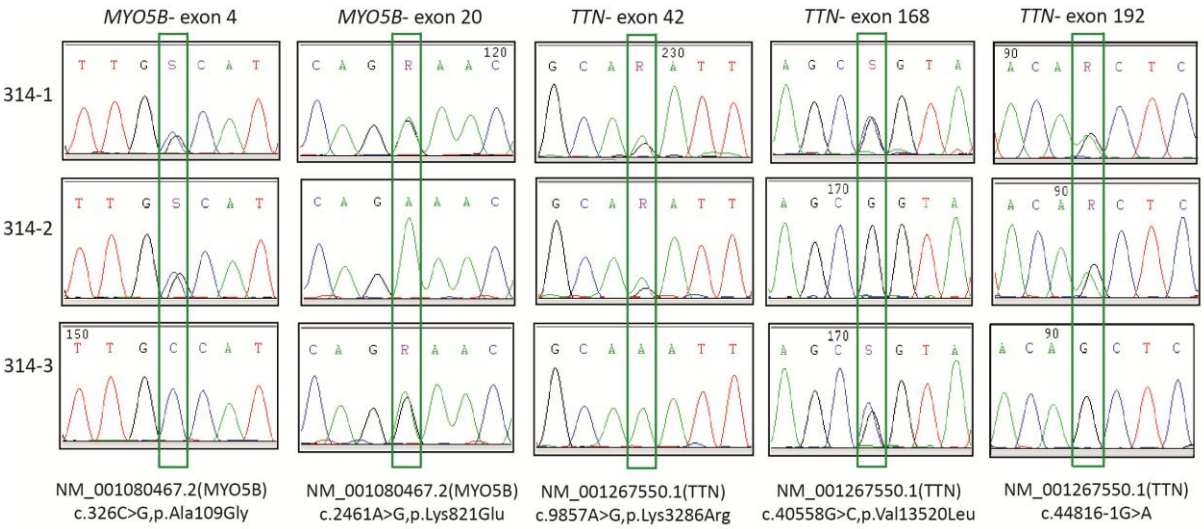


Figure 30: Sanger sequencing results for *MYO5B* and *TTN* genes in AIZ36 and his parents. As shown, two heterozygous variations in *MYO5B* and three variations in *TTN* were detected in this scenario. Mother (APX27) has two *TTN* variations out of three in exons 42 and 192, thus one of these two variations is not pathogenic, since the mother is not affected. The pictures are depicted, using Chromas software.

The missense, GTT>CTT, Val>Leu change in exon 168 is the last nucleotide of this exon in all *TTN* isoforms which prediction tools suggest the disruption of donor splice site due to this change. The position of this change is shown in figure 31. The predictions showing the disruption of splice site are shown in figure 32.

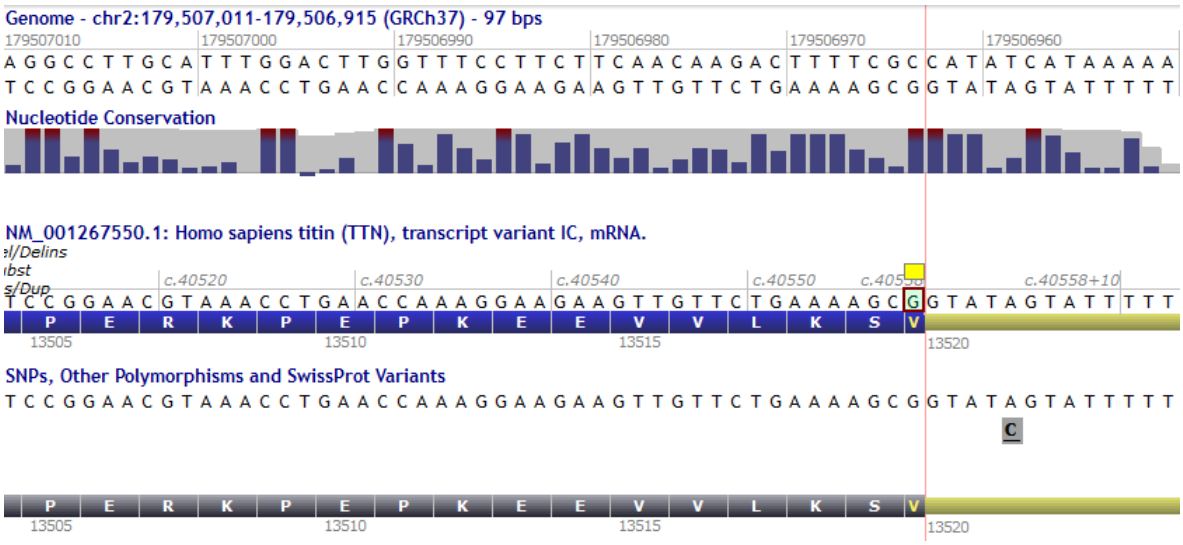


Figure 31: Position of missense, GTT>CTT, V>L change in exon 168 of *TTN*. The red line shows the position of change which is the last nucleotide of this exon leading to donor splice site disruption. The picture is depicted, using ALAMUT software.

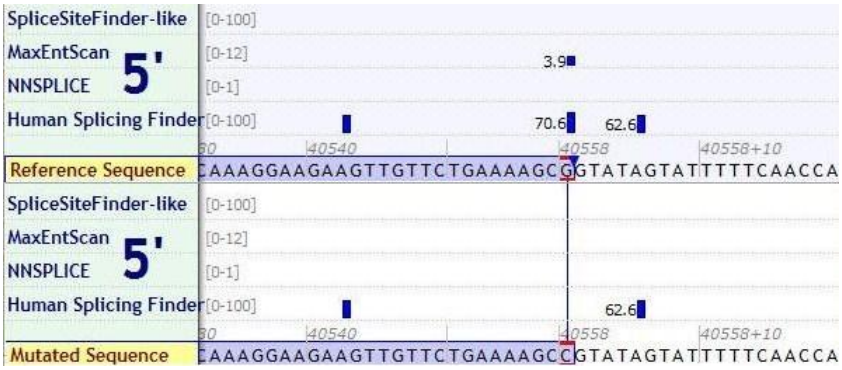


Figure 32: The predictions showing the disruption of donor splice site due to missense, GTT>CTT, V>L change in exon 168 of *TTN*. The upper panel shows the normal splice site (bleu boxes) and the given scores by MaxEntScan and Human Splicing Finder prediction tools. In the lower panel the disruption of the donor splice site predicted by four different prediction tools is shown. The picture is depicted, using ALAMUT software.

Since both of the genes were potential candidates in this scenario, more criteria were checked for these changes which are listed in table 29.

Table 29- Muscle expression of the proteins and presence of variations in Exome Variant Server, NHLBI Exome Sequencing (http://evs.gs.washington.edu/EVS/) for detected variations in *MYO5B* and *TTN*.

Gene	Chr	Muscle expression	Variation; codon; amino acid	EVS
MYO5B	18	Yes but not the highest	Missense; GCC>GGC; A>G	No
MYO5B	18		Missense; AAA>GAA; K>E	G=4/A=10002
TTN	**2**	**Yes and it's the highest**	**Missense ; AAA>AGA; K>R**	**G=3/A=10755**
TTN	**2**		**Missense ; GTT>CTT; V>L**	**No**
TTN	**2**		**Acceptor splice site mutation; AG>AA**	**No**

As mentioned above, the mother (APX27) had two *TTN* variations out of three, thus one of these two variations is not pathogenic, since she is not affected. This non-pathogenic variation can be missense ; AAA>AGA; K>R change, since it is present in Exome Variant Server, NHLBI Exome Sequencing (http://evs.gs.washington.edu/EVS/) with very low frequency.

In this scenario two candidate genes, *TTN* and *MYO5B*, were detected. NGS data of other CNM patients without detected disease-causing mutations were checked to see whether these patients had any unknown variations in these two genes.

TTN is a large gene with a lot of known and unknown variations, listed in different databases such as 1000 genomes or Exome Variant Server, and also it is listed as "black gene" in literature. However this gene is expressed highly in skeletal muscle and has an important role in muscle fibers. Looking to NGS data from several patients showed that almost in all of the patients this gene did not cover and sequence completely, thus all of the variations in this gene were not detected. However, several variations were detected in each patient. Whether these changes are real disease-causing mutations or individual variations or false positives, needs to be verified.

Similar to *TTN*, by analyzing the NGS data from several CNM patients, some variations were detected in *MYO5B.* However, in some patients this gene was not covered and sequenced completely. Whether these changes are real disease-causing mutations or individual variations or false positives, needs to be verified.

3-4-2-2- Recessive scenario due to homozygous change

In this scenario, genes with homozygous variation in the affected child (AIZ36) were selected and crossed to data from the parents (APX27 & APX28), in order to extract genes with homozygous variation in AIZ36 and similar variation in both parents in heterozygous status. After filtering out the variants in introns and UTR regions, synonymous variants, and variants with rs number, no gene remained.

3-4-2-3- *De novo* scenario

In this scenario, the NGS data of the affected child (AIZ36) were crossed to the parents' data (APX27 & APX28), in order to extract the variations that were present in the affected child and absent in both parents. After filtering out the variants in introns and UTR regions, synonymous variants, and variants with Rs number, 153 genes were detected. The data of these 153 genes was compared to exome data of AIZ36, in order to see whether the same variations were detected by exome sequencing. 7 genes were not covered by exome data and 3 genes were detected in exome data. These variations were checked by Sanger sequencing and only one of the non-covered variations and one of the detected variations by exome data were detected by Sanger sequencing and the rest were false

positives. 30 genes among 143 remaining genes, detected by genome data and not detected by exome data, were selected and analyzed by Sanger sequencing. This data showed that all of them were false positives. Analysis of other genes with detected variations in genome and not-detected in exome datasets showed that most of these changes were in GC rich regions of the genes where the probability of being false positives due to mapping or sequencing errors is too high. Therefore, these variations were most probably false positives and were not checked by Sanger sequencing.

Two genes with *de novo* variations in AIZ36 were detected: p.Gln1086His variation in exon 17 of CAP-GLY domain containing linker protein 1 (CLIP1), and p.Gly376Cys variation in exon 5 of FLYWCH-type zinc finger 1 (FLYWCH1). Both parents had wild type alleles for both variations. Sanger sequencing data for the affected child as well as parents are shown in figure 33.

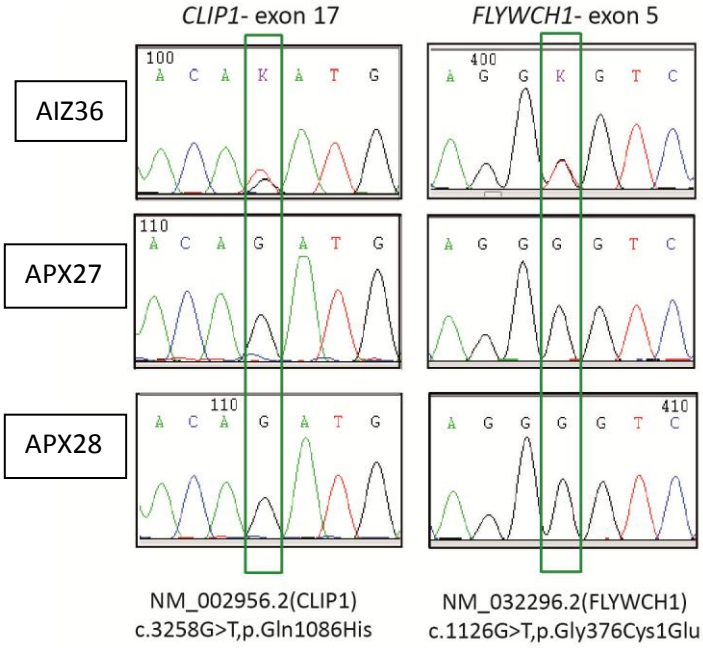


Figure 33: Sanger sequencing results for *CLIP1* and *FLYWCH1* genes in AIZ36 and his parents. As shown, one heterozygous variation in *CLIP1* and one heterozygous variation in *FLYWCH1* were detected in affected child (AIZ36) that were absent in both parents in this scenario.

Variations in both genes were predicted to be deleterious, using polyphen2 and SIFT prediction tools and both genes are expressed in skeletal muscle. Analysis of the NGS data from several CNM patients without detected mutations showed that in some of the patients these two genes did not cover and sequence completely, thus all of the variations in these two genes were not detected. However, some variations were detected in each patient. Whether these changes are real disease-causing mutations or individual variations or false positives, needs to be verified.

Copy number variations (CNV) and structural variations (SV) listed by complete genomics were also analyzed in this family. To ensure that these detected variations were correct and not due to sequencing or mapping errors, this data was compared to similar data from other family (ABJ family). The comparison showed that the same structural or copy number variations were detected in affected and non-affected members in both families suggesting that these variations were detected due to sequencing or mapping errors. Thus, no candidates were detected by CNV or SV analysis.

Therefore, two candidate genes, *TTN* and *MYO5B*, from recessive scenario due to compound heterozygous variations, and two candidate genes, *CLIP1* and *FLYWCH1*, from *de novo* scenario were detected in this family.

Results for aim 2: Improving diagnostic of NMD using NGS

The second aim of my PhD project was to test if massively parallel sequencing can be used as routine technique, in molecular diagnosis of neuromuscular disorders. This part of the project was published as a paper.

Publication 5

Next generation sequencing for molecular diagnosis of neuromuscular diseases

Nasim Vasli**,** Johann Bohm, Stephanie Le Gras, Jean Muller, Cécile Pizot, Bernard Jost, Andoni Echaniz-Laguna, Vincent Laugel, Christine Tranchant, Rafaelle Bernard, Frederic Plewniak, Serge Vicaire, Nicolas Levy, Jamel Chelly, Jean-Louis Mandel, Valerie Biancalana, Jocelyn Laporte

2012, Acta Neuropathol, 124, 273-283

A- Introduction

Inherited neuromuscular disorders (NMD) form a group of genetic diseases which result in chronic long term disability posing a significant burden to the patients, their families and public health care. NMD are often severe and include more than 200 monogenic disorders with a total incidence exceeding 1 in 3000. Despite tremendous research and clinical efforts, the molecular causes of NMD are still unknown for approximately half of patients.

B- Aim of study

The aim was to pilot an efficient screening strategy in an attempt to improve the clinical and molecular investigations of neuromuscular diseases, from a very heterogeneous panel of patients.

C- Results

In this study we used targeted enrichment of 267 known NMD genes followed by NGS in patients affected by different neuromuscular diseases with or without known mutations. DNA multiplexing and blind variant ranking retrieved successfully different mutation types for diseases with different segregations.

D- Conclusion

We conclude that our *NMD-seq* strategy could be implemented into a routine set-up in diagnosis laboratories as a first and reliable screening approach. A faster molecular diagnosis of NMD will have major impacts on patients as it will improve disease management and genetic counseling, and will allow access to therapy or inclusion into therapeutic trials.

Acta Neuropathol (2012) 124:273–283
DOI 10.1007/s00401-012-0982-8

ORIGINAL PAPER

Next generation sequencing for molecular diagnosis of neuromuscular diseases

Nasim Vasli · Johann Böhm · Stéphanie Le Gras · Jean Muller · Cécile Pizot · Bernard Jost · Andoni Echaniz-Laguna · Vincent Laugel · Christine Tranchant · Rafaelle Bernard · Frédéric Plewniak · Serge Vicaire · Nicolas Levy · Jamel Chelly · Jean-Louis Mandel · Valérie Biancalana · Jocelyn Laporte

Received: 4 March 2012 / Revised: 5 April 2012 / Accepted: 5 April 2012 / Published online: 18 April 2012
© The Author(s) 2012. This article is published with open access at Springerlink.com

Abstract Inherited neuromuscular disorders (NMD) are chronic genetic diseases posing a significant burden on patients and the health care system. Despite tremendous research and clinical efforts, the molecular causes remain unknown for nearly half of the patients, due to genetic heterogeneity and conventional molecular diagnosis based on a gene-by-gene approach. We aimed to test next generation sequencing (NGS) as an efficient and cost-effective strategy to accelerate patient diagnosis. We designed a capture library to target the coding and splice site sequences of all known NMD genes and used NGS and DNA multiplexing to retrieve the pathogenic mutations in patients with heterogeneous NMD with or without known mutations. We retrieved all known mutations, including point mutations and small indels, intronic and exonic mutations, and a large deletion in a patient with Duchenne muscular dystrophy, validating the sensitivity and reproducibility of this strategy on a heterogeneous subset of NMD with different genetic inheritance. Most pathogenic mutations were ranked on top in our blind bioinformatic

J. Böhm and S. Le Gras contributed equally to this article.

Electronic supplementary material The online version of this article (doi:10.1007/s00401-012-0982-8) contains supplementary material, which is available to authorized users.

N. Vasli · J. Böhm · S. Le Gras · J. Muller · C. Pizot · B. Jost · F. Plewniak · S. Vicaire · J.-L. Mandel · V. Biancalana · J. Laporte (✉)
IGBMC (Institut de Génétique et de Biologie Moléculaire et Cellulaire), 1, rue Laurent Fries, BP10142, 67404 Illkirch, France
e-mail: jocelyn@igbmc.fr

N. Vasli · J. Böhm · S. Le Gras · J. Muller · C. Pizot · B. Jost · F. Plewniak · S. Vicaire · J.-L. Mandel · V. Biancalana · J. Laporte
U964, Inserm, Illkirch, France

N. Vasli · J. Böhm · S. Le Gras · J. Muller · C. Pizot · B. Jost · F. Plewniak · S. Vicaire · J.-L. Mandel · V. Biancalana · J. Laporte
UMR7104, CNRS, Illkirch, France

N. Vasli · J. Böhm · S. Le Gras · J. Muller · C. Pizot · B. Jost · F. Plewniak · S. Vicaire · J.-L. Mandel · V. Biancalana · J. Laporte
Université de Strasbourg, Illkirch, France

N. Vasli · J. Böhm · J. Muller · C. Pizot · J.-L. Mandel · V. Biancalana · J. Laporte
Chaire de génétique humaine, Collège de France, Illkirch, France

J. Muller · J.-L. Mandel · V. Biancalana
Laboratoire Diagnostic Génétique, Faculté de Médecine, CHRU, Strasbourg, France

A. Echaniz-Laguna · C. Tranchant
Département de Neurologie, Hôpital Civil de Strasbourg, Strasbourg, France

V. Laugel
Service de Pédiatrie, Centre Hospitalier Universitaire (CHU), Strasbourg, France

R. Bernard · N. Levy
Faculté de Médecine de Marseille, Inserm UMRS 910 Génétique Médicale et Génomique Fonctionnelle, Université de la Méditerranée, Marseille, France

J. Chelly
Institut Cochin, INSERM Unité 1016, CNR UMR 1408, Université Paris Descartes, Sorbonne Paris Cité, Paris, France

pipeline. Following the same strategy, we characterized probable *TTN*, *RYR1* and *COL6A3* mutations in several patients without previous molecular diagnosis. The cost was less than conventional testing for a single large gene. With appropriate adaptations, this strategy could be implemented into a routine genetic diagnosis set-up as a first screening approach to detect most kind of mutations, potentially before the need of more invasive and specific clinical investigations. An earlier genetic diagnosis should provide improved disease management and higher quality genetic counseling, and ease access to therapy or inclusion into therapeutic trials.

Keywords Neuromuscular disorder · Sequencing · Molecular diagnosis · DNA barcoding · Myopathy · Neuropathy

Introduction

Inherited neuromuscular disorders (NMD) form a group of genetic diseases which result in chronic long term disability posing a significant burden to the patients, their families and public health care. NMD are often severe and include more than 200 monogenic disorders with a total incidence exceeding 1 in 3,000 [3]. Despite tremendous research and clinical efforts, the molecular causes of NMD are still unknown for approximately half of patients. The precise diagnosis of NMD requires an extensive clinical evaluation in conjunction with targeted complementary tests. To date, routine genetic diagnosis is mainly done on a gene-by-gene basis, starting from the most pertinent one. Diagnostic challenges in this group of diseases include genetic heterogeneity in most of the disorders and lack of segregation data in sporadic cases to orient the screening. As an example, hereditary sensorimotor neuropathies (HSMN) are due to mutations in nearly 50 genes, while congenital myopathies implicate at least 14 different genes [21, 22]. Also, large genes, such as Titin (*TTN*) with 363 exons, are not entirely sequenced even if previously linked to NMD [1]. As a consequence, clinical tests are multiplied, DNA is sent to different laboratories and patients are submitted to thorough examination that includes sometimes invasive investigations. Often genetic diagnosis is delayed, exposing the patient to unnecessary investigations and treatments, precluding the full benefit of a targeted approach to treatment, and increasing recurrence risk in the families.

Current molecular diagnostic approaches are time-consuming and expensive. Recently, massively parallel sequencing using next generation sequencing (NGS) technologies has emerged as a successful approach to interrogate multiple genes simultaneously and is currently mainly used to identify novel disease genes in a research setting [10, 18–20, 31]. A fewer studies reported the use of whole genome (WGS) or whole exome (WES) sequencing for genetic diagnosis of a given monogenic disease. Concerning NMD, Lupski et al. [14] and Montenegro et al. [16], respectively, used WGS and WES in patients with hereditary sensorimotor neuropathies (HSMN) on a research setting. In both cases, they analyzed a single family and focused their variants ranking only on known HSMN genes. Targeted resequencing of known disease genes appears more relevant for routine diagnosis and until now was tested on a few specific disease genes like 5 ataxia genes or 21 breast cancer genes or a single large gene like *DMD* in very homogeneous patient cohorts, or for carrier testing [6, 13, 30].

As no previous large scale sequencing study targeting several NMD genes was reported, our aim here is to pilot an efficient screening strategy in an attempt to improve the clinical and molecular investigations of neuromuscular diseases from a very heterogeneous panel of patients. We used targeted enrichment of 267 known NMD genes followed by NGS in patients affected by different neuromuscular diseases with or without known mutations. DNA multiplexing and blind variant ranking retrieved successfully different mutation types for diseases with different segregations.

Patients and methods

Patients

Two groups of patients with various neuromuscular diseases were selected: eight patients with pathogenic mutations previously identified by conventional Sanger sequencing of candidate genes (patients A to H), and eight random patients without known mutations and different clinical diagnosis encompassing myopathies and neuropathies (patients I to P). Clinical and segregation data are listed in the online resource data. DNA was extracted from venous blood by three different methods: two manual methods, FlexiGene DNA kit (Qiagen GmbH, Hilden, Germany) and Bacc Nucleon 3 (Amersham-Bioscience), and one automated method, the QIA symphony DNA midi kit (Qiagen GmbH, Hilden, Germany). Informed consent was obtained from all individuals, and the study was approved by the comité de protection des personnes (DC-2012-1497).

Targeted massively parallel sequencing

All the 267 NMD genes, known to be implicated in 16 different disease classes (online resource Table 1; http://www.musclegenetable.org/[8]) were targeted for enrichment. Capture design was done using the Agilent eArray (http://earray.chem.agilent.com/earray/). In this pilot study, we

included the 267 genes because genetic heterogeneity exists in all disease classes and several genes are implicated in different classes (e.g. *LMNA* or *DNM2*). Oligonucleotides covered all coding exons and all intron–exon boundaries including at least 50 intronic nucleotides. 5′ and 3′ UTRs and deep intronic sequences were not targeted to avoid increasing the sequence target size that would have strongly decreased the mean sequence coverage. After masking the repetitive elements, the 4,604 targeted exons represented 1.6 Mb (online resource Table 2). A minimum of 3 µg of genomic DNA was sheared to obtain a mean fragment size of 250 nt using Covaris E210 (KBioscience, Herts, UK) followed by automatic library preparation with the SPRI-TE Nucleic Acid Extractor (Beckman Coulter Inc, Brea, CA) using the SPRIworks fragment library cartridge (Beckman Coulter Inc, Brea, CA) and Illumina adapters. Targeted regions were captured using the Agilent SureSelect custom target enrichment kit (Agilent Technologies, Santa Clara, CA) following Agilent protocols. Enriched DNA fragments were barcoded with the Illumina multiplexing sample preparation kit (Illumina, San Diego, CA), pooled by 4, and sequenced on an Illumina Genome Analyzer IIx to generate 72nt paired-end reads for 4 DNAs per channel, following the manufacturer's protocols.

Bioinformatic analysis

The authors implicated in the sequencing and bioinformatic analysis had no information on the patient data, except for the disease class and potential segregation. The bioinformatic analysis pipeline is depicted in Fig. 1. Image analysis and base calling were performed using the Illumina Pipeline RTA (Real-Time Analysis) version 1.9. DNA sequences were aligned to the reference genome GRCh37/hg19 using BWA [11]. Insertions or deletions of up to 50nt were allowed for the alignment to the genome. Reads that mapped to several positions in the genome and reads sharing the same start position and strand were filtered out using Picard (http://picard.sourceforge.net/) and Samtools [12]. From an average of 14 million mapped reads, about 4 million were uniquely mapped in targeted regions. Valid variants had to be seen in both directions with at least 3× coverage and their calling was done using Samtools; minimum mapping quality was 25, consensus quality was 20 and minimum SNV (single nucleotide variation)/indel quality was 20. Variants were defined as homozygous, if present in more than 80 % of the reads. For SNV/indel annotation SVA (v1.02) [4] (http://www.svaproject.org/), Ensembl60 and dbSNP134 were used, and validated non-pathogenic variants present in dbSNP and 1000Genomes databases were removed.

Variants filtering and ranking were done using the VaRank program. Briefly, for each variant, VaRank used

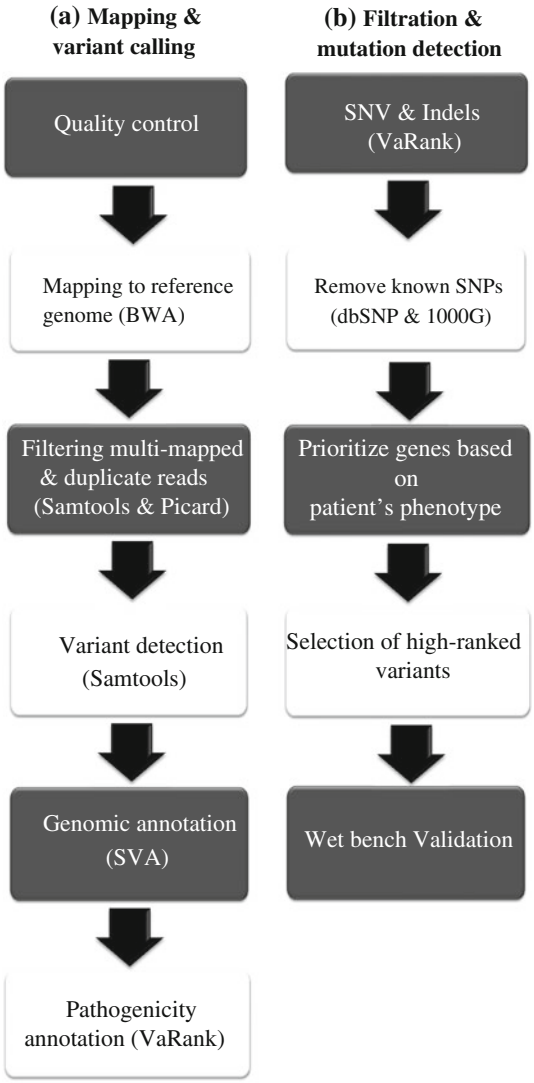


Fig. 1 Bioinformatic filtering and ranking

the Alamut software (Interactive Biosoftware, Rouen, France) to collect genomic annotations and different scores such as the coding status, the nucleotide and amino acid conservation scores and the effect of each change on the protein and splice site, and then compiles them to rank SNVs and indels starting from the most probable pathogenic. The SNVs/indels are characterized using several genomic or functional annotations that VaRank summarizes into a score to produce a list of ranked variants (manuscript in preparation). The probable mutations are ranked starting from the most likely to be pathogenic according to the following list: nonsense, frameshift, essential splice site (affecting the conserved consensus intronic positions), start loss, stop loss, missense, predicted splice site mutation (outside of the consensus sites), in frame indels, and synonymous coding. The scores are modulated according to the genomic conservation based on

the phastcons score [7] and to the SIFT [17] and PolyPhen v2 [23] scores to assess the effect of amino acid change on the protein function. Synonymous coding variants might have an effect on the biosynthesis of the protein [28], and their potential impact on splicing was also scored. Splicing effect is assessed using three different softwares: Human Splicing Finder [2], MaxEntScan [32] and NNSplice [24]. Scores of compound heterozygous mutations in the same gene were added in case of a recessive segregation of the disease to prioritize the best candidate genes. Clinical significance was extracted from dbSNP134 and some of the known mutations were confirmed using locus-specific mutation data bases (LSDB-http://www.umd.be/). The "Clinical significance" field from dbSNP highlights known mutations with an "rs" identifier. Variants annotated as "probably-pathogenic" or "pathogenic" usually corresponded to reported mutations and were weighted to reach a high VaRank score. Indeed, these variants were not filtered as some healthy people are carriers of mutations. In the next step, genes within the patient disease class(es) were extracted and the mode of inheritance of the disease in the family, if known, was matched to the known type of transmission for every selected candidate genes.

In order to detect large deletions, a coverage-based method was used where the number of reads in a sliding window of 20nt was computed across the genome for each patient and coverage compared to three randomly selected patients.

Mutation validation

Sanger sequencing was performed to confirm sequence variants in the original DNA samples and to assess the segregation in the families included in this study (GATC Biotech).

Results

Sequencing results

Following DNA barcoding and pooling by group of 4, targeted sequencing of the 267 known NMD genes (online resource Table 1) was performed in 8 individuals (A to H) with different neuromuscular disorders and known mutations. After alignment with the human reference genome, mean coverage of the targeted exons was 138× and the percentage of nucleotides with at least 10× coverage was 94 (Table 1, online resource Table 2). Average enrichment for targeted exons was 1,410 fold. More than 97 % of the targeted exons were fully covered, while 168 targeted exons were covered <3× in at least half of the patients (online resource Fig. 1 a and online resource Table 2). Most low-covered exons were similar between patients and coverage decreased with increasing GC content (online resource Fig. 1b). Similar findings were obtained in an independent experiment with six of these DNA samples sequenced individually, validating the multiplexing approach (data not shown). DNAs prepared with different extraction protocols gave similar results, supporting the use of these different protocols on a routine diagnosis basis. Reproducibility between different DNAs treated in the same experiment was such that the coverage was similar for given targeted exons (online resource Table 2 and online resource Fig. 2; narrow distribution of the 95th percentile close to the median). This allowed the detection of the copy number to unambiguously determine the gender of patients as a control for the experiment (Fig. 2a, b), and the mapping of a large deletion (see below).

Variants identification and ranking

Variants were identified based on the bioinformatic analysis encompassing sequence mapping, variant calling and filtering, and variant ranking (Fig. 1). On average, we found 1,162 SNVs and 152 indels of which 341 were not reported as SNP (Table 1). 125 variants affecting the essential splice sites or predicted to change the amino acid sequence were found on average in the 267 NMD genes. For prioritization, these variants were ranked using a novel scoring program (VaRank; see methods), then by extracting the different genes fitting the disease class(es) based on the general clinical phenotype of patients, and lastly based on the segregation if known (Table 2 and online resource Table 3). For patients with phenotypes that matched to several disease classes, all genes fitting the different diseases classes were considered.

Identification and confirmation of mutations

We retrieved all ten different known mutations in the eight analyzed DNAs (Table 2; patients A–H). Sequencing data sustaining the mutations are depicted in (Fig. 3a–d and online resource Fig. 4). In particular, we detected homozygous and heterozygous mutations validating the detection of both alleles, point mutations or small insertion or deletions, intronic and exonic mutations. Compound heterozygous mutations in *SETX* were retrieved in the two patients with ataxia (Fig. 3 and online resource Fig. 3). Importantly, our VaRank scoring program blindly ranked the known mutations and implicated genes first in the list when taking into account the disease class and inheritance for most patients (online resource Table 3). Although the clinical data are important to define the disease class, we did not prioritize genes within each disease class based on more detailed pathological data (e.g. even if patient E had myotubular myopathy, all congenital myopathy genes were ranked), suggesting that this approach may be proposed for

Table 1 Sequencing, coverage and variant statistics

	Samples	A	B	C	D	E	F	G	H	Average
Sequencing	Sequenced nucleotides	1179572688	1143805104	749145888	1057816944	1281453552	921756240	912120912	913302000	1019871666
	Sequence after filtering (in nt)[b]	399768120	392297112	314103240	246193344	355696416	351273528	404841528	210318840	334311516
	Sequence in target regions (in nt)	316579968	321670656	249903072	193743144	271231344	274046688	220871448	169312104	252169803
Coverage	Mean coverage (x)	172	176	137	106	148	150	120	96	138
	Median coverage (x)	165	162	126	105	145	144	113	91	131
	% Base ≥3× coverage	98	97	97	98	98	98	97	97	98
	% Base ≥10× coverage	95	94	92	94	95	94	93	93	94
	Fully covered exons	4,477	4,445	4,396	4,443	4,484	4,445	4,429	4,425	4,443
Variants	SNVs	1,097	1,096	1,015	1,148	1,374	1,315	1,127	1,120	1,162
	Indels	208	148	127	139	168	153	127	146	152
	Total heterozygotes	1,018	920	779	973	1,141	1,095	910	859	962
	Total homozygotes	287	324	363	314	401	373	344	407	352
	SNVs + indels	1,305	1,244	1,142	1,287	1,542	1,468	1,254	1,266	1,314
	SNVs + indels without rs number	374	325	259	294	457	403	335	279	341
	Novel coding non-synonymous[a]	111/0	109/0	93/0	99/0	145/0	155/1	116/0	94/1	115/n.a.[c]
	Novel splice site change[a]	10/0	6/0	4/0	4/1	17/0	6/0	5/1	2/0	7/n.a.[c]
	Novel coding stop (gained/lost)[a]	3/0	4/1	0/0	0/0	0/0	1/0	1/0	1/0	1/n.a. [c]
	Novel coding frameshift[a]	3/1	1/0	1/large deletion	2/1	3/1	1/0	2/0	1/1	2/n.a.[c]

Average enrichment is 1,410 fold

[a] Before/after filtering and ranking

[b] After filtering duplicate reads and multiple genomic mapping

[c] Not applicable

patients prior to extensive histological investigations. Moreover, the patients in our cohort were affected by diverse diseases of different segregation (X-linked, autosomal recessive or dominant; Table 2), validating this approach for a wide range of heterogeneous diseases and mutation types.

The large deletion encompassing exons 18–44 of the *DMD* gene was detected in a patient with Duchenne Muscular Dystrophy by comparing the number of reads in these regions with other sequenced DNA samples (Fig. 2b). The mean coverage for exons 18–44 is 0 for this patient and 177 for other patients (online resource Table 2, *DMD* gene for patient C). Unexpectedly, off-target reads from genomic DNA fragments not targeted for enrichment and located in *DMD* introns allowed to restrict the areas containing the upstream and downstream breakpoints from 27 to 11 Kb and 248 to 72 Kb, respectively (Fig. 2c). To assess the accuracy of deletion breakpoints predicted through off-target reads with the precise deleted positions, we analyzed this *DMD* deletion using the custom-designed oligonucleotides CGH-array previously described by Saillour et al. [27] where oligonucleotide probes cover both intronic and exonic *DMD* regions with an average tiling interval of 50 bases. CGH-array indicated that the 3′ breakpoint maps between 32,187,417 (position of the non-deleted probe) and 32,187,427 (position of the deleted probe), and the 5′ breakpoint maps between 32,538,435 and 32,538,443 (Fig. 2d). NGS data are coherent with CGH-array as the off-target reads closer to the deletion mapped at positions 32,072,428 and 32,547,130. The differences between the precise positions based on CGH-array and breakpoints found by NGS data are 115 kb for the 3′ breakpoint and 9 kb for the 5′ breakpoint. For a better precision of NGS method for mapping intronic breakpoints, targeting for enrichment of intronic sequences could be a possibility, but will increase the total targeted sequence length and thus decrease the overall coverage for a given sequencing depth.

Following a similar strategy, we analyzed 8 DNAs (I to P) from patients with heterogeneous neuromuscular disorders without molecular characterization. These patients were not selected based neither on the amount or quality of clinical data nor on the availability of DNA from other members of the family, to mimic the situation of routine

diagnosis. There were no specific inclusion criteria. We identified probable disease-causing mutations in several patients. Sanger sequencing was used to validate the presence of mutations in the original DNA and also confirmed disease segregation (Fig. 3e–f, Table 2, and online resource Fig. 4). Sequencing, coverage and variant statistics were similar to the previous experiment (online resource Table 4). The identified mutations in *RYR1*, *TTN* and *COL6A3* genes were in agreement with the clinical data (online resource data for patient descriptions). Importantly, while variants ranking can be made without

Fig. 2 Detection of copy number and mapping of a deletion in patient C with DMD. **a**, **b** Gender determination: comparison of sequence reads mapping to the X chromosome between two female DNAs in (**a**) and a female (*black*) and a male (*red*) in (**b**). In **b** a deletion of several exons is detected on the X chromosome for the male (*squared*). **c** Next generation sequencing data showing the detection of a 27 exons deletion in patient C with DMD (*middle panel*) compared to two other DNAs (*top and bottom panels*). Random off-target reads allow a more precise mapping of the deletion breakpoints. Off-target reads varied between two different experiments. **d** CGH-array results showing the 5′ and 3′ breakpoints map between 32,538,435 and 32,538,443 and between 32,187,417 and 32,187,427, respectively

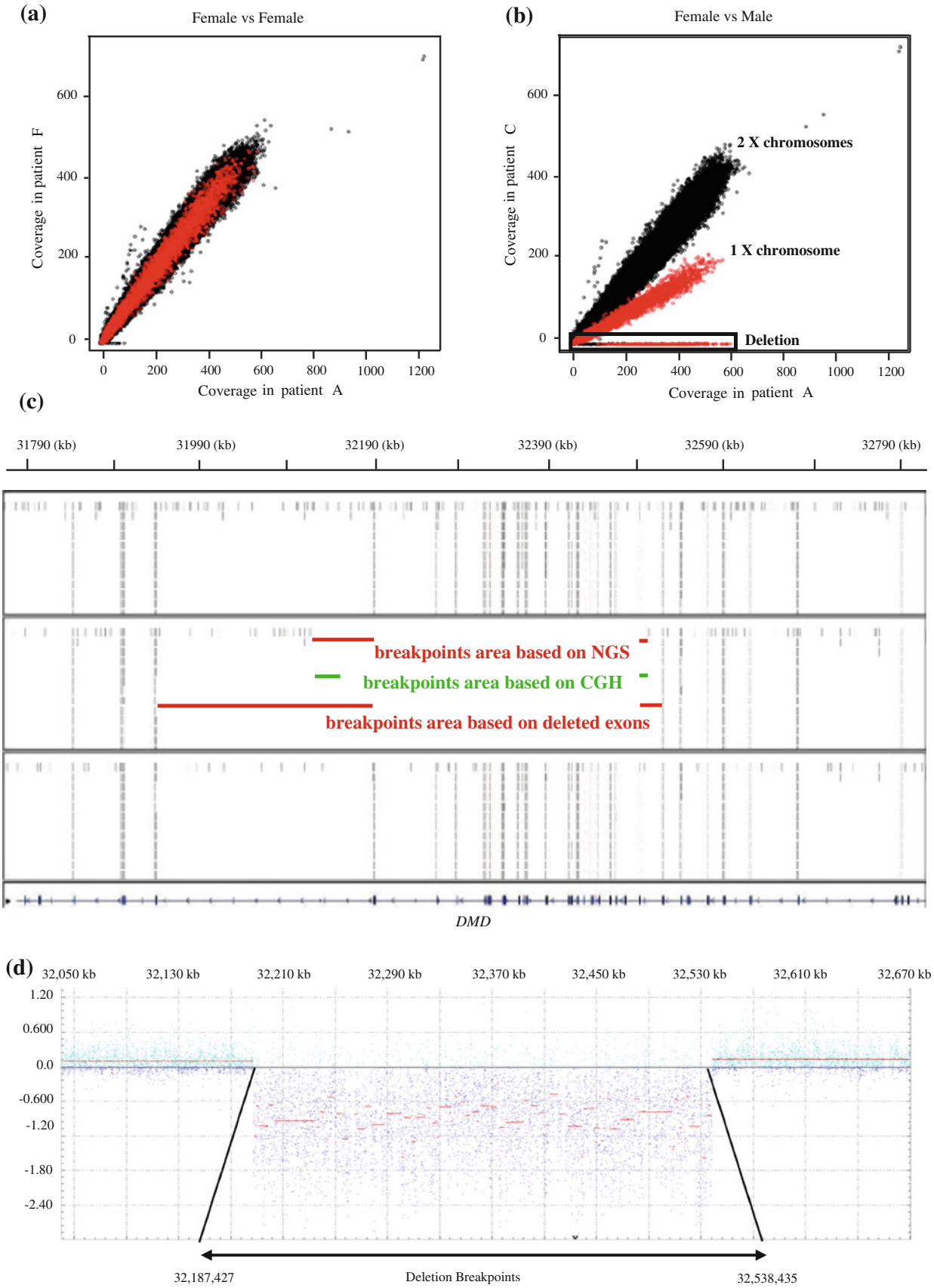
Table 2 Mutations identified in patients with known mutations and probable mutations in patients without previous molecular characterization

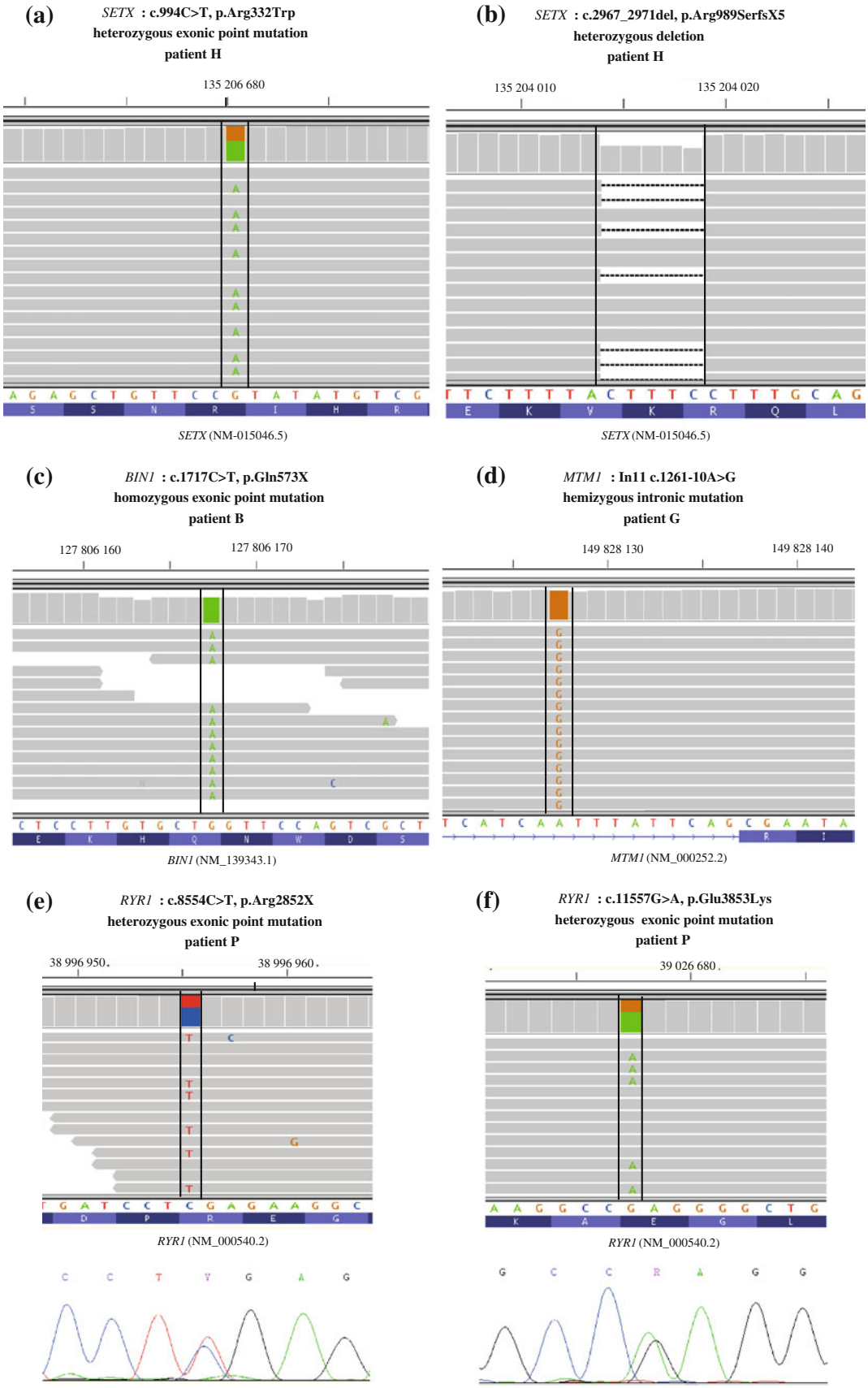
Patient	Gender	Disease (segregation)	Disease class	Gene	Mutation nucleotide (protein)	Haplotype
A	F	Carrier for myotubular myopathy (XL)	Congenital myopathies	*MTM1*	Exon4: c.141–144delAAAG (p.Glu48LeufsX24)	Heterozygous
B	M	Centronuclear myopathy (AR)	Congenital myopathies	*BIN1*	Exon20: c.1717 C > T (p.Gln573X)	Homozygous
C	M	Duchenne muscular dystrophy (XL)	Muscular dystrophies	*DMD*	Deletion ex18-44	Hemizygous
D	F	Ataxia ocular apraxia (AR)	Hereditary ataxias	*SETX*	Exon10: c.3213–3214insT (p.Gln1072SerfsX3); Int10: c.5275-1 G > A	Compound heterozygous
E	M	Myotubular myopathy (XL)	Congenital myopathies	*MTM1*	Exon4: c.156–157insA (p.Cys53MetfsX8)	Hemizygous
F	F	Centronuclear myopathy (AD)	Congenital myopathies	*DNM2*	Exon14: c.1565G>A (p.Arg522His)	Heterozygous
G	M	Myotubular myopathy (XL)	Congenital myopathies	*MTM1*	Int11: c.1261–10A>G	Hemizygous
H	F	Ataxia ocular apraxia (AR)	Hereditary ataxias	*SETX*	Exon10: c.2967-2971delGAAAG (p.Arg989SerfsX5); Exon8: c.994C>T (p.Arg332Trp)	Compound heterozygous
I	M	HMSN, demyelinating CMT neuropathy (AR)[a]	n.a.[a]	None		
J	M	Myopathy with cytoplasmic aggregates	All myopathies	*TTN*[c]	Exon292: c.68576C>T (p.Pro22859Leu)	Heterozygous
K	F	Bethlem dystrophy or myofibrillar myopathy (AD)	Muscular dystrophies, other myopathies	*COL6A3*[c]	Exon27: c.6812G>A (p.Arg2271Lys)	Heterozygous
L	M	Hereditary spastic paraplegia (sporadic)	Hereditary paraplegias	None		
M	M	Vacuolar myopathy (sporadic)	Congenital myopathies, distal myopathies, other myopathies	None		
N	M	HMSN, axonal CMT (AR)	Hereditary neuropathies	None (LMNA)	Exon11: c.1928C>A (p.Thr643Asn); c.1930C>T (p.Arg644Cys)[b]	Compound heterozygous
O	F	Muscular dystrophy (AR)	Muscular dystrophies	*TTN*[c]	Exon18: c.3100G>A (p.Val1034Met); Exon240: c.49243G>A (p.Ala16415Thr)	Compound heterozygous
P	M	Muscular dystrophy and arthrogryposis (AR)	Muscular dystrophies, congenital myopathies, other NMD diseases	*RYR1*[c]	Exon55: c.8554C>T (p.Arg2852X); Exon81: c.11557G>A (p.Glu3853Lys)	Compound heterozygous

[a] *HMSN* hereditary motor and sensory neuropathy, *CMT* Charcot–Marie–Tooth; patient I was later re-diagnosed as having a mitochondrial disease for which genes were not targeted

[b] Previously reported as pathogenic; probable monoallelic compound heterozygous

[c] Confirmed by segregation analysis


(a)
Female vs Female
Coverage in patient F
Coverage in patient A
(b)
Female vs Male
Coverage in patient C
2 X chromosomes
1 X chromosome
Deletion
Coverage in patient A
(c)
31790 (kb)
31990 (kb)
32190 (kb)
32390 (kb)
32590 (kb)
32790 (kb)
breakpoints area based on NGS
breakpoints area based on CGH
breakpoints area based on deleted exons
DMD
(d)
32,050 kb
32,130 kb
32,210 kb
32,290 kb
32,370 kb
32,450 kb
32,530 kb
32,610 kb
32,670 kb
32,187,427
Deletion Breakpoints
32,538,435


(a)
SETX : c.994C>T, p.Arg332Trp
heterozygous exonic point mutation
patient H
135 206 680
SETX (NM-015046.5)
(b)
SETX : c.2967_2971del, p.Arg989SerfsX5
heterozygous deletion
patient H
135 204 010
135 204 020
SETX (NM-015046.5)
(c)
BIN1 : c.1717C>T, p.Gln573X
homozygous exonic point mutation
patient B
127 806 160
127 806 170
BIN1 (NM_139343.1)
(d)
MTM1 : In11 c.1261-10A>G
hemizygous intronic mutation
patient G
149 828 130
149 828 140
MTM1 (NM_000252.2)
(e)
RYR1 : c.8554C>T, p.Arg2852X
heterozygous exonic point mutation
patient P
38 996 950
38 996 960
RYR1 (NM_000540.2)
(f)
RYR1 : c.11557G>A, p.Glu3853Lys
heterozygous exonic point mutation
patient P
39 026 680
RYR1 (NM_000540.2)

◀**Fig. 3** Detection of different types of mutations from patients with previously known and unknown molecular diagnosis. Compound heterozygous exonic point mutation (**a**) and heterozygous indel mutation (**b**) in the *SETX* gene in patient H with ataxia. **c** Homozygous exonic point mutation in the *BIN1* gene in patient B with centronuclear myopathy. **d** Intronic mutation in the *MTM1* gene in patient G with myotubular myopathy. **e**, **f** Novel compound heterozygous mutations detected in patient P with muscular dystrophy and arthrogryposis in the *RYR1* gene by next generation sequencing and confirmed by Sanger sequencing. Displayed with the integrative genomics viewer IGV [25]. The normal nucleotide and protein sequences are depicted at the *bottom*

detailed clinical and histological data to suggest probable mutated genes, such data are very valuable to validate the molecular findings. For example, we identified compound heterozygous mutations (including a nonsense mutation) in the ryanodine receptor (*RYR1)* in patient P presenting with muscular dystrophy and arthrogryposis (Fig. 3e–f). Indeed *RYR1* mutations have previously been linked to several congenital myopathies, [29] and also to severe neonatal arthrogryposis [26]. The mutations were found in his affected twin brother and each parent was found to be heterozygous for one mutation. We also identified probable mutations in the large *TTN* gene in patients J and O with myopathy with cytoplasmic aggregates and limb girdle muscular dystrophy, respectively, widening the clinical and molecular spectra for this gene that is not routinely sequenced on a diagnosis setting due to its large size.

We did not have false negative in the eight patients with known mutations as we retrieved all mutations. We checked the rate of false positive, i.e. variant not present in the starting DNA, in patients with unknown mutations where we found the probable disease-causing mutations. We found that the probability of being false positive due to sequencing or mapping errors is high when the percentage of reads showing the change is less than 25 or when the number of reads showing the change is less than 8. Even with these cut-offs, 16.5 % of false positive variants were found out of 40 variants tested, calling for validation of the mutations by Sanger sequencing.

Discussion

In this study, we performed targeted sequencing of the coding sequences and all intron–exon boundaries including at least 50 intronic nucleotides of the known NMD genes through massively parallel sequencing in a cohort of patients with heterogeneous neuromuscular diseases. We were able to retrieve all the known mutations in previously characterized patients and we identified several novel pathogenic mutations in patients lacking molecular diagnosis.

We demonstrate that this strategy can detect several types of mutations including intronic and exonic changes as well as small indel and a large deletion. All mutations were detected from the massively parallel sequencing and analysis of a single proband, unlike previous studies where comparative sequencing of several individual exomes was used to retrieve the causative mutations in a family with HSMN [16]. A main challenge in NGS data analysis is the identification of the pathogenic change among the large list of variants. Our blind analysis based on variant ranking and disease class allowed the identification of all known mutations. However, detailed clinical, histological and molecular data were necessary for the confirmation steps, i.e., matching the genetic data with the phenotype.

Such targeted parallel sequencing of all candidate genes is especially suitable for diseases with high genetic heterogeneity, as it is the case for NMD, and should ease the identification of allelic diseases, i.e., different diseases caused by mutations of the same gene. In addition, this strategy allows the analysis of large genes, such as *TTN*, that are routinely not fully tested by conventional Sanger sequencing even if known to be implicated in diseases. For example, our identification of a probable *TTN* mutation in a patient with myopathy with cytoplasmic aggregates and respiratory insufficiency widen the clinical spectrum compared to previous studies [5].

We did not find disease-causing mutations among the coding sequences of the NMD genes in four patients with unknown genetic cause. Patient I was first clinically diagnosed with demyelinating polyneuropathy, but clinical and biochemical re-analyses in parallel to NGS suggested he had a mitochondrial disease which implicated genes are not covered by our present design. Patient N showed two missense changes in *LMNA* including the p.Arg644Cys change, previously linked to various laminopathies. Both changes are on the same allele, as they were always found in the same reads/fragments (online resource Fig. 4e), and thus cannot be the sole cause of the axonal neuropathy. We did not have access to parent DNAs to investigate this further. For the other two patients, who were previously excluded for several candidate genes by Sanger sequencing, mutations were also missed by our approach. The disease-causing mutation may be a deep intronic change, repeat expansions or translocation for which detection has not been tested in this study. Concerning specifically repeat expansions, they cannot be mapped back to a reference genome unambiguously due to their repetitive nature. Alternatively, these patients may also be mutated in a gene not linked to NMD at the time of our targeting library design.

WGS or WES would in principle allow re-analysis of variants list once newly discovered genes are identified, while these genes should be added to update the NMD capture library. However, these approaches have several disadvantages for routine molecular diagnosis compared to

the *NMD-seq* strategy described in this study, especially concerning design, coverage, variant analysis and validation, incidental findings, and throughput and price. WES capture library should also be updated to incorporate novel gene and exon predictions, and cannot be customized to increase the enrichment of specific exons difficult to capture or incorporate known intronic mutation hotspots. *NMD-seq* has a higher coverage and leads to a smaller list of variants as it focuses on a subset of genes, whereas the sensitivity and heterozygosity assessment decrease following WGS or WES due to lower coverage [31]. Sequencing more genes at a lower coverage leads to an increased risk of false negative and an increased number of false positive variants that are time-consuming to validate. Indeed, WES at 50× mean coverage results in about 20 % of targeted regions covered less than 10 times, outputs not suited for routine diagnosis. WES or WGS also potentiate incidental finding, i.e. the discovery of an unrelated disease not targeted by the diagnosis measure; this could be an ethical issue. Moreover, WGS or WES have lower throughput and a higher cost, both are important issues for routine diagnosis. We validated DNA multiplexing for four DNAs in one channel to increase throughput and decrease the cost in comparison with conventional Sanger approaches which is about 500–1,000 € per sample for one gene but can increase to 5,000 € or more depending on gene size. Testing several candidates on a gene-by-gene basis may exceed 8,000 € [9]. With recent developments in sequencers and DNA barcoding, we estimate the total cost of *NMD-seq* from a pool of 12 barcoded DNAs to about 500 € per patient for at least 140× coverage, while WES and WGS cost about 1,500 € and 5,000 € for a 50× coverage, respectively. It takes about 2 months to perform the NMD-seq approach for 267 genes (excluding validation of the data by Sanger sequencing), a similar turnaround time to what is proposed by diagnosis laboratories to test a gene with 20 exons under current routine diagnosis.

Major conditions for further use of the *NMD-seq* strategy as a routine approach in genetic diagnostic labs are the reproducibility, detection sensitivity and the study of heterogeneous cohort of patients with sometimes incomplete clinical characterization as it was the case in our study. After validation of other types of mutations not tested in this study, this strategy could be implemented as a first screening approach, potentially before the need of more invasive and time-consuming investigations such as biopsies.

Our strategy does not require the knowledge of detailed clinical data for proposing candidate mutations; however, this knowledge is necessary for the final validation of the diagnosis that can only be performed matching both clinical and genetic data. NGS will not replace clinical investigations but rather direct clinicians towards the most adequate investigations, while excluding unnecessary costly and time-consuming tests. As a general rule for all NGS approaches, the targeted regions are not homogeneously covered. This point could most probably be enhanced by increasing the amount of oligonucleotides targeting regions more difficult to enrich. For an exclusion diagnosis, conventional Sanger sequencing of specific exons might be necessary if the best candidate genes for a specific disorder are not well covered by NGS. In any case, mutations found by NGS must be validated by Sanger sequencing to rule out sequence errors introduced during the NGS protocol, to provide a second independent confirmation and a basis for a simplified test for further counseling within the family. Importantly, variant ranking and confirmation depend on clinical data and knowledge on NMD, and are thus probably not directly applicable for direct-to-consumer testing.

A faster molecular diagnosis of NMD will have major impacts on patients as it will improve disease management and genetic counseling, and will allow access to therapy or inclusion into therapeutic trials. As an example, the identification of *RYR1* mutations in patient P is of major medical importance as the treatment of *RYR1* patients with salbutamol has shown significant amelioration of muscle weakness [15]. In conclusion, we provided the first proof-of-principle that next generation sequencing could be apply for molecular diagnosis of neuromuscular disorders.

Acknowledgments We thank Michel Koenig and Mathieu Anheim for DNA samples with known mutations, Ngoc-Hoan Nguyen and Céline Keime for help in the bioinformatics setup and analysis, Claudia Strafella, Nicolas Dondaine, Gabrielle Rudolf, Juliette Nectoux, Céline Leroux, Isabelle Atlan and France Leturcq for technical assistance, and Gisèle Bonne for discussions. This study was supported by the Institut National de la Santé et de la Recherche Médicale (INSERM), the Centre National de la Recherche Scientifique (CNRS), University of Strasbourg (UdS), Collège de France and grants from the Agence Nationale de la Recherche, Muscular Dystrophy Association and Myotubular Trust.

Conflict of interest The authors declare that they have no conflict of interest.

Open Access This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided the original author(s) and the source are credited.

References

1. Bang ML, Centner T, Fornoff F et al (2001) The complete gene sequence of titin, expression of an unusual approximately 700-kDa titin isoform, and its interaction with obscurin identify a novel Z-line to I-band linking system. Circ Res 89:1065–1072
2. Desmet FO, Hamroun D, Lalande M et al (2009) Human Splicing Finder: an online bioinformatics tool to predict splicing signals. Nucleic Acids Res 37:e67

3. Emery AE (1991) Population frequencies of inherited neuromuscular diseases—a world survey. Neuromuscul Disord 1:19–29
4. Ge D, Ruzzo EK, Shianna KV et al (2011) SVA: software for annotating and visualizing sequenced human genomes. Bioinformatics 27:1998–2000
5. Hackman P, Vihola A, Haravuori H et al (2002) Tibial muscular dystrophy is a titinopathy caused by mutations in TTN, the gene encoding the giant skeletal-muscle protein titin. Am J Hum Genet 71:492–500
6. Hoischen A, Gilissen C, Arts P et al (2010) Massively parallel sequencing of ataxia genes after array-based enrichment. Hum Mutat 31:494–499
7. Hubisz MJ, Pollard KS, Siepel A (2011) PHAST and RPHAST: phylogenetic analysis with space/time models. Brief Bioinform 12:41–51
8. Kaplan JC (2010) The 2011 version of the gene table of neuromuscular disorders. Neuromuscul Disord 20:852–873
9. Kingsmore SF, Saunders CJ (2011) Deep sequencing of patient genomes for disease diagnosis: when will it become routine? Sci Transl Med 3:87ps23
10. Levy S, Sutton G, Ng PC et al (2007) The diploid genome sequence of an individual human. PLoS Biol 5:e254
11. Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25:1754–1760
12. Li H, Handsaker B, Wysoker A et al (2009) The Sequence Alignment/Map format and SAMtools. Bioinformatics 25:2078–2079
13. Lim BC, Lee S, Shin JY et al (2011) Genetic diagnosis of Duchenne and Becker muscular dystrophy using next-generation sequencing technology: comprehensive mutational search in a single platform. J Med Genet 48:731–736
14. Lupski JR, Reid JG, Gonzaga-Jauregui C et al (2010) Whole-genome sequencing in a patient with Charcot–Marie–Tooth neuropathy. N Engl J Med 362:1181–1191
15. Messina S, Hartley L, Main M et al (2004) Pilot trial of salbutamol in central core and multi-minicore diseases. Neuropediatrics 35:262–266
16. Montenegro G, Powell E, Huang J et al (2011) Exome sequencing allows for rapid gene identification in a Charcot–Marie–Tooth family. Ann Neurol 69:464–470
17. Ng PC, Henikoff S (2003) SIFT: predicting amino acid changes that affect protein function. Nucleic Acids Res 31:3812–3814
18. Ng SB, Buckingham KJ, Lee C et al (2010) Exome sequencing identifies the cause of a mendelian disorder. Nat Genet 42:30–35
19. Ng SB, Nickerson DA, Bamshad MJ, Shendure J (2010) Massively parallel sequencing and rare disease. Hum Mol Genet 19:R119–R124
20. Ng SB, Turner EH, Robertson PD et al (2009) Targeted capture and massively parallel sequencing of 12 human exomes. Nature 461:272–276
21. North K (2008) What's new in congenital myopathies? Neuromuscul Disord 18:433–442
22. Pareyson D, Marchesi C (2009) Diagnosis, natural history, and management of Charcot–Marie–Tooth disease. Lancet Neurol 8:654–667
23. Ramensky V, Bork P, Sunyaev S (2002) Human non-synonymous SNPs: server and survey. Nucleic Acids Res 30:3894–3900
24. Reese MG, Eeckman FH, Kulp D, Haussler D (1997) Improved splice site detection in Genie. J Comput Biol 4:311–323
25. Robinson JT, Thorvaldsdottir H, Winckler W et al (2011) Integrative genomics viewer. Nat Biotechnol 29:24–26
26. Romero NB, Monnier N, Viollet L et al (2003) Dominant and recessive central core disease associated with RYR1 mutations and fetal akinesia. Brain 126:2341–2349
27. Saillour Y, Cossee M, Leturcq F et al (2008) Detection of exonic copy-number changes using a highly efficient oligonucleotide-based comparative genomic hybridization-array method. Hum Mutat 29:1083–1090
28. Sauna ZE, Kimchi-Sarfaty C (2011) Understanding the contribution of synonymous mutations to human disease. Nat Rev Genet 12:683–691
29. Treves S, Jungbluth H, Muntoni F, Zorzato F (2008) Congenital muscle disorders with cores: the ryanodine receptor calcium channel paradigm. Curr Opin Pharmacol 8:319–326
30. Walsh T, Lee MK, Casadei S et al (2010) Detection of inherited mutations for breast and ovarian cancer using genomic capture and massively parallel sequencing. Proc Natl Acad Sci USA 107:12629–12633
31. Wheeler DA, Srinivasan M, Egholm M et al (2008) The complete genome of an individual by massively parallel DNA sequencing. Nature 452:872–876
32. Yeo G, Burge CB (2004) Maximum entropy modeling of short sequence motifs with applications to RNA splicing signals. J Comput Biol 11:377–394

ActaNeuropathologica

Next generation sequencing for molecular diagnosis of neuromuscular diseases

Nasim Vasli[1,2,3,4,5], Johann Böhm[1,2,3,4,5,$](PhD), Stéphanie Le Gras[1,2,3,4,$], Jean Muller[1,2,3,4,5,6](PhD), Cécile Pizot[1,2,3,4,5], Bernard Jost[1,2,3,4](PhD), Andoni Echaniz-Laguna[7](MD), Vincent Laugel[8](MD), Christine Tranchant[7](MD), Rafaelle Bernard[9](MD), Frédéric Plewniak[1,2,3,4](PhD), Serge Vicaire[1,2,3,4], Nicolas Levy[9](MD, PhD), Jamel Chelly[10](MD, PhD), Jean-Louis Mandel[1,2,3,4,5,6](MD, PhD), Valérie Biancalana[1,2,3,4,5,6](PhD), Jocelyn Laporte[1,2,3,4,5*](PhD).

[1]IGBMC (Institut de Génétique et de Biologie Moléculaire et Cellulaire), Illkirch, France
[2]Inserm, U964, Illkirch, France
[3]CNRS, UMR7104, Illkirch, France
[4]Université de Strasbourg, Illkirch, France
[5]Collège de France, chaire de génétique humaine, Illkirch, France
[6]Laboratoire Diagnostic Génétique, Faculté de Médecine, CHRU, Strasbourg, France
[7]Département de Neurologie, Hôpital Civil de Strasbourg, Strasbourg, France
[8]Service de Pédiatrie, Centre Hospitalier Universitaire (CHU), Strasbourg, France
[9]Faculté de Médecine de Marseille, Université de la Méditerranée, Inserm UMRS 910 Génétique Médicale et Génomique Fonctionnelle, Marseille, France
[10]Institut Cochin, INSERM Unité 1016, CNR UMR 1408, Université Paris Descartes, Sorbonne Paris Cité, Paris, France

[$]Equal contributors
Corresponding author: Dr Jocelyn Laporte

Corresponding author's address: 1, rue Laurent Fries, BP10142, 67404 Illkirch, France.
Corresponding author's phone and fax: Phone: +33 3 88653412- fax: +33 3 88653246

Corresponding author's e-mail address: jocelyn@igbmc.fr

Online Resource data

Enrichment factor

Enrichment factor for the targeted NMD genes, following targeted capture and sequencing, was calculated as the ratio between the total number of sequenced nucleotides related to the total size of the human genome versus the total number of nucleotides on target regions related to the total size of targeted regions.

Clinical and segregation data for patients I-P without previous molecular characterization

Online resource table 3 lists the sequencing data for all patients, the found mutations, the predicted amino acid change effect and the rank of the mutation among all found variants.

Patient I is a 43 year old Algerian man, born from a consanguineous marriage, with an affected sister. He first presented with polyneuropathy since he was 8 years old and he was diagnosed

with recessive demyelinating Charcot-Marie-Tooth disease (CMT4). However, additional clinical investigations showed he was affected by a mitochondrial disease. We did not find any probable mutations in the NMD genes targeted by next generation sequencing, and genes implicated in such diseases were not targeted by the *NMD-seq* capture library.

Patient J is a 56 years-old Portuguese man with isolated respiratory insufficiency since the age of 46. He had no muscle weakness, no muscle wasting, and no dysphagia. CK levels were normal. He had a sister who also presented with isolated respiratory insufficiency. Muscle biopsy demonstrated cytoplasmic body myopathy in both patients. Edstrom et al described patients with similar clinical signs and an autosomal dominant mutation in the kinase domain of TTN [1, 2]. This myopathy was called hereditary myopathy with early respiratory failure (HMERF). In our patient the probable mutation was in the *TTN* gene, thus in accordance with previous findings in these other patients. However, in our patient the mutation is outside of the kinase domain and this may explain the milder presentation of disease in this patient. In our patient, the respiratory insufficiency appeared later in life, widening the clinical severity that can be associated to dominant *TTN* mutation.

Patient K is a 35-year old French woman with a muscular dystrophy resembling either a limb girdle muscular dystrophy or Bethlem/Ullrich syndrome. She developed progressive moderate proximal and also distal muscle weakness since age 20. CK level is normal. Histological data suggested myofibrillar myopathy. Her father is affected with a milder myopathy and her brother also has difficulties to rise from a chair, that may be due to either a congenital hip dislocation or to muscle weakness. This brother has two daughters showing congenital hip dislocation and a very severe neonatal muscle weakness. Assuming they all share the same disease with different clinical expression, none of the candidate genes in patient K were found similarly mutated in all four other affected relatives. However, a probable heterozygous mutation in *COL6A3* is common to patient K, his father and his brother, all sharing similar age of onset and ambulation difficulties. This variant is absent in the unaffected mother. Mutations in the *COL6A3* gene have been previously implicated in other patients with Bethlem/Ullrich syndrome. Based on our sequencing findings, complementary clinical analyses are ongoing to determine whether the different family members suffer from the same disease with strong clinical variability, or whether two diseases segregate in the family.

Patient L is a 5-year old boy affected with familial spastic paraplegia. An affected cousin lives in Turkey and could not be examined. He has walking problems, increased deep tendon reflexes and bilateral Babinski's sign. He also shows some signs of progressive muscle weakness and amyotrophy. Cerebrospinal MRI and CK levels were normal. Muscle biopsy was refused by parents. We couldn't find any variations in genes known to be implicated in spastic paraplegias.

Patient M is a 47-year old French man with vacuolar myopathy. From 30 years old he showed muscle weakness in his legs. He is a sporadic case. We excluded all candidate variants by checking non-affected parents and sister.

Patient N is a 38-year old Algerian man from a consanguineous family. He has axonal hereditary sensorimotor neuropathies (HSMN). One brother and two sisters are affected by the same disease. Following our next generation sequencing and data analysis protocol, two probable heterozygous variations were found in the Lamin A/C gene, including the p.Arg644Cys missense

previously reported as pathogenic in different laminopathies and never found in control populations. However, both variants are on the same allele based on NGS data, as the two changes were always found in the same reads/fragments (Online resource Fig 4e), we favor the implication of a novel gene not targeted by the *NMD-seq* library. Parents DNAs were not available to investigate this further.

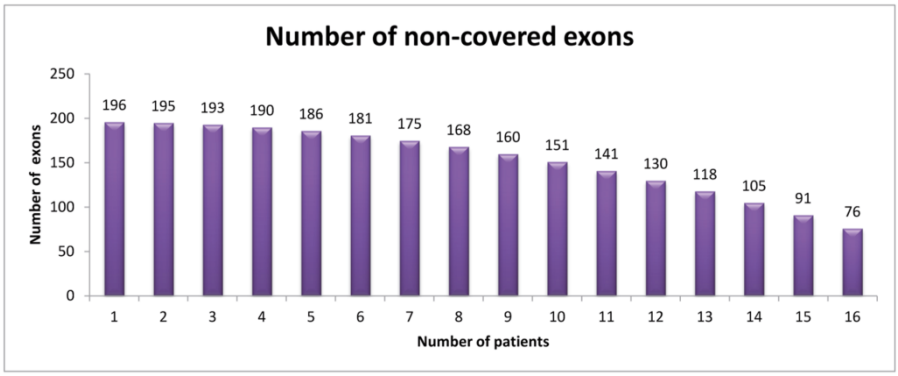
Patient O is a 50-year old French woman with limb girdle muscular dystrophy. She has one affected brother. She developed proximal upper and lower limbs weakness since age 35. Symptoms are stable. We found two probable heterozygous mutations in the *TTN* gene by next generation sequencing that were confirmed to be both present in her affected brother by Sanger sequencing. Her mother was carrier of one of the changes in *TTN*, while DNA from the father was not available.

Patient P is a French 13-year old boy with congenital muscular dystrophy and arthrogryposis. He has an affected twin brother. He showed congenital muscle weakness, stiff ankles and knees, feeding problems and respiratory distress at birth. He was never able to walk and later developed severe scoliosis and chronic restrictive respiratory failure. Muscle biopsy showed extensive muscle fiber degeneration and fibrosis, as well as numerous rod inclusions. We found two probable heterozygous mutations in *RYR1* in both twins, including a stop codon, and each parent was heterozygous carrier of one of the variants.

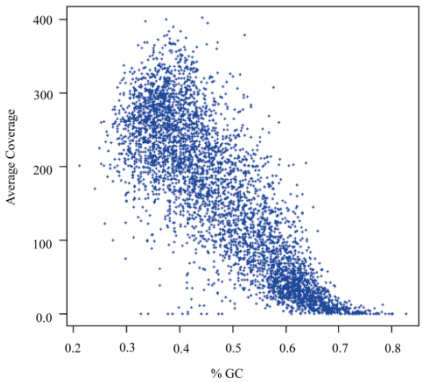
Online resource figures:

Online resource Fig 1: Sequence coverage. a) Number of non-covered exons is depicted for 1 to all 16 patients tested. Non covered exons are exons with coverage less or equal to 2x. Most non-covered exons are similar between samples and GC rich. A full list is found in online resource table 2. b) Coverage depends on GC content as increasing percentage of GC parallels with decreasing nucleotide coverage. Displays patient A data.

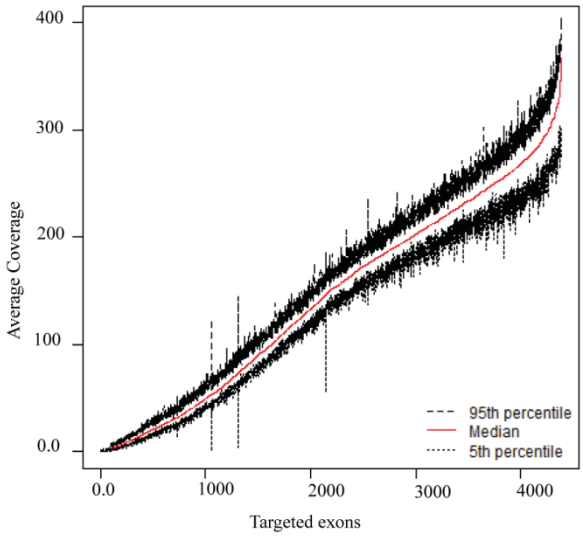
a



b

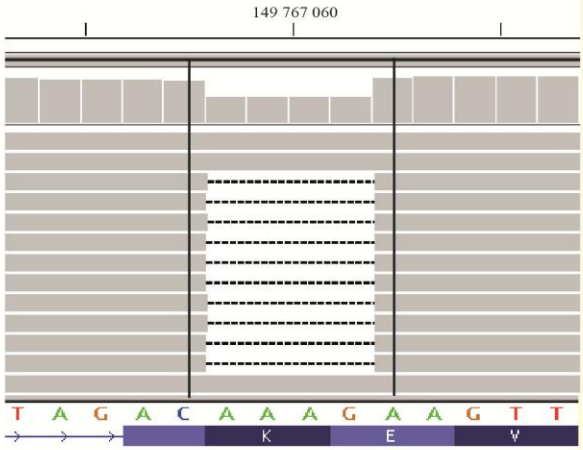


Online resource Fig 2: Coverage in targeted exons
Distribution of average coverage within exons in autosomal chromosomes across all samples tested. Coverage is not homogeneous across all regions; however, coverage is similar across samples at a given position as shown by the narrow distribution. The median has been computed based on the average coverage of all samples.



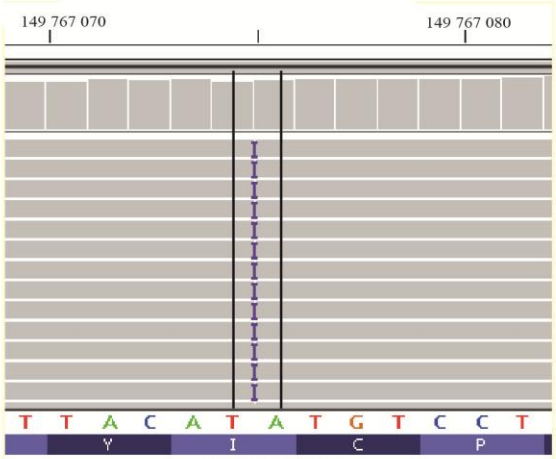
Online resource Fig 3: Detection of different types of mutations. a) Detection of an exonic heterozygous deletion in the *MTM1* gene in a female carrier for X-linked myotubular myopathy (patient A). The mutation in exon 4 is c.141-144delAAAG; p.Glu48fsX24. b) Detection of a hemizygous small insertion in the *MTM1* gene in a patient with X-linked myotubular myopathy (patient E). The mutation in exon 4 is c.156insA; p.Cys53fsX8. c and d) Detection of compound heterozygous mutations in the *SETX* gene in a patient with autosomal recessive ataxia (patient D). The mutations in exons 10 and 11 are c.3213_3214insT; p.Q1072fsX3 and c.5275-1G>A, respectively. e) Detection of a heterozygous exonic point mutation in the *DNM2* gene in a patient with autosomal dominant centronuclear myopathy (patient F). The mutation in exon 14 is c. 1565G>A, p.Arg522His. Figures displayed with the integrative genomics viewer IGV. The normal nucleotide and protein sequences are depicted at the bottom.

a *MTM1*: c.141-144delAAAG, p.Glu48LeufsX24
heterozygous deletion
patient A



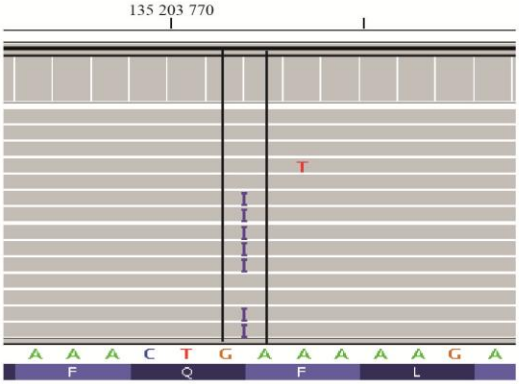
MTM1 (NM_000252.2)

b *MTM1*: c.156-157insA, p.Cys53MetfsX8
hemizygous insertion
patient E



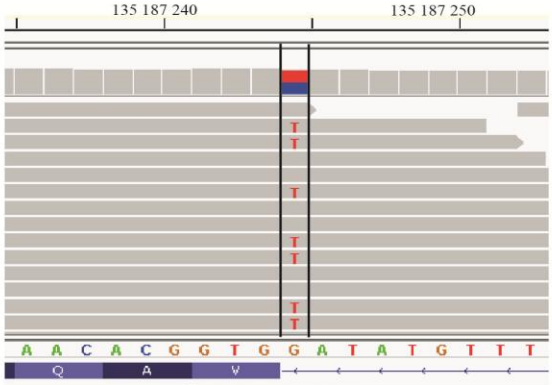
MTM1 (NM_000252.2)

c *SETX*: c.3213-3214insT, p.Gln1072SerfsX3
heterozygous insertion
patient D



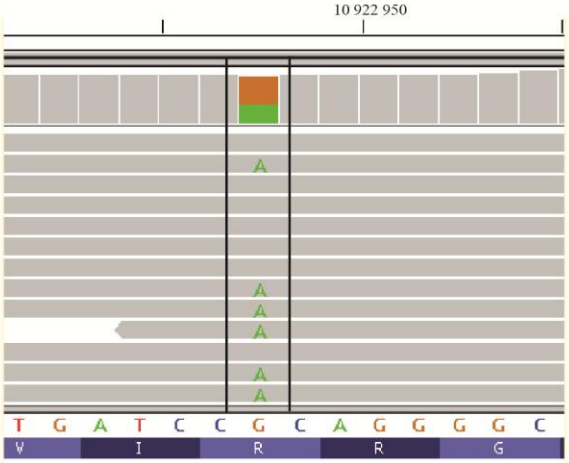
SETX (NM-015046.5)

d *SETX*: c.5275-1G>A
heterozygous intronic mutation
patient D



SETX (NM-015046.5)

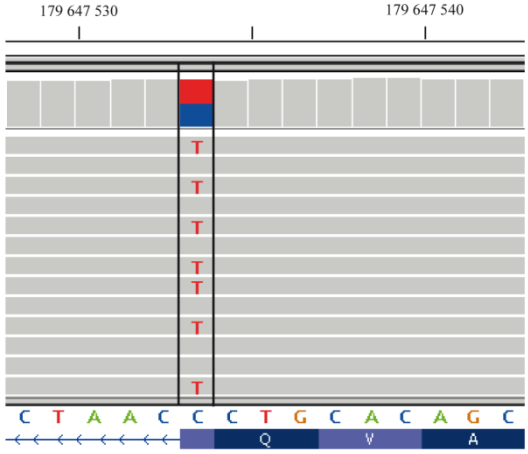
e *DNM2*: c.1565G>A, p.Arg522His
heterozygous exonic point mutation
patient F



DNM2 (NM_001190716)

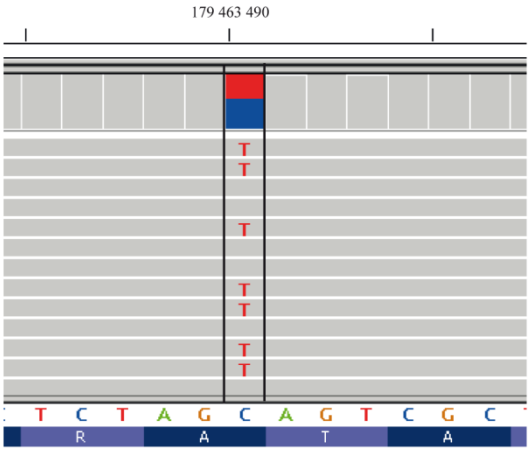
Online resource Fig 4: Detection of novel mutations. a) and b) Detection of compound heterozygous mutations in the *TTN* gene in a patient with limb girdle muscular dystrophy (patient O). The mutations in exons 18 and 240 are c.3100G>A, p.Val1034Met and c.49243G>A, p.Ala16415Thr. c) Detection of a heterozygous mutation in the *TTN* gene in a patient with myopathy with cytoplasmic aggregates (patient J). The mutations in exon 292 is c.68576C>T, p.Pro22859Leu. d) Detection of an exonic heterozygous point mutation in the *COL6A3* gene in a patient with autosomal dominant Bethlem/Ullrich syndrome (patient K). The mutation in exon 27 is c.6812G>A, p.Arg2271Lys. e) Detection of monoallelic heterozygous variations in the *LMNA* gene in a patient with axonal neuropathy (patient N). The variations in exon 11 are c.1928C>A, p.Thr643Asn and c.1930C>T, p.Arg644Cys. The p.Arg644Cys change was previously reported as pathogenic in laminopathies. Figures displayed with the integrative genomics viewer IGV. The normal nucleotide and protein sequences are depicted at the bottom.

a *TTN*: c.3100G>A, p.Val1034Met
heterozygous point mutation
patient O



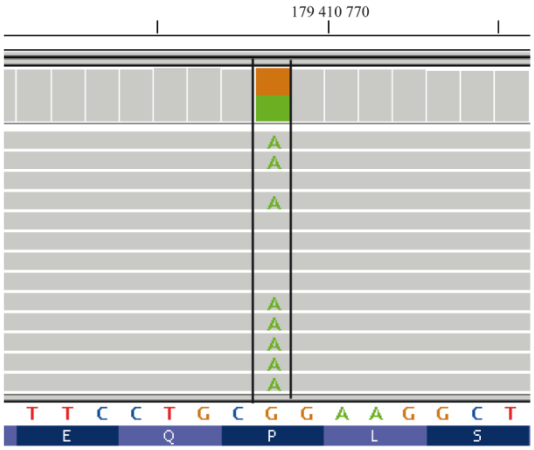
TTN (NM_133378.4)

b *TTN*: c.49243G>A, p.Ala16415Thr
heterozygous point mutation
patient O



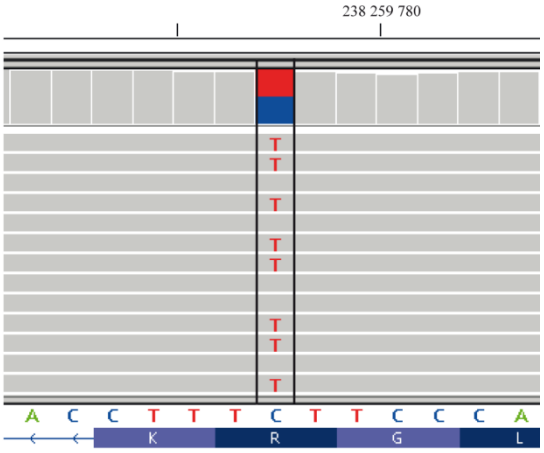
TTN (NM_133378.4)

c *TTN*: c.87491C>T, p.Pro29164Leu
heterozygous point mutation
patient J



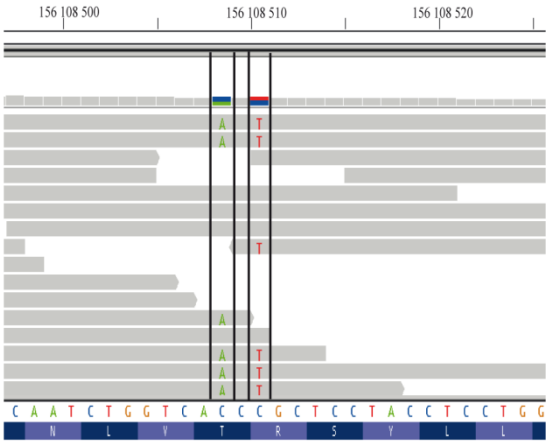
TTN (NM_133378.4)

d *COL6A3*: c.6812G>A, p.Arg2271Lys
heterozygous point mutation
patient K



COL6A3 (NM_004369.3)

e *LMNA*: c.1928C>A, p.Thr643Asn; c.1930C>T, p.Arg644Cys
monoallelic heterozygous
patient N



LMNA (NM_170707.2)

Online resource Table 1: List of the 267 targeted neuromuscular disease genes and associated diseases.

Online resource Table 2: Coordinates, GC content and mean coverage of targeted exons in each patient.

Online resource Table 3: Variants ranking, conservation scores and amino acid change scores.

Online resource Table 4: Sequencing, coverage and variant statistics for patients with previously unknown mutations.

References

1. Edstrom, L., Thornell, L.E., Albo, J., Landin, S., and Samuelsson, M. (1990) Myopathy with respiratory failure and typical myofibrillar lesions. *J Neurol Sci* 96, 211-228

2. Lange, S., Xiang, F., Yakovenko, A., *et al.* (2005) The kinase domain of titin controls muscle gene expression and protein turnover. *Science* 308, 1599-1603

Supplementary Table 1: List of the 267 targeted neuromuscular disease genes and associated diseases

DISEASE CLASS	DISEASE NAME	OMIM No.	GENE NAME	CHROMOSOMAL LOCATION	INHERITANCE	TYPE OF MUTATION
1.Muscular dystrophies	Duchenne Muscular Dystrophy	310200	DMD	Xp21.2	XR	truncation, deletions, substitution mutation, frameshifts
1.Muscular dystrophies	Becker Muscular Dystrophy	300376	DMD	Xp21.2	XR	deletions, substitution, nonsense mutations
1.Muscular dystrophies	Emery-Dreifuss muscular dystrophy X-linked , type 1	310300	EMD	Xq28	XR	insertion, deletions, frameshifts
1.Muscular dystrophies	Emery-Dreifuss muscular dystrophy, X-linked , type 2	300696	FHL1	Xq27.2	XR	substitution mutation, frameshift
1.Muscular dystrophies	Emery-Dreifuss muscular dystrophy	181350	LMNA	1q21.2	AD	deletion, substitution mutations
1.Muscular dystrophies	Emery-Dreifuss Autosomal recessive	181350	LMNA	1q21.2	AR	substitution mutations
1.Muscular dystrophies	Emery-Dreifuss with nesprin-1 defect	612998	SYNE1	6q25	AD	substitution mutations, splice site mutation/premature stop
1.Muscular dystrophies	Emery-Dreifuss with nesprin-2 defect	612999	SYNE2	14q23	AD	substitution mutations
1.Muscular dystrophies	Generalized lipodystrophy, congenital, with muscular dystrophy type 4	613327	PTRF	17q21	AD	insertion, deletion/premature truncation, duplication truncation
1.Muscular dystrophies	Muscular dystrophy,Limb-Girdle, type 1A	159000	MYOT	5q31	AD	substitution mutations
1.Muscular dystrophies	Muscular dystrophy,Limb-Girdle, type 1B	159001	LMNA	1q21.2	AD	substitution mutations, premature truncation
1.Muscular dystrophies	Muscular dystrophy, Limb-Girdle, type 1C	607801	CAV3	3p25	AD	missense, substitution mutations, microdeletion
1.Muscular dystrophies	Muscular dystrophy, Limb-Girdle, type 2A	253600	CAPN3	15q15.1-q21.1	AR	nonsense,splice site, missense, frameshift, substitutions, deletion
1.Muscular dystrophies	Muscular dystrophy, Limb-Girdle, type 2B	253601	DYSF	2p13.3-p13.1	AR	substitution, missense mutations, deletions, insertions
1.Muscular dystrophies	Muscular dystrophy, Limb-Girdle, type 2C	253700	SGCG	13q12	AR	substitution mutations, deletions/missense mutation, insertions/missense mutation
1.Muscular dystrophies	Muscular dystrophy, Limb-Girdle, type 2D	608099	SGCA	17q12-q21.33	AR	substitution mutations
1.Muscular dystrophies	Muscular dystrophy, Limb-Girdle, type 2E	604286	SGCB	4q12	AR	nonsense, substitution mutations, deletion/protein truncation, insertion, duplication, duplication/premature truncation
1.Muscular dystrophies	Muscular dystrophy, Limb-Girdle, type 2F	601287	SGCD	5q33	AR	substitution mutations, deletion/premature truncation
1.Muscular dystrophies	Muscular dystrophy, Limb-Girdle, type 2G	601954	TCAP	17q12	AR	truncating mutation, deletion
1.Muscular dystrophies	Muscular dystrophy, Limb-Girdle, type 2H	254110	TRIM32	9q31-q34.1	AR	missense, substitution mutations, deletion/premature truncation
1.Muscular dystrophies	Muscular dystrophy-dystroglycanopathy type C5 (Limb-Girdle, type 2I)	607155	FKRP	19q13.3	AR	substitution mutations, insertions
1.Muscular dystrophies	Muscular dystrophy, Limb-Girdle, type 2J	608807	TTN	2q31	AR	deletion/insertion
1.Muscular dystrophies	Muscular dystrophy-dystroglycanopathy,type C4 (Limb-Girdle, type 2K)	609308	POMT1	9q34.1	AR	substitution mutation
1.Muscular dystrophies	Muscular dystrophy, Limb-Girdle, type 2L	611307	ANO5	11p14.3	AR	frameshift, substitution mutations
1.Muscular dystrophies	Muscular dystrophy-dystroglycanopathy,type C4 (Limb-Girdle, type 2M)	611588	FKTN	9q31	AR	substitution mutations,deletions, insertion
1.Muscular dystrophies	Muscular dystrophy-dystroglycanopathy,type C2 (Limb-Girdle, type 2N)	613158	POMT2	14q24.3	AR	substitution mutation
1.Muscular dystrophies	Muscular dystrophy-dystroglycanopathy type C3 (Limb-Girdle, type 2O)	613157	POMGNT1	1p34-p33	AR	substitution mutation
1.Muscular dystrophies	Muscular dystrophy, Limb-Girdle, type Q	613723	PLEC1	8q24	AR	deletion
1.Muscular dystrophies	Miyoshi muscular dystrophy 3	613319	ANO5	11p14.3	AR	substituion mutations
2. Congenital muscular dystrophies	Congenital muscular dystrophy, LMNA-related	613205	LMNA	1q21.2		substitution, missense mutations
2. Congenital muscular dystrophies	Congenital muscular dystrophy with merosin deficiency	607855	LAMA2	6q22-q23	AR	substitution, nonsense mutations, deletions, deletion/frameshift, splice site mutation/deletion, substitution/premature truncation
2. Congenital muscular dystrophies	Congenital muscular dystrophy-dystroglycanopathy, type A1 (Walker-Warburg syndrome or muscle-eye-brain disease POMT1-related)	236670	POMT1	9q34.1	AR	deletion/frameshift, insertion/frameshift, duplication/frameshift, substitution mutations
2. Congenital muscular dystrophies	Congenital muscular dystrophy-dystroglycanopathy, type A2 (Walker-Warburg syndrome or muscle-eye-brain disease POMT2-related)	613150	POMT2	14q24.3	AR	splicing, substitution mutations, deletion/premature stop codon
2. Congenital muscular dystrophies	Congenital muscular dystrophy-dystroglycanopathy, type A3 (Walker-Warburg syndrome or muscle-eye-brain disease POMGNT1-related)	253280	POMGNT1	1p34-p33	AR	splice site/deletion,deletions/premature truncations, substitution, missense, splice site mutations
2. Congenital muscular dystrophies	Muscular dystrophy-dystroglycanopathy (congenital with mental retardation), type B, 3	613161	POMGNT1	1p34-p33	AR	Missense, Nonsense & Frameshift
2. Congenital muscular dystrophies	Congenital muscular dystrophy-dystroglycanopathy, type A6	613154	LARGE	22q12.3-13.1	AR	deletion, frameshift,substitution mutation, substitution mutation
2. Congenital muscular dystrophies	Congenital muscular dystrophy-dystroglycanopathy, type B1 (with congenital mental retardation)	613155	POMT1	9q34.1	AR	substitution, splice site mutations
2. Congenital muscular dystrophies	Congenital muscular dystrophy-dystroglycanopathy, type B2 (with congenital mental retardation)	613156	POMT2	14q24.3	AR	substitution mutations, premature truncation
2. Congenital muscular dystrophies	Congenital muscular dystrophy-dystroglycanopathy, type B3 (with congenital mental retardation)	613151	POMGNT1	1p34-p33	AR	substitution, splice site mutations
2. Congenital muscular dystrophies	Congenital muscular dystrophy-dystroglycanopathy, type B5	606612	FKRP	19q13.3	AR	missense, nonsense, substitution mutations
2. Congenital muscular dystrophies	Congenital muscular dystrophy-dystroglycanopathy, type B6	608840	LARGE	22q12.3-13.1	AR	substitution,insertion/frameshift
2. Congenital muscular dystrophies	Muscle dystrophy dystroglycanopathy, congenital, type 4A, (Fukuyama congenital muscular dystrophy)	253800	FKTN	9q31	AR	transposonal insertion, deletions, insertions, nonsense, substitution mutations, frameshifts
2. Congenital muscular dystrophies	Muscle dystrophy dystroglycanopathy, congenital, congenital, type 4B	613152	FKTN	9q31	AR	substitution mutation and deletion
2. Congenital muscular dystrophies	Muscle dystrophy dystroglycanopathy, congenital, congenital, type 4C (limb-girdle)	611588	FKTN	9q31	AR	insertions, deletions, substitution mutations, frameshifts
2. Congenital muscular dystrophies	Walker-Warburg syndrome		FKTN	9q31	AR	substitution mutation, deletion
2. Congenital muscular dystrophies	Walker-Warburg syndrome		FKRP	19q13.3	AR	substitution mutation
2. Congenital muscular dystrophies	Muscle-eye-brain disease	613153	FKRP	19q13.3	AR	substitution mutation
2. Congenital muscular dystrophies	Rigid spine muscular dystrophy 1 (minicore myopathy, severe classic form; multiminicore disease, severe classic form; desmin-related myopathy with mallory bodies included)	602771	SEPN1	1p36-p35	AR	substitution mutations, deletion, stop codon mutation
2. Congenital muscular dystrophies	Ullrich syndrome	254090	COL6A1	21q22.3	AR	substitution mutations, deletions, frameshifts
2. Congenital muscular dystrophies	Ullrich syndrome	254090	COL6A1	21q22.3	AD	deletions, substitution mutations
2. Congenital muscular dystrophies	Ullrich syndrome	254090	COL6A2	21q22.3	AR	insertions,deletions, substitution,frameshift, splice site mutations
2. Congenital muscular dystrophies	Ullrich syndrome	254090	COL6A2	21q22.3	AD	deletions, substitution mutations
2. Congenital muscular dystrophies	Ullrich syndrome	254090	COL6A3	2q37	AR	nonsense,splice site mutations
2. Congenital muscular dystrophies	Ullrich syndrome	254090	COL6A3	2q37	AD	splice site mutation
2. Congenital muscular dystrophies	Bethlem myopathy	158810	COL6A1	21q22.3	AD	missense, substitution mutations,deletions
2. Congenital muscular dystrophies	Bethlem myopathy	158810	COL6A2	21q22.3	AD	missense, substitution mutations

2. Congenital muscular dystrophies	Bethlem myopathy	158810	COL6A3	21q22.3	AD	substitution mutations
2. Congenital muscular dystrophies	Myosclerosis	255600	COL6A2	21q22.3	AR	substitution mutation
2. Congenital muscular dystrophies	Congenital muscular dystrophy with integrin defect	613204	ITGA7	12q13	AR	insertion, frameshift deletions
3. Congenital myopathies	Nemaline myopathy1	609284	TPM3	1q22-q23	AD	substitutions
3. Congenital myopathies	Nemaline myopathy1		TPM3	1q22-q23	AR	deletion
3. Congenital myopathies	Nemaline myopathy2	256030	NEB	2q22	AR	deletions, duplication/insertion, substitution
3. Congenital myopathies	Nemaline myopathy3	161800	ACTA1	1q42.1	AD	substitutions
3. Congenital myopathies	Nemaline myopathy4	609285	TPM2	9p13.2-p13.1	AD	substitutions, deletions
3. Congenital myopathies	Nemaline myopathy5	605355	TNNT1	19q13.4	AR	transversion
3. Congenital myopathies	Nemaline myopathy7	610687	CFL2	14q12	AR	substituion mutations
3. Congenital myopathies	Myopathy, congenital, with fiber-type disproportion	255310	ACTA1	1q42.1	AD	substitution mutations
3. Congenital myopathies	Myopathy, congenital, with fiber-type disproportion		SEPN1	1p36-p35	AD	substitution mutation
3. Congenital myopathies	Myopathy congenital with cores		ACTA1	1q42.1	AD	substitution mutation
3. Congenital myopathies	Myopathy congenital with cores		TPM3	1q22-q23	AR	substituion mutations
3. Congenital myopathies	Myopathy, congenital, with excess of thin myophilaments	161800	ACTA1	1q42.1	AD	substitutions
3. Congenital myopathies	Myotubular myopathy	310400	MTM1	Xq28	XR	substitution, missense mutations, frameshifts,deletions, splice site mutations
3. Congenital myopathies	Centronuclear myopathy	160150	DNM2	19p13.2	AD	substitution mutations
3. Congenital myopathies	Centronuclear myopathy	255200	BIN1	2q14	AR	substitution mutations
3. Congenital myopathies	Centronuclear myopathy	160150	MTMR14	3p25.3	AD	substitution mutations
3. Congenital myopathies	Centronuclear myopathy		RYR1	19q13.1	AR	substitution mutation
3. Congenital myopathies	Central core disease (congenital neuromuscular disease with uniform type 1 fiber included)	117000	RYR1	19q13.1	AD	substitution mutations, deletions-insertions/substitution, deletion/insertion, deletion/premature truncation
3. Congenital myopathies	Central core disease, recessive (transient multiminicore myopathy)	117000	RYR1	19q13.1	AR	substitution mutations
3. Congenital myopathies	Multiminicore disease with external ophtalmopegia	255320	RYR1	19q13.1	AR	substitution, splice site mutations, insertion-transition/frameshift
3. Congenital myopathies	Myosin storage myopathy (Hyaline body myopathy,dominant)	608358	MYH7	14q12	AD	substitution mutation
3. Congenital myopathies	Cap myopathy	609285	TPM2	9p13.2-p13.1	AD	deletions, substitution mutations
3. Congenital myopathies	Cap myopathy	609284	TPM3	1q22-q23	AD	substitution mutations
3. Congenital myopathies	Cap myopathy	161800	ACTA1	1q42.1	AD	substitution mutation
3. Congenital myopathies	Myopathy, early onset with fatal cardiomyopathy	611705	TTN	2q31	AR	deletions
3. Congenital myopathies	Congenital lethal myopathy	612540	CNTN1	12q11-q12	AR	duplication
4. Distal myopathies	Distal recessive myopathy (Miyoshi myopathy)	254130	DYSF	2p13.3-p13.1	AR	substitution, missense mutations
4. Distal myopathies	Tibial muscular dystrophy (Udd myopathy)	600334	TTN	2q31	AD	deletion/insertion, substitution mutations
4. Distal myopathies	Inclusion body myopathy	600737	GNE	9p13.3	AR	missense, substitution mutations
4. Distal myopathies	Inclusion body myopathy 3	605637	MYH2	17p13.1	AD	substitution mutation
4. Distal myopathies	Nonaka myopathy, (distal, with rimmed vacuoles)	605820	GNE	9p13.3	AR	substitution mutations
4. Distal myopathies	Distal myopathy 1 (Laing distal myopathy)	160500	MYH7	14q12	AD	substitution mutations, deletions
4. Distal myopathies	Distal myopathy 2(with vocal cord and pharyngeal dysfuncti0n)	606070	MATR3	5q31.2	AD	missense mutations
4. Distal myopathies	Distal myopathy with caveolin defect		CAV3	3p25	AD	substitution mutation
5. Other myopathies	Myofibrillar myopathy, alpha-B crystallin related	608810	CRYAB	11q22.3-q23.1	AD	deletion, substitution mutations
5. Other myopathies	Myofibrillar myopathy, desmin-related myopathy	601419	DES	2q35	AD	substitution mutations, frameshifts, deltions
5. Other myopathies	Myopathy myofibrillar, ZASP related	609452	LDB3	10q22.2-23.3	AD	substitution mutations
5. Other myopathies	Myotilinopathy	609200	MYOT	5q31	AD	substitution mutations
5. Other myopathies	Spheroid body myopathy	182920	MYOT	5q31	AD	substitution mutations
5. Other myopathies	Myofibrillar myopathy, filamin-C related	609524	FLNC	7q32	AD	substitution mutation, deletion
5. Other myopathies	Myofibrillar myopathy with BAG3 defect	612954	BAG3	10q25.2-q26.2	AD	substitution mutations
5. Other myopathies	Danon disease (Glycogen storage IIB)	300257	LAMP2	Xq24	XD	deletion/frameshift, insertion/frameshift, insertions, substitution mutations
5. Other myopathies	Myopathy with excessive autophagia	310440	VMA21	Xq28	XR	
5. Other myopathies	Oculopharyngeal muscular dystrophy	164300	PABPN1	14q11.2-q13	AD	expansions, substitution mutation
5. Other myopathies	Hereditary myopathy with early respiratory failure (Edstrom myopathy)	603689	TTN	2q31	AD	substitution mutation
5. Other myopathies	Epidermolysis bullosa simplex associated with muscular dystrophy	226670	PLEC1	8p24	AR	deletions, insertion, deletion/frameshift, substitution
5. Other myopathies	Muscle hypertrophy		MSTN	2q32.2	AR	splice site mutation
5. Other myopathies	Fibrodysplasia ossificans progressiva	135100	ACVR1	2q23-q24	AD	substitution mutation
5. Other myopathies	Hyperckemia, idiopathic	123320	CAV3	3p25	AD	substitution mutation, missense mutation
5. Other myopathies	X-linked myopathy with postural muscle atrophy	300696	FHL1	Xq27.2	XR	substitution mutations, insertions, premature truncation
5. Other myopathies	Reducing body myopathy	300717-300718	FHL1	Xq27.2	XL-D	substitution mutations, deletions
5. Other myopathies	Scapuloperoneal myopathy	300695	FHL1	Xq27.2	XD	substitution mutation
5. Other myopathies	Inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia	167320	VCP	9p13-p12	AD	substitution mutations
5. Other myopathies	Myopathy with exercise intolerance, Swedish type	255125	ISCU	12q24.1	AR	intronic transversion/stop codon mutation, substitution mutation
5. Other myopathies	SCAD deficiency	201470	ACADS	12q22-qter	AR	substitution mutations, deletion
5. Other myopathies	Stuve-Widemann syndrome/Schwartz-Jampel type 2 syndrome	601559	LIFR	5p13.1	AR	insertion/premature stop, deletion/premature stop
5. Other myopathies	Hypertrophic cardiomyopathy	115197	MYBPC2	19q13.33		frameshift deletion/protein truncation
5. Other myopathies	Congenital insensitivity to pain with anhidrosis	256800	NTRK1	1q21-q22	AR	deletion/premature truncation, deletion, substitution, nonsense, missense mutations
5. Other myopathies	Cerebellar ataxia		PLEKHG4	16q22.1	AD	transition mutation
5. Other myopathies	Waardenburg-Shah syndrome, type 4C	613266	SOX10	22q13	AD	deletion/insertion, deletions, missense, truncating mutations
5. Other myopathies	Waardenburg-Shah syndrome, type 2E	611584	SOX10	22q13	AD	truncating, substitution mutations
5. Other myopathies	Peripheral demyelinating neuropathy, central desmyelination, Wanderburg syndrome and Hirschsprung disease	609136	SOX10	22q13	AD	deletions/premature truncation, truncating, substitution mutations
5. Other myopathies	X-linked infantile spinal muscular atrophy	301830	UBA1	Xp11.23	X-LINKED -(probably multiple loci)	substitution mutations
5. Other myopathies	Adrenoleukodystrophy	300100	ABCD1	Xq28	X-LINKED	deletions, substitutions, missense, nonsense,fraeshift, splice mutations
5. Other myopathies	Leukodystrophy hypomyelinating 4	612233	HSPD1	2q33.1	AR	substitution mutation
5. Other myopathies	Chanarin-Dorfman syndrome	275630	ABHD5	3p21	AR	splice mutation
5. Other myopathies	Multiple acyl-co dehydrogenase deficiency, MADD (glutaric aciduria IIa)	231680	ETFA	15q23-q25	AR	substitution mutations, deletions
5. Other myopathies	Multiple acyl-co dehydrogenase deficiency, MADD (glutaric aciduria IIb)	231680	ETFB	19q13.3	AR	substitution mutations, deletions
5. Other myopathies	Multiple acyl-co dehydrogenase deficiency, MADD (glutaric aciduria IIc)	231680	ETFDH	4q32-qter	AR	transition, deletion/framshift, substitution mutations

5. Other myopathies	VLCAD deficiency	201475	ACADVL	17p13	AR	deletions, missense, fraeshift, mutations
5. Other myopathies	Scapuloperoneal myopathy, MYH7 related	181430	MYH7	14q12	AD	substitution mutations
5. Other myopathies			RYR1	19q13.1		
6. Myotonic syndromes	Myotonic, dystrophy (Steinert)	160900	DMPK	19q13.2-q13.3	AD	expansion
6. Myotonic syndromes	Myotonic dystrophy type 2	602668	CNBP	3q13.3q24	AD	expansion
6. Myotonic syndromes	Rippling muscle disease	606072	CAV3	3p25	AD	missense, substitution mutations
6. Myotonic syndromes	Rippling muscle disease	606072	CAV3	3p25	AR	substitution mutations
6. Myotonic syndromes	Schwartz-Jampel syndrome	255800	HSPG2	1p36.1	AR	frameshift/protein truncation, truncating mutations, exon fusion mutations, deletions
6. Myotonic syndromes	Brody disease	601003	ATP2A1	16p12	AR (AD in one family)	substitutions, splice mutations, deletions
7. Ion channel muscle diseases	Myotonia congenita (Thomsen)	160800	CLCN1	7q35	AD	substitution mutations
7. Ion channel muscle diseases	Myotonia (Becker)	255700	CLCN1	7q35	AR	substitution mutations, insertions, deletions
7. Ion channel muscle diseases	Normokalemic periodic paralysis type	170600	SCN4A	17q23.1-q25.3	AD	substitution mutations
7. Ion channel muscle diseases	Hypokalemic periodic paralysis type	170500	SCN4A	17q23.1-q25.3	AD	substitution mutations
7. Ion channel muscle diseases	Hypokalemic periodic paralysis type 2	613345	SCN4A	17q23.1-q25.3	AD	substitution mutations
7. Ion channel muscle diseases	Paramyotonia congenita of Von Eulenburg	168300	SCN4A	17q23.1-q25.3	AD	substitution mutations
7. Ion channel muscle diseases	Potassium aggravated myotonia		SCN4A	17q23.1-q25.3	AD	substitution mutations
7. Ion channel muscle diseases	Hypokalaemic periodic paralysis type 1	170400	CACNA1S	1q32	AD	substitution mutations
7. Ion channel muscle diseases	Episodic ataxia type-2	108500	CACNA1A	19p13	AD	frameshifts, splice site, deletions, duplications
7. Ion channel muscle diseases	Hypokalemic periodic paralysis type 3		KCNE3	11q13-q14	AD	substitution mutations
7. Ion channel muscle diseases	Hypokalemic periodic paralysis type 3		KCNE3	11q13-q14	AD	substitution mutations
7. Ion channel muscle diseases	Periodic paralysis, potassium-sensitive cardiodysrhythmic Andersen-Tawil syndrome (Long QT syndrome 7)	170390	KCNJ2	17q23.1-24.2	AD	substitution, missense mutations, deletions
7. Ion channel muscle diseases	Jervell and Lange-Nielsen cardioauditory syndrome 1	220400	KCNQ1	11p15.5	AR	deletion-insertion/premature stop, deletions/premature stop, insertion/premature stop, substitution, missense mutations
7. Ion channel muscle diseases	Jervell and Lange-Nielsen cardioauditory syndrome 2	612347	KCNE1	21q22.1-q22.2	AR	substitution mutations
8. Malignant hyperthermia	Malignant hyperthermia susceptibility 1	145600	RYR1	19q13.1	AD	substitution mutations
8. Malignant hyperthermia	Malignant hyperthermia susceptibility 5	601887	CACNA1S	1q32	AD	substitution mutation
8. Malignant hyperthermia	Susceptibility to thyrotoxic periodic paralysis	188580	CACNA1S	1q32	AD	polymorphisms
9. Metabolic myopathies	Glycogen storage disease Type II (Pompe), infantile form	232300	GAA	17q25.2-q25.3	AR	substitution mutations, deletions
9. Metabolic myopathies	Glycogen storage disease Type II (Pompe), adult form	232300	GAA	17q25.2-q25.3	AR	substitution mutations, deletions
9. Metabolic myopathies	Glycogen storage disease type IIIa	232400	AGL	1p21	AR	insertions,deletions, substitution mutations
9. Metabolic myopathies	Glycogen storage disease type IV	232500	GBE1	3p12	AR	substitution mutations, deletions, insertion/premature truncation, frameshift
9. Metabolic myopathies	Glycogen storage disease Type V (McArdle)	232600	PYGM	11q13	AR	substitution, nonsense, missense, splice site mutations, deletions
9. Metabolic myopathies	Glycogen storage disease Type VII (Tarui)	232800	PFKM	12q13.3	AR	expansion
9. Metabolic myopathies	Glycogen storage disease type 0	611556	GYS1	19q13.3	AR	stop codon mutation
9. Metabolic myopathies	Glycogen storage disease of heart, lethal congenital	261740	PRKAG2	7q36	AD	substitution mutation
9. Metabolic myopathies	Phosphoglycerate kinase deficiency	300653	PGK1	Xq13	XR	substitution, splice site mutations, deletions
9. Metabolic myopathies	Glycogen storage X (phosphoglycerate mutase deficiency)	261670	PGAM2	7p13-p12.3	AR	substitution mutations
9. Metabolic myopathies	Glycogen storage disease XI (Lactate dehydrogenase-A deficiency)	612933	LDHA	11p15.4	AR	deletion/premature truncation, substitution mutation
9. Metabolic myopathies	Glycogen disease XIII	612932	ENO3	17pter-p12	AD	substitution mutations
9. Metabolic myopathies	Carnitine palmitoyl-transferase II deficiency, infantile (with hypoketonic hypoglycemia and cardiomyopathy)	255110	CPT2	1p32	AR	substituion mutations
9. Metabolic myopathies	Carnitine palmitoyl-transferase II deficiency, adult onset	600649	CPT2	1p32	AR	substitution mutations, deletions
9. Metabolic myopathies	Carnitine palmitoyl-transferase II deficiency, lethal neonatal	608836	CPT2	1p32	AR	insertion/deletion, substitution, splice site mutations
10. Hereditary cardiomyopathies	Familial hypertrophic cardiomyopathy, 1	192600	MYH7	14q12	AD	substitution, missense mutations, deletion
10. Hereditary cardiomyopathies	Familial hypertrophic cardiomyopathy, 2	115195	TNNT2	1q32	AD	substitution, missense mutations
10. Hereditary cardiomyopathies	Familial hypertrophic cardiomyopathy, 3	115196	TPM1	15q22.1	AD	substitution mutations
10. Hereditary cardiomyopathies	Familial hypertrophic cardiomyopathy, 6 (with Wolff-Parkinson -whyte syndrome)	600858	PRKAG2	7q36	AD	missense, substitution mutations, insertion
10. Hereditary cardiomyopathies	Familial hypertrophic cardiomyopathy, 7	613690	TNNI3	19q13.4	AD	missense, substitution mutations, deletion
10. Hereditary cardiomyopathies	Familial hypertrophic cardiomyopathy, 8	608751	MYL3	3p	AD	substitution mutations
10. Hereditary cardiomyopathies	Familial hypertrophic cardiomyopathy, 9	613765	TTN	2q31	AD	substitution mutation
10. Hereditary cardiomyopathies	Familial hypertrophic cardiomyopathy, 10	608758	MYL2	12q23-q24.3	AD	substitution mutations
10. Hereditary cardiomyopathies	Familial hypertrophic cardiomyopathy, 11	612098	ACTC1	15q11-q14	AD	substitution mutation
10. Hereditary cardiomyopathies	Familial hypertrophic cardiomyopathy, 12	612124	CSRP3	11p15.1	AD	substitution mutation
10. Hereditary cardiomyopathies	Familial hypertrophic cardiomyopathy, 13	613243	TNNC1	3p21.3-14.3	AD	substitution mutations
10. Hereditary cardiomyopathies	Familial hypertrophic cardiomyopathy, 14	613251	MYH6	14q12	AD	substitution mutations
10. Hereditary cardiomyopathies	Familial hypertrophic cardiomyopathy 15	613255	VCL	10q22.1-q23		substitution mutations
10. Hereditary cardiomyopathies	Hypertrophic cardiomyopathy	192600	MYLK2	20q13.3	AD	substitution mutations
10. Hereditary cardiomyopathies	Hypertrophic cardiomyopathy	192600	CAV3	3p25	AD	substitution mutations
10. Hereditary cardiomyopathies	Hypertrophic cardiomyopathy, early-onset fatal		COX15	10q24	AR	deletion, substitution mutations
10. Hereditary cardiomyopathies	Dilated cardiomyopathy, 1A	115200	LMNA	1q21.2	AD	substitution mutations, insertion
10. Hereditary cardiomyopathies	Dilated cardiomyopathy, 1AA	612158	ACTN2	1q42-q43	AD	substitution mutation
10. Hereditary cardiomyopathies	Dilated cardiomyopathy, 1C with left ventricular noncompaction	601493	LDB3	10q22.2-23.3	AD	substitution mutations
10. Hereditary cardiomyopathies	Dilated cardiomyopathy, 1D	601494	TNNT2	1q32	AD	substitution mutations,deletion
10. Hereditary cardiomyopathies	Dilated cardiomyopathy, 1E with conduction disorder and arrythmia	601154	SCN5A	3p21	AD	substitution mutations, insertion/premature truncation
10. Hereditary cardiomyopathies	Dilated cardiomyopathy, 1G	604145	TTN	2q31	AD	insertion/protein truncation, missense, substitution, nonsense mutations
10. Hereditary cardiomyopathies	Dilated cardiomyopathy, 1I	604765	DES	2q35	AD	substitution mutation
10. Hereditary cardiomyopathies	Dilated cardiomyopathy, 1J	605362	EYA4	6q23	AD	deletion/frameshift
10. Hereditary cardiomyopathies	Dilated cardiomyopathy, 1L	606685	SGCD	5q33	AD	deletion, deletion/premature truncation, substitution mutations
10. Hereditary cardiomyopathies	Dilated cardiomyopathy, 1M	607482	CSRP3	11p15.1	AD	missense, substitution mitations
10. Hereditary cardiomyopathies	Dilated cardiomyopathy, 1N	607487	TCAP	17q12	AD	substitution mutation
10. Hereditary cardiomyopathies	Dilated cardiomyopathy, 1O	608569	ABCC9	12p12.1	AD	insertion/deletion, substitution mutations
10. Hereditary cardiomyopathies	Dilated cardiomyopathy, 1P	609909	PLN	6q22.1	AD	substitution mutations, deletion
10. Hereditary cardiomyopathies	Dilated cardiomyopathy, 1R	613424	ACTC1	15q11-q14	AD	missense mutations
10. Hereditary cardiomyopathies	Dilated cardiomyopathy, 1S	613426	MYH7	14q12	AD	substitution, missense mutations
10. Hereditary cardiomyopathies	Dilated cardiomyopathy, 1T	613740	TMPO	12q22	AD	substitution mutation
10. Hereditary cardiomyopathies	Dilated cardiomyopathy, 1W	611407	VCL	10q22.1-q23	AD	deletion
10. Hereditary cardiomyopathies	Dilated cardiomyopathy, 1Y	611878	TPM1	15q22.1	AD	substitution mutation
10. Hereditary cardiomyopathies	Dilated cardiomyopathy, 1Z	611879	TNNC1	3p21.3-14.3	AD	substitution mutation
10. Hereditary cardiomyopathies	Dilated cardiomyopathy 1X, due to fukutin defect	611615	FKTN	9p31	AR	retrotransposon insertion, substitution mutation

10. Hereditary cardiomyopathies	Dilated cardiomyopathy 2A	611880	TNNI3	19q13.4	AR	substitution mutation
10. Hereditary cardiomyopathies	Dilated cardiomyopathy 3A	300069	TAZ	Xq28	XR	deletion/premature stop
10. Hereditary cardiomyopathies	Dilated cardiomyopathy IIIB, due to dystrophin defect	302045	DMD	Xp21.2	XR	substitution mutations, deletions, splice site mutations
10. Hereditary cardiomyopathies	Dilated cardiomyopathy due to laminin-alpha4 defect		LAMA4	6q21	AD	nonsense,missense mutations
10. Hereditary cardiomyopathies	Dilated cardiomyopathy due to integrin-linked kinase defect		ILK	11p15.5-p15.4	AD	point mutations
10. Hereditary cardiomyopathies	Dilated cardiomyopathy due to myopalladin defect		MYPN	10q21.3	AD	substitution mutations
10. Hereditary cardiomyopathies	Dilated cardiomyopathy 1DD	613172	RBM20	10q25.2	AD	substitution mutations
10. Hereditary cardiomyopathies	Dilated cardiomyopathy due to cardiac ankyrin repeat protein defect		ANKRD1	10q23.31	AD	missense mutations
10. Hereditary cardiomyopathies	Dilated cardiomyopathy 1FF	613286	TNNI3	19q13.4	AD	substitution mutations
10. Hereditary cardiomyopathies	Dilated cardiomyopathy 1EE	613252	MYH6	14q12	AD	substitution mutations
10. Hereditary cardiomyopathies	Dilated cardiomyopathy with woolly hair and keratoderma	605676	DSP	6p24	AR	deletion
10. Hereditary cardiomyopathies	Restrictive cardiomyopathy, 1	115210	TNNI3	19q13.4	AD	substitution mutations
10. Hereditary cardiomyopathies	Cardioskeletal myopathy with neutropenia and abnormal mitochondria (Barth syndrome)	302060	TAZ	Xq28	XR	insertion/premature stop, substitution, splicing, missense mutations, deletion
10. Hereditary cardiomyopathies	Noncompaction of left ventricular myocardium with congenital heart defects	604169	DTNA	18q12.1-q12.2	AD	substitution mutation
10. Hereditary cardiomyopathies	Cardiovalvular dysplasia, X-linked (Myxomatous valvular dystrophy)	314400	FLNA	Xq28	XR	substitution mutations, deletions
10. Hereditary cardiomyopathies	Arrhythmogenic right ventricular dysplasia, 1	107970	TGFB3	14q24	AD	transition mutations
10. Hereditary cardiomyopathies	Arrhythmogenic right ventricular dysplasia, 2	600996	RYR2	1q42.1-q43	AD	missense mutations
10. Hereditary cardiomyopathies	Arrhythmogenic right ventricular dysplasia, 5	604400	TMEM43	3p25	AD	substitution mutation
10. Hereditary cardiomyopathies	Arrhythmogenic right ventricular dysplasia, 8	607450	DSP	6p24	AD	substitution, missense mutations
10. Hereditary cardiomyopathies	Arrhythmogenic right ventricular dysplasia, 9	609040	PKP2	12p11	AD	splice site, stop codon mutations
10. Hereditary cardiomyopathies	Arrhythmogenic right ventricular dysplasia, 10	610193	DSG2	18q12.1-q12.2	AD	substitution mutations
10. Hereditary cardiomyopathies	Arrhythmogenic right ventricular dysplasia, 11	610476	DSC2	18q12.1	AD	insertion, deletion, splice site mutation
10. Hereditary cardiomyopathies	Arrhythmogenic right ventricular dysplasia, 12	611528	JUP	17q21	AD	insertion
10. Hereditary cardiomyopathies	Ventricular tachycardia, catecholaminergic polymorphic	604772	RYR2	1q42.1-q43	AD	missense, substitution mutations
10. Hereditary cardiomyopathies	Ventricular tachycardia, catecholaminergic polymorphic, recessive	611938	CASQ2	1p13.3-p11	AR	missense mutations, deletions
10. Hereditary cardiomyopathies	Naxos disease	601214	JUP	17q21	AR	deletion/frameshift
10. Hereditary cardiomyopathies	Long QT syndrome 1 (Romano-Ward syndrome)	192500	KCNQ1	11p15.5	AD	deletions, substitution, missense, splice site mutations, substitution/premature truncation
10. Hereditary cardiomyopathies	Long QT syndrome 2	613688	KCNH2	7q35-q36	AD	substitution mutations, deletions
10. Hereditary cardiomyopathies	Long QT syndrome 3	603830	SCN5A	3p21	AD	deletion, insertion, substitution, missense mutations
10. Hereditary cardiomyopathies	Long QT syndrome 4	600919	ANK2	4q25-q27	AD	substitution mutations
10. Hereditary cardiomyopathies	Long QT syndrome 5	613695	KCNE1	21q22.1-q22.2	AD	substitution mutations
10. Hereditary cardiomyopathies	Long QT syndrome 6	613693	KCNE2	22q22.1	AD	substitution mutations
10. Hereditary cardiomyopathies	Long QT syndrome 8 (Timothy syndrome)	601005	CACNA1C	12p13.3	AD	substitution mutations
10. Hereditary cardiomyopathies	Long QT syndrome 9	611818	CAV3	3p25	AD	missense mutations
10. Hereditary cardiomyopathies	Short QT syndrome 1	609620	KCNH2	7q35-q36	AD	substitution mutations
10. Hereditary cardiomyopathies	Short QT syndrome 2	609621	KCNQ1	11p15.5	AD	substitution mutation
10. Hereditary cardiomyopathies	Short QT syndrome 3	609622	KCNJ2	17q23.1-24.2	AD	substitution mutation
10. Hereditary cardiomyopathies	Atrial fibrillation, 3	607554	KCNQ1	11p15.5	AD	substitution mutation
10. Hereditary cardiomyopathies	Atrial fibrillation, 4	611493	KCNE2	22q22.1	AD	missense mutations
10. Hereditary cardiomyopathies	Atrial fibrillation, 6	612201	NNPPA	1p36.2	AD	deletion/fusion protein generation
10. Hereditary cardiomyopathies	Atrial fibrillation, 7	612240	KCNA5	12p13	AD	subsitution mutations
10. Hereditary cardiomyopathies	Atrial fibrillation	614049	GJA5	1q21.1	AD	substitution mutation
10. Hereditary cardiomyopathies	Atrial standstill		GJA5	1q21.1	AD	substitution mutation
10. Hereditary cardiomyopathies	Atrial standstill	614022	SCN5A	3p21	AD	substitution mutation
10. Hereditary cardiomyopathies	Brugada syndrome 2	611777	GPD1L	3p22.3	AD	substitution mutations
10. Hereditary cardiomyopathies	Brugada syndrome 3	611875	CACNA1C	12p13.3	AD	substitution mutations
10. Hereditary cardiomyopathies	Brugada syndrome 4	611876	CACNB2	10p12	AD	missense mutation
10. Hereditary cardiomyopathies	Brugada syndrome 6	613119	KCNE3	11q13-q14	AD	substitution mutation
10. Hereditary cardiomyopathies	Sick sinus syndrome	163800	HCN4	15q24-q25	AD	missense, substitution mutations, deletion/frameshift
10. Hereditary cardiomyopathies	Sick sinus syndrome	608567	SCN5A	3p21	AR	substitution mutations
11. Congenital myasthenic syndromes	Slow channel syndromes	601462	CHRNA1	2q24-q32	AD	substitution mutations
11. Congenital myasthenic syndromes	Slow channel syndromes	601462	CHRNB1	17p12-p11	AD	substitution mutations
11. Congenital myasthenic syndromes	Slow channel syndromes	601462	CHRND	2q33-q34	AD	substitution mutations
11. Congenital myasthenic syndromes	Slow channel syndromes	601462	CHRNE	17p13-p12	AD	substitution mutations
11. Congenital myasthenic syndromes	Slow channel syndromes	601462	CHRNE	17p13-p12	AR	substitution mutations
11. Congenital myasthenic syndromes	Fast channel syndrome	608930	CHRNA1	2q24-q32	AR	substitution and null mutations
11. Congenital myasthenic syndromes	Fast channel syndrome	608930	CHRND	2q33-q34	AR	substitution mutation and deletion
11. Congenital myasthenic syndromes	Fast channel syndrome	608930	CHRNE	17p13-p12	AD	null mutations
11. Congenital myasthenic syndromes	Fast channel syndrome	608930	CHRNE	17p13-p12	AR	null mutations
11. Congenital myasthenic syndromes	Congenital mystenic syndrome with acetylcholine receptor deficiency	608931	CHRNB1	17p12-p11	AR	deletions
11. Congenital myasthenic syndromes	Congenital mystenic syndrome with acetylcholine receptor deficiency	608931	CHRNE	17p13-p12	AR	substitution, transition mutations, deletions, insertions
11. Congenital myasthenic syndromes	Congenital myasthenic syndrome with rapsyn deficiency	608931	RAPSN	11p11.2-p11.1	AR	substitution mustations
11. Congenital myasthenic syndromes	Congenital myasthenic syndrome with choline acetyltransferase deficiency	254210	CHAT	10q11.2	AR	missense, substitution mutations
11. Congenital myasthenic syndromes	End-plate acetylcholin-esterase deficiency(Congenital myasthenic syndrome Engel type)	603034	COLQ	3p25	AR	deletion, splice site, substitution mutations,frameshifts
11. Congenital myasthenic syndromes	Congenital myasthenic syndrome with MuSK deficiency	608931	MUSK	9q31.3-32	AR	substitution mutations, insertion/frameshift
11. Congenital myasthenic syndromes	Familial limb-girdle myasthenia	254300	DOK7	4p16.2	AR	frameshift,nonsense, splice site, missense mutations, deletions
11. Congenital myasthenic syndromes	Myasthenic syndrome due to mutation in SCN4A		SCN4A	17q23.1-q25.3	AR	substitution mutation
11. Congenital myasthenic syndromes	Escobar syndrome (multiple pterygium syndrome)	265000	CHRNG	2q33.34	AR	substitution, nonsense mutations
12. Spinal muscular atrophies	Spinal muscular atrophy , type I (Werdnig-Hoffman)	253300	SMN1	5q12.2-13.3	AR	duplication/premature truncation, deletion/premature stop, missense, substitution mutations, frameshift deletion
12. Spinal muscular atrophies	Spinal muscular atrophy, type II (intermediate)	253550	SMN1	5q12.2-13.3	AR	substitution, missense,nonsense mutations, frameshift deletion, deletion
12. Spinal muscular atrophies	Spinal muscular atrophy, type III (Kugelberg-Welander)	253400	SMN1	5q12.2-13.3	AR	substitution, missense, nonsense mutations, frameshift deletion, deletion
12. Spinal muscular atrophies	Spinal muscular atrophy, type IV, adult form	271150	SMN1	5q12.2-13.3	AR	frameshift deletion

12. Spinal muscular atrophies	Spinal muscular atrophy, distal autosomal recessive 1 (with respiratory distress)	604320	IGHMBP2	11q13.2-q13.4	AR	substitution mutations, transversions, substitution/frameshift, frameshift/nonsense mutation
12. Spinal muscular atrophies	Spinal muscular atrophy, distal autosomal recessive 4 (with respiratory distress)	611067	PLEKHG5	1p36	AR	substitution mutations
12. Spinal muscular atrophies	Neuronopathy, distal hereditary motor, type IIA, juvenile	158590	HSPB8	12q24	AD	substitution mutations
12. Spinal muscular atrophies	Neuronopathy, distal hereditary motor, type IIB, adult juvenile	608634	HSPB1	7q11.23	AD	substitution mutations
12. Spinal muscular atrophies	Distal hereditary motor neuropathy type V (HMN5)	600794	GARS	7p15	AD	substitution mutations
12. Spinal muscular atrophies	Distal spinal muscular atrophy type V	600794	BSCL2	11q13	AD	transition mutation
12. Spinal muscular atrophies	Neuroparhy, distal hereditary motor type VIIB	607641	DCTN1	2p13	AD	substitution mutation
12. Spinal muscular atrophies	Susceptibility to amyotrophic lateral sclerosis	105400	DCTN1	2p13	AD	substitution mutations
12. Spinal muscular atrophies	Familial amyotrophic lateral sclerosis 1	105400	SOD1	21q22.1	AD	substitution, splicing, truncating mutations, deletion
12. Spinal muscular atrophies	Familial amyotrophic lateral sclerosis 1	105400	SOD1	21q22.1	AR	substitution mutations
12. Spinal muscular atrophies	Familial amyotrophic lateral sclerosis 1		VAPB	20q13.3		substitution mutation
12. Spinal muscular atrophies	Amyotrophic lateral sclerosis 2, juvenile	205100	ALS2	2q33.1	AR	deletions
12. Spinal muscular atrophies	Familial amyotrophic lateral sclerosis 4	602433	SETX	9q34	AD	substitution, missense mutations
12. Spinal muscular atrophies	Familial amyotrophic lateral sclerosis 6	608030	FUS	16p11.2	AD	substitution mutation
12. Spinal muscular atrophies	Familial amyotrophic lateral sclerosis 8	608627	VAPB	20q13.3	AD	substitution mutation
12. Spinal muscular atrophies	Familial amyotrophic lateral sclerosis	611895	ANG	14q11.1-q11.2	AD	substitution mutations
12. Spinal muscular atrophies	Familial amyotrophic lateral sclerosis 10 (with or without frontotemporal dementia with TDP43 inclusions)	612069	TARDBP	1p36.2	AD	substitution mutations
12. Spinal muscular atrophies	Amyotrophic lateral sclerosis 11	612577	FIG4	6q21	AD	substitution mutations, premature truncation
12. Spinal muscular atrophies	Spinal and bulbar muscular atrophy (kennedy disease)	313200	AR	Xq11-q12	XR	expansion
12. Spinal muscular atrophies	Lethal Congenital Contracture Syndrome 1	253310	GLE1	9q34	AR	substitution/insertion,substitution mutations
12. Spinal muscular atrophies	Lethal Congenital Contracture Syndrome 2	607598	ERBB3	12q13	AR	substitution mutation
12. Spinal muscular atrophies	Lethal Congenital Contracture Syndrome 3	611369	PIP5K1C	19p13.3	AR	substitution mutation
12. Spinal muscular atrophies	Spinal muscular atrophy with pontocerebellar hypoplasia	607596	VRK1	14q32	AR	substitution mutation
12. Spinal muscular atrophies	Spinal muscular atrophy proximal, late onset (Finkel type)	182980	VAPB	20q13.3	AD	substitution mutation
13. Hereditary ataxias	Spinocerebellar ataxia 1	601556	ATXN1	6p23	AD	expansion
13. Hereditary ataxias	Spinocerebellar ataxia 2	183090	ATXN2	12q24	AD	expansion
13. Hereditary ataxias	Spinocerebellar ataxia 3 (Machado-Joseph disease)	109150	ATXN3	14q24.3-q31	AD	expansion
13. Hereditary ataxias	Spinocerebellar ataxia 5	600224	SPTBN2	11q13	AD	deletions, substitution mutation
13. Hereditary ataxias	Spinocerebellar ataxia 6	183086	CACNA1A	19p13	AD	expansion
13. Hereditary ataxias	Spinocerebellar ataxia 7 (olivopontocerebellar atrophy III)	164500	ATXN7	3p21.1-p12	AD	expansion
13. Hereditary ataxias	Spinocerebellar ataxia 10	603516	ATXN10	22q13	AD	expansion
13. Hereditary ataxias	Spinocerebellar ataxia 11	604432	TTBK2	15q15.2	AD	insertion/premature stop, frameshift deletion/premature stop
13. Hereditary ataxias	Spinocerebellar ataxia 12	604326	PPP2R2B	5q31-q33	AD	expansion
13. Hereditary ataxias	Spinocerebellar ataxia 13	605259	KCNC3	19q13.3-q13.4	AD	substitution mutations
13. Hereditary ataxias	Spinocerebellar ataxia 14	605361	PRKCG	19q13.4	AD	missense, substitution mutations, deletion/substitution, expansion
13. Hereditary ataxias	Spinocerebellar ataxia 15	606658	ITPR1	3p26-p25	AD	deletion, substitution mutation
13. Hereditary ataxias	Spinocerebellar ataxia 17 (Huntington disease-like)	607136	TBP	6q27	AD	expansion
13. Hereditary ataxias	Spinocerebellar ataxia 27	609307	FGF14	13q34	AD	deletion, substitution mutation
13. Hereditary ataxias	Episodic ataxia type-1 , with myokimia	160120	KCNA1	12p13	AD	missense, substitution mutations
13. Hereditary ataxias	Familial hemiplegic migraine	141500	CACNA1A	19p13	AD	missense mutations, deletions
13. Hereditary ataxias	Episodic ataxia type-5	613855	CACNB4	2q22-q23	AD	missense mutations
13. Hereditary ataxias	Episodic ataxia type-6	612656	SLC1A3	5p13	AD	substitution mutations
13. Hereditary ataxias	Friedreich ataxia 1	229300	FXN	9q13	AR	expansions,frameshifts, substitution mutations
13. Hereditary ataxias	Friedreich ataxia 1 with retained reflexes	229300	FXN	9q13	AR	expansion
13. Hereditary ataxias	Friedreich ataxia with selective vitamin E deficiency	277460	TTPA	8q13.1-13.3	AR	deletion/premature stop, insertion/premature stop, substitution, splicing mutations
13. Hereditary ataxias	Mitochondrial DNA depletion syndrome 7 (Hepatocerebral type)	271245	C10orf2	10q24	AR	substitution mutations
13. Hereditary ataxias	Mitochondrial DNA depletion syndrome 4A (Alpers type)	203700	POLG	15q25	AR	substitution mutations
13. Hereditary ataxias	Mitochondrial DNA depletion syndrome 4B (Mingie type)	613662	POLG	15q25	AR	substitution mutations
13. Hereditary ataxias	Ataxia, early onset, with oculomotor apraxia and hypoalbuminemia (EAOH)	208920	APTX	9q13.3	AR	deletions, insertions, splice site, substitution mutations
13. Hereditary ataxias	Autosomal recessive spinocerebellar ataxia, 1		SETX	9q34	AR	truncating, substitution mutations, deletion/frameshift, deletions
13. Hereditary ataxias	Autosomal recessive spinocerebellar ataxia, 8	610743	SYNE1	6q25	AR	deletion/premature truncation, truncating, splicing,nonsense, substitution mutations
13. Hereditary ataxias	Autosomal recessive spinocerebellar ataxia, 9 (with ubiquinone deficiency)	612016	ADCK3	1q42.2	AR	missense,splice site,insertion/deletion, substitution mutations
13. Hereditary ataxias	Spinocerebellar ataxia with axonal neuropathy	607250	TDP1	14q31-q32	AR	missense mutation
13. Hereditary ataxias	Marinesco-Sjögren syndrome (cerebellar ataxia with cataract and myopathy)	248800	SIL1	5q31	AR	splice site, substitution, nonsense, stop codon mutations, duplication/premature truncation, deletion
13. Hereditary ataxias	Sensory ataxic neuropathy, dysarthria and ophtalmoparesis (spinocerebellar ataxia with epilepsy included)	258450	POLG	15q25	AR	substitution mutations
13. Hereditary ataxias	Ataxia telangiectasia	208900	ATM	11q22.3	AR	deletions, insertions, splice site, substitution mutations
13. Hereditary ataxias	Ataxia telangiectasia-like disorder	604391	MRE11A	11q21	AR	substitution mutations
13. Hereditary ataxias	Spastic ataxia, Charlevoix-Saguenay type	270550	SACS	13q12	AR	deletions, deletion/frameshift, deletion/preature truncation, insertions/premature truancation, insertion/frameshift, substitution mutations
13. Hereditary ataxias	Refsum disease-1 (adult)	266500	PHYH	10pter-p11.2	AR	deletions, insertion, deletion/frameshift, substitution mutations
13. Hereditary ataxias	Refsum disease-2 (adult)	266500	PEX7	6q22-q24	AR	truncating mutations, substitution-duplication/frameshift, substitution mutations
13. Hereditary ataxias	Abetalipoproteinemia		MTTP	4q22-q24	AR	substitution, missense, splice site mutations, frameshift/premature truncation, deletions
14. Hereditary motor and sensory neuropathies (HMSN)	Charcot-Marie-Tooth neuropathy Type 1A, demyelinating	118220	PMP22	17p11.2	AD	duplication, substitution mutations
14. Hereditary motor and sensory neuropathies (HMSN)	Charcot-Marie-Tooth neuropathy Type 1A, demyelinating	118220	PMP22	17p11.2	AR	substitution mutation
14. Hereditary motor and sensory neuropathies (HMSN)	Charcot-Marie-Tooth neuropathy Type 1B, demyelinating	118200	MPZ	1q22	AD	substitution mutations,deletions,transversion
14. Hereditary motor and sensory neuropathies (HMSN)	Charcot-Marie-Tooth neuropathy Type 1C	601098	LITAF	16p13.3-p12	AD	substitution mutations
14. Hereditary motor and sensory neuropathies (HMSN)	Charcot-Marie-Tooth neuropathy Type 1D	607678	EGR2	10q21.1-q22.1	AD	substitution mutations
14. Hereditary motor and sensory neuropathies (HMSN)	Charcot-Marie-Tooth neuropathy and deafness (Type 1E)	118300	PMP22	17p11.2	AD	substitution mutations, deletion
14. Hereditary motor and sensory neuropathies (HMSN)	Charcot-Marie-Tooth neuropathy Type 1F	607734	NEFL	8p21	AD	substitution mutations, deletion

14. Hereditary motor and sensory neuropathies (HMSN)	Hereditary neuropathy with liability to pressure palsies	162500	PMP22	17p11.2	AD	deletions, insertion/frameshft, deltion/premature truncation, substitution mutations
14. Hereditary motor and sensory neuropathies (HMSN)	Charcot-Marie-Tooth disease with foot deformity and congenital vertical talus	192950	HOXD10	2q31-q32	AD	substitution mutation
14. Hereditary motor and sensory neuropathies (HMSN)	Slowed nerve conduction velocity	608236	ARHGEF10	8p23	AD	transition mutation
14. Hereditary motor and sensory neuropathies (HMSN)	Charcot-Marie-Tooth neuropathy, dominant intermediate B	606482	DNM2	19p13.2	AD	substitution mutations, deletions
14. Hereditary motor and sensory neuropathies (HMSN)	Charcot-Marie-Tooth neuropathy, dominant intermediate C	608323	YARS	1p35	AD	missense mutations, deletion
14. Hereditary motor and sensory neuropathies (HMSN)	Charcot-Marie-Tooth neuropathy, dominant intermediate D	607791	MPZ	1q22	AD	substitution mutation
14. Hereditary motor and sensory neuropathies (HMSN)	Charcot-Marie-Tooth neuropathy Type 4A	214400	GDAP1	8q13-q21.1	AR	nonsense, substitution mutations, premature truncation
14. Hereditary motor and sensory neuropathies (HMSN)	Charcot-Marie-Tooth neuropathy Type 4B1	601382	MTMR2	11q22	AR	substitution mutations, substitution/deletion, deletion-insertion/frameshift premature truncation
14. Hereditary motor and sensory neuropathies (HMSN)	Charcot-Marie-Tooth neuropathy Type 4B2	604563	SBF2	11p15	AR	deletion, nonsense mutations/premature truncation
14. Hereditary motor and sensory neuropathies (HMSN)	Charcot-Marie-Tooth neuropathy Type 4C	601569	SH3TC2	5q32	AR	nonsense, substitution mutations, deletion/premature stop
14. Hereditary motor and sensory neuropathies (HMSN)	Charcot-Marie-Tooth neuropathy Type 4D (HMSN Lom, with deafness)	601455	NDRG1	8q24.3	AR	nonsense, substitution mutations
14. Hereditary motor and sensory neuropathies (HMSN)	Charcot-Marie-Tooth neuropathy Type 4E (congenital hypomyelinating myopathy)	605253	EGR2	10q21.1-q22.1	AD	substitution mutations
14. Hereditary motor and sensory neuropathies (HMSN)	Charcot-Marie-Tooth neuropathy Type 4E (congenital hypomyelinating myopathy)	605253	EGR2	10q21.1-q22.1	AR	substitution mutations
14. Hereditary motor and sensory neuropathies (HMSN)	Charcot-Marie-Tooth neuropathy Type 4E (congenital hypomyelinating myopathy)	605253	MPZ	1q22	AD	substitutions,insertion/deletion
14. Hereditary motor and sensory neuropathies (HMSN)	Charcot-Marie-Tooth neuropathy Type 4F	145900	PRX	19q13.1-q13.2	AR	insertion, deletion/frameshift, nonsense mutation
14. Hereditary motor and sensory neuropathies (HMSN)	Charcot-Marie-Tooth neuropathy Type 4H	609311	FGD4	12p11.2	AR	nonsense, substitution mutation, deletion/frameshift
14. Hereditary motor and sensory neuropathies (HMSN)	Charcot-Marie-Tooth neuropathy Type 4J	611228	FIG4	6q21	AR	substitution, frameshifts, deletions
14. Hereditary motor and sensory neuropathies (HMSN)	Charcot-Marie-Tooth neuropathy X-linked 1	302800	GJB1	Xq13.1	XD	substitution mutations, deletion/frameshifts, deletions
14. Hereditary motor and sensory neuropathies (HMSN)	Charcot-Marie-Tooth neuropathy X-linked 5 (with hearing loss and optic neuropathy)	311070	PRPS1	Xq22-q24	XR	substitution mutations
14. Hereditary motor and sensory neuropathies (HMSN)	Déjerine-Sottas hypertrophic neuropathy	145900	PMP22	17p11.2	AD	substitution mutation, deletions
14. Hereditary motor and sensory neuropathies (HMSN)	Déjerine-Sottas hypertrophic neuropathy	145900	PMP22	17p11.2	AR	substitution mutation, deletions
14. Hereditary motor and sensory neuropathies (HMSN)	Déjerine-Sottas hypertrophic neuropathy	145900	MPZ	1q22	AD	substitution mutations
14. Hereditary motor and sensory neuropathies (HMSN)	Déjerine-Sottas hypertrophic neuropathy	145900	MPZ	1q22	AR	deletion
14. Hereditary motor and sensory neuropathies (HMSN)	Déjerine-Sottas hypertrophic neuropathy	145900	EGR2	10q21.1-q22.1	AD	substitution mutation
14. Hereditary motor and sensory neuropathies (HMSN)	Déjerine-Sottas hypertrophic neuropathy	145900	EGR2	10q21.1-q22.1	AR	substitution mutation
14. Hereditary motor and sensory neuropathies (HMSN)	Déjerine-Sottas hypertrophic neuropathy		GJB1	10q21.1-q22.1	AD	substitution mutation
14. Hereditary motor and sensory neuropathies (HMSN)	Déjerine-Sottas hypertrophic neuropathy		GJB1	10q21.1-q22.1	AR	substitution mutation
14. Hereditary motor and sensory neuropathies (HMSN)	Déjerine-Sottas hypertrophic neuropathy	145900	PRX	19q13.1-q13.2	AR	substitution, nonsense mutations, deletions/frameshifts
14. Hereditary motor and sensory neuropathies (HMSN)	Charcot-Marie-Tooth neuropathy Type 2A1	118210	KIF1B	1p36	AD	substitution mutation
14. Hereditary motor and sensory neuropathies (HMSN)	Charcot-Marie-Tooth neuropathy Type 2A2	609260	MFN2	1p36.2	AD	substitution, missense mutations
14. Hereditary motor and sensory neuropathies (HMSN)	Charcot-Marie-Tooth neuropathy Type 2B	600882	RAB7A	3q21	AD	substitution, missense mutations
14. Hereditary motor and sensory neuropathies (HMSN)	Charcot-Marie-Tooth neuropathy Type 2D	601472	GARS	7p15	AD	substitution mutations
14. Hereditary motor and sensory neuropathies (HMSN)	Charcot-Marie-Tooth neuropathy Type 2E	607684	NEFL	8p21	AD	substitution mutations, duplication/insertion
14. Hereditary motor and sensory neuropathies (HMSN)	Charcot-Marie-Tooth neuropathy Type 2F	606595	HSPB1	7q11.23	AD	substitution mutations
14. Hereditary motor and sensory neuropathies (HMSN)	Charcot-Marie-Tooth neuropathy Type 2I (late onset)	607677	MPZ	1q22	AD	missense, substitution mutations
14. Hereditary motor and sensory neuropathies (HMSN)	Charcot-Marie-Tooth neuropathy Type 2J (with hearing loss and pupillary abnormality)	607736	MPZ	1q22	AD	substitution mutations
14. Hereditary motor and sensory neuropathies (HMSN)	Charcot-Marie-Tooth neuropathy Type 2K	607831	GDAP1	8q13-q21.1	AD	substitution mutations
14. Hereditary motor and sensory neuropathies (HMSN)	Charcot-Marie-Tooth neuropathy Type 2K	607831	GDAP1	8q13-q21.1	AR	substitution mutations
14. Hereditary motor and sensory neuropathies (HMSN)	Charcot-Marie-Tooth neuropathy Type 2L	608673	HSPB8	12q24	AD	substitution mutations
14. Hereditary motor and sensory neuropathies (HMSN)	Charcot-Marie-Tooth neuropathy recessive intermediate A	608340	GDAP1	8q13-q21.1	AR	substituion, splice site mutations, insertion
14. Hereditary motor and sensory neuropathies (HMSN)	Charcot-Marie-Tooth neuropathy Autosomal recessive CMT2B1	605588	LMNA	1q21.2	AR	substitution mutation
14. Hereditary motor and sensory neuropathies (HMSN)	Charcot Marie-Tooth neuropathy, axonal, with vocal cord paresis	607706	GDAP1	8q13-q21.1	AR	substitution mutations, insertion/frameshift
14. Hereditary motor and sensory neuropathies (HMSN)	Hereditary sensory (and autonomic) neuropathy type I	162400	SPTLC1	9q22.1-22.3	AD	substitution mutations
14. Hereditary motor and sensory neuropathies (HMSN)	Hereditary sensory and autonomic neuropathy type II	201300	WNK1	12p13	AR	deletions/premature truncation, insertions/premature truncation, substitution/premature truncation, deletion
14. Hereditary motor and sensory neuropathies (HMSN)	Hereditary sensory and autonomic neuropathy type III (Familial dysautonomia, Riley-Day syndrome)	223900	IKBKAP	9q31	AR	splice site mutation/deletion, substitution mutation
14. Hereditary motor and sensory neuropathies (HMSN)	Hereditary sensory and autonomic neuropathy type V	608654	NGF		AR	substitution mutations,deletion
14. Hereditary motor and sensory neuropathies (HMSN)	Distal hereditary motor neuropathy type IIA	158590	HSPB8	12q24	AD	substitution mutations
14. Hereditary motor and sensory neuropathies (HMSN)	Hereditary motor and sensory neuropathy, type VI	601152	MFN2	1p36.2	AD	substitution mutations
14. Hereditary motor and sensory neuropathies (HMSN)	Agenesis of the corpus callosum with peripheral neuropathy (Charlevoix disease)	218000	SLC12A6	15q13-q14	AR	deletion/premature truncation, deletion-insertion/premature truncation, substitution mutations
14. Hereditary motor and sensory neuropathies (HMSN)	Giant axonal neuropathy	256850	GAN	16q24.1	AR	substitution and nonsense mutations
14. Hereditary motor and sensory neuropathies (HMSN)	Congenital cataracts, facial dysmorphism and neuropathy	604168	CTDP1	18q23	AR	insertion
14. Hereditary motor and sensory neuropathies (HMSN)	Roussy-Levy syndrome	180800	PMP22	17p11.2	AD	duplication
15. Hereditary paraplegias	Spastic paraplegia 3 (Strumpell disease)	182600	SPG3A or ATL1	14q11-q21	AD	substitution mutations, insertion/premature stop, deletions
15. Hereditary paraplegias	Spastic paraplegia 4	182601	SPAST	2p22-p21	AD	substitution, truncating, splicing/frameshift, missense mutations, insertion/premature truncation, deletions, duplication
15. Hereditary paraplegias	Spastic paraplegia 6	600363	NIPA1	15q11.1	AD	substitution mutations
15. Hereditary paraplegias	Spastic paraplegia 7	607259	SPG7	16q24.3	AR	substitution mutations, deletion/premature truncation, insertion-deletion/premature truncation, deletions
15. Hereditary paraplegias	Spastic paraplegia 8	603563	KIAA0196	8q24.13	AD	substitution mutations
15. Hereditary paraplegias	Spastic paraplegia 10	604187	KIF5A	12q13	AD	substitution mutations
15. Hereditary paraplegias	Spastic paraplegia 11	604360	SPG11	15q21.1	AR	deletion/premature truncation,substitution, truncating, splice site mutations, duplication/premature truncation
15. Hereditary paraplegias	Spastic paraplegia 13	605280	HSPD1	2q33.1	AD	substitution mutation

15. Hereditary paraplegias	Spastic paraplegia 15 (Kjellin syndrome)	270700	ZFYVE26	14q24.1	AR	substitution, splice site mutations, deletion/premature truncation
15. Hereditary paraplegias	Spastic paraplegia 17 (Silver)	270685	BSCL2	11q13	AD	transition mutation
15. Hereditary paraplegias	Spastic paraplegia 20 (Troyer)	275900	SPG20	13q12.3	AR	deletions/premature truncation
15. Hereditary paraplegias	Spastic paraplegia 21 (Mast)	248900	SPG21	15q21-q22	AR	insertion/prematute truncation
15. Hereditary paraplegias	Spastic paraplegia 31	610250	REEP1	2p11.2	AD	deletion, splice site, substitution mutation
15. Hereditary paraplegias	Spastic paraplegia 33	610244	ZFYVE27	10q24.2	AD	substitution mutation
15. Hereditary paraplegias	Spastic paraplegia 39	612020	PNPLA6	19p13.3	AR	missense, substitution mutations, insertion, insertion/premature truncation
15. Hereditary paraplegias	Spastic paraplegia, complicated recessive (Sjögren-Larsson syndrome)	270200	ALDH3A2	17p11.2	AR	insertions, deletions, substitution mutations
15. Hereditary paraplegias	Infantile-onset ascending spastic paraplegia	607225	ALS2	2q33.1	AR	deletions, substitution mutations
15. Hereditary paraplegias	Spastic paraplegias, X-linked type 1 (MASA syndrome)	303350	L1CAM	Xq28	XR	deletions, substitution mutations
15. Hereditary paraplegias	Spastic paraplegias, X-linked type 2	312920	PLP1	Xq22	XR	substitution mutations
15. Hereditary paraplegias	Autosomal recessive spastic ataxia of Charlevoix-Saguenay	270550	SACS	13q12	AR	deletion/premature truncation, insertion/premature truncation, substitution mutations
16. Other neuromuscular disorders	Torsion dystonia, early onset	128100	TOR1A	9q34	AD	substitution mutations, deletions
16. Other neuromuscular disorders	Myoclonus-dystonia syndrome	159900	SGCE	7q21	AD	deletion/premature stop, substitution, nonsense mutations, insertion/premature stop, deletion
16. Other neuromuscular disorders	Amylodosis, hereditary, transthyrene-related	105210	TTR	18q11.2-12-1	AD	substitution mutations, deletion
16. Other neuromuscular disorders	Congenital fibrosis of the extraocular muscles 1	135700	KIF21A	12q12	AD	substitution mutations
16. Other neuromuscular disorders	Congenital fibrosis of the extraocular muscles 3B	135700	KIF21A	12q12	AD	substitution mutations
16. Other neuromuscular disorders	Congenital fibrosis of the extraocular muscles	602078	PHOX2A	11q13.3-13.4	AR	splice site, substitution mutations
16. Other neuromuscular disorders	Distal arthrogryposis type 1	108120	TPM2	9p13.2-p13.1	AD	substitution mutations
16. Other neuromuscular disorders	Distal arthrogryposis type 2A, Freeman-Sheldon syndrome	193700	MYH3	17p13.1	AD	subsitution mutations
16. Other neuromuscular disorders	Distal arthrogryposis type 2B, Sheldon-Hall syndrome	601680	TNNI2	11p15.5	AD	deletions
16. Other neuromuscular disorders	Distal arthrogryposis type 2B, Sheldon-Hall syndrome	601680	TNNT3	11p15.5	AD	substitution mutation
16. Other neuromuscular disorders	Distal arthrogryposis type 2B, Sheldon-Hall syndrome	601680	MYH3	17p13.1	AD	deletion,substitution mutations
16. Other neuromuscular disorders	Distal arthrogryposis type 2B, Sheldon-Hall syndrome	601680	TPM2	9p13.2-p13.1	AD	substituion mutations
16. Other neuromuscular disorders	Trismus- pseudocamptodactyly syndrome	158300	MYH8	17p13.1	AD	substitution mutation
16. Other neuromuscular disorders	Progressive external ophthalmoplegia with mitochondrial DNA deletions, autosomal dominant, 1	157640	POLG	15q25	AD	substitution mutations
16. Other neuromuscular disorders	Progressive external ophthalmoplegia with mitochondrial DNA deletions, autosomal Recessive,	258450	POLG	15q25	AR	substitution mutations
16. Other neuromuscular disorders	Progressive external ophthalmoplegia with mitochondrial DNA deletions, autosomal dominant, 2	609283	SLC25A4	4p35	AD	substitution mutations
16. Other neuromuscular disorders	Progressive external ophthalmoplegia with mitochondrial DNA deletions, autosomal dominant, 3	609286	C10orf2	10q24	AD	substitution mutations, duplication
16. Other neuromuscular disorders	Progressive external ophthalmoplegia with mitochondrial DNA deletions, autosomal dominant, 4; PEOA4	610131	POLG2	17q23-q24	AD	substitution mutation
16. Other neuromuscular disorders	Progressive external ophthalmoplegia with mitochondrial DNA deletions, autosomal dominant, 4	613077	RRM2B	8q23.1	AD	substitution mutations/premature truncation
16. Other neuromuscular disorders	Mitochondrial DNA depletion syndrome 2	609560	TK2	16q22	AR	substitution mutations
16. Other neuromuscular disorders	Mitochondrial DNA depletion syndrome (encephalomyopathic type with methylmalonic aciduria)	612073	SUCLA2	13q12.2-q13	AR	insertion/deletion, truncating, substitution mutations
16. Other neuromuscular disorders	Mitochondrial DNA depletion syndrome 8A (encephalomyopathic type with renal tubulopathy)	612075	RRM2B	8q23.1	AR	substitutions/deletions, substitution, splice site mutations, deletions,
16. Other neuromuscular disorders	Mitochondrial DNA depletion syndrome 8B (Mngie type)	612075	RRM2B	8q23.1	AR	substitution mutations
16. Other neuromuscular disorders	Optic atrophy 1 with deafness (with external ophthalmoplegia and myopathy)	125250	OPA1	3q28-q29	AD	substitution mutations, deletion/premature truncation
16. Other neuromuscular disorders	Amyloid polyneuropathy-nephropathy, Iowa type	105200	APOA1	11q23	AD	substitution mutations
16. Other neuromuscular disorders	Apolipoproteins A-I and C-III, combined deficiency		APOA1/APOC3	11q23	AD	inversion and fusion of the two genes
16. Other neuromuscular disorders	Apolipoprotein A-I deficiency		APOA1	11q23	AD	nonsense mutation
16. Other neuromuscular disorders	Hypoalphalipoproteinemia, primary	604091	APOA1	11q23	AD	insertion
16. Other neuromuscular disorders	Congenital generalized lipodystrophy	269700	BSCL2	11q13	AR	frameshifts,substituitions,deletion/insertion, deletions
16. Other neuromuscular disorders	Familial lipodystrophy type 2, partial	151660	LMNA	1q21.2	AD	splicing, substitution mutations
16. Other neuromuscular disorders	Coenzyme Q(10) deficiency	607426	ADCK3	1q42.2	AR	substitution mutations, insertions
16. Other neuromuscular disorders	Multiple pterygium syndrome	253290	CHRNA1	2q24-q32	AR	duplication
16. Other neuromuscular disorders	Multiple pterygium syndrome	253290	CHRND	2q33-q34	AR	substitution, nonsense, missense mutations
16. Other neuromuscular disorders	Multiple pterygium syndrome	253290	CHRNG	2q33-q34	AR	missense mutations, deletions
16. Other neuromuscular disorders	Ossification of the posterior longitudinal ligament of spine	602475	COL6A1	21q22.3	AR	SNP
16. Other neuromuscular disorders	scapuloperenal syndrome neurogenic, Kaeser type	181400	DES	2q35	AD	substitution mutation
16. Other neuromuscular disorders	lethal arthrogryposis with anterior horn cell disease	611890	GLE1	9q34		substitution/insertion, missense mutations
16. Other neuromuscular disorders	Amyloidosis, familial, finnish-type	105120	GSN	9q34	AD	substitution mutations
16. Other neuromuscular disorders	X-linked hydrocephalus	307000	L1CAM	Xq28	XR	insertion/deletion, substitution mutations, deletions, frameshift/premature truncation
16. Other neuromuscular disorders	Mandibuloacral dysplasia with type A lipodystrophy	248370	LMNA	1q21.2	AD	substitution mutations
16. Other neuromuscular disorders	Heart-hand syndrome, slovenian type	610140	LMNA	1q21.2	AD	frameshift/premature truncation
16. Other neuromuscular disorders	Hutchinson-Gilford progeria syndrome	176670	LMNA	1q21.2	AD	substitution mutations, substitution/deletion
16. Other neuromuscular disorders	Pelizaeus-Merzbacher disease	312080	PLP1	Xq22	XR	substitution, splicing mutations, duplication/insertion
16. Other neuromuscular disorders	Neutral lipid storage disease with myopathy	610717	PNPLA2	11p15.5		deletions/frameshifts, missense, substitution mutations
16. Other neuromuscular disorders	Fetal akinesia deformation sequence	208150	RAPSN	11p11.2-p11.1	AR	deletions, substitution mutations
16. Other neuromuscular disorders	Carnitine deficiency systemic primary	212140	SLC22A5	5q31.1	AR	deletion/protein truncation, insertion/frameshift, substitution, missense, mutations, splice site mutation/premature stop
16. Other neuromuscular disorders	Carnitine-acylcarnitine translocase deficiency	212138	SLC25A20	3p21.31	AR	substitution, splicing mutations, insertion, deletion/premature stop, stop codon mutation

Supplementary Table 2: Coordinates, GC content and mean coverage of targeted exons in each patient

Chr	Start	End	Gene	GC %	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
chr1	100316519	100316759	AGL	0,34	241	278	181	152	202	213	181	124	265	253	236	222	232	225	256	195
chr1	100326968	100327328	AGL	0,32	273	321	243	151	214	255	212	159	283	290	303	248	289	313	318	203
chr1	100327715	100328075	AGL	0,33	261	282	211	152	220	220	194	127	276	255	260	217	253	270	272	204
chr1	100329863	100330223	AGL	0,33	333	366	289	191	257	284	249	169	362	367	357	278	360	344	387	254
chr1	100335866	100336226	AGL	0,34	291	305	223	172	226	225	195	135	306	310	284	236	295	300	302	230
chr1	100336249	100336489	AGL	0,28	216	261	176	132	191	198	164	120	258	242	223	208	239	252	240	173
chr1	100340184	100340424	AGL	0,32	229	252	201	160	200	219	172	126	261	239	250	211	240	244	265	175
chr1	100340640	100341082	AGL	0,33	260	300	211	163	218	216	187	140	294	274	261	224	279	267	267	198
chr1	100342009	100342225	AGL	0,37	226	233	180	135	183	195	154	125	221	238	233	208	227	215	220	177
chr1	100343110	100343470	AGL	0,36	276	283	221	144	219	227	196	134	296	280	254	228	251	267	289	203
chr1	100345420	100345660	AGL	0,35	255	272	209	136	206	195	176	123	281	252	224	201	238	241	279	202
chr1	100346089	100346449	AGL	0,36	304	328	253	168	247	264	209	156	333	320	284	256	307	319	321	229
chr1	100346562	100347276	AGL	0,37	319	336	249	164	253	259	230	153	320	334	322	268	309	329	354	229
chr1	100349601	100350311	AGL	0,32	315	363	261	174	241	266	235	158	335	336	332	281	339	337	347	233
chr1	100353478	100353718	AGL	0,39	227	243	168	121	173	194	155	120	246	234	228	190	222	242	239	175
chr1	100356723	100356963	AGL	0,35	221	262	183	150	196	190	167	124	257	232	224	187	230	220	240	190
chr1	100357108	100357348	AGL	0,37	285	309	243	153	229	248	207	142	364	314	290	245	307	306	313	224
chr1	100357895	100358255	AGL	0,33	282	331	244	163	230	260	217	140	324	315	299	251	298	298	332	246
chr1	100361772	100362012	AGL	0,37	188	193	162	127	176	168	136	97	218	195	198	166	210	193	212	157
chr1	100366124	100366484	AGL	0,37	243	272	238	149	220	221	179	120	297	262	265	207	256	261	271	207
chr1	100368174	100368414	AGL	0,37	183	202	145	113	178	154	145	98	235	205	202	162	179	183	190	155
chr1	100376231	100376471	AGL	0,33	176	194	147	106	164	172	133	111	226	183	197	168	198	190	175	152
chr1	100377896	100378136	AGL	0,33	219	231	167	118	180	182	134	116	208	205	215	170	209	219	198	157
chr1	100379008	100379368	AGL	0,35	276	325	224	159	219	236	194	137	306	303	284	241	289	287	314	213
chr1	100380873	100381113	AGL	0,33	246	253	201	140	214	215	190	126	268	251	259	219	270	263	257	173
chr1	100381886	100382366	AGL	0,30	300	332	247	174	229	246	217	159	327	282	306	263	297	299	316	227
chr1	100387028	100387268	AGL	0,33	257	252	199	138	194	227	180	133	280	252	274	227	251	249	253	202
chr1	10292319	10292559	KIF1B	0,42	176	176	157	98	168	160	142	100	197	166	185	160	193	171	174	127
chr1	10316222	10316462	KIF1B	0,39	310	326	254	175	260	254	230	142	312	308	320	259	315	288	328	238
chr1	10318460	10318820	KIF1B	0,35	276	310	221	158	213	222	203	133	282	287	268	226	279	250	302	227
chr1	10321875	10322115	KIF1B	0,41	265	280	217	165	232	252	185	136	282	255	262	234	278	263	278	205
chr1	10327370	10327706	KIF1B	0,44	168	193	154	125	171	148	135	102	208	180	196	166	181	183	189	166
chr1	10328145	10328385	KIF1B	0,43	166	169	118	107	158	170	119	85	178	149	163	130	174	167	175	140
chr1	10331473	10331689	KIF1B	0,37	235	238	181	130	202	179	166	125	233	234	225	201	212	213	222	184
chr1	10332211	10332451	KIF1B	0,32	228	257	191	131	194	195	172	121	271	236	238	200	255	241	230	180
chr1	10335403	10335643	KIF1B	0,33	206	217	155	128	146	183	152	117	219	223	223	174	198	201	207	162
chr1	10336297	10336537	KIF1B	0,38	256	273	224	152	213	234	177	126	294	263	271	216	270	264	248	198
chr1	10337934	10338294	KIF1B	0,35	257	301	214	148	212	207	203	135	277	244	254	208	259	259	280	189
chr1	10342393	10342633	KIF1B	0,46	215	224	184	131	204	189	151	121	244	205	213	196	213	198	223	169
chr1	10351059	10351299	KIF1B	0,37	180	212	147	107	168	141	130	90	190	189	186	152	166	174	172	147
chr1	10352022	10352262	KIF1B	0,39	145	137	108	89	125	124	99	76	144	128	138	104	141	122	146	121
chr1	10355063	10355303	KIF1B	0,41	286	306	223	144	236	245	197	154	325	317	290	233	329	278	311	212
chr1	10355650	10355890	KIF1B	0,46	84	87	60	55	84	79	62	55	99	85	90	88	77	77	78	75
chr1	10356561	10356801	KIF1B	0,44	140	128	106	90	127	111	86	79	168	124	136	122	132	128	129	115
chr1	10356923	10357379	KIF1B	0,42	226	243	176	134	220	190	162	117	267	227	231	194	230	224	238	189
chr1	10363204	10364764	KIF1B	0,46	196	200	157	121	186	170	144	110	223	192	206	176	202	207	203	167
chr1	10380098	10380290	KIF1B	0,39	105	109	75	76	110	105	77	63	110	106	106	90	103	111	105	87
chr1	10381660	10382020	KIF1B	0,45	169	177	136	100	163	139	131	98	187	184	164	141	161	170	171	136
chr1	10383850	10384210	KIF1B	0,43	267	284	250	161	265	262	213	134	319	306	295	250	294	284	314	227
chr1	10384764	10385004	KIF1B	0,41	122	127	78	75	125	100	86	69	131	111	132	105	120	116	112	104
chr1	10386112	10386472	KIF1B	0,55	167	163	130	109	145	142	119	84	187	154	153	148	161	169	174	136
chr1	10394516	10394756	KIF1B	0,50	113	124	99	82	123	115	80	65	140	119	132	114	105	107	119	89
chr1	10396637	10396877	KIF1B	0,41	225	265	190	144	216	216	172	123	284	246	246	201	249	233	255	187
chr1	10397076	10397316	KIF1B	0,42	236	244	199	156	234	207	180	125	257	249	243	208	237	255	254	184
chr1	10397329	10397689	KIF1B	0,47	327	337	257	180	275	279	224	164	348	344	311	273	317	326	354	236
chr1	10399751	10399991	KIF1B	0,42	223	239	183	138	200	209	160	123	251	227	231	194	217	216	226	175
chr1	10402046	10402286	KIF1B	0,36	276	326	243	164	253	263	220	149	314	308	295	264	292	280	318	212

chr1	10403197	10403437	KIF1B	0,42	268	266	218	151	215	215	177	126	280	276	291	233	279	283	275	190
chr1	10405836	10406076	KIF1B	0,38	214	215	161	125	182	178	146	111	231	203	196	182	196	214	207	156
chr1	10407731	10407971	KIF1B	0,41	123	142	108	90	129	115	103	80	149	131	143	105	144	136	108	115
chr1	10408626	10408842	KIF1B	0,46	152	158	111	110	140	121	106	79	179	127	152	134	139	156	143	111
chr1	10412621	10412861	KIF1B	0,44	220	212	150	114	197	176	132	112	235	201	199	155	204	209	207	157
chr1	10420923	10421163	KIF1B	0,48	237	261	178	137	225	202	169	134	266	251	242	199	256	242	234	196
chr1	10421696	10421936	KIF1B	0,50	206	201	167	130	178	184	141	102	233	220	210	186	232	213	243	179
chr1	10423251	10423467	KIF1B	0,34	192	189	155	106	157	142	134	110	229	181	180	158	213	187	202	138
chr1	10425074	10425766	KIF1B	0,49	182	192	149	117	167	160	137	102	210	180	188	161	174	192	183	156
chr1	10428464	10428680	KIF1B	0,45	184	213	155	113	173	182	134	106	218	190	191	169	216	206	205	137
chr1	10431139	10431379	KIF1B	0,47	230	248	192	132	214	232	170	137	259	256	230	201	229	232	252	184
chr1	10434268	10434628	KIF1B	0,43	269	268	229	172	254	226	196	137	304	296	273	235	251	269	294	219
chr1	10434827	10435187	KIF1B	0,45	225	236	161	129	201	184	158	112	232	220	213	186	213	200	217	165
chr1	10435231	10435447	KIF1B	0,46	195	201	152	119	160	163	142	111	211	193	173	176	187	179	193	170
chr1	10436503	10436743	KIF1B	0,51	194	176	157	121	166	173	124	110	220	209	195	162	196	189	190	160
chr1	11073723	11074083	TARDBP	0,45	101	111	76	73	108	83	69	54	112	82	99	91	90	92	94	81
chr1	11076802	11077138	TARDBP	0,35	254	256	222	159	230	230	189	131	285	256	256	226	264	248	263	205
chr1	11078679	11079039	TARDBP	0,39	244	272	205	146	203	216	181	142	283	252	251	224	249	263	270	192
chr1	11080379	11080691	TARDBP	0,43	186	183	163	122	193	174	166	116	212	196	209	190	208	212	195	160
chr1	11082085	11082805	TARDBP	0,44	208	214	160	130	183	163	144	114	216	200	210	164	209	200	206	153
chr1	115828633	115829473	NGF	0,56	153	136	115	101	146	126	103	87	173	144	152	133	149	158	160	126
chr1	116243949	116244141	CASQ2	0,51	79	70	64	62	77	67	53	47	90	75	87	63	74	70	63	74
chr1	116245458	116245698	CASQ2	0,50	99	81	64	71	102	80	60	56	99	79	90	74	95	92	93	68
chr1	116247742	116247982	CASQ2	0,54	43	39	29	30	51	38	31	25	46	42	46	28	40	44	34	40
chr1	116260367	116260607	CASQ2	0,47	107	102	88	83	112	109	70	69	127	112	120	99	109	96	98	99
chr1	116268068	116268188	CASQ2	0,45	32	45	25	27	40	39	18	20	58	25	41	34	31	31	28	31
chr1	116269557	116269797	CASQ2	0,45	141	144	90	101	121	130	88	78	155	143	156	108	130	142	142	121
chr1	116275438	116275678	CASQ2	0,37	174	187	138	110	152	155	123	83	193	148	166	144	180	170	162	133
chr1	116280780	116281020	CASQ2	0,44	241	232	170	137	205	176	171	129	261	248	257	212	230	246	266	188
chr1	116283278	116283518	CASQ2	0,45	191	207	150	118	170	167	130	84	244	193	191	154	199	196	198	158
chr1	116287370	116287610	CASQ2	0,40	278	308	246	151	224	244	198	156	328	291	287	238	287	277	312	203
chr1	116310865	116311225	CASQ2	0,45	226	247	189	134	202	200	147	116	254	241	239	202	228	240	243	186
chr1	11906008	11906128	NPPA	0,54	44	49	38	33	52	45	47	42	53	45	48	41	58	53	56	45
chr1	11907092	11907796	NPPA	0,57	137	120	108	99	132	121	106	75	149	119	130	113	133	125	124	112
chr1	12049132	12049492	MFN2	0,52	187	159	136	111	168	146	127	97	191	168	173	158	178	168	180	141
chr1	12052559	12052799	MFN2	0,53	128	115	93	83	133	97	80	72	130	110	130	116	123	117	109	88
chr1	12056113	12056473	MFN2	0,57	96	102	77	69	100	85	69	61	115	90	98	84	88	103	98	81
chr1	12057295	12057535	MFN2	0,56	130	136	101	83	126	112	88	86	153	121	123	122	137	125	137	112
chr1	12058760	12059218	MFN2	0,56	143	125	105	100	133	115	103	80	147	118	138	122	132	124	138	115
chr1	12061354	12061714	MFN2	0,54	70	70	54	59	76	73	51	42	87	74	75	65	74	74	68	68
chr1	12061739	12062219	MFN2	0,56	93	92	75	77	101	95	68	49	118	91	110	90	101	101	91	87
chr1	12063991	12064231	MFN2	0,48	103	101	90	69	105	101	68	53	111	94	112	91	105	111	99	82
chr1	12064497	12064737	MFN2	0,51	94	107	74	75	96	97	76	62	114	98	103	87	100	112	89	88
chr1	12064812	12065052	MFN2	0,57	54	47	39	50	49	48	40	37	54	44	50	53	58	49	44	46
chr1	12065697	12066057	MFN2	0,55	201	210	167	140	189	184	144	122	238	196	209	183	227	220	211	164
chr1	12066492	12066852	MFN2	0,62	98	96	81	82	98	87	70	64	107	91	108	87	104	100	80	91
chr1	12067027	12067387	MFN2	0,61	33	29	30	26	40	30	29	26	37	29	41	31	26	36	35	35
chr1	12069595	12069835	MFN2	0,57	66	69	58	59	74	55	55	38	71	61	68	56	70	72	69	57
chr1	12071491	12071707	MFN2	0,56	29	30	21	28	41	28	17	22	37	24	31	32	27	27	28	23
chr1	147230207	147231407	GJA5	0,56	111	108	94	82	114	104	81	67	136	107	119	105	116	118	112	104
chr1	154130035	154130275	TPM3	0,52	132	120	95	90	118	108	86	72	135	115	115	107	120	112	121	99
chr1	154131355	154131571	TPM3	0,49	77	86	50	50	100	75	48	52	104	75	86	70	88	86	75	72
chr1	154140354	154140474	TPM3	0,45	11	16	10	11	18	9	10	7	16	12	17	17	15	8	14	16
chr1	154141699	154141939	TPM3	0,51	154	162	122	107	141	142	109	83	170	140	159	127	144	141	131	129
chr1	154142790	154143030	TPM3	0,42	340	342	274	156	249	278	225	164	326	336	319	283	325	301	341	238
chr1	154143035	154143275	TPM3	0,35	350	359	289	205	283	300	253	171	344	369	357	297	363	343	379	256
chr1	154143806	154144046	TPM3	0,49	134	139	107	94	130	119	95	79	159	123	134	110	136	138	137	122
chr1	154144449	154144665	TPM3	0,47	201	234	173	126	183	176	150	117	224	204	208	177	217	214	208	165
chr1	154145298	154145738	TPM3	0,45	291	312	257	183	254	251	231	147	304	326	303	261	316	308	308	226
chr1	154148537	154148777	TPM3	0,51	230	243	193	138	199	204	173	138	250	230	231	193	246	230	233	184

chr1	154155409	154155649	TPM3	0,65	83	93	82	67	97	91	70	60	107	79	85	98	90	98	91	84
chr1	154163604	154163844	TPM3	0,46	320	348	292	182	257	287	233	167	372	339	309	301	351	318	360	241
chr1	154164315	154164555	TPM3	0,49	278	317	233	167	256	237	200	152	352	316	287	250	299	283	333	229
chr1	156084643	156085123	LMNA	0,70	12	10	9	9	9	14	9	8	12	7	10	10	11	10	10	14
chr1	156100305	156100665	LMNA	0,60	45	35	35	36	49	45	27	23	49	34	45	45	43	41	33	37
chr1	156104144	156104384	LMNA	0,56	38	43	33	31	46	38	33	29	52	39	43	38	46	57	39	36
chr1	156104500	156104860	LMNA	0,59	59	52	38	41	58	43	36	32	54	50	53	50	52	49	53	48
chr1	156104920	156105160	LMNA	0,65	10	12	10	16	20	14	8	10	19	10	14	11	16	14	15	12
chr1	156105599	156106319	LMNA	0,63	30	28	24	22	33	26	19	18	35	23	29	25	27	30	24	28
chr1	156106627	156107107	LMNA	0,62	75	66	51	55	87	61	46	46	82	61	77	67	76	74	77	58
chr1	156107379	156107619	LMNA	0,65	3	4	3	7	5	7	1	4	4	2	6	4	6	5	2	6
chr1	156108173	156108653	LMNA	0,67	11	11	10	10	15	14	8	9	14	14	16	11	13	12	12	12
chr1	156108763	156109003	LMNA	0,63	24	25	19	18	31	23	20	18	31	16	29	19	22	24	13	25
chr1	156785565	156785685	NTRK1	0,60	4	3	2	5	8	6	4	6	7	2	5	3	6	6	7	4
chr1	156811854	156812070	NTRK1	0,57	57	48	42	45	59	56	40	37	89	55	56	53	54	61	56	45
chr1	156830652	156831012	NTRK1	0,75	1	1	0	1	1	2	0	1	2	0	2	1	0	0	0	1
chr1	156834062	156834302	NTRK1	0,61	23	25	17	21	33	30	18	21	38	27	32	20	29	22	26	30
chr1	156834435	156834675	NTRK1	0,64	23	18	19	13	28	19	17	12	25	21	22	23	26	23	28	19
chr1	156836615	156836855	NTRK1	0,57	58	46	40	47	65	48	39	32	59	47	58	58	56	59	51	50
chr1	156837788	156838148	NTRK1	0,63	91	71	59	52	81	73	57	54	97	79	85	71	83	71	79	76
chr1	156838187	156838547	NTRK1	0,64	10	8	4	10	11	9	10	5	11	5	10	10	8	10	11	6
chr1	156841360	156841600	NTRK1	0,58	44	47	43	39	54	52	36	26	59	46	45	40	45	43	50	51
chr1	156843347	156843827	NTRK1	0,63	25	27	19	22	31	28	20	19	32	23	28	24	29	21	25	27
chr1	156844123	156844243	NTRK1	0,61	41	40	31	32	50	36	38	28	48	37	43	39	46	37	46	42
chr1	156844270	156844510	NTRK1	0,56	54	41	45	38	53	45	39	30	61	49	60	47	55	46	50	43
chr1	156844628	156844868	NTRK1	0,57	145	126	116	99	136	121	99	77	162	137	137	125	150	136	152	122
chr1	156845256	156845496	NTRK1	0,62	27	15	17	18	38	17	18	15	28	26	32	23	32	19	20	20
chr1	156845816	156846056	NTRK1	0,65	19	15	12	12	17	9	7	7	19	13	11	13	12	9	12	12
chr1	156846097	156846457	NTRK1	0,65	33	26	25	25	33	23	22	21	34	24	30	29	25	33	27	37
chr1	156848853	156849213	NTRK1	0,59	53	51	37	45	58	46	31	40	63	45	52	54	45	43	49	46
chr1	156849689	156850049	NTRK1	0,62	38	31	23	32	36	24	23	19	40	25	35	26	28	30	27	33
chr1	156851229	156851541	NTRK1	0,65	3	4	3	2	2	6	1	2	8	3	5	3	3	6	5	3
chr1	160160440	160160920	CASQ1	0,57	85	92	73	66	92	83	68	59	102	83	100	87	90	88	88	76
chr1	160162513	160162753	CASQ1	0,52	53	65	41	39	57	51	39	32	72	42	56	45	54	45	37	49
chr1	160163466	160163706	CASQ1	0,55	106	112	79	83	114	89	83	67	119	89	123	97	99	121	117	95
chr1	160164737	160164977	CASQ1	0,50	142	141	106	93	148	131	91	84	181	137	140	121	160	133	136	121
chr1	160165167	160165407	CASQ1	0,55	233	261	210	165	207	221	168	140	281	272	273	233	255	255	277	200
chr1	160165631	160165871	CASQ1	0,57	140	144	98	96	140	111	107	91	174	124	141	119	149	144	151	124
chr1	160167261	160167501	CASQ1	0,56	88	77	69	68	96	73	58	45	90	74	88	79	91	78	82	66
chr1	160168373	160168613	CASQ1	0,53	94	79	72	71	102	94	82	60	111	94	103	88	93	95	98	86
chr1	160168672	160168912	CASQ1	0,50	236	242	199	143	221	194	158	129	250	228	252	193	229	248	258	180
chr1	160169557	160169797	CASQ1	0,50	178	155	114	112	165	148	111	89	190	149	176	144	171	168	144	126
chr1	160170980	160171220	CASQ1	0,55	62	53	42	42	57	46	42	36	69	66	67	46	54	53	62	45
chr1	161275596	161276047	MPZ	0,62	159	139	124	111	147	133	90	95	156	142	143	127	145	137	134	119
chr1	161276066	161276306	MPZ	0,63	126	103	86	96	115	100	86	72	129	121	124	108	126	134	112	108
chr1	161276424	161276784	MPZ	0,51	206	218	152	124	181	174	150	112	227	206	210	188	208	190	193	170
chr1	161276950	161277310	MPZ	0,55	136	140	112	88	140	126	98	77	144	142	133	124	154	140	128	122
chr1	161279541	161279781	MPZ	0,61	7	4	3	3	4	4	3	2	5	3	7	6	6	4	4	3
chr1	201008904	201009610	CACNA1S	0,60	32	29	24	28	39	33	23	26	39	32	33	32	27	34	29	33
chr1	201009675	201009915	CACNA1S	0,61	11	11	9	10	16	17	12	4	16	13	13	10	13	11	9	15
chr1	201010554	201010794	CACNA1S	0,56	47	43	40	45	68	52	36	34	59	54	59	50	61	57	54	55
chr1	201012353	201012713	CACNA1S	0,58	70	72	57	59	76	71	49	50	78	62	78	65	67	67	82	67
chr1	201013399	201013639	CACNA1S	0,61	75	83	70	65	92	84	65	56	104	77	83	71	73	83	78	73
chr1	201016184	201016424	CACNA1S	0,53	112	108	89	83	117	113	84	59	132	108	119	97	116	105	105	94
chr1	201016583	201016823	CACNA1S	0,53	107	124	86	87	116	90	79	65	136	99	113	92	105	112	97	90
chr1	201017640	201017880	CACNA1S	0,59	28	36	25	24	35	22	21	22	49	27	30	29	33	36	28	28
chr1	201018058	201018298	CACNA1S	0,59	32	33	25	26	32	29	24	19	34	29	32	25	31	31	28	29
chr1	201019460	201019700	CACNA1S	0,54	66	66	50	44	77	61	36	41	79	69	70	60	63	74	62	53
chr1	201020011	201020371	CACNA1S	0,59	84	67	69	60	84	74	52	46	93	69	91	67	69	77	71	68
chr1	201021610	201021850	CACNA1S	0,55	101	104	72	84	98	94	74	80	111	92	110	79	104	96	97	89

chr1	201022249	201022489	CACNA1S	0,62	38	34	26	30	40	34	28	32	39	36	42	31	30	37	37	34
chr1	201022530	201022770	CACNA1S	0,62	27	22	19	18	34	21	16	18	35	24	23	22	23	29	24	24
chr1	201023578	201023698	CACNA1S	0,63	0	0	0	1	0	0	0	0	0	0	1	1	0	1	0	0
chr1	201027481	201027697	CACNA1S	0,54	52	60	39	46	56	43	45	30	61	50	58	50	61	52	54	48
chr1	201028251	201028491	CACNA1S	0,59	28	31	19	22	31	25	15	25	37	27	29	20	27	30	30	27
chr1	201029684	201030044	CACNA1S	0,56	118	123	90	86	118	98	78	70	119	96	123	100	123	107	117	97
chr1	201030315	201030675	CACNA1S	0,57	105	98	79	79	93	83	70	60	114	103	107	89	101	109	100	76
chr1	201030964	201031324	CACNA1S	0,62	40	42	37	42	48	30	36	30	49	31	44	38	43	41	46	41
chr1	201031537	201031657	CACNA1S	0,61	26	26	28	26	36	34	21	30	40	30	34	29	36	37	31	29
chr1	201034899	201035139	CACNA1S	0,63	15	15	17	15	16	11	11	10	21	15	20	15	14	22	15	14
chr1	201035280	201035520	CACNA1S	0,66	38	36	36	39	50	37	28	39	51	35	51	39	46	45	46	44
chr1	201035947	201036187	CACNA1S	0,60	35	26	22	22	38	27	22	26	39	26	33	29	24	24	35	34
chr1	201038174	201038414	CACNA1S	0,57	47	49	37	41	52	54	36	29	61	43	55	49	55	45	42	41
chr1	201038542	201038662	CACNA1S	0,64	8	5	4	5	7	7	9	5	9	6	6	5	7	7	8	6
chr1	201038670	201038790	CACNA1S	0,61	4	1	2	3	5	3	5	5	7	2	4	6	7	5	4	5
chr1	201039345	201039585	CACNA1S	0,59	56	61	45	44	69	48	44	43	72	49	65	53	63	64	66	47
chr1	201041797	201042037	CACNA1S	0,51	97	119	75	72	103	91	73	60	123	97	109	95	118	117	97	85
chr1	201042603	201042843	CACNA1S	0,54	96	96	76	71	107	89	73	75	129	109	120	96	121	106	129	96
chr1	201043570	201043810	CACNA1S	0,58	43	40	35	34	46	47	26	30	51	52	52	39	48	55	45	39
chr1	201044562	201044802	CACNA1S	0,55	94	104	70	73	96	95	68	60	117	90	97	98	102	93	99	88
chr1	201046066	201046306	CACNA1S	0,57	22	21	13	22	29	22	20	19	34	21	25	21	20	34	33	28
chr1	201046939	201047299	CACNA1S	0,61	64	41	39	54	62	42	46	48	79	54	72	55	60	64	62	57
chr1	201052189	201052549	CACNA1S	0,56	57	52	41	45	69	55	43	41	71	57	63	54	57	58	57	59
chr1	201053998	201054238	CACNA1S	0,56	84	72	64	67	83	80	64	55	108	74	83	74	84	88	81	83
chr1	201054456	201054816	CACNA1S	0,55	118	119	88	77	109	92	81	76	130	111	114	101	111	112	115	95
chr1	201056918	201057134	CACNA1S	0,50	101	114	89	78	109	100	79	69	142	100	124	104	117	99	103	86
chr1	201058308	201058668	CACNA1S	0,60	46	35	33	34	39	38	32	36	49	31	51	37	42	40	46	41
chr1	201060663	201061350	CACNA1S	0,59	48	53	45	42	51	46	34	32	61	47	56	47	56	60	49	48
chr1	201062958	201063198	CACNA1S	0,54	78	81	55	57	70	65	54	54	99	84	86	69	74	81	82	66
chr1	201079224	201079464	CACNA1S	0,60	36	40	29	35	40	35	24	24	46	38	50	39	38	45	43	47
chr1	201081211	201081571	CACNA1S	0,62	85	75	63	59	96	77	54	57	105	70	78	74	81	84	90	74
chr1	201328240	201328480	TNNT2	0,65	9	11	10	11	12	10	14	9	15	12	14	10	11	16	9	11
chr1	201328650	201328890	TNNT2	0,58	40	42	34	39	45	37	37	34	57	46	49	47	46	50	40	40
chr1	201330331	201330571	TNNT2	0,59	76	85	58	69	93	75	69	58	111	78	97	75	93	91	90	74
chr1	201330961	201331321	TNNT2	0,59	202	210	177	132	186	177	139	120	216	216	189	182	209	212	220	159
chr1	201332358	201332598	TNNT2	0,53	294	287	224	162	231	241	189	157	304	285	274	236	265	306	291	219
chr1	201333344	201333584	TNNT2	0,65	9	4	6	8	7	12	7	7	14	5	9	8	10	12	9	9
chr1	201334256	201334496	TNNT2	0,56	158	159	124	111	167	139	95	92	169	139	171	136	141	134	156	135
chr1	201334647	201334887	TNNT2	0,58	64	61	50	53	68	57	45	39	76	52	73	61	63	66	79	55
chr1	201335862	201336102	TNNT2	0,60	87	79	58	77	98	79	54	58	90	81	96	78	84	91	73	80
chr1	201336796	201337036	TNNT2	0,51	325	370	278	216	292	294	252	180	388	368	305	276	352	354	364	261
chr1	201337202	201337442	TNNT2	0,61	83	77	70	76	93	73	64	56	104	73	91	81	91	89	92	80
chr1	201338405	201338525	TNNT2	0,61	10	13	8	8	9	7	7	5	11	12	11	10	8	8	6	9
chr1	201338838	201339054	TNNT2	0,63	42	49	40	42	51	40	31	37	64	45	52	43	47	47	50	46
chr1	201341101	201341328	TNNT2	0,52	85	81	66	54	83	65	52	53	87	78	68	74	67	68	76	61
chr1	201342241	201342457	TNNT2	0,56	139	132	110	92	138	100	89	87	185	136	155	132	152	148	158	125
chr1	22149738	22150280	HSPG2	0,67	9	7	5	8	12	7	6	7	7	7	11	11	10	8	7	10
chr1	22150535	22150953	HSPG2	0,67	20	18	20	17	25	22	12	15	24	19	26	22	22	22	22	21
chr1	22150970	22151330	HSPG2	0,63	23	20	18	12	23	18	11	17	23	19	22	18	22	22	17	20
chr1	22154263	22155013	HSPG2	0,62	34	31	26	26	36	32	24	26	35	31	32	31	32	34	32	31
chr1	22155269	22155629	HSPG2	0,70	4	5	5	3	4	2	1	3	4	4	4	5	6	5	4	4
chr1	22155806	22156166	HSPG2	0,66	12	19	12	12	19	14	12	8	12	16	16	14	16	13	17	16
chr1	22156414	22156654	HSPG2	0,68	4	4	2	3	6	1	3	3	8	5	5	5	8	7	3	4
chr1	22157408	22158362	HSPG2	0,65	18	17	16	16	22	17	15	12	22	17	23	18	17	20	19	18
chr1	22158923	22159163	HSPG2	0,66	22	24	18	20	25	21	17	15	29	16	25	16	20	25	23	26
chr1	22159693	22160173	HSPG2	0,62	51	50	41	44	62	51	38	39	57	50	58	51	57	55	55	50
chr1	22160243	22160483	HSPG2	0,57	20	28	20	21	32	22	20	19	30	24	20	22	27	27	27	19
chr1	22161064	22161544	HSPG2	0,62	40	33	28	33	47	38	24	26	49	32	38	33	35	39	34	36
chr1	22161961	22162201	HSPG2	0,51	85	97	75	71	100	84	71	61	110	88	111	101	103	91	92	84
chr1	22163216	22163576	HSPG2	0,64	24	20	16	22	24	19	19	15	30	15	29	26	23	24	27	22

chr1	22165207	22165687	HSPG2	0,65	16	17	9	17	19	15	12	13	25	15	20	14	16	17	16	19
chr1	22165773	22166133	HSPG2	0,62	39	40	28	25	38	37	29	36	41	37	40	35	38	40	39	41
chr1	22166232	22166592	HSPG2	0,59	100	102	80	82	105	89	79	64	123	97	97	100	108	97	113	96
chr1	22167505	22167865	HSPG2	0,64	16	15	8	10	16	12	10	8	17	12	12	14	14	14	10	15
chr1	22167983	22168223	HSPG2	0,61	28	25	17	18	36	25	20	18	30	23	31	24	25	27	26	25
chr1	22168443	22168923	HSPG2	0,66	35	33	25	30	35	29	26	20	36	30	31	37	34	37	35	33
chr1	22169158	22169518	HSPG2	0,64	14	16	13	11	15	17	10	11	19	13	21	14	19	17	15	16
chr1	22169680	22170040	HSPG2	0,64	11	18	9	15	16	14	14	12	21	12	15	18	11	12	18	16
chr1	22170536	22170896	HSPG2	0,64	13	12	11	13	16	13	8	9	16	11	17	14	16	15	16	13
chr1	22172494	22173196	HSPG2	0,63	25	26	22	22	31	26	22	18	29	22	34	23	27	26	24	27
chr1	22173795	22174035	HSPG2	0,64	21	18	15	20	27	19	19	13	25	21	24	18	20	21	21	25
chr1	22174077	22174638	HSPG2	0,64	43	38	36	32	52	38	30	32	52	35	49	39	36	42	40	42
chr1	22175029	22175629	HSPG2	0,64	47	36	37	38	47	35	33	29	49	40	50	38	38	47	51	39
chr1	22176453	22176789	HSPG2	0,65	29	30	27	28	39	34	25	23	48	22	32	28	31	31	33	31
chr1	22176803	22177043	HSPG2	0,67	16	17	10	21	23	16	11	15	19	15	16	13	21	21	14	16
chr1	22177928	22178833	HSPG2	0,65	22	22	17	20	25	20	19	20	28	18	29	18	22	26	22	22
chr1	22179137	22179617	HSPG2	0,67	8	10	7	12	12	9	6	8	13	7	13	11	10	10	8	13
chr1	22180580	22180940	HSPG2	0,63	6	5	6	7	5	5	5	6	7	5	5	6	4	5	5	4
chr1	22181049	22181529	HSPG2	0,66	12	14	12	13	18	11	12	13	20	11	20	14	15	17	16	16
chr1	22181742	22182458	HSPG2	0,67	8	8	5	9	8	9	6	7	10	9	11	8	8	9	9	9
chr1	22183452	22183932	HSPG2	0,62	58	48	40	50	66	50	39	38	68	47	58	49	59	59	53	54
chr1	22185993	22186818	HSPG2	0,62	32	30	28	26	39	31	27	26	45	29	37	39	35	33	31	34
chr1	22188172	22188412	HSPG2	0,58	57	65	50	55	74	67	51	48	82	59	75	66	57	64	63	61
chr1	22188413	22188653	HSPG2	0,61	23	21	20	21	22	27	18	16	32	20	28	20	21	25	21	16
chr1	22190529	22190769	HSPG2	0,67	3	2	1	4	5	7	4	1	5	5	4	2	5	4	7	5
chr1	22191270	22191630	HSPG2	0,68	5	6	3	4	7	6	5	4	8	8	8	5	5	9	7	8
chr1	22191697	22191937	HSPG2	0,61	25	27	17	25	31	32	19	22	37	26	27	22	26	27	29	32
chr1	22192135	22192375	HSPG2	0,60	13	17	11	18	16	15	13	12	17	11	14	12	9	14	15	15
chr1	22198594	22198954	HSPG2	0,63	30	28	24	27	35	27	21	19	38	26	34	27	27	28	29	24
chr1	22199002	22199362	HSPG2	0,67	17	14	13	17	17	20	15	12	21	17	17	16	15	18	21	21
chr1	22199416	22199656	HSPG2	0,65	11	8	5	10	13	10	7	13	12	13	10	8	8	9	13	9
chr1	22199772	22200012	HSPG2	0,57	49	51	30	36	51	41	34	37	64	40	45	43	50	41	47	43
chr1	22200340	22200580	HSPG2	0,65	9	4	7	7	10	7	4	5	8	5	8	5	5	11	7	7
chr1	22200820	22201514	HSPG2	0,67	10	9	6	10	10	10	7	7	12	6	12	8	11	8	7	13
chr1	22202103	22202601	HSPG2	0,65	22	21	16	19	22	18	22	15	26	22	26	19	22	20	18	21
chr1	22202721	22203254	HSPG2	0,62	44	42	36	39	59	48	36	38	63	47	51	52	54	44	43	45
chr1	22204592	22204832	HSPG2	0,63	6	6	4	6	6	6	2	2	6	4	8	3	6	5	4	4
chr1	22204860	22205220	HSPG2	0,65	41	44	29	33	52	36	35	30	48	37	45	41	42	43	39	40
chr1	22205435	22205675	HSPG2	0,62	20	24	12	23	29	15	15	17	32	19	22	20	21	28	24	20
chr1	22206517	22207391	HSPG2	0,62	46	43	30	42	46	43	35	31	51	39	44	44	37	46	48	38
chr1	22207733	22208093	HSPG2	0,61	45	37	31	34	41	31	27	28	51	39	38	35	41	35	41	37
chr1	22210915	22211755	HSPG2	0,66	17	15	13	16	22	16	14	11	21	15	18	13	17	16	16	18
chr1	22211758	22211998	HSPG2	0,63	30	29	15	29	34	28	24	23	35	26	27	31	24	29	33	31
chr1	22213669	22214245	HSPG2	0,64	33	30	21	23	36	28	21	24	38	30	35	31	33	32	28	33
chr1	22214374	22214614	HSPG2	0,64	21	18	13	13	22	15	15	15	18	14	19	21	21	22	25	16
chr1	22216425	22216737	HSPG2	0,63	33	25	24	24	37	26	18	18	40	22	35	27	27	35	42	25
chr1	22216813	22217293	HSPG2	0,60	93	90	75	71	104	88	70	59	121	94	102	87	90	95	99	87
chr1	22222306	22222498	HSPG2	0,59	21	12	11	11	20	21	14	11	21	13	18	19	15	18	18	16
chr1	22222615	22222855	HSPG2	0,56	123	125	112	107	146	121	103	85	170	140	147	121	128	133	146	119
chr1	227148994	227149354	ADCK3	0,62	22	18	14	21	23	22	11	17	29	21	27	20	21	15	22	19
chr1	227152605	227153205	ADCK3	0,62	36	29	26	32	37	32	22	20	35	29	34	28	30	36	28	31
chr1	227153284	227153524	ADCK3	0,64	12	9	8	11	12	14	5	7	12	9	9	8	11	10	7	9
chr1	227165066	227165306	ADCK3	0,73	0	1	0	0	0	1	0	0	2	0	1	0	0	1	0	1
chr1	227169668	227169908	ADCK3	0,68	10	8	2	7	9	4	4	4	9	5	11	8	7	5	7	8
chr1	227170301	227170844	ADCK3	0,65	33	36	30	33	48	32	25	27	45	37	41	38	41	32	41	38
chr1	227171173	227171628	ADCK3	0,65	48	50	36	38	50	50	37	40	63	43	54	40	60	45	45	43
chr1	227171685	227172045	ADCK3	0,64	39	31	27	29	43	29	29	26	50	31	37	33	35	44	31	39
chr1	227172182	227172422	ADCK3	0,61	90	80	73	74	100	69	63	51	105	87	91	79	95	97	95	72
chr1	227172489	227172729	ADCK3	0,59	57	55	39	46	67	54	47	31	77	50	60	50	46	60	47	51
chr1	227172877	227173117	ADCK3	0,62	24	26	19	21	26	20	15	15	31	20	24	16	23	26	25	22

chr1	227174055	227174535	ADCK3	0,61	92	100	77	80	106	88	62	58	115	86	112	86	90	98	97	84
chr1	229567139	229567691	ACTA1	0,62	46	41	36	39	52	35	32	28	53	36	44	41	44	42	43	40
chr1	229567713	229567977	ACTA1	0,67	26	17	15	22	24	20	17	12	20	17	20	16	16	23	18	14
chr1	229568001	229568241	ACTA1	0,67	10	8	6	8	11	6	5	5	8	6	10	8	8	9	6	8
chr1	229568291	229568917	ACTA1	0,64	55	43	35	39	60	44	35	36	53	41	52	47	51	49	46	48
chr1	236849894	236850110	ACTN2	0,67	11	9	10	10	21	10	10	12	14	12	11	17	14	8	13	13
chr1	236881094	236881334	ACTN2	0,46	151	143	100	105	129	129	97	87	169	134	164	123	164	137	142	121
chr1	236882133	236882373	ACTN2	0,50	119	128	89	82	115	123	86	77	160	114	137	110	122	124	139	117
chr1	236883327	236883567	ACTN2	0,32	252	268	204	155	206	212	154	127	269	256	253	221	226	241	263	187
chr1	236889156	236889396	ACTN2	0,47	150	142	109	111	157	135	104	81	193	155	158	145	154	171	152	141
chr1	236890896	236891136	ACTN2	0,54	136	139	110	83	139	136	91	100	166	150	137	150	154	141	145	117
chr1	236894453	236894693	ACTN2	0,37	213	252	176	133	207	204	154	116	235	220	207	183	239	211	234	169
chr1	236898857	236899097	ACTN2	0,52	174	183	122	104	155	147	118	99	209	166	172	153	193	165	173	134
chr1	236900347	236900587	ACTN2	0,51	49	58	56	41	67	42	37	26	72	51	53	39	45	48	56	40
chr1	236902536	236902896	ACTN2	0,57	39	37	25	30	41	32	28	27	44	33	43	30	42	42	28	38
chr1	236906089	236906449	ACTN2	0,49	123	125	97	84	122	104	89	82	149	120	125	100	113	124	123	103
chr1	236907820	236908180	ACTN2	0,59	34	30	20	27	29	24	26	20	38	32	31	27	33	31	23	26
chr1	236910900	236911140	ACTN2	0,39	123	139	99	87	111	112	99	78	147	131	120	125	115	124	122	106
chr1	236912313	236912673	ACTN2	0,37	222	233	178	138	191	201	158	121	267	226	241	193	236	235	234	190
chr1	236914680	236915040	ACTN2	0,54	66	63	52	45	74	60	46	37	79	61	72	49	52	74	56	55
chr1	236917193	236917433	ACTN2	0,59	36	34	32	26	37	37	20	23	55	27	49	41	39	35	30	26
chr1	236918228	236918588	ACTN2	0,56	108	105	91	68	102	91	68	63	132	90	119	96	114	117	89	89
chr1	236920678	236921038	ACTN2	0,46	211	202	165	130	178	190	148	123	228	239	203	185	214	226	229	166
chr1	236922936	236923176	ACTN2	0,41	203	223	175	124	188	207	155	115	253	222	208	199	210	234	211	167
chr1	236924213	236924573	ACTN2	0,48	187	210	154	122	177	181	134	111	248	191	211	155	214	199	217	166
chr1	236925659	236926019	ACTN2	0,48	93	96	74	72	94	86	72	59	119	86	102	77	94	101	96	86
chr1	237205802	237205922	RYR2	0,69	1	0	1	1	2	0	1	0	2	1	0	1	1	1	0	3
chr1	237433736	237433976	RYR2	0,44	195	210	170	114	179	178	139	108	211	199	215	174	219	207	190	154
chr1	237494109	237494349	RYR2	0,49	111	114	94	80	111	101	79	61	114	116	125	107	132	112	107	97
chr1	237519154	237519394	RYR2	0,34	263	298	224	151	207	212	184	147	295	276	287	226	256	272	293	206
chr1	237532820	237533012	RYR2	0,47	134	124	97	84	118	104	72	62	149	107	116	98	114	107	119	101
chr1	237537959	237538175	RYR2	0,48	183	206	157	111	180	160	141	105	226	199	189	173	209	198	201	147
chr1	237540558	237540798	RYR2	0,40	135	143	104	89	151	127	99	73	151	132	143	110	136	127	130	108
chr1	237550510	237550750	RYR2	0,49	136	153	118	97	141	135	101	102	175	152	158	139	166	177	153	123
chr1	237551345	237551561	RYR2	0,45	98	109	83	61	112	88	72	61	120	91	105	82	112	108	83	91
chr1	237580265	237580505	RYR2	0,41	209	213	150	124	181	175	126	95	227	211	192	168	186	206	206	144
chr1	237586289	237586649	RYR2	0,38	232	249	184	141	206	215	169	130	261	244	225	199	242	238	251	195
chr1	237604520	237604880	RYR2	0,35	203	190	161	119	171	166	138	110	205	184	195	167	186	192	210	153
chr1	237608641	237608881	RYR2	0,39	183	197	160	115	189	161	138	93	232	195	214	172	204	200	209	160
chr1	237617602	237617962	RYR2	0,40	260	278	216	146	219	225	171	137	275	273	267	220	295	271	282	219
chr1	237619847	237620087	RYR2	0,42	148	150	112	92	151	141	104	98	189	145	159	138	168	162	160	131
chr1	237632319	237632559	RYR2	0,37	253	275	193	152	226	204	173	130	284	253	253	219	271	254	256	172
chr1	237655044	237655284	RYR2	0,28	258	265	184	139	195	227	185	126	265	240	256	214	258	245	276	180
chr1	237656211	237656451	RYR2	0,45	156	193	137	107	156	149	120	93	176	160	176	156	184	167	170	134
chr1	237659751	237660111	RYR2	0,46	203	179	147	121	182	171	141	104	209	186	197	169	205	195	204	141
chr1	237663926	237664286	RYR2	0,40	334	351	286	193	279	291	234	168	368	377	340	299	353	377	381	278
chr1	237666516	237666876	RYR2	0,46	160	173	121	112	172	137	121	86	190	152	175	151	162	165	169	134
chr1	237669941	237670181	RYR2	0,34	206	195	159	114	177	163	134	100	219	201	181	186	206	197	196	141
chr1	237674915	237675131	RYR2	0,44	170	182	133	112	183	160	125	107	208	176	172	149	196	172	168	135
chr1	237693648	237693888	RYR2	0,37	193	226	195	137	180	166	153	117	236	209	227	191	213	208	215	167
chr1	237711630	237711990	RYR2	0,40	243	261	208	141	215	218	173	140	282	261	246	210	260	271	252	198
chr1	237713737	237714097	RYR2	0,45	213	218	191	132	189	185	143	115	245	212	235	179	241	217	228	178
chr1	237729790	237730150	RYR2	0,52	140	137	110	94	135	128	85	83	160	125	138	118	141	125	136	116
chr1	237732351	237732711	RYR2	0,49	171	163	137	99	153	143	114	95	185	159	173	142	178	165	171	136
chr1	237753016	237753376	RYR2	0,37	294	329	259	179	252	258	226	150	330	315	303	241	309	314	332	221
chr1	237753875	237754355	RYR2	0,45	223	224	169	132	195	194	151	132	246	225	227	197	225	222	241	179
chr1	237754975	237755215	RYR2	0,38	247	276	218	148	218	234	183	139	286	253	253	223	261	290	265	195
chr1	237756675	237757035	RYR2	0,37	271	334	224	171	238	241	199	141	311	284	303	244	283	302	314	209
chr1	237758697	237759057	RYR2	0,43	151	157	109	101	141	121	96	73	166	142	139	122	134	152	155	112
chr1	237765247	237765487	RYR2	0,29	209	226	168	116	164	173	138	103	248	210	224	179	217	207	226	175

chr1	237773994	237774354	RYR2	0,52	125	111	104	88	130	105	75	70	123	112	120	115	117	121	127	104
chr1	237777260	237778220	RYR2	0,49	241	246	187	144	210	210	167	127	272	239	251	204	243	245	253	185
chr1	237780505	237780865	RYR2	0,39	267	282	218	164	238	235	172	135	302	264	266	225	289	278	289	207
chr1	237786997	237787237	RYR2	0,33	258	265	200	172	216	221	166	140	284	251	250	221	255	253	240	196
chr1	237788852	237789212	RYR2	0,39	236	255	194	143	235	213	159	129	267	248	254	213	261	232	260	194
chr1	237791003	237791483	RYR2	0,43	181	184	140	128	178	168	120	97	220	179	183	162	192	178	179	142
chr1	237794663	237794903	RYR2	0,43	192	186	141	131	170	158	141	114	213	167	204	169	196	180	176	164
chr1	237796823	237797063	RYR2	0,34	239	248	185	135	192	214	171	130	246	221	250	203	222	230	223	184
chr1	237798120	237798360	RYR2	0,40	212	222	167	131	178	177	146	109	214	206	219	195	209	209	216	160
chr1	237801604	237801844	RYR2	0,41	144	157	117	95	139	140	96	76	163	141	161	123	140	153	145	121
chr1	237802227	237802587	RYR2	0,40	198	187	144	117	188	177	141	103	216	181	192	163	188	190	197	167
chr1	237804129	237804369	RYR2	0,42	129	139	91	86	127	121	94	92	157	137	148	115	145	144	137	116
chr1	237806566	237806806	RYR2	0,41	153	172	132	102	167	159	120	98	191	168	182	158	190	179	168	134
chr1	237811648	237812008	RYR2	0,43	273	286	214	153	231	258	200	139	299	301	272	248	284	299	293	222
chr1	237813106	237813466	RYR2	0,43	310	311	249	180	245	262	215	152	333	344	308	263	329	315	329	222
chr1	237814635	237814875	RYR2	0,31	302	334	264	160	219	264	211	153	322	320	294	259	336	307	309	241
chr1	237817463	237817823	RYR2	0,36	230	232	187	120	209	191	156	113	239	238	223	190	231	230	221	177
chr1	237819022	237819382	RYR2	0,38	266	278	219	155	214	235	182	135	301	275	281	232	271	278	277	206
chr1	237821162	237821402	RYR2	0,31	225	230	174	139	166	192	149	115	244	210	237	197	226	217	236	173
chr1	237823209	237823449	RYR2	0,27	197	237	181	130	157	186	169	131	248	231	202	186	235	223	212	172
chr1	237824054	237824294	RYR2	0,37	129	131	112	80	124	107	91	58	151	115	135	113	119	126	115	107
chr1	237829730	237829970	RYR2	0,35	232	237	189	134	208	192	151	121	248	232	234	203	230	237	264	185
chr1	237831100	237831340	RYR2	0,29	181	193	129	115	150	150	122	105	194	173	182	150	196	177	174	159
chr1	237837337	237837577	RYR2	0,40	197	209	176	126	184	193	145	107	236	212	235	172	216	216	208	165
chr1	237837968	237838208	RYR2	0,40	298	304	230	151	235	235	190	148	313	291	274	240	285	289	285	228
chr1	237841259	237841499	RYR2	0,36	238	289	217	152	229	224	161	144	296	249	275	208	265	238	263	187
chr1	237843696	237843936	RYR2	0,39	206	258	193	122	197	208	147	109	264	235	226	191	245	227	230	186
chr1	237850683	237850899	RYR2	0,41	321	332	267	197	267	270	237	179	347	346	323	256	330	334	309	257
chr1	237862174	237862414	RYR2	0,31	257	259	208	149	200	223	191	133	281	247	244	210	265	259	255	202
chr1	237863467	237863827	RYR2	0,41	219	223	163	129	209	184	140	110	252	212	226	177	209	225	213	173
chr1	237865198	237865438	RYR2	0,32	226	251	196	122	168	193	163	120	260	242	238	194	238	223	258	182
chr1	237868457	237868697	RYR2	0,37	253	268	202	155	216	230	175	124	303	263	267	238	268	263	284	192
chr1	237870168	237870648	RYR2	0,42	199	222	168	127	188	182	143	111	233	205	218	178	206	214	212	175
chr1	237872097	237872457	RYR2	0,42	239	249	184	133	193	192	154	123	258	223	226	205	240	243	251	179
chr1	237872703	237872943	RYR2	0,42	105	110	103	72	109	89	65	63	131	103	104	85	126	106	114	91
chr1	237874970	237875210	RYR2	0,31	269	281	234	149	195	223	180	138	320	269	253	237	268	255	278	213
chr1	237880402	237880762	RYR2	0,47	275	266	229	148	232	228	186	139	286	274	280	225	267	276	292	209
chr1	237881671	237881911	RYR2	0,30	262	258	207	144	209	226	165	131	266	244	244	229	251	261	246	188
chr1	237886374	237886614	RYR2	0,38	243	296	229	160	236	233	196	130	296	288	288	224	279	272	294	218
chr1	237889470	237889710	RYR2	0,30	209	218	162	122	144	169	141	98	210	206	222	192	191	196	213	171
chr1	237890322	237890562	RYR2	0,37	234	240	186	126	193	202	154	116	256	246	239	195	249	241	244	169
chr1	237893487	237893727	RYR2	0,38	250	279	217	153	208	226	171	144	282	275	259	229	261	258	287	181
chr1	237895277	237895517	RYR2	0,40	120	128	87	67	137	104	90	70	141	121	112	100	129	109	121	101
chr1	237896910	237897150	RYR2	0,34	245	293	203	161	222	207	176	143	284	278	266	229	239	253	288	214
chr1	237905585	237905705	RYR2	0,35	88	106	72	65	98	87	81	58	108	100	112	87	97	96	91	97
chr1	237919517	237919757	RYR2	0,46	117	119	89	71	122	93	79	60	141	118	117	103	120	115	120	101
chr1	237920916	237921156	RYR2	0,35	212	266	208	140	185	211	169	144	273	277	239	239	236	257	248	198
chr1	237922993	237923233	RYR2	0,40	169	184	145	93	165	156	127	100	198	158	168	129	177	186	163	142
chr1	237924171	237924411	RYR2	0,38	173	183	134	120	153	136	105	90	210	173	176	156	194	154	167	131
chr1	237934026	237934266	RYR2	0,49	92	99	67	71	96	80	61	55	97	91	108	88	98	94	98	81
chr1	237935235	237935475	RYR2	0,34	234	269	194	150	195	213	169	128	249	231	230	209	248	238	266	196
chr1	237936763	237937003	RYR2	0,34	239	239	191	136	185	192	170	124	231	232	242	195	241	239	264	202
chr1	237941897	237942137	RYR2	0,43	148	147	135	102	155	137	101	93	170	139	155	139	161	168	149	116
chr1	237944785	237945025	RYR2	0,32	278	287	233	157	196	227	182	134	289	262	253	231	266	286	281	210
chr1	237946903	237948343	RYR2	0,48	220	227	183	135	200	195	157	121	257	219	231	200	229	228	243	177
chr1	237951181	237951541	RYR2	0,39	223	244	184	147	218	207	169	120	266	218	238	199	231	230	234	189
chr1	237954651	237954891	RYR2	0,33	280	339	262	172	243	255	203	150	346	325	318	267	323	317	329	257
chr1	237955321	237955657	RYR2	0,44	198	212	149	128	185	156	138	107	208	200	204	158	202	197	199	168
chr1	237957111	237957351	RYR2	0,34	236	241	181	128	188	204	143	127	219	218	231	170	219	232	209	158
chr1	237958489	237958729	RYR2	0,25	225	261	194	156	177	210	167	123	262	242	241	184	248	207	228	195

chr1	237961283	237961523	RYR2	0,42	155	156	114	100	138	119	98	68	159	144	138	112	152	154	133	104
chr1	237965065	237965305	RYR2	0,40	171	165	134	102	153	152	117	84	188	167	181	143	170	172	187	139
chr1	237969377	237969689	RYR2	0,36	330	366	286	191	267	278	277	188	372	392	343	305	367	385	381	248
chr1	237972147	237972387	RYR2	0,36	296	305	240	162	255	247	184	138	335	296	275	258	302	279	310	233
chr1	237982233	237982593	RYR2	0,41	252	249	211	123	215	219	165	129	275	245	257	211	244	253	263	180
chr1	237991592	237991832	RYR2	0,39	197	207	160	132	180	167	133	104	221	217	199	148	221	194	207	150
chr1	237993759	237993999	RYR2	0,45	252	237	203	145	211	207	178	133	285	265	253	219	269	260	269	193
chr1	237994719	237994959	RYR2	0,33	243	265	225	146	202	211	188	148	276	247	259	213	270	236	255	204
chr1	237995779	237996019	RYR2	0,41	269	290	239	176	250	205	175	152	309	290	285	265	293	296	300	201
chr1	26126846	26126966	SEPN1	0,83	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chr1	26127472	26127712	SEPN1	0,59	41	47	38	44	49	38	36	36	56	42	52	48	57	41	43	43
chr1	26131579	26131819	SEPN1	0,60	21	25	22	20	29	19	15	16	27	17	24	24	17	18	24	20
chr1	26134995	26135355	SEPN1	0,66	23	30	23	28	30	28	21	20	43	24	28	26	29	30	31	31
chr1	26135458	26135698	SEPN1	0,66	39	35	32	36	47	31	24	34	52	30	51	34	39	38	34	35
chr1	26136122	26136362	SEPN1	0,63	137	134	117	105	141	110	84	87	154	131	137	125	142	148	135	134
chr1	26137865	26138455	SEPN1	0,61	41	36	29	38	38	40	28	25	43	34	36	33	35	37	30	38
chr1	26139110	26139350	SEPN1	0,62	6	8	4	4	7	9	4	6	6	4	7	5	5	6	6	6
chr1	26140280	26140760	SEPN1	0,63	47	51	41	40	59	38	36	31	66	44	56	52	57	55	54	47
chr1	26141943	26142303	SEPN1	0,61	138	122	98	83	123	112	94	76	151	122	134	119	136	116	130	104
chr1	33241516	33241756	YARS	0,49	114	116	87	81	106	91	84	70	122	99	113	90	107	113	97	103
chr1	33244873	33245233	YARS	0,54	137	144	109	106	131	116	93	91	160	119	144	120	128	128	144	122
chr1	33245602	33245962	YARS	0,56	142	145	130	110	145	129	101	94	187	139	154	147	160	151	163	131
chr1	33246648	33246840	YARS	0,52	46	47	34	42	49	58	31	32	65	49	44	43	51	48	54	38
chr1	33247952	33248192	YARS	0,50	133	127	97	91	134	112	88	71	149	128	142	115	124	114	136	103
chr1	33251885	33252125	YARS	0,40	282	298	242	169	251	245	199	143	328	295	307	234	308	289	311	210
chr1	33252488	33252728	YARS	0,44	243	261	189	143	194	224	168	123	258	229	261	189	228	219	245	173
chr1	33256688	33256928	YARS	0,44	209	223	165	128	193	175	140	111	212	215	223	192	205	197	219	158
chr1	33263275	33263491	YARS	0,38	186	184	172	122	168	172	140	105	206	204	209	179	193	197	187	172
chr1	33272027	33272267	YARS	0,54	106	104	78	74	115	94	73	66	114	104	84	90	101	99	103	79
chr1	33276099	33276764	YARS	0,48	294	297	246	168	239	259	212	145	334	295	302	254	297	301	327	235
chr1	33282696	33282936	YARS	0,64	36	41	28	34	52	32	27	27	50	43	45	44	49	42	34	33
chr1	46654850	46655330	POMGNT1	0,57	46	46	35	37	46	46	33	27	54	38	50	34	42	44	50	45
chr1	46655473	46655713	POMGNT1	0,55	59	54	44	50	61	49	49	39	78	48	66	52	55	63	58	53
chr1	46656070	46656286	POMGNT1	0,53	201	205	149	132	183	178	154	115	224	186	221	178	227	223	223	163
chr1	46656303	46656543	POMGNT1	0,54	174	174	151	113	191	154	132	100	203	156	187	152	209	181	181	151
chr1	46657728	46658328	POMGNT1	0,56	68	72	54	51	82	68	53	44	91	66	73	64	65	75	80	65
chr1	46658480	46658720	POMGNT1	0,53	136	156	123	113	155	132	107	94	194	144	162	149	163	158	175	139
chr1	46658772	46659132	POMGNT1	0,57	115	99	85	81	113	99	73	66	132	98	119	94	116	112	118	106
chr1	46659153	46659393	POMGNT1	0,59	143	140	116	106	152	144	104	81	180	147	158	132	146	149	153	147
chr1	46659441	46659681	POMGNT1	0,54	82	69	60	66	89	73	48	49	100	77	80	72	75	75	83	69
chr1	46659889	46660129	POMGNT1	0,61	48	49	44	39	56	47	33	29	58	45	49	48	47	50	55	44
chr1	46660153	46660393	POMGNT1	0,59	104	87	75	76	97	85	66	68	120	95	106	97	101	98	89	82
chr1	46660454	46660694	POMGNT1	0,62	47	32	36	32	50	34	33	29	58	34	44	42	37	42	47	36
chr1	46661375	46661831	POMGNT1	0,55	70	66	58	51	75	77	49	43	81	62	92	61	77	78	72	68
chr1	46662341	46662818	POMGNT1	0,58	178	168	136	126	172	160	127	102	184	159	189	149	176	182	178	155
chr1	46663313	46663553	POMGNT1	0,59	12	5	10	13	15	8	8	9	11	8	10	8	10	8	7	13
chr1	53662511	53662871	CPT2	0,72	8	5	3	2	8	5	3	5	7	5	6	6	6	5	3	6
chr1	53666310	53666550	CPT2	0,47	203	215	196	139	200	173	154	119	247	201	221	208	227	218	246	174
chr1	53667927	53668167	CPT2	0,39	167	185	130	111	154	154	125	106	198	181	182	148	188	181	178	150
chr1	53675618	53677058	CPT2	0,50	193	200	150	121	173	166	134	96	209	189	192	166	194	188	198	150
chr1	53678898	53679330	CPT2	0,52	127	130	102	89	120	126	79	73	144	112	137	115	137	126	125	113
chr1	6527567	6527687	PLEKHG5	0,61	2	5	4	2	4	3	2	6	4	3	4	3	5	3	3	2
chr1	6527785	6528745	PLEKHG5	0,66	17	10	12	16	19	13	12	11	19	14	14	16	14	13	14	14
chr1	6528992	6529184	PLEKHG5	0,60	37	24	24	28	39	34	20	27	35	32	36	32	30	34	31	28
chr1	6529236	6529812	PLEKHG5	0,61	77	78	60	64	83	72	58	54	93	75	81	66	74	76	74	69
chr1	6530235	6530475	PLEKHG5	0,66	23	22	17	22	31	30	18	18	33	22	25	20	28	35	18	23
chr1	6530514	6531189	PLEKHG5	0,69	22	15	16	17	20	15	14	16	25	15	16	16	22	21	20	20
chr1	6531442	6531962	PLEKHG5	0,71	9	8	8	12	10	9	7	8	14	10	9	9	12	9	9	12
chr1	6532514	6532754	PLEKHG5	0,64	10	9	6	11	15	10	9	9	16	6	10	11	13	11	9	9
chr1	6532979	6533579	PLEKHG5	0,67	30	27	24	26	34	25	21	19	34	28	39	27	34	26	28	25

chr1	6533968	6534328	PLEKHG5	0,68	10	7	7	7	6	8	8	7	9	8	9	5	7	9	8	13
chr1	6534458	6534698	PLEKHG5	0,61	21	19	26	25	38	29	15	21	37	27	33	24	25	27	32	27
chr1	6535029	6535245	PLEKHG5	0,59	103	99	80	85	101	97	80	68	133	116	106	111	105	102	107	95
chr1	6535431	6535671	PLEKHG5	0,68	6	6	5	7	11	10	5	6	15	3	7	5	8	6	9	5
chr1	6535923	6536163	PLEKHG5	0,65	67	59	54	63	83	69	54	51	93	73	88	72	77	79	76	68
chr1	6537533	6537773	PLEKHG5	0,63	18	16	12	15	17	13	12	11	18	14	22	17	15	17	17	14
chr1	6556470	6556710	PLEKHG5	0,68	4	5	4	5	6	4	4	4	9	5	8	5	2	4	3	5
chr1	6579417	6579657	PLEKHG5	0,53	180	162	138	116	187	148	126	111	197	162	165	143	185	160	183	130
chr10	101474289	101474529	COX15	0,51	39	32	32	34	41	40	22	22	44	36	37	42	45	38	34	28
chr10	101476041	101476281	COX15	0,46	115	109	91	79	125	99	76	62	128	109	116	97	132	104	116	93
chr10	101477999	101478359	COX15	0,46	216	249	182	130	208	191	159	130	239	229	219	187	220	214	221	180
chr10	101480664	101480904	COX15	0,46	64	67	56	54	75	70	45	39	77	66	77	70	81	64	70	58
chr10	101483696	101483936	COX15	0,49	118	115	88	75	114	104	77	67	130	113	127	117	107	121	115	96
chr10	101486661	101486997	COX15	0,46	171	170	145	124	183	163	133	100	213	179	176	160	185	172	168	146
chr10	101487138	101487378	COX15	0,40	280	274	226	170	249	237	187	136	316	280	286	242	284	270	296	225
chr10	101489220	101489580	COX15	0,48	205	219	198	148	194	207	165	121	245	232	220	204	228	222	232	182
chr10	101491641	101491881	COX15	0,60	36	32	27	26	38	28	20	29	46	24	35	36	39	27	25	28
chr10	102747868	102749308	C10orf2	0,56	137	128	105	96	135	122	102	81	155	121	142	120	135	134	127	119
chr10	102749340	102749700	C10orf2	0,53	117	115	95	95	131	116	93	73	154	101	116	108	121	132	123	112
chr10	102750126	102750366	C10orf2	0,49	167	188	147	113	165	160	127	98	209	186	189	150	182	160	181	145
chr10	102750523	102750883	C10orf2	0,49	143	149	117	100	147	126	103	83	164	150	144	123	145	143	145	112
chr10	102752866	102753346	C10orf2	0,55	110	105	87	85	108	91	76	72	133	101	109	102	105	108	106	93
chr10	112404131	112404347	RBM20	0,76	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
chr10	112540500	112541700	RBM20	0,58	132	121	109	100	137	118	95	80	159	131	144	122	134	139	133	119
chr10	112543027	112543219	RBM20	0,51	104	132	95	86	132	113	77	81	141	101	119	100	117	129	110	103
chr10	112544061	112544277	RBM20	0,51	45	50	35	35	52	53	37	38	58	46	59	37	48	49	56	42
chr10	112544477	112544693	RBM20	0,37	208	227	179	131	180	202	176	126	238	216	226	186	221	249	246	178
chr10	112557155	112557515	RBM20	0,44	180	182	127	118	177	153	132	101	196	170	212	159	176	179	187	139
chr10	112559474	112559714	RBM20	0,41	200	190	140	128	172	167	124	113	204	185	191	158	197	196	200	150
chr10	112570060	112570300	RBM20	0,56	60	60	55	41	61	54	38	38	68	57	69	48	73	67	59	54
chr10	112571950	112572790	RBM20	0,57	66	56	52	44	66	52	53	39	73	55	68	58	63	62	55	60
chr10	112579761	112580001	RBM20	0,46	90	100	88	72	96	77	76	52	124	97	107	96	92	105	103	79
chr10	112580942	112581782	RBM20	0,53	137	129	108	84	134	121	97	81	157	128	147	124	143	146	146	117
chr10	112583199	112583439	RBM20	0,41	150	148	117	111	137	126	107	103	154	130	148	131	150	151	142	121
chr10	112590759	112590999	RBM20	0,58	44	47	36	35	52	37	28	27	62	34	48	32	55	44	40	43
chr10	112595533	112595749	RBM20	0,56	51	49	47	47	65	45	39	37	63	50	63	54	59	47	46	48
chr10	121411097	121411433	BAG3	0,75	8	5	3	6	9	7	4	4	6	4	8	6	8	8	5	8
chr10	121429285	121429765	BAG3	0,63	52	53	33	37	54	52	37	36	69	46	58	47	54	44	54	44
chr10	121431667	121432267	BAG3	0,64	54	57	45	47	68	51	43	44	67	57	68	55	63	54	60	59
chr10	121435904	121436864	BAG3	0,52	164	165	126	107	153	157	122	90	175	160	167	145	169	168	183	135
chr10	13320207	13320447	PHYH	0,34	255	279	224	158	195	258	182	136	265	272	264	236	249	267	309	213
chr10	13322922	13323162	PHYH	0,39	252	296	215	153	227	226	183	128	287	265	258	200	265	269	288	196
chr10	13325632	13325944	PHYH	0,46	230	241	179	141	181	188	161	128	247	239	222	188	218	225	214	171
chr10	13330262	13330574	PHYH	0,55	50	52	35	41	54	43	33	36	63	44	56	57	52	51	41	47
chr10	13333775	13333991	PHYH	0,35	205	222	172	141	189	187	160	135	240	220	221	196	232	212	222	171
chr10	13336355	13336691	PHYH	0,47	251	272	190	139	220	221	168	124	274	252	248	221	263	243	275	185
chr10	13337429	13337669	PHYH	0,33	198	251	171	125	201	197	149	116	249	225	232	208	230	222	227	179
chr10	13340090	13340282	PHYH	0,32	182	185	129	113	143	156	137	107	185	160	182	150	168	157	162	143
chr10	13341884	13342124	PHYH	0,77	0	0	0	0	0	2	1	1	1	0	0	1	0	0	0	1
chr10	18429605	18429845	CACNB2	0,70	7	4	2	8	6	3	2	5	5	5	6	3	5	6	2	4
chr10	18430006	18430246	CACNB2	0,68	3	5	2	2	4	3	3	3	6	3	2	2	7	6	4	4
chr10	18439809	18440001	CACNB2	0,34	209	207	136	115	175	163	111	107	195	165	187	134	181	161	163	138
chr10	18550102	18550342	CACNB2	0,52	46	51	46	36	51	33	38	30	60	46	43	45	50	57	48	43
chr10	18629760	18630000	CACNB2	0,47	168	155	130	108	151	142	111	90	180	157	161	138	154	153	149	124
chr10	18689874	18690114	CACNB2	0,56	84	81	56	55	73	68	48	43	89	74	73	62	78	82	68	53
chr10	18690792	18691032	CACNB2	0,50	80	91	61	68	96	81	62	51	108	80	88	72	80	78	82	78
chr10	18787224	18787464	CACNB2	0,42	201	246	172	120	191	175	142	108	236	221	232	176	209	201	217	161
chr10	18789688	18789928	CACNB2	0,39	138	136	107	103	136	118	95	77	164	137	142	116	142	139	145	114
chr10	18795317	18795557	CACNB2	0,34	210	221	158	127	157	183	135	105	242	232	220	178	196	205	221	168
chr10	18803111	18803351	CACNB2	0,43	287	299	230	157	233	238	188	147	307	307	280	217	265	290	290	217

chr10	18803389	18803509	CACNB2	0,40	146	136	119	87	142	126	118	91	171	146	153	125	149	159	149	116
chr10	18803819	18804059	CACNB2	0,35	247	286	191	156	211	210	178	138	273	237	255	228	268	259	269	202
chr10	18807184	18807424	CACNB2	0,50	182	195	154	109	171	184	140	96	225	200	188	168	189	186	196	149
chr10	18807747	18807987	CACNB2	0,35	151	184	131	109	160	161	123	90	201	165	184	149	181	164	194	122
chr10	18816451	18816691	CACNB2	0,43	257	272	199	150	228	224	187	139	276	264	274	226	268	279	271	193
chr10	18822900	18823260	CACNB2	0,35	264	308	233	155	232	231	189	147	309	300	294	237	279	276	289	228
chr10	18824955	18825171	CACNB2	0,37	191	190	168	127	190	171	121	112	218	172	194	163	182	171	172	169
chr10	18827021	18827381	CACNB2	0,51	241	214	189	138	192	183	162	128	243	227	213	197	229	220	242	185
chr10	18828105	18828705	CACNB2	0,52	141	146	110	97	145	132	102	77	174	146	148	129	149	148	136	115
chr10	50822315	50822627	CHAT	0,74	0	1	0	0	1	1	0	0	0	1	2	0	1	1	1	1
chr10	50823998	50824238	CHAT	0,52	85	90	74	63	78	81	66	44	93	81	100	75	76	99	88	76
chr10	50824477	50824717	CHAT	0,56	89	98	81	67	97	87	75	60	115	99	103	92	92	103	88	88
chr10	50827686	50828046	CHAT	0,60	41	27	23	28	42	29	24	25	37	28	41	35	34	38	35	38
chr10	50828479	50828719	CHAT	0,62	30	32	28	28	45	31	23	27	51	30	33	37	37	29	34	28
chr10	50830100	50830268	CHAT	0,62	16	15	10	12	20	14	7	10	19	16	18	18	18	17	14	16
chr10	50833428	50833788	CHAT	0,58	187	181	164	120	172	165	132	110	211	189	178	173	182	181	185	146
chr10	50835562	50835922	CHAT	0,57	145	152	113	81	137	138	98	83	168	144	145	137	152	142	149	114
chr10	50854455	50854815	CHAT	0,66	19	25	14	16	24	22	17	15	24	12	26	24	20	22	23	21
chr10	50856482	50856722	CHAT	0,61	38	32	28	34	41	27	26	25	50	29	37	37	35	26	31	31
chr10	50857497	50857737	CHAT	0,63	19	24	13	20	32	22	16	18	33	24	23	28	21	33	19	20
chr10	50859870	50860110	CHAT	0,45	158	142	101	98	146	144	106	85	155	139	143	123	139	134	139	132
chr10	50863031	50863582	CHAT	0,56	87	91	77	70	93	83	70	58	120	92	103	83	102	94	94	77
chr10	50870639	50870879	CHAT	0,57	100	100	72	75	97	82	78	59	116	100	103	80	106	107	93	90
chr10	50872717	50873197	CHAT	0,55	168	158	122	118	159	151	120	104	201	165	193	149	166	182	179	146
chr10	64572877	64574317	EGR2	0,61	61	62	50	43	66	57	40	41	78	52	65	58	67	66	61	60
chr10	64575524	64575884	EGR2	0,60	54	58	52	44	65	54	41	37	68	52	62	49	54	54	50	49
chr10	69881106	69882186	MYPN	0,46	248	259	205	150	220	215	184	132	283	264	255	216	255	255	276	195
chr10	69902600	69902912	MYPN	0,42	273	267	203	152	231	214	188	133	288	277	284	224	255	257	274	215
chr10	69905137	69905377	MYPN	0,33	240	224	171	140	176	188	174	107	250	222	244	188	222	230	223	159
chr10	69908093	69908309	MYPN	0,50	191	199	144	131	179	186	145	105	218	213	221	179	194	213	208	153
chr10	69909712	69909952	MYPN	0,35	312	311	262	178	250	261	223	162	341	324	316	274	304	315	330	214
chr10	69918133	69918493	MYPN	0,29	216	232	163	131	167	185	155	120	233	217	210	172	217	224	218	175
chr10	69921377	69921617	MYPN	0,30	262	268	211	153	203	225	179	129	288	255	283	231	244	240	253	204
chr10	69925396	69925636	MYPN	0,41	218	213	184	128	212	186	153	127	275	239	233	202	225	235	237	167
chr10	69925996	69926476	MYPN	0,54	131	127	113	94	132	127	96	84	162	125	133	125	136	145	132	115
chr10	69933757	69934477	MYPN	0,50	322	338	269	173	260	251	222	163	355	352	325	271	338	345	350	249
chr10	69935028	69935268	MYPN	0,39	176	192	121	115	159	143	112	101	193	160	190	137	172	174	168	144
chr10	69948592	69948952	MYPN	0,44	197	215	166	110	170	176	134	111	233	183	198	169	193	216	210	169
chr10	69954014	69954374	MYPN	0,47	192	210	159	128	177	172	141	108	198	186	211	168	198	196	194	145
chr10	69955127	69955367	MYPN	0,44	169	186	134	111	171	141	121	98	209	169	182	145	158	167	162	142
chr10	69957051	69957291	MYPN	0,44	106	112	87	83	105	96	83	67	131	99	119	95	99	114	111	93
chr10	69959048	69959408	MYPN	0,51	142	146	99	93	142	117	116	86	169	135	130	115	126	150	128	118
chr10	69961563	69961803	MYPN	0,55	101	121	88	68	104	81	68	65	120	97	95	77	108	97	99	95
chr10	69966473	69966713	MYPN	0,46	117	115	91	91	115	111	92	76	148	106	126	104	110	124	116	96
chr10	69969947	69970307	MYPN	0,50	173	174	134	121	177	146	116	102	197	160	176	151	180	176	184	131
chr10	75757869	75758229	VCL	0,71	15	17	14	15	21	15	12	14	18	15	18	16	17	15	14	21
chr10	75802755	75802995	VCL	0,29	221	219	166	131	169	202	162	112	241	221	232	201	238	224	227	163
chr10	75830322	75830906	VCL	0,41	242	253	194	152	218	211	176	126	252	254	242	212	234	250	255	196
chr10	75832428	75832668	VCL	0,46	150	168	135	94	135	124	111	87	177	147	169	133	148	135	156	119
chr10	75834400	75834760	VCL	0,35	242	246	194	141	194	204	171	129	254	226	227	207	248	216	236	182
chr10	75842136	75842376	VCL	0,43	129	140	122	101	151	152	119	92	174	152	169	136	146	143	150	123
chr10	75843017	75843377	VCL	0,42	202	222	156	123	176	173	136	113	244	193	203	167	202	204	211	150
chr10	75848850	75849210	VCL	0,43	158	149	118	90	136	135	110	81	170	134	153	129	158	156	141	123
chr10	75849729	75849969	VCL	0,44	160	179	149	117	153	156	120	96	204	171	170	156	180	178	179	147
chr10	75853943	75854303	VCL	0,50	106	110	87	76	113	95	79	56	120	109	116	86	108	109	98	93
chr10	75855333	75855693	VCL	0,52	85	84	58	67	101	77	44	61	100	68	87	61	80	84	83	66
chr10	75856905	75857145	VCL	0,46	130	122	89	81	121	105	82	77	132	112	114	103	123	131	113	102
chr10	75860600	75860960	VCL	0,41	167	179	125	122	169	141	125	90	196	159	177	141	161	154	165	131
chr10	75863511	75863751	VCL	0,41	212	235	178	137	188	199	177	122	226	222	241	196	241	227	220	195
chr10	75864719	75865199	VCL	0,48	124	130	93	85	117	105	90	70	138	121	141	102	125	118	106	99

chr10	75866929	75867169	VCL	0,52	62	47	44	46	69	56	39	47	84	53	67	54	56	66	61	54
chr10	75868641	75869001	VCL	0,47	126	126	111	105	138	120	100	77	167	124	138	120	129	143	138	123
chr10	75871588	75871948	VCL	0,55	149	161	128	101	136	139	105	92	194	142	165	145	151	142	161	132
chr10	75873847	75874159	VCL	0,55	77	63	53	46	75	64	52	44	79	64	70	64	77	76	67	50
chr10	75874526	75874742	VCL	0,47	56	54	35	43	57	54	37	37	65	50	61	51	56	58	53	63
chr10	75877673	75878033	VCL	0,52	104	110	76	78	100	86	73	58	120	93	107	90	97	101	89	75
chr10	88428374	88428614	LDB3	0,65	21	13	13	12	15	12	13	13	20	16	16	14	11	13	16	12
chr10	88439019	88439379	LDB3	0,59	92	79	63	68	88	76	71	59	108	77	100	86	90	93	96	77
chr10	88439756	88439996	LDB3	0,63	73	70	55	58	86	63	51	39	90	66	83	74	74	77	73	65
chr10	88441136	88441616	LDB3	0,68	15	14	9	12	16	10	12	10	23	13	19	15	14	16	17	13
chr10	88445325	88445565	LDB3	0,57	115	98	76	71	114	102	77	69	105	88	114	100	108	96	105	81
chr10	88446747	88447107	LDB3	0,64	86	91	69	73	97	80	68	65	113	80	94	81	95	89	95	94
chr10	88451557	88451917	LDB3	0,63	60	59	44	45	65	52	41	34	70	52	66	51	57	59	58	51
chr10	88452189	88452429	LDB3	0,61	73	79	51	63	98	65	50	49	102	75	72	76	80	86	68	69
chr10	88459007	88459223	LDB3	0,53	150	141	122	101	160	155	100	100	182	160	183	151	162	160	160	124
chr10	88466201	88466561	LDB3	0,67	28	28	22	21	27	25	17	17	32	25	31	28	25	30	28	24
chr10	88469554	88469914	LDB3	0,65	13	11	15	13	19	14	14	14	19	11	16	11	20	20	14	13
chr10	88476025	88476145	LDB3	0,52	32	30	26	35	34	24	22	28	40	28	44	36	39	41	35	28
chr10	88476235	88476595	LDB3	0,66	34	34	29	27	37	30	22	26	46	35	37	32	34	36	31	33
chr10	88477683	88477995	LDB3	0,54	112	123	93	88	108	86	90	69	127	115	116	97	132	110	102	98
chr10	88478423	88478663	LDB3	0,58	44	51	40	52	51	51	37	31	63	44	53	48	50	53	41	50
chr10	88485831	88486071	LDB3	0,57	48	57	44	38	57	49	41	44	63	51	49	56	54	55	62	40
chr10	88492568	88492808	LDB3	0,56	43	39	28	30	43	35	28	27	50	43	35	35	30	34	37	42
chr10	92672557	92672797	ANKRD1	0,45	224	229	155	130	201	174	152	120	236	226	217	179	204	225	223	162
chr10	92675228	92675707	ANKRD1	0,51	104	86	65	69	89	78	69	57	109	84	97	73	83	94	87	76
chr10	92675890	92676106	ANKRD1	0,49	75	82	63	57	90	66	61	53	103	78	84	84	90	73	83	61
chr10	92677417	92677657	ANKRD1	0,38	268	288	218	159	231	212	200	131	273	280	278	221	287	266	302	197
chr10	92678535	92678751	ANKRD1	0,38	220	213	203	144	216	176	108	117	201	206	180	191	209	169	185	148
chr10	92678836	92679076	ANKRD1	0,32	216	218	175	124	173	171	120	95	223	197	194	163	206	171	204	158
chr10	92679835	92680195	ANKRD1	0,52	292	300	246	161	248	259	211	159	353	310	308	253	316	329	333	234
chr10	92680650	92680890	ANKRD1	0,44	250	277	190	154	211	220	169	142	276	239	252	217	261	264	286	189
chr10	99498223	99498535	ZFYVE27	0,54	194	191	141	126	174	167	131	98	232	183	202	184	197	195	188	150
chr10	99502765	99503005	ZFYVE27	0,54	83	84	79	70	96	84	70	58	117	92	92	74	80	84	93	79
chr10	99504414	99504702	ZFYVE27	0,59	21	20	16	16	20	23	16	15	24	16	26	22	23	16	21	18
chr10	99507953	99508193	ZFYVE27	0,62	44	40	31	36	33	40	24	23	51	35	37	32	35	39	34	37
chr10	99509166	99509406	ZFYVE27	0,54	199	206	161	130	186	180	140	117	229	187	214	159	183	186	198	155
chr10	99510037	99510277	ZFYVE27	0,58	42	45	32	33	39	38	24	22	45	36	47	44	38	42	33	31
chr10	99511055	99511295	ZFYVE27	0,57	35	35	31	33	38	34	34	28	42	35	57	32	38	39	37	37
chr10	99512503	99513046	ZFYVE27	0,63	16	14	13	9	16	11	11	10	16	10	17	12	15	14	13	14
chr10	99516924	99517164	ZFYVE27	0,60	49	55	37	44	52	56	33	35	53	46	58	43	58	43	45	46
chr10	99517319	99517559	ZFYVE27	0,60	38	32	28	25	40	29	27	21	42	27	34	37	39	44	48	30
chr10	99518904	99519144	ZFYVE27	0,58	39	42	38	41	36	41	25	31	42	42	52	40	46	46	49	49
chr11	10011016	10011232	SBF2	0,31	253	293	240	159	214	232	189	141	281	270	259	239	261	265	266	205
chr11	10013905	10014145	SBF2	0,36	246	275	210	156	217	215	196	140	271	276	242	225	267	251	282	185
chr11	10014473	10014713	SBF2	0,30	224	253	198	148	182	195	188	119	265	258	242	209	237	246	244	190
chr11	10015386	10015626	SBF2	0,29	196	223	163	120	181	192	138	107	222	205	216	185	203	176	208	156
chr11	10019749	10019989	SBF2	0,31	264	270	212	154	204	223	192	131	300	258	270	240	264	265	300	214
chr11	10022394	10022634	SBF2	0,33	179	214	134	100	157	153	126	95	194	168	184	152	161	186	182	137
chr11	10024049	10024289	SBF2	0,34	209	232	180	134	171	194	172	118	267	230	229	184	226	219	229	190
chr11	10049929	10050169	SBF2	0,39	211	211	168	122	201	178	141	94	211	187	199	150	189	215	196	157
chr11	10051246	10051486	SBF2	0,41	154	172	148	120	153	153	126	98	193	170	177	155	164	163	173	134
chr11	10052535	10052775	SBF2	0,35	271	266	230	140	207	238	188	130	318	284	273	228	272	270	313	210
chr11	10064339	10064579	SBF2	0,39	116	121	98	86	124	107	85	77	130	107	129	104	117	128	115	100
chr11	10215371	10215611	SBF2	0,32	328	364	265	165	261	278	230	161	329	339	317	280	329	321	335	257
chr11	108098307	108098667	ATM	0,30	265	286	207	143	198	218	186	135	271	250	262	197	265	250	258	210
chr11	108099797	108100157	ATM	0,31	258	301	230	152	213	250	195	133	286	269	280	227	272	248	286	212
chr11	108106298	108106658	ATM	0,33	274	307	232	156	236	246	191	144	296	299	278	247	297	269	285	216
chr11	108114666	108114906	ATM	0,34	261	305	204	165	199	225	182	133	264	214	254	206	260	266	269	190
chr11	108115453	108115813	ATM	0,35	300	347	256	168	228	263	206	149	333	346	300	242	338	321	343	228
chr11	108117592	108117952	ATM	0,33	268	309	223	149	213	233	200	141	305	279	254	215	267	261	291	216

chr11	108119625	108119937	ATM	0,33	285	302	256	165	207	256	224	151	298	326	283	254	307	294	318	248
chr11	108121362	108121842	ATM	0,37	279	272	220	158	228	226	185	144	298	265	282	230	267	271	279	218
chr11	108122480	108122840	ATM	0,38	306	337	262	176	250	272	226	165	343	330	332	281	325	312	325	240
chr11	108123471	108123711	ATM	0,31	225	243	189	129	170	214	166	118	246	247	255	209	253	231	269	179
chr11	108124473	108124833	ATM	0,35	260	287	204	138	204	226	204	126	297	281	271	213	272	254	283	204
chr11	108126925	108127141	ATM	0,35	161	188	119	109	154	152	117	96	176	147	161	148	150	146	164	118
chr11	108128128	108128344	ATM	0,33	192	210	174	119	190	186	145	109	219	197	189	164	209	200	193	157
chr11	108129624	108129840	ATM	0,32	263	253	203	157	206	198	166	127	257	264	246	223	234	247	240	186
chr11	108137803	108138163	ATM	0,39	275	278	217	142	244	235	200	132	297	296	287	226	281	279	287	212
chr11	108139056	108139416	ATM	0,37	284	315	224	164	231	243	192	134	320	315	293	231	307	285	291	226
chr11	108141711	108142235	ATM	0,35	320	349	275	188	271	297	237	167	350	353	328	294	317	331	353	262
chr11	108143176	108143633	ATM	0,33	276	315	223	168	238	247	223	157	318	300	294	247	289	306	322	223
chr11	108150203	108150419	ATM	0,36	182	228	144	119	158	175	135	111	218	194	203	177	222	185	210	164
chr11	108151628	108151988	ATM	0,32	239	281	206	136	204	220	171	154	277	257	251	206	275	258	283	187
chr11	108153341	108153701	ATM	0,29	313	330	268	173	233	254	198	155	309	296	292	247	304	301	316	233
chr11	108154896	108155256	ATM	0,33	238	249	197	151	199	213	179	125	259	250	239	202	240	244	228	193
chr11	108158307	108158523	ATM	0,37	206	222	165	129	192	184	147	111	209	186	211	161	202	190	192	151
chr11	108159646	108159886	ATM	0,32	262	294	227	143	185	247	211	146	303	287	282	247	265	266	301	222
chr11	108160248	108160584	ATM	0,30	256	274	204	146	190	222	188	150	263	246	260	220	247	267	257	207
chr11	108163252	108163612	ATM	0,35	282	296	244	153	238	237	202	148	308	295	271	246	280	277	304	212
chr11	108164026	108164266	ATM	0,29	272	288	217	154	205	235	202	139	295	260	264	236	257	250	277	222
chr11	108165599	108165839	ATM	0,35	252	266	212	152	221	236	192	123	302	263	258	230	262	253	276	201
chr11	108167920	108168136	ATM	0,32	199	202	164	119	170	184	135	104	221	188	204	171	199	188	191	146
chr11	108170346	108170706	ATM	0,33	232	265	197	147	204	212	170	131	272	264	269	217	265	262	267	189
chr11	108172265	108172625	ATM	0,32	282	314	233	157	228	240	210	145	297	281	279	234	268	277	299	216
chr11	108173487	108173847	ATM	0,32	277	319	235	166	214	249	201	140	310	306	302	251	285	288	296	235
chr11	108175310	108175670	ATM	0,34	292	291	227	148	226	238	204	148	299	297	294	247	294	270	294	208
chr11	108178547	108178787	ATM	0,39	196	209	163	119	173	160	138	99	223	211	189	180	207	194	207	154
chr11	108180784	108181144	ATM	0,31	292	326	252	163	218	253	213	138	311	321	309	246	303	310	326	222
chr11	108183061	108183301	ATM	0,32	207	214	171	133	161	198	141	120	230	200	218	186	211	193	221	169
chr11	108186454	108186934	ATM	0,37	267	275	220	146	227	234	202	134	302	289	279	221	261	276	286	207
chr11	108187993	108188353	ATM	0,32	235	301	235	142	245	222	172	128	277	271	247	260	245	289	296	199
chr11	108190612	108190852	ATM	0,30	238	298	205	134	211	225	189	121	284	261	255	242	257	256	262	200
chr11	108191967	108192207	ATM	0,41	185	174	140	114	182	175	134	108	218	163	178	173	189	175	182	152
chr11	108195973	108196333	ATM	0,35	283	296	219	157	235	236	210	140	287	276	282	248	280	278	283	195
chr11	108196688	108197024	ATM	0,35	214	219	166	119	190	195	148	115	234	214	225	182	229	212	197	173
chr11	108198308	108198548	ATM	0,39	255	248	227	138	216	226	167	133	268	253	237	216	241	251	258	181
chr11	108199676	108200036	ATM	0,34	204	217	157	125	180	187	156	109	230	207	207	170	198	196	207	170
chr11	108200861	108201197	ATM	0,38	248	274	206	131	208	220	169	126	276	250	239	212	271	257	249	205
chr11	108202095	108202311	ATM	0,34	198	251	162	135	167	186	149	133	226	220	227	190	217	211	220	169
chr11	108202504	108202864	ATM	0,30	282	306	224	161	224	246	192	137	291	295	276	250	277	294	316	224
chr11	108203437	108203677	ATM	0,34	156	189	121	113	131	146	112	85	183	156	176	142	169	166	160	121
chr11	108204532	108204748	ATM	0,30	249	296	208	151	205	236	187	122	293	266	282	216	238	244	271	193
chr11	108205585	108205945	ATM	0,33	274	281	224	168	198	233	178	138	290	264	258	230	261	263	291	199
chr11	108206509	108206749	ATM	0,35	184	195	147	115	161	152	120	89	182	171	186	166	176	199	170	159
chr11	108213843	108214203	ATM	0,35	231	267	195	137	209	219	168	122	271	256	250	209	248	259	257	197
chr11	108216396	108216732	ATM	0,31	253	281	223	158	209	228	182	131	289	252	269	215	255	248	265	204
chr11	108217928	108218168	ATM	0,31	218	217	162	131	170	173	156	121	232	207	218	188	210	193	234	177
chr11	108224429	108224669	ATM	0,40	233	262	184	150	214	221	172	135	253	248	256	206	273	234	258	207
chr11	108225449	108225689	ATM	0,36	223	243	181	144	199	207	191	122	259	225	226	191	242	233	237	180
chr11	108235756	108236323	ATM	0,39	300	307	239	161	261	249	196	142	324	292	300	243	289	303	302	240
chr11	111779409	111779769	CRYAB	0,48	98	96	81	64	100	79	73	48	119	88	97	87	95	93	87	84
chr11	111780991	111781231	CRYAB	0,44	179	193	158	124	173	169	149	104	218	198	200	183	196	208	215	172
chr11	111782167	111782527	CRYAB	0,58	44	35	30	38	40	44	31	26	49	43	44	30	44	46	41	37
chr11	116706465	116707185	APOA1	0,65	75	67	54	53	75	64	45	39	76	65	65	65	73	72	69	61
chr11	116707614	116708201	APOA1	0,62	49	50	39	42	57	40	42	34	71	48	57	45	57	56	46	48
chr11	18418005	18418245	LDHA	0,50	265	290	218	155	239	236	194	143	295	276	263	234	278	256	283	212
chr11	18418260	18418620	LDHA	0,40	370	376	296	195	280	316	269	175	370	374	344	307	361	330	361	267
chr11	18420913	18421153	LDHA	0,36	209	244	174	124	182	185	141	120	233	234	225	189	209	213	220	153
chr11	18422290	18422626	LDHA	0,37	285	304	222	168	241	233	201	146	302	265	287	243	294	279	272	232

chr11	18424293	18424653	LDHA	0,39	182	197	141	122	179	163	132	108	189	187	175	143	170	174	169	146
chr11	18425179	18425419	LDHA	0,35	178	182	131	116	162	145	136	94	190	176	169	136	187	195	172	136
chr11	18426937	18427177	LDHA	0,42	206	236	171	139	188	180	170	111	246	214	218	196	223	225	211	171
chr11	18428613	18428973	LDHA	0,39	233	234	185	140	213	215	184	130	257	243	254	205	250	242	230	171
chr11	1860872	1860992	TNNI2	0,61	8	10	4	11	14	13	7	10	12	9	11	7	11	9	12	8
chr11	1861400	1861520	TNNI2	0,65	2	0	0	0	0	1	0	0	0	3	0	3	3	1	1	1
chr11	1861578	1861938	TNNI2	0,70	7	5	3	7	7	5	5	4	6	4	6	6	7	4	6	5
chr11	1861954	1862146	TNNI2	0,64	10	10	6	6	8	8	8	5	14	6	7	5	10	10	6	8
chr11	1862192	1862528	TNNI2	0,68	10	11	6	7	8	9	4	7	12	6	11	7	6	9	5	7
chr11	1862613	1862853	TNNI2	0,62	14	13	7	13	16	17	9	10	18	10	12	13	12	12	10	12
chr11	19204134	19204374	CSRP3	0,44	175	176	137	124	161	147	125	100	209	187	165	159	185	184	179	157
chr11	19206425	19206665	CSRP3	0,45	163	186	150	96	168	138	119	100	201	184	186	149	191	185	173	129
chr11	19207708	19207948	CSRP3	0,50	154	155	125	106	156	132	100	85	172	152	161	146	152	155	147	138
chr11	19209586	19209946	CSRP3	0,55	94	106	83	68	104	94	78	56	117	85	111	86	104	103	101	94
chr11	19213819	19214059	CSRP3	0,49	123	124	99	91	120	119	96	72	142	120	139	106	126	119	121	102
chr11	1944066	1944186	TNNT3	0,61	56	51	43	45	70	48	35	38	58	41	51	49	57	60	52	53
chr11	1944733	1944853	TNNT3	0,56	7	6	5	6	7	9	6	5	10	7	8	7	9	11	8	7
chr11	1946277	1946615	TNNT3	0,65	75	82	65	69	90	79	49	66	82	71	88	84	82	89	74	75
chr11	1947645	1947765	TNNT3	0,60	13	5	4	7	12	10	6	6	4	9	11	5	10	9	9	10
chr11	1947871	1947991	TNNT3	0,63	35	41	22	33	47	35	24	31	42	37	46	29	43	32	40	43
chr11	1950241	1950481	TNNT3	0,67	12	12	10	14	13	20	9	8	17	14	14	10	14	12	11	13
chr11	1950988	1951108	TNNT3	0,65	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chr11	1953601	1953841	TNNT3	0,59	77	78	64	75	75	68	55	49	111	74	86	81	87	77	72	71
chr11	1954854	1955334	TNNT3	0,63	27	26	19	20	32	27	22	19	30	20	20	22	20	27	22	20
chr11	1955483	1955963	TNNT3	0,60	26	19	21	19	29	21	15	14	29	19	27	26	28	23	24	26
chr11	1955983	1956223	TNNT3	0,62	45	35	28	31	40	31	30	26	43	36	36	33	29	40	29	39
chr11	1957343	1957583	TNNT3	0,62	22	25	21	24	30	29	26	20	38	20	27	29	24	21	25	24
chr11	1958092	1958332	TNNT3	0,67	3	6	2	3	6	6	3	2	5	4	6	4	3	1	3	2
chr11	1959574	1959814	TNNT3	0,66	5	5	4	4	2	3	1	2	6	2	4	3	4	6	4	2
chr11	22214938	22215178	ANO5	0,67	34	26	24	25	37	30	17	18	34	29	38	32	32	33	35	28
chr11	22225252	22225492	ANO5	0,27	220	255	198	123	190	197	162	120	245	250	240	225	228	234	240	202
chr11	22232714	22232954	ANO5	0,41	307	308	220	167	240	258	196	140	304	286	285	259	286	305	304	216
chr11	22239692	22239932	ANO5	0,40	167	187	141	106	146	142	104	101	205	156	174	153	162	192	177	139
chr11	22242579	22242819	ANO5	0,33	192	253	171	126	188	199	142	114	238	209	225	179	232	214	217	160
chr11	22247443	22247683	ANO5	0,33	226	224	162	127	186	186	142	105	218	221	224	168	210	224	233	161
chr11	22248749	22249229	ANO5	0,41	319	322	263	170	249	265	214	152	339	337	327	274	307	318	352	228
chr11	22257645	22257885	ANO5	0,39	264	282	200	145	231	215	185	143	305	284	271	226	270	275	293	219
chr11	22261052	22261292	ANO5	0,37	297	321	262	146	230	264	223	149	316	325	303	275	298	297	342	259
chr11	22271729	22271969	ANO5	0,34	214	256	167	114	180	185	152	98	249	223	233	194	248	246	234	163
chr11	22272219	22272646	ANO5	0,35	292	316	245	146	229	234	208	141	301	301	287	255	291	286	296	218
chr11	22276893	22277133	ANO5	0,39	136	159	105	87	152	106	99	86	154	128	147	126	136	140	141	107
chr11	22279142	22279382	ANO5	0,38	226	234	171	145	190	182	148	104	262	219	236	195	236	245	203	178
chr11	22280995	22281355	ANO5	0,37	338	382	286	182	241	295	238	170	385	359	342	297	336	356	379	246
chr11	22283579	22283939	ANO5	0,35	281	288	226	159	226	243	184	133	318	281	283	246	279	290	326	230
chr11	22284420	22284660	ANO5	0,35	294	306	239	163	246	246	191	159	315	302	295	243	268	304	325	246
chr11	22291802	22292042	ANO5	0,40	193	208	167	126	182	152	129	100	212	185	216	166	194	204	220	146
chr11	22294276	22294612	ANO5	0,40	301	318	252	171	234	249	206	134	342	297	314	248	317	312	313	236
chr11	22296023	22296383	ANO5	0,35	297	333	262	177	257	256	218	145	340	341	312	269	335	325	356	243
chr11	22297572	22297812	ANO5	0,34	281	312	226	149	203	229	194	141	297	297	290	233	257	293	293	208
chr11	22301020	22301380	ANO5	0,38	283	307	226	157	246	241	177	147	291	281	283	228	287	288	291	219
chr11	2466245	2466461	KCNQ1	0,80	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	1
chr11	2466536	2466776	KCNQ1	0,70	6	9	8	10	16	11	8	10	16	10	15	12	12	10	8	15
chr11	2549082	2549322	KCNQ1	0,63	68	56	49	40	64	68	49	46	64	64	67	63	61	64	71	61
chr11	2591800	2592040	KCNQ1	0,65	13	9	4	8	10	5	7	6	12	9	9	7	8	7	9	9
chr11	2592473	2592713	KCNQ1	0,67	21	12	16	14	22	17	10	12	27	17	23	16	21	23	22	21
chr11	2593170	2593410	KCNQ1	0,69	2	5	4	4	7	5	2	2	3	3	6	1	6	4	2	5
chr11	2593965	2594325	KCNQ1	0,62	32	31	19	24	37	32	24	21	43	29	34	25	32	33	30	26
chr11	2604599	2604839	KCNQ1	0,63	75	72	55	51	85	64	59	48	84	68	79	68	68	70	63	62
chr11	2606369	2606609	KCNQ1	0,61	76	54	45	51	67	55	45	45	72	59	65	65	60	62	61	56
chr11	2608740	2608980	KCNQ1	0,66	28	21	21	24	36	22	13	16	27	22	29	20	22	24	25	24

chr11	2609833	2610193	KCNQ1	0,58	46	52	46	46	48	52	44	37	60	52	61	52	51	62	53	45
chr11	2683130	2683370	KCNQ1	0,58	80	77	61	59	77	74	51	48	103	85	83	72	83	76	76	69
chr11	2789991	2790231	KCNQ1	0,60	76	63	57	63	79	57	55	54	90	62	74	69	70	72	68	65
chr11	2797116	2797356	KCNQ1	0,63	19	16	8	11	19	12	8	9	17	11	14	13	10	12	10	11
chr11	2798118	2798358	KCNQ1	0,60	46	47	36	42	53	36	33	24	58	44	52	50	43	38	39	42
chr11	2799116	2799356	KCNQ1	0,63	40	28	27	30	49	28	25	27	49	36	37	34	37	32	35	26
chr11	2868934	2869294	KCNQ1	0,69	7	6	5	3	7	4	6	7	6	4	6	6	4	5	8	4
chr11	47459441	47459681	RAPSN	0,64	3	2	3	6	5	4	3	4	8	3	5	7	5	5	5	7
chr11	47460199	47460535	RAPSN	0,63	19	19	15	20	25	16	17	13	20	19	15	22	17	17	11	16
chr11	47462616	47462856	RAPSN	0,62	111	102	85	79	105	98	69	68	119	90	108	88	103	107	101	91
chr11	47463077	47463557	RAPSN	0,66	5	4	3	4	7	5	3	7	9	5	7	5	5	6	7	10
chr11	47464106	47464466	RAPSN	0,62	35	26	20	24	30	26	23	21	38	32	34	28	28	28	34	29
chr11	47469292	47469772	RAPSN	0,63	26	18	17	21	31	20	16	18	27	20	27	26	20	23	19	26
chr11	47470240	47470600	RAPSN	0,65	52	65	44	56	63	45	45	36	71	55	67	50	56	67	59	61
chr11	62457728	62458448	BSCL2	0,57	45	39	36	43	56	45	37	29	63	44	46	42	47	46	47	51
chr11	62458459	62459002	BSCL2	0,53	154	151	117	104	148	128	108	87	162	141	164	134	152	149	150	119
chr11	62459776	62460016	BSCL2	0,62	31	23	30	26	30	38	29	23	37	31	29	29	34	32	32	28
chr11	62460081	62460321	BSCL2	0,56	65	65	64	54	76	67	50	41	90	57	63	60	68	71	68	65
chr11	62461979	62462291	BSCL2	0,50	193	204	170	155	196	207	155	129	231	204	219	207	226	219	227	157
chr11	62469860	62470100	BSCL2	0,48	196	173	150	121	164	164	142	109	208	209	200	159	207	204	195	170
chr11	62472720	62473176	BSCL2	0,58	25	25	21	18	28	23	14	16	26	22	28	20	28	22	23	25
chr11	62474503	62474743	BSCL2	0,54	281	294	228	173	233	252	196	142	280	267	272	253	292	259	286	201
chr11	64514025	64514545	PYGM	0,58	95	91	72	68	113	87	68	66	108	92	108	89	101	89	87	87
chr11	64514642	64514882	PYGM	0,59	51	60	36	46	60	45	50	39	78	53	50	42	62	56	56	56
chr11	64517771	64518131	PYGM	0,59	177	172	139	113	169	172	120	88	196	177	179	139	196	169	177	140
chr11	64518687	64519217	PYGM	0,57	103	108	81	86	104	98	73	68	121	98	107	93	105	104	95	91
chr11	64519313	64519625	PYGM	0,54	70	69	56	61	74	65	49	51	93	65	69	65	79	65	70	65
chr11	64519805	64520045	PYGM	0,58	139	141	111	112	151	123	104	89	184	141	148	130	162	127	145	138
chr11	64520481	64520721	PYGM	0,58	86	86	72	67	105	79	56	47	107	73	87	75	87	80	77	76
chr11	64520892	64521603	PYGM	0,67	14	13	12	11	17	13	7	9	16	10	16	15	11	14	10	13
chr11	64521650	64521890	PYGM	0,62	17	12	12	9	16	14	10	13	12	7	12	12	16	11	11	13
chr11	64522056	64522416	PYGM	0,62	23	24	17	23	29	22	14	17	28	20	22	19	21	23	21	22
chr11	64522647	64523127	PYGM	0,61	64	54	46	50	67	51	43	37	69	51	58	48	55	52	57	50
chr11	64525196	64525436	PYGM	0,62	50	50	35	40	63	45	35	44	66	41	64	47	48	48	47	52
chr11	64525646	64526246	PYGM	0,60	87	74	61	62	77	69	64	48	89	69	78	64	85	74	73	65
chr11	64527068	64527428	PYGM	0,62	62	67	51	45	68	59	49	41	71	60	60	59	61	59	50	66
chr11	6625425	6625665	ILK	0,60	35	37	31	19	40	29	22	20	32	27	35	30	29	35	28	34
chr11	6629202	6629538	ILK	0,53	166	171	127	127	164	140	112	90	178	162	160	137	174	164	150	147
chr11	6629551	6629791	ILK	0,49	158	157	123	117	151	136	124	85	197	158	163	149	170	144	154	141
chr11	6629820	6631563	ILK	0,52	248	257	199	154	221	219	175	133	260	253	244	219	256	249	244	202
chr11	6631587	6631947	ILK	0,51	160	155	110	101	136	117	102	92	162	146	138	118	149	137	146	128
chr11	66453278	66453638	SPTBN2	0,60	38	44	33	31	44	32	29	28	53	43	45	36	43	45	46	36
chr11	66453769	66454009	SPTBN2	0,54	65	54	51	39	70	60	52	46	78	62	72	55	66	68	64	53
chr11	66454371	66454731	SPTBN2	0,62	175	165	143	104	149	147	114	87	179	169	180	140	174	177	178	131
chr11	66454827	66455187	SPTBN2	0,65	20	14	11	13	19	15	16	15	21	15	19	14	20	17	16	19
chr11	66455272	66455752	SPTBN2	0,62	48	50	43	43	53	49	38	34	58	43	55	47	49	48	56	44
chr11	66456041	66456401	SPTBN2	0,61	69	60	52	59	69	68	49	42	79	62	71	66	71	61	65	69
chr11	66456488	66456728	SPTBN2	0,63	32	36	28	27	37	37	24	23	41	34	32	32	36	30	31	34
chr11	66457214	66457814	SPTBN2	0,65	60	51	43	41	59	41	39	35	67	53	60	48	54	51	49	46
chr11	66458701	66459181	SPTBN2	0,64	19	19	14	17	21	15	16	18	26	18	19	21	19	19	20	22
chr11	66459928	66460288	SPTBN2	0,64	25	25	20	21	32	29	16	20	31	25	23	17	26	29	24	22
chr11	66460378	66460978	SPTBN2	0,64	28	27	20	24	39	24	23	20	33	26	31	28	29	28	29	30
chr11	66461167	66461407	SPTBN2	0,58	22	25	21	20	36	28	18	19	35	23	23	24	26	23	27	28
chr11	66461541	66461901	SPTBN2	0,62	77	78	60	67	95	70	54	59	104	72	86	81	92	86	82	85
chr11	66463639	66464119	SPTBN2	0,58	76	73	57	55	94	69	54	46	89	75	82	73	74	76	76	72
chr11	66466002	66466362	SPTBN2	0,58	89	88	66	72	93	85	67	71	116	86	101	86	93	94	91	87
chr11	66466387	66466627	SPTBN2	0,54	110	110	97	79	121	102	81	78	123	96	119	93	113	127	125	94
chr11	66466797	66467157	SPTBN2	0,55	107	112	88	91	117	95	96	72	138	101	128	102	112	109	118	98
chr11	66467894	66468854	SPTBN2	0,65	60	59	51	44	60	52	43	40	70	52	70	51	65	60	62	54
chr11	66469003	66469243	SPTBN2	0,56	76	88	62	58	96	71	60	50	101	74	90	73	92	88	76	71

chr11	66471963	66473043	SPTBN2	0,68	32	25	23	26	32	26	25	19	41	29	33	32	33	31	32	32
chr11	66473051	66473411	SPTBN2	0,64	89	91	73	69	92	85	80	60	113	94	109	83	92	95	104	83
chr11	66474897	66475377	SPTBN2	0,65	31	26	23	22	36	27	20	24	36	27	31	28	28	32	32	29
chr11	66475510	66475870	SPTBN2	0,63	25	26	21	22	33	30	18	20	29	23	31	22	24	27	25	29
chr11	66476311	66476551	SPTBN2	0,61	39	45	30	28	44	38	27	25	43	32	54	38	43	42	44	42
chr11	66477966	66478563	SPTBN2	0,58	71	70	49	55	76	64	52	44	93	65	75	62	79	68	69	63
chr11	66481046	66481286	SPTBN2	0,50	170	179	140	130	162	151	120	96	202	171	179	152	167	192	178	143
chr11	66481481	66481952	SPTBN2	0,53	142	136	110	100	127	118	103	76	158	124	147	111	144	155	142	119
chr11	66482678	66482918	SPTBN2	0,57	51	41	43	34	56	42	36	27	55	37	51	42	45	47	45	46
chr11	66483196	66483556	SPTBN2	0,58	133	113	86	75	119	104	80	73	141	112	124	109	110	125	131	103
chr11	66488452	66488812	SPTBN2	0,57	106	131	93	87	124	101	83	68	138	101	131	112	119	124	118	106
chr11	68671343	68671583	IGHMBP2	0,73	2	3	2	1	2	3	1	1	3	1	0	2	1	2	2	1
chr11	68673441	68673801	IGHMBP2	0,50	134	131	105	82	127	118	97	73	142	121	153	108	125	126	122	102
chr11	68675528	68675888	IGHMBP2	0,46	147	145	124	116	138	134	108	90	172	135	149	121	143	151	153	133
chr11	68675930	68676170	IGHMBP2	0,47	153	155	121	107	139	137	113	86	162	150	166	136	154	157	142	126
chr11	68678809	68679169	IGHMBP2	0,56	156	158	120	106	135	132	112	92	157	140	160	135	157	162	161	124
chr11	68682210	68682570	IGHMBP2	0,53	108	107	77	84	120	92	93	66	132	93	109	100	117	111	123	94
chr11	68685146	68685386	IGHMBP2	0,43	102	102	67	74	99	92	74	61	111	94	100	90	90	92	94	78
chr11	68696557	68696917	IGHMBP2	0,64	98	92	78	72	95	76	75	58	108	99	102	90	90	94	103	90
chr11	68700677	68701037	IGHMBP2	0,63	32	33	27	23	38	31	27	22	41	23	41	36	32	35	33	35
chr11	68701201	68701441	IGHMBP2	0,59	29	20	18	25	30	16	16	14	28	23	21	16	28	21	26	18
chr11	68701855	68702071	IGHMBP2	0,59	81	81	63	58	78	73	57	45	97	76	89	73	75	84	75	71
chr11	68702708	68702948	IGHMBP2	0,56	35	33	24	36	38	32	25	28	47	34	38	36	34	39	39	32
chr11	68703651	68704611	IGHMBP2	0,60	61	50	51	50	62	52	44	41	76	55	63	52	62	60	63	52
chr11	68705555	68705915	IGHMBP2	0,60	25	18	18	27	29	22	16	17	28	18	24	21	23	24	30	18
chr11	68706920	68707280	IGHMBP2	0,65	12	10	10	14	14	12	9	7	16	11	15	9	12	13	10	12
chr11	71950710	71951166	PHOX2A	0,74	1	0	1	1	2	2	1	2	2	0	2	1	1	1	2	2
chr11	71951177	71951297	PHOX2A	0,71	0	0	0	0	1	0	0	0	1	1	0	1	3	1	0	0
chr11	71952059	71952419	PHOX2A	0,61	61	47	47	41	65	55	47	35	73	49	66	62	54	56	61	53
chr11	71954738	71955074	PHOX2A	0,73	1	1	1	2	1	0	1	2	2	1	1	1	2	2	1	1
chr11	74168212	74168692	KCNE3	0,55	109	104	74	75	99	90	77	56	126	104	112	93	103	110	105	98
chr11	819717	819909	PNPLA2	0,70	0	0	0	1	0	0	0	1	1	0	0	0	0	0	0	0
chr11	821525	822125	PNPLA2	0,61	83	71	62	61	80	69	55	58	89	66	85	63	77	70	66	65
chr11	822321	822681	PNPLA2	0,61	68	72	54	58	75	68	45	42	74	57	70	54	71	64	63	72
chr11	823436	823874	PNPLA2	0,69	6	7	4	7	9	7	5	6	7	5	6	6	6	6	6	5
chr11	823943	824183	PNPLA2	0,71	10	14	13	12	9	6	8	7	11	9	11	11	11	8	10	10
chr11	824227	824947	PNPLA2	0,71	7	7	5	7	9	5	4	4	8	5	8	8	6	8	7	6
chr11	94153198	94153438	MRE11A	0,31	254	293	213	159	181	207	186	139	299	267	280	242	268	255	299	202
chr11	94168911	94169151	MRE11A	0,32	208	216	173	121	180	180	157	118	212	204	231	167	203	208	215	156
chr11	94170251	94170491	MRE11A	0,28	276	287	222	167	203	241	189	142	300	255	265	226	260	252	282	211
chr11	94178897	94179137	MRE11A	0,37	234	230	190	147	206	216	161	135	253	218	242	199	231	256	245	191
chr11	94180314	94180674	MRE11A	0,46	277	320	264	176	217	246	203	148	328	322	302	239	306	287	325	208
chr11	94189352	94189592	MRE11A	0,32	266	309	201	145	201	224	188	130	310	289	273	238	280	277	281	203
chr11	94192504	94192840	MRE11A	0,36	371	388	308	202	270	299	250	178	387	377	368	301	359	364	397	270
chr11	94194031	94194271	MRE11A	0,31	244	292	209	135	198	228	177	120	258	250	268	218	251	274	265	211
chr11	94197221	94197461	MRE11A	0,34	267	275	220	137	204	220	189	142	297	269	275	231	258	260	265	201
chr11	94200898	94201138	MRE11A	0,34	224	235	194	132	181	189	151	115	236	207	216	198	228	215	216	171
chr11	94203542	94203902	MRE11A	0,35	278	299	244	149	221	225	190	138	293	282	278	235	283	283	297	201
chr11	94204652	94205012	MRE11A	0,33	251	291	206	140	199	236	194	140	280	255	257	235	264	256	271	200
chr11	94209391	94209631	MRE11A	0,34	290	306	224	150	234	240	200	147	311	312	290	249	290	273	307	227
chr11	94211791	94212151	MRE11A	0,34	280	303	210	173	229	249	191	142	292	321	294	259	293	287	296	225
chr11	94212763	94213003	MRE11A	0,36	242	268	213	150	205	227	173	132	274	267	241	214	241	232	268	191
chr11	94218989	94219349	MRE11A	0,32	293	338	246	181	217	264	213	155	331	300	302	274	321	323	334	228
chr11	94223944	94224184	MRE11A	0,30	264	333	234	169	213	256	185	140	274	288	288	232	264	298	285	212
chr11	94225897	94226017	MRE11A	0,47	58	65	49	51	67	48	35	38	76	48	55	56	50	45	35	52
chr11	95568354	95568714	MTMR2	0,42	275	297	227	147	237	256	196	135	294	273	273	233	284	292	288	211
chr11	95569219	95569579	MTMR2	0,41	268	305	236	159	223	236	197	149	301	278	282	245	286	275	277	214
chr11	95571194	95571434	MTMR2	0,35	201	203	153	129	181	181	137	108	242	213	222	180	205	206	200	167
chr11	95574706	95574946	MTMR2	0,38	240	267	208	136	214	217	184	121	276	252	257	202	241	260	255	188
chr11	95578039	95578399	MTMR2	0,39	288	298	225	152	238	247	201	142	291	298	276	243	278	286	288	214

chr11	95580790	95581150	MTMR2	0,35	299	294	237	159	235	254	188	153	330	296	289	245	318	296	324	228
chr11	95582775	95583111	MTMR2	0,40	279	299	239	166	241	250	191	154	315	289	280	254	290	276	318	217
chr11	95583658	95584018	MTMR2	0,38	319	358	265	189	277	279	238	175	365	357	328	296	352	345	376	248
chr11	95590637	95590877	MTMR2	0,33	220	257	186	128	175	190	164	116	247	243	251	202	214	235	256	179
chr11	95591625	95591865	MTMR2	0,31	272	311	222	146	207	234	195	145	306	292	277	232	257	275	288	204
chr11	95595090	95595330	MTMR2	0,32	303	319	257	166	241	256	233	164	330	319	316	277	302	306	348	225
chr11	95595362	95595602	MTMR2	0,34	330	348	262	178	263	271	229	158	349	351	338	302	320	321	353	248
chr11	95598682	95598922	MTMR2	0,38	235	250	168	124	198	228	165	124	251	224	249	203	237	226	252	171
chr11	95621252	95621492	MTMR2	0,35	207	233	176	139	184	184	160	118	245	223	223	210	220	213	243	180
chr11	95656958	95657198	MTMR2	0,69	4	3	2	3	2	3	3	4	7	2	5	3	7	2	5	5
chr11	9801893	9802133	SBF2	0,47	152	137	113	92	129	124	95	85	148	140	139	121	149	147	146	118
chr11	9802999	9803239	SBF2	0,46	104	114	77	87	124	109	80	68	119	108	108	95	105	108	100	96
chr11	9805821	9806061	SBF2	0,41	183	174	134	116	187	148	129	111	210	178	197	160	191	185	168	146
chr11	9806569	9806881	SBF2	0,51	92	80	77	63	89	77	63	54	101	87	91	76	83	90	87	78
chr11	9809112	9809352	SBF2	0,47	194	207	157	117	172	170	154	102	227	181	192	157	196	197	199	155
chr11	9810592	9810952	SBF2	0,52	114	128	89	82	127	112	95	75	141	125	115	118	130	119	120	104
chr11	9812046	9812286	SBF2	0,29	319	344	246	170	252	273	224	154	292	303	317	259	304	301	327	211
chr11	9817320	9817560	SBF2	0,35	191	198	150	113	164	165	125	105	220	195	213	158	205	200	187	162
chr11	9829459	9829819	SBF2	0,43	245	253	193	142	206	211	170	130	252	234	231	210	234	252	240	181
chr11	9830420	9830636	SBF2	0,42	202	226	156	104	186	182	143	112	222	201	223	190	214	199	224	167
chr11	9833986	9834346	SBF2	0,40	248	250	208	145	230	206	172	134	286	261	251	211	268	237	273	200
chr11	9838298	9838658	SBF2	0,46	161	169	123	121	144	164	125	87	181	166	158	145	166	156	162	125
chr11	9850792	9851104	SBF2	0,37	205	210	177	133	188	186	163	112	250	230	211	193	223	227	223	183
chr11	9853669	9853981	SBF2	0,46	178	179	137	125	157	162	125	98	190	166	172	150	185	184	200	139
chr11	9860963	9861323	SBF2	0,45	243	254	195	159	230	222	177	130	273	269	253	211	250	263	254	215
chr11	9864064	9864424	SBF2	0,36	328	361	290	189	256	290	230	154	349	352	329	287	340	326	357	264
chr11	9867019	9867379	SBF2	0,38	267	306	231	167	231	245	193	141	285	282	291	254	283	294	317	222
chr11	9868446	9868686	SBF2	0,40	214	245	199	134	215	192	177	129	249	244	231	217	224	238	229	203
chr11	9871469	9871781	SBF2	0,47	182	186	153	127	168	164	141	112	214	179	195	159	197	168	203	166
chr11	9874139	9874379	SBF2	0,38	218	243	199	141	195	200	179	123	264	217	232	212	218	248	237	181
chr11	9874992	9875352	SBF2	0,38	316	349	283	185	262	262	237	161	342	359	332	286	341	325	369	232
chr11	9877895	9878351	SBF2	0,42	260	240	199	139	216	205	178	140	248	227	235	206	237	234	244	184
chr11	9879677	9880037	SBF2	0,44	245	215	180	148	202	181	156	117	225	218	227	190	226	224	234	184
chr11	9917403	9917643	SBF2	0,33	252	277	231	149	184	223	184	150	287	287	287	223	267	258	266	180
chr11	9983398	9983758	SBF2	0,38	209	226	163	136	179	172	152	113	241	216	230	186	214	218	224	167
chr11	9985254	9985494	SBF2	0,31	260	285	210	150	212	234	177	148	283	255	270	225	262	240	278	194
chr11	9989809	9990169	SBF2	0,40	251	260	206	137	221	211	169	120	274	254	245	212	239	262	257	193
chr12	1003644	1003884	WNK1	0,39	206	215	157	121	196	183	152	120	230	199	222	174	197	200	237	175
chr12	1005147	1005987	WNK1	0,43	219	211	192	143	195	198	149	120	244	214	234	207	224	212	227	171
chr12	1006565	1006925	WNK1	0,45	190	185	154	130	192	160	143	114	206	176	187	182	195	183	198	156
chr12	1009558	1009918	WNK1	0,50	214	227	165	114	190	188	165	105	242	212	228	187	208	204	218	174
chr12	1016926	1017286	WNK1	0,48	155	176	148	114	167	150	131	97	202	177	191	156	180	180	170	146
chr12	1017559	1018039	WNK1	0,54	183	168	138	116	154	148	125	104	199	181	182	148	176	175	167	152
chr12	108956335	108956575	ISCU	0,73	3	1	0	2	2	3	1	1	5	1	2	1	2	3	1	1
chr12	108957815	108958225	ISCU	0,39	182	192	148	114	159	167	139	104	201	165	164	163	185	175	177	157
chr12	108959030	108959270	ISCU	0,44	164	180	133	98	148	130	124	84	175	146	170	134	133	158	157	124
chr12	108960884	108961124	ISCU	0,47	230	217	193	139	221	196	174	120	256	238	226	221	222	211	219	192
chr12	108962529	108962769	ISCU	0,48	107	118	89	85	114	101	83	67	124	95	116	93	117	117	93	89
chr12	111348809	111349049	MYL2	0,62	63	64	56	52	58	52	43	44	74	59	83	70	64	67	64	60
chr12	111350803	111351208	MYL2	0,59	93	92	70	78	106	87	68	67	120	101	108	92	109	112	111	89
chr12	111351966	111352182	MYL2	0,47	227	203	159	127	198	186	150	120	249	239	217	187	225	202	238	157
chr12	111353436	111353676	MYL2	0,53	88	97	67	62	83	86	57	59	93	93	101	77	84	88	83	80
chr12	111356832	111357072	MYL2	0,50	63	61	45	50	66	53	47	33	88	58	63	60	63	62	65	55
chr12	111358271	111358391	MYL2	0,60	23	34	29	26	33	20	15	31	38	33	35	35	36	31	29	26
chr12	111890509	111890749	ATXN2	0,43	100	99	89	66	111	76	73	61	100	87	93	93	95	88	80	83
chr12	111891384	111891744	ATXN2	0,57	110	99	82	71	108	93	74	63	119	86	109	90	113	95	93	83
chr12	111893766	111894126	ATXN2	0,53	92	94	82	69	95	83	73	48	105	79	99	79	98	88	94	90
chr12	111894910	111895270	ATXN2	0,51	108	136	96	91	130	110	96	73	124	110	140	99	139	121	117	116
chr12	111902372	111902612	ATXN2	0,32	221	230	158	137	166	196	159	105	226	210	208	187	210	191	220	163
chr12	111907857	111908097	ATXN2	0,43	168	193	137	119	155	146	114	102	185	166	170	154	163	161	159	135

chr12	111908273	111908609	ATXN2	0,45	100	97	75	70	105	85	79	67	119	99	110	88	108	94	101	88
chr12	111922984	111923224	ATXN2	0,35	252	244	200	146	195	215	172	123	263	228	258	207	252	245	244	180
chr12	111923412	111923772	ATXN2	0,41	234	245	174	131	191	199	164	115	261	215	244	186	232	214	233	175
chr12	111924476	111924716	ATXN2	0,34	163	202	126	117	153	162	132	91	187	164	179	147	167	163	167	126
chr12	111926191	111926671	ATXN2	0,38	298	297	236	170	246	258	214	141	310	300	282	256	283	282	292	211
chr12	111947267	111947507	ATXN2	0,24	170	187	127	106	138	148	136	97	188	166	188	147	160	172	169	135
chr12	111947628	111947868	ATXN2	0,33	273	286	219	163	204	232	198	149	287	278	277	215	271	279	285	206
chr12	111948107	111948467	ATXN2	0,43	172	184	137	114	169	161	135	100	190	175	174	161	171	165	172	153
chr12	111951071	111951407	ATXN2	0,48	205	205	174	115	188	169	172	120	246	205	220	186	217	207	218	169
chr12	111953882	111954242	ATXN2	0,47	53	49	37	41	46	40	31	35	50	47	49	48	53	51	44	46
chr12	111955961	111956321	ATXN2	0,37	264	299	204	145	214	217	189	133	275	269	261	224	264	265	255	202
chr12	111957601	111957961	ATXN2	0,39	366	361	303	172	273	294	244	168	383	368	361	303	365	353	400	244
chr12	111958611	111958851	ATXN2	0,29	261	308	218	147	208	233	196	147	310	285	278	248	271	257	282	204
chr12	111962937	111963177	ATXN2	0,42	264	272	211	151	223	223	175	138	290	280	232	228	270	251	277	213
chr12	111989978	111990338	ATXN2	0,34	321	367	283	163	261	273	219	154	348	361	303	269	308	344	359	249
chr12	111990625	111990865	ATXN2	0,29	281	313	231	185	203	253	220	163	311	292	297	235	269	283	307	225
chr12	111991866	111992058	ATXN2	0,27	277	302	229	149	213	234	213	161	301	267	290	262	275	281	276	211
chr12	111993623	111993743	ATXN2	0,35	107	120	99	84	114	114	85	70	122	127	118	101	109	93	102	100
chr12	112036559	112036751	ATXN2	0,79	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chr12	112036983	112037127	ATXN2	0,73	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
chr12	112037223	112037415	ATXN2	0,73	1	1	0	3	1	1	0	2	2	2	1	1	1	1	1	0
chr12	119617105	119617561	HSPB8	0,64	74	58	57	57	70	65	55	52	88	60	73	62	66	80	61	67
chr12	119624765	119624981	HSPB8	0,52	63	72	48	54	77	82	46	56	81	71	92	57	76	84	64	60
chr12	119631403	119631763	HSPB8	0,44	265	278	229	162	226	232	192	138	315	268	274	231	263	272	277	210
chr12	121163591	121163831	ACADS	0,76	0	0	0	0	2	0	0	0	1	1	1	0	0	0	1	1
chr12	121164754	121165090	ACADS	0,57	105	107	93	77	124	97	72	69	132	113	120	101	113	124	101	102
chr12	121174683	121175043	ACADS	0,68	7	4	5	6	8	6	2	5	11	7	8	5	7	7	9	7
chr12	121175094	121175334	ACADS	0,63	10	14	9	14	15	10	9	7	15	10	17	13	13	14	11	14
chr12	121175535	121175895	ACADS	0,62	13	21	15	11	16	15	11	12	22	14	18	15	13	14	17	18
chr12	121175977	121176790	ACADS	0,65	23	25	18	21	29	21	16	18	29	20	27	22	24	22	20	22
chr12	121176856	121177336	ACADS	0,68	23	18	17	20	26	19	16	12	25	19	21	23	18	20	21	17
chr12	2162632	2162848	CACNA1C	0,58	58	49	50	52	64	49	37	41	71	44	73	56	58	53	57	44
chr12	21953926	21954166	ABCC9	0,39	220	216	194	129	187	179	153	112	259	239	227	175	220	234	226	169
chr12	21958056	21958296	ABCC9	0,42	103	108	78	69	113	85	69	62	112	93	97	90	94	110	100	86
chr12	21958842	21959082	ABCC9	0,34	282	316	254	172	249	249	190	149	296	302	282	243	294	310	322	221
chr12	21960226	21960466	ABCC9	0,41	251	275	212	163	221	241	164	140	295	282	286	241	268	297	280	198
chr12	21962716	21962956	ABCC9	0,31	219	222	186	130	199	198	150	97	237	220	207	192	225	217	236	166
chr12	21964916	21965156	ABCC9	0,34	197	245	174	132	185	183	161	117	242	228	219	180	213	218	227	158
chr12	21967496	21967736	ABCC9	0,37	226	248	175	127	188	170	151	129	240	219	222	188	235	225	212	189
chr12	21968641	21968881	ABCC9	0,40	233	224	177	130	199	195	155	116	237	223	214	205	209	229	230	188
chr12	21970041	21970257	ABCC9	0,38	211	250	174	126	220	196	157	102	266	242	244	205	234	236	235	176
chr12	21971056	21971272	ABCC9	0,39	225	228	183	150	225	200	164	136	266	237	244	213	257	254	240	175
chr12	21981822	21982062	ABCC9	0,42	130	134	105	81	122	128	103	78	155	127	150	121	139	140	137	107
chr12	21990937	21991177	ABCC9	0,40	118	118	90	86	118	107	87	65	129	118	130	106	108	125	102	85
chr12	21995198	21995510	ABCC9	0,40	148	141	107	102	142	133	109	84	169	139	163	132	144	158	167	125
chr12	21997331	21997571	ABCC9	0,34	192	205	147	133	168	176	138	119	216	203	220	167	211	198	201	159
chr12	21997594	21997954	ABCC9	0,36	267	288	219	154	226	232	176	131	305	283	272	204	275	278	311	213
chr12	21998471	21998831	ABCC9	0,40	319	337	254	175	271	269	209	160	328	329	316	292	314	327	366	232
chr12	22001011	22001251	ABCC9	0,41	241	257	206	142	227	209	166	133	299	277	250	226	270	263	284	204
chr12	22004973	22005213	ABCC9	0,38	289	289	257	165	247	249	207	148	323	326	296	278	322	296	313	228
chr12	22005250	22005490	ABCC9	0,37	297	317	235	157	243	269	206	133	323	319	281	267	335	311	337	220
chr12	22012439	22012679	ABCC9	0,38	320	328	290	177	266	257	220	158	347	350	317	265	323	310	364	244
chr12	22013826	22014066	ABCC9	0,32	280	311	206	144	223	218	203	146	328	308	296	257	298	293	296	225
chr12	22015817	22016057	ABCC9	0,34	263	267	203	151	196	206	154	123	269	240	237	217	251	252	272	190
chr12	22017271	22017511	ABCC9	0,30	222	237	194	132	190	191	160	122	233	225	225	209	212	208	227	166
chr12	22025491	22025731	ABCC9	0,40	201	240	165	132	204	170	145	107	234	209	208	170	208	207	194	160
chr12	22028503	22028743	ABCC9	0,29	277	295	230	145	218	260	205	142	293	278	278	241	295	273	305	208
chr12	22035633	22035873	ABCC9	0,37	281	289	209	160	238	230	185	129	286	262	253	232	299	294	293	194
chr12	22040693	22040933	ABCC9	0,39	264	274	198	147	229	218	191	134	265	255	252	207	264	275	248	192
chr12	22046930	22047170	ABCC9	0,45	139	153	122	100	158	140	104	77	179	123	155	145	165	146	161	129

chr12	22048108	22048348	ABCC9	0,32	229	240	191	144	200	186	158	114	260	244	222	197	227	239	231	178
chr12	22058959	22059271	ABCC9	0,35	250	272	203	143	203	212	176	129	283	258	249	234	272	252	289	206
chr12	22060957	22061197	ABCC9	0,39	191	220	163	125	177	178	143	110	228	209	218	179	211	218	198	160
chr12	22062988	22063348	ABCC9	0,32	247	274	211	148	226	215	174	141	285	270	263	240	253	257	284	196
chr12	22063655	22064015	ABCC9	0,35	210	248	158	131	185	190	154	106	261	214	210	193	226	215	228	174
chr12	22065722	22066082	ABCC9	0,35	205	209	160	115	177	161	132	106	222	202	202	157	200	190	189	150
chr12	22068542	22068902	ABCC9	0,37	275	299	227	170	226	227	201	147	305	286	280	252	270	274	286	197
chr12	22069773	22070133	ABCC9	0,37	280	300	232	159	230	239	204	137	299	307	285	229	286	284	314	215
chr12	22078816	22079056	ABCC9	0,39	166	189	126	123	167	137	111	101	187	170	177	139	164	156	177	158
chr12	22086606	22086966	ABCC9	0,39	233	244	179	128	199	190	150	116	241	247	237	189	234	251	239	182
chr12	22089357	22089717	ABCC9	0,33	274	295	232	159	220	236	194	148	299	277	269	241	301	281	304	197
chr12	2224310	2224790	CACNA1C	0,63	19	23	22	22	31	21	19	19	23	22	25	19	18	24	27	23
chr12	2229423	2229663	CACNA1C	0,39	314	343	261	183	267	279	213	171	351	348	319	259	323	336	339	251
chr12	2558086	2558326	CACNA1C	0,40	287	310	230	160	227	222	211	141	310	305	275	231	290	287	314	208
chr12	2566682	2566922	CACNA1C	0,54	74	75	60	56	81	74	57	52	115	79	89	79	73	75	73	77
chr12	2595216	2595528	CACNA1C	0,60	90	96	75	69	102	80	68	66	109	85	100	95	87	89	98	88
chr12	2602273	2602633	CACNA1C	0,59	136	133	117	90	136	117	100	78	165	131	132	124	141	140	139	100
chr12	2613533	2613773	CACNA1C	0,49	229	245	188	141	187	188	167	122	256	224	245	196	251	242	254	169
chr12	2613939	2614179	CACNA1C	0,45	200	202	151	123	149	144	122	102	192	170	186	156	195	184	170	159
chr12	2621883	2622243	CACNA1C	0,56	68	67	53	60	78	55	46	47	80	55	75	59	73	68	71	63
chr12	2659033	2659273	CACNA1C	0,58	97	98	78	72	85	91	69	56	120	85	106	83	80	101	102	78
chr12	2666009	2666249	CACNA1C	0,49	265	287	230	157	240	231	190	132	286	286	264	215	259	268	287	207
chr12	2675487	2675847	CACNA1C	0,58	76	69	55	57	69	66	56	49	75	72	87	81	74	78	79	63
chr12	2676667	2677027	CACNA1C	0,60	89	75	59	62	85	70	60	56	113	76	87	84	84	83	79	68
chr12	2690701	2690821	CACNA1C	0,60	41	33	25	26	36	34	24	21	40	25	45	27	26	38	37	24
chr12	2690866	2691058	CACNA1C	0,56	58	71	49	54	75	64	42	48	79	66	68	55	60	56	57	53
chr12	2691927	2692167	CACNA1C	0,54	90	108	66	75	92	82	69	61	106	84	96	91	80	100	89	80
chr12	2693559	2693751	CACNA1C	0,53	62	76	54	64	91	66	54	46	88	80	80	72	68	66	65	69
chr12	2694481	2694721	CACNA1C	0,56	11	11	8	9	15	12	8	6	14	10	16	9	15	18	11	10
chr12	2694915	2695155	CACNA1C	0,49	140	159	124	116	146	140	113	86	178	159	159	135	164	154	152	115
chr12	2702324	2702564	CACNA1C	0,53	62	66	47	47	77	54	37	37	69	54	65	47	65	51	59	53
chr12	2704984	2705224	CACNA1C	0,58	307	289	251	186	228	263	221	144	356	343	303	286	307	324	380	237
chr12	2706305	2706752	CACNA1C	0,45	394	409	326	212	282	308	280	192	411	418	416	328	417	397	427	304
chr12	2710952	2711192	CACNA1C	0,59	64	60	57	55	80	55	47	40	75	61	79	61	66	73	69	55
chr12	2714170	2714410	CACNA1C	0,57	107	96	81	77	96	92	77	63	111	94	106	93	103	101	91	84
chr12	2714778	2715018	CACNA1C	0,58	82	76	62	64	84	69	55	44	102	67	83	67	81	84	82	82
chr12	2715682	2715922	CACNA1C	0,53	69	74	61	64	77	81	60	49	111	85	73	73	85	79	79	58
chr12	2716042	2716402	CACNA1C	0,56	129	123	113	96	140	111	94	82	155	125	154	114	138	119	128	111
chr12	2717624	2717744	CACNA1C	0,60	41	46	26	30	50	36	35	27	50	45	43	38	34	41	38	42
chr12	2717816	2717936	CACNA1C	0,60	59	59	38	43	67	57	49	36	70	54	66	53	55	62	55	55
chr12	2719605	2719965	CACNA1C	0,59	142	138	117	99	132	116	97	86	162	123	142	122	131	146	153	119
chr12	2721003	2721243	CACNA1C	0,57	171	182	117	115	173	142	133	99	185	157	179	138	181	173	179	126
chr12	2742716	2742956	CACNA1C	0,46	319	323	276	200	254	259	225	179	350	336	318	291	337	334	354	230
chr12	2743408	2743624	CACNA1C	0,48	230	231	179	162	209	196	154	122	268	228	221	203	228	228	253	190
chr12	2757536	2757776	CACNA1C	0,57	268	249	212	185	235	213	193	146	281	253	258	242	264	253	273	207
chr12	2760656	2761016	CACNA1C	0,63	136	129	116	97	135	116	96	75	148	132	144	127	138	148	142	126
chr12	2762913	2763153	CACNA1C	0,64	30	22	17	17	30	23	17	20	33	23	25	23	25	22	21	29
chr12	2764238	2764478	CACNA1C	0,51	186	187	167	119	183	166	140	103	222	192	201	173	194	194	196	148
chr12	2773917	2774253	CACNA1C	0,61	72	49	42	41	71	63	40	38	69	58	72	61	61	63	65	66
chr12	2774690	2774930	CACNA1C	0,50	136	155	116	104	162	154	115	90	181	161	165	130	156	149	153	120
chr12	2775779	2776019	CACNA1C	0,58	40	46	31	38	45	41	34	32	53	39	48	43	45	43	41	43
chr12	2778029	2778269	CACNA1C	0,59	105	98	76	67	108	83	71	64	117	85	107	84	97	99	97	94
chr12	2783572	2783908	CACNA1C	0,61	25	18	18	20	24	20	17	16	30	16	28	21	23	23	21	30
chr12	2786203	2786443	CACNA1C	0,59	128	128	107	92	128	104	95	75	145	116	124	108	134	145	136	117
chr12	2786845	2787085	CACNA1C	0,59	15	19	17	20	25	15	11	15	31	19	28	20	20	21	22	17
chr12	2788545	2789025	CACNA1C	0,67	51	57	47	52	66	46	42	44	70	53	67	52	48	56	54	58
chr12	2789454	2789814	CACNA1C	0,59	58	55	44	48	70	56	47	45	79	62	70	65	66	56	69	57
chr12	2791047	2791287	CACNA1C	0,55	35	28	29	25	43	25	24	23	41	28	33	28	33	31	26	33
chr12	2791659	2791899	CACNA1C	0,56	18	18	15	18	25	17	13	15	23	21	19	17	22	26	22	18
chr12	2794834	2795074	CACNA1C	0,58	115	127	75	75	112	99	79	67	113	114	126	102	120	102	103	100

chr12	2795263	2795503	CACNA1C	0,58	76	59	65	68	74	62	57	49	100	65	87	74	73	76	73	70
chr12	2797538	2798018	CACNA1C	0,67	59	50	46	49	55	52	36	38	62	51	60	51	51	54	58	50
chr12	2799975	2800455	CACNA1C	0,60	32	40	29	36	43	35	28	24	43	34	41	38	44	38	32	41
chr12	32729217	32729457	FGD4	0,42	218	259	167	132	194	216	166	112	250	219	229	187	208	218	222	178
chr12	32734787	32735507	FGD4	0,45	208	214	165	129	185	191	156	106	236	221	213	170	216	221	225	166
chr12	32751355	32751595	FGD4	0,30	273	309	245	167	231	246	204	146	288	301	258	217	276	257	290	230
chr12	32754233	32754449	FGD4	0,36	115	120	90	80	102	116	81	69	145	107	132	109	118	125	118	103
chr12	32755047	32755359	FGD4	0,32	267	271	220	161	194	222	188	134	275	268	263	236	286	252	291	211
chr12	32760839	32761079	FGD4	0,39	210	241	205	141	208	214	156	128	256	232	244	193	239	252	240	178
chr12	32763618	32763858	FGD4	0,34	268	327	232	165	227	248	215	150	292	288	287	241	301	284	314	224
chr12	32763963	32764275	FGD4	0,35	294	277	228	157	223	216	180	129	294	271	265	223	269	259	273	195
chr12	32772561	32772897	FGD4	0,40	276	284	236	155	223	228	200	141	299	272	275	226	285	271	306	202
chr12	32777293	32777413	FGD4	0,30	123	109	88	75	107	108	93	60	142	102	111	83	121	125	101	93
chr12	32777835	32778075	FGD4	0,32	248	276	238	157	213	225	179	134	299	264	277	247	268	277	275	192
chr12	32778530	32778770	FGD4	0,35	207	215	176	122	181	185	153	112	229	187	198	178	225	194	224	153
chr12	32786425	32786665	FGD4	0,28	198	214	165	118	174	196	160	104	236	192	204	174	210	207	196	166
chr12	32791478	32791838	FGD4	0,43	179	187	137	111	150	154	117	90	206	175	193	147	174	179	167	150
chr12	32793158	32793518	FGD4	0,49	137	134	108	90	127	129	103	84	168	147	148	130	143	149	152	118
chr12	32945271	32945741	PKP2	0,44	207	209	151	123	193	187	139	100	231	197	211	186	198	213	223	158
chr12	32948957	32949317	PKP2	0,43	193	199	153	124	179	171	131	106	217	194	203	175	195	200	198	134
chr12	32955233	32955593	PKP2	0,41	296	311	230	150	232	244	203	158	308	289	292	243	295	297	309	227
chr12	32974196	32974556	PKP2	0,45	244	274	198	153	206	209	156	119	271	241	255	218	248	243	261	205
chr12	32975326	32975662	PKP2	0,40	306	343	260	166	250	266	225	146	333	330	322	259	312	331	356	237
chr12	32976917	32977157	PKP2	0,39	272	289	226	159	236	238	182	154	319	270	282	247	273	274	298	199
chr12	32993870	32994230	PKP2	0,43	197	225	170	134	175	189	139	111	252	216	221	175	222	213	201	159
chr12	32996195	32996315	PKP2	0,50	39	33	31	31	39	29	19	32	40	31	31	26	34	40	33	32
chr12	33003623	33003983	PKP2	0,42	274	265	203	148	231	237	189	136	280	270	256	216	268	266	268	190
chr12	33021808	33022048	PKP2	0,50	93	102	77	69	93	93	69	65	123	95	105	80	108	93	96	81
chr12	33030708	33031548	PKP2	0,59	86	75	66	55	83	70	61	52	90	75	86	67	78	93	82	66
chr12	33031789	33032029	PKP2	0,36	225	216	162	138	183	181	148	124	227	196	220	185	212	204	210	159
chr12	33049373	33049733	PKP2	0,74	3	1	2	3	4	1	1	2	2	2	2	2	2	1	2	3
chr12	39688152	39688392	KIF21A	0,35	284	304	246	165	255	254	203	159	310	304	305	234	331	318	332	216
chr12	39695201	39695561	KIF21A	0,39	232	258	200	141	216	214	159	130	262	254	278	224	243	247	258	183
chr12	39696671	39697031	KIF21A	0,37	293	301	245	175	252	268	222	143	337	334	299	279	313	332	327	233
chr12	39698561	39698801	KIF21A	0,32	169	163	131	106	159	159	126	84	175	166	165	148	185	169	178	139
chr12	39701268	39701628	KIF21A	0,41	227	239	187	145	209	210	155	127	272	237	229	208	247	237	254	181
chr12	39703297	39703633	KIF21A	0,33	308	328	238	163	256	270	232	163	346	314	321	273	330	331	307	235
chr12	39705174	39705414	KIF21A	0,38	142	159	117	107	143	136	122	86	186	141	145	125	139	141	133	115
chr12	39708976	39709096	KIF21A	0,41	42	51	33	36	53	43	27	34	61	49	45	39	53	55	43	31
chr12	39709632	39709872	KIF21A	0,38	125	137	104	85	115	118	88	78	148	132	139	110	139	135	134	99
chr12	39711818	39712058	KIF21A	0,38	150	158	111	84	134	125	93	85	171	134	162	128	154	138	146	119
chr12	39713641	39713881	KIF21A	0,35	287	297	228	143	225	223	199	148	316	289	276	254	272	284	302	223
chr12	39716396	39716756	KIF21A	0,44	207	186	165	132	171	173	155	110	226	203	213	179	199	202	210	160
chr12	39719547	39719787	KIF21A	0,30	259	275	206	148	210	206	168	131	265	270	261	226	255	258	290	189
chr12	39719975	39720215	KIF21A	0,33	287	317	235	165	226	250	189	151	294	278	294	264	299	266	313	217
chr12	39723933	39724173	KIF21A	0,32	183	196	146	105	150	157	121	102	215	181	185	159	202	188	190	146
chr12	39724450	39724786	KIF21A	0,39	294	320	253	159	250	270	208	156	357	346	319	256	316	310	330	232
chr12	39725422	39725662	KIF21A	0,37	220	242	194	134	202	198	157	128	273	210	236	177	228	248	227	193
chr12	39725932	39727167	KIF21A	0,39	336	349	279	190	266	284	233	167	349	339	337	285	351	354	357	258
chr12	39730831	39731071	KIF21A	0,32	299	346	274	169	204	258	224	140	353	341	314	256	326	309	371	244
chr12	39733886	39734246	KIF21A	0,33	296	355	277	191	256	279	228	157	378	357	350	296	369	336	367	253
chr12	39734621	39734981	KIF21A	0,33	261	313	231	151	214	241	207	125	314	299	299	259	275	294	314	213
chr12	39735249	39735369	KIF21A	0,37	184	234	179	132	175	197	154	114	235	211	203	203	206	242	222	171
chr12	39735760	39736000	KIF21A	0,34	315	345	267	173	224	276	233	171	347	335	307	276	327	343	351	230
chr12	39740166	39740406	KIF21A	0,35	229	282	198	139	236	210	166	129	300	275	255	212	253	238	246	193
chr12	39745477	39745789	KIF21A	0,34	249	256	198	152	207	218	173	125	283	259	254	225	260	252	273	200
chr12	39750545	39750785	KIF21A	0,28	247	282	205	158	212	227	177	126	278	268	246	237	273	247	276	198
chr12	39750964	39751324	KIF21A	0,40	335	337	287	176	282	300	228	172	358	361	335	303	344	366	381	235
chr12	39751897	39752257	KIF21A	0,37	304	365	258	176	252	268	234	156	351	335	338	263	317	330	359	264
chr12	39756837	39757077	KIF21A	0,36	186	204	151	112	169	153	129	100	216	171	188	161	181	190	177	152

chr12	39760055	39760415	KIF21A	0,35	314	363	275	176	280	287	235	166	374	371	362	275	330	341	382	260
chr12	39760778	39761018	KIF21A	0,38	196	197	155	118	197	190	143	104	212	215	218	175	201	214	202	156
chr12	39761579	39761939	KIF21A	0,29	316	354	248	179	204	271	214	159	359	330	318	291	315	326	338	250
chr12	39763441	39764131	KIF21A	0,33	319	353	273	187	252	285	231	161	361	341	333	268	336	329	364	253
chr12	39836708	39836828	KIF21A	0,74	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chr12	41302144	41302384	CNTN1	0,29	234	232	193	140	188	192	160	115	237	219	221	197	214	239	233	171
chr12	41303770	41304010	CNTN1	0,31	241	284	204	162	168	217	177	119	278	264	262	202	248	261	275	196
chr12	41312386	41312626	CNTN1	0,39	253	256	182	141	205	187	163	134	269	242	252	221	245	240	258	187
chr12	41315963	41316323	CNTN1	0,41	213	237	165	120	199	170	147	121	240	212	214	185	216	228	211	165
chr12	41318286	41318526	CNTN1	0,38	223	232	177	135	191	197	148	126	241	221	231	193	247	231	245	169
chr12	41323520	41323880	CNTN1	0,35	292	320	264	159	227	258	213	150	308	332	297	266	300	299	336	219
chr12	41327192	41327769	CNTN1	0,31	285	305	234	145	228	238	189	140	297	270	284	229	275	268	295	215
chr12	41330524	41330764	CNTN1	0,44	156	161	125	105	158	147	108	91	193	166	165	129	161	152	155	132
chr12	41331310	41331550	CNTN1	0,31	250	278	217	144	201	209	178	128	285	246	261	216	246	261	276	196
chr12	41333031	41333391	CNTN1	0,34	247	249	199	138	213	215	173	123	262	222	229	203	237	241	246	188
chr12	41337342	41337582	CNTN1	0,31	192	211	156	124	151	163	167	97	215	181	191	169	197	202	195	161
chr12	41337704	41338064	CNTN1	0,33	283	311	245	163	223	260	211	152	316	301	305	260	299	308	322	217
chr12	41352855	41353095	CNTN1	0,37	200	205	160	122	186	177	129	109	228	217	200	172	205	220	229	163
chr12	41374609	41374969	CNTN1	0,36	274	288	226	157	215	228	186	148	294	284	275	243	276	276	275	229
chr12	41386816	41387176	CNTN1	0,34	190	221	168	123	189	173	146	116	221	195	224	167	201	191	208	163
chr12	41407944	41408184	CNTN1	0,38	139	149	109	108	155	129	94	90	186	151	156	125	140	137	148	112
chr12	41410420	41410780	CNTN1	0,40	287	288	236	163	249	231	190	149	297	295	286	229	289	283	298	206
chr12	41414070	41414310	CNTN1	0,29	231	274	187	130	171	209	181	127	285	243	253	220	248	236	253	192
chr12	41418864	41419224	CNTN1	0,43	177	173	138	102	152	134	117	94	177	166	179	136	171	169	175	140
chr12	41421594	41421834	CNTN1	0,39	259	251	225	159	230	215	160	125	288	254	268	214	262	249	272	187
chr12	41422762	41423122	CNTN1	0,41	206	219	163	113	195	159	146	114	225	202	200	175	219	209	217	159
chr12	41463678	41463918	CNTN1	0,47	286	296	241	147	254	219	195	130	323	314	279	261	318	308	319	219
chr12	48501077	48501317	PFKM	0,44	190	214	172	135	186	169	138	111	224	191	204	179	219	222	173	163
chr12	48501795	48502035	PFKM	0,46	212	209	174	134	165	175	165	111	250	201	208	177	221	196	214	138
chr12	48516475	48516715	PFKM	0,46	143	146	114	87	139	121	86	78	157	139	147	129	149	127	139	116
chr12	48524062	48524302	PFKM	0,46	315	323	276	188	282	272	221	148	359	334	312	271	306	302	328	237
chr12	48525017	48525257	PFKM	0,48	148	133	94	100	137	134	97	85	168	144	146	134	135	154	153	126
chr12	48526565	48526925	PFKM	0,54	116	120	94	72	109	98	83	74	134	105	121	108	122	120	119	100
chr12	48527000	48527360	PFKM	0,50	114	105	89	75	109	101	80	67	131	99	119	88	111	113	117	95
chr12	48527916	48528156	PFKM	0,50	128	140	102	87	138	124	96	81	169	138	141	132	142	146	147	125
chr12	48528437	48528893	PFKM	0,48	250	270	214	153	211	201	177	134	288	254	252	213	245	245	295	211
chr12	48528999	48529239	PFKM	0,55	119	123	98	84	111	102	83	68	133	128	126	93	121	118	120	95
chr12	48531446	48531686	PFKM	0,58	71	64	51	62	71	61	48	38	80	66	78	60	74	74	76	62
chr12	48532979	48533219	PFKM	0,54	71	78	62	50	82	64	40	44	80	69	82	61	78	70	71	58
chr12	48533543	48533783	PFKM	0,51	229	235	191	130	197	211	166	127	261	225	234	201	230	216	248	162
chr12	48534399	48534759	PFKM	0,55	189	190	149	122	176	160	130	120	201	186	202	178	188	190	202	159
chr12	48535008	48535248	PFKM	0,54	58	45	37	43	52	46	33	28	58	40	55	41	51	47	42	36
chr12	48535430	48535646	PFKM	0,49	315	327	284	176	260	277	250	176	349	348	325	303	355	342	383	250
chr12	48535676	48535916	PFKM	0,51	182	179	151	132	173	165	134	115	205	185	196	170	199	191	205	161
chr12	48536466	48536826	PFKM	0,51	121	122	95	88	123	106	79	77	131	118	130	112	130	116	119	107
chr12	48537446	48537686	PFKM	0,42	191	202	152	133	176	163	141	98	239	190	194	179	192	203	197	164
chr12	48537764	48538004	PFKM	0,51	128	115	110	88	123	115	95	80	144	127	142	117	130	134	115	110
chr12	48538715	48539195	PFKM	0,48	226	223	184	146	201	182	156	115	264	220	228	193	233	226	218	170
chr12	48539238	48539598	PFKM	0,46	212	203	171	141	193	179	153	113	235	202	212	178	216	204	214	165
chr12	5020448	5022128	KCNA1	0,56	156	152	122	103	145	132	109	88	176	148	166	135	152	161	163	121
chr12	5153214	5155254	KCNA5	0,65	56	50	41	44	56	49	39	37	67	49	61	49	58	54	55	51
chr12	56078776	56079136	ITGA7	0,65	21	17	13	14	16	12	11	11	20	13	17	15	16	18	17	16
chr12	56081697	56082154	ITGA7	0,57	116	109	96	81	117	101	80	71	127	105	129	108	119	124	108	106
chr12	56082564	56082804	ITGA7	0,59	12	11	11	16	22	16	8	11	17	14	19	11	12	12	12	11
chr12	56086573	56086813	ITGA7	0,62	39	35	26	30	32	33	21	24	39	30	35	31	37	40	35	35
chr12	56086829	56087141	ITGA7	0,58	96	97	88	78	108	94	85	66	129	96	119	79	117	116	104	90
chr12	56087735	56088850	ITGA7	0,60	85	82	70	69	98	78	67	60	98	84	88	76	99	92	89	83
chr12	56089243	56089483	ITGA7	0,59	34	35	25	25	46	29	25	29	36	29	40	34	40	29	30	26
chr12	56089961	56090321	ITGA7	0,58	30	32	21	23	37	31	21	22	39	24	26	28	27	26	32	31
chr12	56090587	56091150	ITGA7	0,60	35	39	30	32	44	37	26	30	45	34	42	41	39	41	37	40

chr12	56091162	56091402	ITGA7	0,57	57	75	53	51	76	56	49	42	74	67	64	61	69	67	62	60
chr12	56091422	56091885	ITGA7	0,61	106	82	78	68	104	83	75	63	112	91	103	83	90	98	92	89
chr12	56092107	56092777	ITGA7	0,62	32	27	19	28	38	30	22	19	38	28	30	28	27	32	27	30
chr12	56093593	56093833	ITGA7	0,59	65	58	50	46	59	61	55	38	71	56	64	59	57	61	64	54
chr12	56093991	56094231	ITGA7	0,65	4	4	3	3	5	3	3	2	3	3	3	2	5	3	1	3
chr12	56094630	56094990	ITGA7	0,58	96	97	75	73	100	82	69	60	124	94	111	84	99	96	85	90
chr12	56095061	56095277	ITGA7	0,51	274	263	249	168	241	239	199	160	329	309	300	261	306	285	326	200
chr12	56096599	56097055	ITGA7	0,59	141	124	104	89	114	111	94	81	140	129	128	111	136	127	134	106
chr12	56101183	56101543	ITGA7	0,67	9	8	7	6	19	12	7	7	11	9	11	10	11	13	11	8
chr12	56105762	56106106	ITGA7	0,49	206	238	187	142	199	196	166	123	246	235	238	190	241	219	247	177
chr12	56474005	56474245	ERBB3	0,69	6	3	4	4	4	3	5	4	4	4	6	4	5	3	3	6
chr12	56477430	56477790	ERBB3	0,54	199	226	165	134	194	178	159	116	246	228	226	167	217	222	217	167
chr12	56478697	56479177	ERBB3	0,54	210	218	156	133	187	189	155	113	232	204	207	193	219	208	222	164
chr12	56480257	56480497	ERBB3	0,51	154	163	120	101	164	141	117	94	176	143	185	129	156	146	156	140
chr12	56481273	56481513	ERBB3	0,53	154	147	124	92	136	130	103	92	167	142	167	144	162	142	149	129
chr12	56481517	56482055	ERBB3	0,50	193	180	143	119	181	169	126	111	222	181	195	164	191	189	185	148
chr12	56482249	56482729	ERBB3	0,52	194	198	153	127	186	177	151	109	237	186	204	177	197	188	200	157
chr12	56486476	56486692	ERBB3	0,47	145	157	117	108	143	127	113	88	164	139	156	142	161	150	140	124
chr12	56486694	56486934	ERBB3	0,48	179	212	160	125	174	168	159	112	232	199	231	171	230	185	181	154
chr12	56487051	56487411	ERBB3	0,50	241	247	190	147	193	211	183	122	254	240	233	200	240	227	259	190
chr12	56487493	56487733	ERBB3	0,59	51	47	35	52	53	53	42	34	58	53	65	50	51	54	49	53
chr12	56487807	56488047	ERBB3	0,55	125	122	98	99	123	105	88	70	146	118	138	110	126	117	124	117
chr12	56488082	56488442	ERBB3	0,52	109	104	79	87	110	95	79	70	141	111	124	103	108	112	116	108
chr12	56488947	56489187	ERBB3	0,46	98	90	80	69	92	89	72	52	119	108	108	89	112	105	90	87
chr12	56489339	56489699	ERBB3	0,43	212	218	175	125	206	189	165	115	258	230	212	186	223	214	216	170
chr12	56490226	56490700	ERBB3	0,48	132	143	113	100	140	127	102	89	161	137	157	122	146	147	140	122
chr12	56490741	56491101	ERBB3	0,54	96	90	67	63	99	83	68	56	105	87	92	77	84	82	85	81
chr12	56491466	56491826	ERBB3	0,52	95	100	74	63	94	79	67	50	115	80	92	77	96	94	85	76
chr12	56492201	56492441	ERBB3	0,41	200	227	176	141	207	177	180	123	234	196	206	195	233	199	209	164
chr12	56492486	56492726	ERBB3	0,51	179	186	122	112	174	136	134	99	197	141	164	138	158	177	148	143
chr12	56493370	56494113	ERBB3	0,48	218	210	172	138	201	193	164	119	248	210	222	189	232	203	218	168
chr12	56494754	56495935	ERBB3	0,52	223	211	182	142	199	191	163	119	249	217	220	198	232	215	224	180
chr12	57943998	57944238	KIF5A	0,61	40	32	31	29	43	34	27	23	53	33	32	35	34	36	33	37
chr12	57957168	57957528	KIF5A	0,45	365	386	284	186	285	293	256	180	387	372	348	304	351	347	390	257
chr12	57957822	57958062	KIF5A	0,52	209	208	186	124	171	177	155	111	232	210	182	195	207	230	224	164
chr12	57958146	57958386	KIF5A	0,43	227	242	181	134	190	191	162	114	257	216	216	205	244	229	241	167
chr12	57958608	57958848	KIF5A	0,53	68	71	56	52	77	52	44	37	88	63	69	65	66	68	68	56
chr12	57960832	57961072	KIF5A	0,44	132	143	107	88	131	124	82	78	142	127	133	111	138	113	121	101
chr12	57961215	57961455	KIF5A	0,54	38	31	23	30	38	43	28	31	51	33	46	29	43	39	37	33
chr12	57962677	57962917	KIF5A	0,57	99	82	83	66	100	95	74	59	100	85	90	78	93	91	100	70
chr12	57962932	57963380	KIF5A	0,51	221	215	180	144	198	190	151	110	247	226	214	180	210	206	233	163
chr12	57963410	57963530	KIF5A	0,60	34	23	22	11	23	19	21	17	32	30	24	19	30	25	26	24
chr12	57963677	57964037	KIF5A	0,57	48	50	36	41	62	49	39	31	59	45	58	45	55	50	53	44
chr12	57965017	57965257	KIF5A	0,47	152	156	129	117	131	130	108	80	156	149	151	138	149	151	138	123
chr12	57965766	57966126	KIF5A	0,57	29	33	26	23	35	31	24	18	35	25	30	23	28	30	28	30
chr12	57966255	57966615	KIF5A	0,58	88	81	69	58	82	71	64	52	93	86	93	66	87	93	80	81
chr12	57968780	57969140	KIF5A	0,53	70	77	59	61	90	69	61	48	89	77	88	71	93	77	79	62
chr12	57969361	57969601	KIF5A	0,54	118	106	80	89	114	93	71	71	126	102	109	95	119	104	111	89
chr12	57969781	57970226	KIF5A	0,56	92	87	68	64	93	88	78	60	111	86	94	79	94	94	103	86
chr12	57970474	57970714	KIF5A	0,50	161	162	131	106	155	146	121	98	187	152	156	146	169	166	163	117
chr12	57971405	57971645	KIF5A	0,49	76	76	64	56	91	70	58	53	89	80	73	63	78	67	74	62
chr12	57971707	57971947	KIF5A	0,51	166	170	140	105	162	146	120	95	197	166	155	159	153	179	174	132
chr12	57971952	57972192	KIF5A	0,49	146	145	98	108	136	119	91	87	156	131	142	130	137	149	153	122
chr12	57974666	57975026	KIF5A	0,55	107	112	89	79	101	99	85	61	132	97	105	97	106	111	112	100
chr12	57975094	57975454	KIF5A	0,53	217	200	184	133	191	187	171	123	240	216	241	194	237	229	239	179
chr12	57975573	57975813	KIF5A	0,53	67	71	49	64	60	65	51	36	74	58	71	67	68	66	60	60
chr12	57976278	57976518	KIF5A	0,46	224	225	169	139	181	186	150	119	259	214	229	178	241	222	241	174
chr12	57976802	57977042	KIF5A	0,55	90	100	84	70	96	90	71	68	122	94	112	106	117	93	99	80
chr12	862630	863590	WNK1	0,65	61	60	44	46	67	54	44	35	73	56	62	53	64	68	60	53
chr12	922713	923073	WNK1	0,32	246	287	219	131	195	217	193	139	285	260	258	229	272	251	272	203

chr12	936137	936497	WNK1	0,42	215	237	184	125	195	200	152	105	234	238	235	194	224	235	249	171
chr12	939067	939427	WNK1	0,45	238	260	202	148	213	199	165	133	254	235	238	215	270	249	251	185
chr12	966250	966490	WNK1	0,35	209	221	179	120	184	176	150	114	259	211	219	198	226	191	220	156
chr12	968340	968700	WNK1	0,30	217	228	200	134	176	209	146	117	245	234	221	193	225	226	230	179
chr12	970103	970583	WNK1	0,44	217	210	154	122	180	180	158	116	224	209	211	181	228	200	206	180
chr12	971162	971522	WNK1	0,43	245	258	196	142	212	223	175	128	262	239	241	208	253	247	254	194
chr12	974222	974582	WNK1	0,49	284	291	246	163	245	237	206	139	315	293	278	254	294	273	302	230
chr12	976930	978370	WNK1	0,46	322	328	277	177	267	277	229	164	349	347	324	289	332	333	352	248
chr12	980352	980592	WNK1	0,41	244	244	200	150	204	199	164	130	265	254	250	190	245	245	226	179
chr12	987272	987632	WNK1	0,39	308	326	271	178	273	276	229	168	327	343	327	275	324	340	332	247
chr12	988699	989275	WNK1	0,50	277	273	226	174	233	230	178	148	302	280	274	237	291	273	273	217
chr12	98909558	98909894	TMPO	0,69	19	15	9	20	21	17	16	15	19	17	25	20	19	22	12	16
chr12	98921606	98921846	TMPO	0,34	225	253	166	147	193	191	179	113	255	250	230	179	245	226	228	168
chr12	98925356	98925716	TMPO	0,34	258	295	213	153	207	216	188	147	288	278	284	238	265	280	271	209
chr12	98926520	98928200	TMPO	0,42	286	287	230	160	232	237	206	136	305	290	282	243	292	290	298	222
chr12	98931181	98931421	TMPO	0,37	186	190	155	116	151	165	125	102	209	189	196	159	191	201	169	146
chr12	98937921	98938401	TMPO	0,36	240	241	196	138	195	211	161	126	253	224	231	190	229	226	233	174
chr12	98938663	98938903	TMPO	0,34	235	265	193	147	230	196	178	126	274	232	239	231	236	253	236	203
chr12	98940060	98940300	TMPO	0,30	200	206	169	123	174	180	157	108	228	194	219	176	196	191	207	152
chr12	98941253	98941733	TMPO	0,38	298	323	243	175	254	254	215	154	314	306	296	255	297	306	330	229
chr12	989785	990265	WNK1	0,45	313	320	252	168	260	272	205	155	320	314	326	257	306	332	330	239
chr12	990786	991288	WNK1	0,40	232	254	180	135	194	188	153	120	241	216	216	194	232	218	226	177
chr12	992046	992286	WNK1	0,36	183	186	122	114	148	148	127	101	195	168	190	145	185	173	177	137
chr12	992467	992827	WNK1	0,37	273	296	238	159	243	231	195	143	304	276	278	235	267	278	281	221
chr12	992846	993062	WNK1	0,38	249	242	194	152	235	207	175	137	265	239	235	201	231	269	258	204
chr12	993227	993467	WNK1	0,37	235	258	205	150	222	206	190	131	270	241	249	213	237	263	253	214
chr12	993752	995312	WNK1	0,47	368	373	303	191	286	305	256	175	396	383	355	310	372	373	392	270
chr12	996308	996548	WNK1	0,47	228	234	195	148	219	224	170	127	254	230	251	214	242	234	250	172
chr12	998227	998467	WNK1	0,39	254	289	210	165	234	233	189	141	296	274	286	239	282	263	311	192
chr12	999528	999768	WNK1	0,45	148	155	119	105	144	141	97	89	171	142	147	135	147	147	151	117
chr13	102375128	102375368	FGF14	0,47	221	221	172	127	190	188	124	114	232	216	221	184	211	205	241	164
chr13	102378880	102379240	FGF14	0,35	301	320	249	185	255	245	208	156	318	308	301	241	310	308	329	225
chr13	102521006	102521246	FGF14	0,43	249	265	215	148	215	247	188	141	272	275	262	224	263	280	286	196
chr13	102527470	102527710	FGF14	0,43	190	229	168	139	213	204	158	114	230	212	216	197	209	224	221	175
chr13	102568718	102569078	FGF14	0,71	16	17	17	19	20	15	16	11	19	13	27	17	23	20	14	18
chr13	103053744	103054104	FGF14	0,41	255	277	208	163	237	244	187	144	286	255	254	230	271	283	263	214
chr13	23777750	23778110	SGCG	0,40	278	290	226	166	213	242	198	149	315	281	273	248	282	285	297	220
chr13	23808680	23808920	SGCG	0,32	177	219	143	97	152	184	134	92	220	183	190	156	198	203	200	154
chr13	23824692	23824932	SGCG	0,37	259	266	217	145	221	220	172	135	283	268	231	221	255	276	255	184
chr13	23853437	23853677	SGCG	0,38	192	166	147	104	165	152	136	105	202	195	184	149	184	173	181	118
chr13	23869469	23869709	SGCG	0,39	183	199	157	116	160	151	135	106	238	182	204	171	189	213	180	144
chr13	23894755	23894971	SGCG	0,44	127	137	94	100	119	114	101	92	148	118	123	123	128	129	123	102
chr13	23898413	23898773	SGCG	0,57	90	85	74	72	85	85	63	54	99	103	93	79	105	103	89	84
chr13	23904177	23914341	SACS	0,38	377	402	314	205	292	317	265	184	398	407	375	325	383	387	416	279
chr13	23914413	23915925	SACS	0,37	364	393	302	205	305	313	269	184	395	393	380	321	366	374	410	285
chr13	23927849	23928089	SACS	0,32	156	167	139	136	144	158	115	89	193	145	167	134	175	170	160	119
chr13	23928561	23930241	SACS	0,44	303	314	235	165	239	250	211	144	323	317	301	249	298	298	317	229
chr13	23932366	23932726	SACS	0,39	271	295	236	169	228	240	184	144	313	302	296	245	294	288	311	220
chr13	23939240	23939480	SACS	0,31	297	357	278	167	229	258	204	155	320	319	304	271	311	318	327	250
chr13	23942463	23942703	SACS	0,36	238	280	213	157	227	218	172	142	256	262	265	217	259	257	263	195
chr13	23945164	23945380	SACS	0,32	239	265	203	142	195	212	182	132	292	262	244	218	257	278	253	188
chr13	23949152	23949512	SACS	0,76	5	7	4	2	6	3	3	5	7	3	6	6	7	5	4	4
chr13	23985308	23985428	SACS	0,35	207	213	165	146	194	195	147	119	243	215	214	193	225	219	242	169
chr13	36878395	36878875	SPG20	0,37	360	374	285	189	270	306	233	172	380	368	330	274	337	349	400	269
chr13	36886239	36886695	SPG20	0,35	325	341	249	181	247	265	213	151	330	334	322	267	319	328	352	254
chr13	36888280	36888640	SPG20	0,37	332	353	275	189	262	286	233	161	373	350	354	276	341	329	359	253
chr13	36900653	36900893	SPG20	0,35	227	239	205	128	168	199	171	114	275	241	230	189	234	227	229	181
chr13	36903396	36903756	SPG20	0,37	317	351	254	179	268	282	226	163	333	343	320	275	335	328	360	234
chr13	36905454	36905814	SPG20	0,37	219	242	179	130	189	188	132	119	235	220	221	193	245	212	242	181
chr13	36909064	36910000	SPG20	0,42	304	325	255	173	263	256	208	158	330	302	300	263	317	311	313	233

chr13	48517422	48517662	SUCLA2	0,34	296	294	231	152	230	251	186	139	278	285	293	236	276	267	291	206
chr13	48522998	48523238	SUCLA2	0,33	321	358	260	177	264	274	214	163	332	308	341	267	333	318	332	233
chr13	48523557	48523797	SUCLA2	0,34	275	292	221	148	205	230	167	135	312	295	277	227	284	286	297	196
chr13	48528242	48528791	SUCLA2	0,36	315	354	262	174	261	261	221	163	344	345	323	266	330	313	347	245
chr13	48542678	48542918	SUCLA2	0,34	278	346	250	178	234	240	199	145	330	301	294	252	277	281	294	235
chr13	48547342	48547582	SUCLA2	0,35	260	290	233	147	237	231	189	143	303	286	268	251	269	255	289	210
chr13	48562576	48562936	SUCLA2	0,31	333	389	300	198	252	304	266	180	372	381	356	320	371	357	439	273
chr13	48562946	48563186	SUCLA2	0,34	324	371	277	191	273	313	232	156	347	346	344	284	329	380	387	268
chr13	48570887	48571247	SUCLA2	0,37	296	346	256	176	235	277	223	160	335	315	306	266	298	298	319	223
chr13	48575240	48575480	SUCLA2	0,71	6	9	6	9	11	11	5	7	10	6	11	11	10	10	10	14
chr14	21161645	21162245	ANG	0,53	164	159	123	118	160	143	114	99	187	164	154	147	172	163	166	135
chr14	23790918	23791038	PABPN1	0,74	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chr14	23791326	23791566	PABPN1	0,55	35	31	32	34	46	44	35	28	52	36	48	34	34	42	41	39
chr14	23792145	23792361	PABPN1	0,44	143	142	106	93	132	135	100	83	154	129	150	121	144	147	126	104
chr14	23792518	23792758	PABPN1	0,42	167	209	143	118	179	186	131	103	203	169	176	168	189	191	196	146
chr14	23793130	23793586	PABPN1	0,51	211	226	190	136	198	179	163	125	242	218	222	187	234	218	221	180
chr14	23794404	23794524	PABPN1	0,45	39	42	35	42	58	43	31	30	53	38	42	45	42	32	46	38
chr14	23851140	23851380	MYH6	0,54	80	85	54	64	76	71	59	53	97	64	77	62	74	83	77	65
chr14	23851583	23851823	MYH6	0,59	105	105	92	87	118	105	76	67	155	113	134	106	115	113	144	97
chr14	23852361	23852601	MYH6	0,53	45	42	29	28	36	33	24	22	43	32	37	38	33	38	26	28
chr14	23853548	23854028	MYH6	0,62	130	126	104	96	126	112	96	79	153	116	136	122	128	123	122	114
chr14	23854067	23854307	MYH6	0,51	141	134	121	102	138	119	88	87	174	137	148	137	144	127	130	121
chr14	23855058	23855418	MYH6	0,62	73	68	64	64	78	58	51	48	82	68	89	71	81	84	76	65
chr14	23855437	23855917	MYH6	0,62	96	72	67	70	83	71	60	54	89	76	88	68	89	88	81	81
chr14	23856679	23857229	MYH6	0,61	161	157	119	116	150	137	116	107	190	155	180	152	173	166	175	130
chr14	23857275	23857635	MYH6	0,60	124	118	96	101	128	112	83	89	144	119	130	124	114	133	135	112
chr14	23857985	23858345	MYH6	0,62	61	62	48	48	64	59	44	38	75	61	64	53	62	67	55	51
chr14	23858524	23859004	MYH6	0,59	103	92	69	68	111	91	73	59	120	95	104	95	99	102	100	90
chr14	23859202	23859634	MYH6	0,66	56	46	38	32	51	40	35	30	47	45	57	43	47	53	57	49
chr14	23861695	23861935	MYH6	0,51	160	163	133	109	144	153	111	100	197	166	159	141	174	165	170	124
chr14	23862013	23862373	MYH6	0,52	252	250	181	150	204	204	168	124	271	241	235	206	243	249	258	195
chr14	23862458	23863175	MYH6	0,52	297	301	244	173	243	248	218	139	318	311	285	260	287	289	307	235
chr14	23863224	23863584	MYH6	0,57	157	164	129	100	155	139	117	91	186	181	161	154	164	175	176	133
chr14	23865440	23865680	MYH6	0,60	97	92	72	73	100	72	60	63	115	86	96	82	96	92	89	93
chr14	23865844	23866558	MYH6	0,57	83	83	66	70	88	78	59	56	96	81	88	78	72	86	80	76
chr14	23866654	23866846	MYH6	0,54	26	17	13	18	25	22	15	11	24	20	21	18	15	15	27	19
chr14	23867851	23868331	MYH6	0,56	129	128	100	89	130	113	97	77	145	119	137	107	124	122	127	104
chr14	23869369	23869729	MYH6	0,56	203	201	160	114	175	164	130	106	218	205	194	165	188	179	196	149
chr14	23869864	23870224	MYH6	0,56	125	113	90	84	107	108	80	69	130	104	122	116	119	123	112	94
chr14	23871579	23872035	MYH6	0,63	100	94	79	68	109	86	66	57	108	87	110	90	89	86	99	81
chr14	23872485	23872725	MYH6	0,58	36	31	23	26	38	26	22	22	49	32	40	32	33	37	36	32
chr14	23872835	23873075	MYH6	0,59	43	34	32	35	42	34	31	28	42	35	36	39	38	42	33	36
chr14	23873430	23873670	MYH6	0,59	37	24	28	25	41	29	28	22	42	24	36	43	41	47	30	33
chr14	23873855	23874095	MYH6	0,58	86	86	55	65	91	73	58	57	90	78	104	70	88	88	85	70
chr14	23874208	23874688	MYH6	0,60	89	82	65	66	90	81	61	49	107	67	97	79	94	84	94	82
chr14	23874727	23875087	MYH6	0,60	75	67	54	56	76	59	53	43	81	77	85	74	72	69	67	63
chr14	23876151	23876511	MYH6	0,59	182	185	140	128	167	161	129	112	204	195	194	168	190	196	219	162
chr14	23882011	23882131	MYH7	0,50	14	12	12	9	11	11	7	5	19	11	14	13	13	8	10	10
chr14	23882914	23883383	MYH7	0,63	55	53	39	49	62	57	37	39	68	51	57	47	60	58	53	49
chr14	23884101	23885119	MYH7	0,60	126	120	103	99	128	116	91	81	148	120	139	114	126	128	137	113
chr14	23885126	23885606	MYH7	0,62	84	83	65	67	105	74	63	54	104	79	94	81	86	90	93	83
chr14	23886018	23886258	MYH7	0,60	143	129	90	90	123	109	93	75	149	110	150	111	123	137	132	107
chr14	23886264	23886983	MYH7	0,61	125	107	96	89	117	117	85	74	140	109	132	105	120	131	128	112
chr14	23887336	23887696	MYH7	0,64	45	62	44	49	60	54	42	32	67	47	61	45	58	50	55	49
chr14	23888324	23888564	MYH7	0,59	95	80	65	75	102	87	70	65	109	102	100	88	87	95	97	93
chr14	23888634	23888874	MYH7	0,59	49	38	35	38	53	41	41	26	48	42	40	41	44	53	36	43
chr14	23888966	23889422	MYH7	0,63	67	54	46	38	56	48	52	37	69	73	68	54	67	67	66	54
chr14	23890091	23890331	MYH7	0,58	53	44	28	39	59	53	27	31	68	52	52	41	52	39	44	45
chr14	23891281	23891641	MYH7	0,56	134	135	118	84	140	120	102	85	147	135	147	129	136	146	150	119
chr14	23892687	23893023	MYH7	0,54	222	225	183	167	206	210	169	129	268	235	237	203	234	236	249	198

chr14	23893056	23893416	MYH7	0,53	283	292	240	158	240	242	200	145	319	317	299	243	301	307	325	222
chr14	23893925	23894285	MYH7	0,57	141	124	106	95	127	118	94	81	150	128	133	114	138	126	131	116
chr14	23894438	23894678	MYH7	0,59	69	60	49	49	67	70	49	35	71	59	70	52	63	65	71	54
chr14	23894845	23895085	MYH7	0,51	162	157	120	122	158	136	123	85	176	159	169	127	152	172	164	134
chr14	23895102	23895318	MYH7	0,61	77	68	61	63	92	81	57	43	99	75	78	62	94	91	89	71
chr14	23895909	23896149	MYH7	0,49	54	67	46	41	69	54	51	39	72	49	65	54	58	66	51	47
chr14	23896362	23896602	MYH7	0,56	39	43	31	34	43	34	37	29	60	43	43	31	40	38	44	40
chr14	23896708	23897188	MYH7	0,54	179	172	137	124	162	160	138	102	210	172	174	147	185	195	195	148
chr14	23897613	23897973	MYH7	0,55	200	197	150	117	163	179	143	103	200	220	203	165	198	219	219	164
chr14	23898106	23898614	MYH7	0,55	159	158	123	101	157	144	124	93	178	151	168	140	159	169	161	140
chr14	23898932	23899172	MYH7	0,54	216	208	172	136	192	181	142	125	214	187	195	180	210	195	197	178
chr14	23899700	23899940	MYH7	0,58	103	92	84	75	99	77	73	62	120	79	103	90	94	104	98	89
chr14	23900038	23900278	MYH7	0,46	148	157	117	105	130	130	98	90	176	145	156	128	147	147	162	128
chr14	23900562	23901175	MYH7	0,56	106	97	79	78	110	94	72	59	130	101	114	100	106	119	109	98
chr14	23901581	23902105	MYH7	0,58	66	64	52	53	83	64	46	45	94	66	74	63	74	74	68	67
chr14	23902184	23902544	MYH7	0,56	78	76	54	57	90	77	54	50	88	86	86	76	77	82	89	68
chr14	23902660	23903020	MYH7	0,59	56	61	45	44	62	52	36	37	80	57	70	57	62	57	58	46
chr14	35181966	35182940	CFL2	0,34	349	375	282	197	262	295	260	167	371	359	343	300	352	341	377	256
chr14	35183684	35183804	CFL2	0,74	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0
chr14	51026880	51027120	ATL1	0,66	28	31	31	30	40	28	17	24	41	38	36	30	38	35	31	25
chr14	51054492	51054852	ATL1	0,42	183	212	156	118	188	165	129	105	217	188	215	167	189	190	201	157
chr14	51057605	51057845	ATL1	0,34	168	213	139	109	145	163	141	100	188	178	213	148	191	187	185	137
chr14	51058184	51058424	ATL1	0,34	293	332	224	156	220	237	181	133	295	281	289	246	294	275	278	209
chr14	51060468	51060708	ATL1	0,32	233	218	186	131	192	181	162	121	256	240	249	217	221	232	231	188
chr14	51062258	51062450	ATL1	0,37	234	253	184	133	190	218	153	134	247	222	226	187	215	236	235	171
chr14	51079902	51080142	ATL1	0,35	153	164	128	92	138	150	126	87	152	153	170	137	168	153	164	126
chr14	51081039	51081279	ATL1	0,35	273	313	219	187	250	261	217	136	298	305	257	250	291	306	319	214
chr14	51087260	51087500	ATL1	0,39	196	200	169	111	182	176	147	113	213	201	208	187	191	211	186	152
chr14	51088468	51088708	ATL1	0,31	185	217	178	127	156	182	140	125	237	201	218	177	205	205	197	166
chr14	51089810	51090050	ATL1	0,36	207	194	140	126	168	168	118	102	203	195	183	153	181	166	193	145
chr14	51094652	51095204	ATL1	0,43	234	263	188	158	216	205	167	130	261	258	260	206	248	263	265	191
chr14	51098880	51099120	ATL1	0,31	234	267	210	159	212	227	198	136	252	245	244	208	251	242	283	191
chr14	64375785	64376025	SYNE2	0,41	208	219	166	125	193	172	138	116	242	200	202	172	222	214	214	167
chr14	64407242	64407482	SYNE2	0,42	187	188	157	119	162	157	136	99	208	195	192	163	185	203	195	145
chr14	64408336	64408936	SYNE2	0,33	367	398	317	202	290	303	249	182	375	415	358	331	365	362	385	275
chr14	64416453	64416813	SYNE2	0,45	282	300	253	150	256	251	190	151	351	313	277	264	294	296	317	211
chr14	64421354	64421714	SYNE2	0,35	248	272	205	145	202	216	166	127	266	236	232	191	245	243	249	194
chr14	64428172	64428412	SYNE2	0,38	227	223	181	123	211	190	155	115	242	238	241	191	229	226	238	177
chr14	64430547	64430787	SYNE2	0,30	179	196	153	110	164	156	134	100	204	184	175	155	156	182	182	131
chr14	64434367	64434607	SYNE2	0,37	214	206	183	128	197	190	157	108	237	208	228	181	212	206	205	171
chr14	64443182	64443542	SYNE2	0,35	251	262	204	140	209	216	194	132	275	253	254	203	244	246	263	186
chr14	64444558	64444798	SYNE2	0,37	252	298	234	153	229	226	213	132	284	296	283	231	273	287	267	198
chr14	64445470	64445830	SYNE2	0,34	286	333	270	173	252	250	230	159	336	326	322	277	313	316	338	232
chr14	64447290	64447530	SYNE2	0,31	267	299	203	159	197	217	188	140	309	276	269	237	263	263	263	203
chr14	64447646	64447982	SYNE2	0,30	211	265	177	144	200	203	171	121	259	245	244	192	240	227	243	183
chr14	64449295	64449535	SYNE2	0,37	211	248	189	138	208	203	163	137	271	228	246	205	237	235	234	192
chr14	64450349	64450685	SYNE2	0,26	241	248	176	127	183	194	156	132	220	214	231	189	217	216	216	162
chr14	64453098	64453434	SYNE2	0,34	232	228	181	139	202	193	145	109	257	233	226	197	231	236	222	188
chr14	64457027	64457387	SYNE2	0,36	169	180	125	100	147	148	118	82	182	164	177	153	165	182	162	134
chr14	64457566	64457926	SYNE2	0,37	217	231	176	124	182	177	149	113	237	212	218	197	211	210	210	169
chr14	64460495	64460735	SYNE2	0,32	193	230	176	136	188	170	139	119	232	210	207	191	208	205	207	154
chr14	64461660	64462020	SYNE2	0,28	237	248	198	131	199	208	173	117	251	241	234	196	244	229	251	180
chr14	64463590	64464183	SYNE2	0,40	263	267	215	143	228	235	180	137	302	264	261	242	280	286	288	208
chr14	64464871	64465111	SYNE2	0,34	256	298	210	135	218	213	182	141	285	264	272	220	277	256	265	191
chr14	64465574	64465814	SYNE2	0,34	234	260	222	155	198	218	183	123	259	239	239	222	218	228	241	183
chr14	64467178	64467514	SYNE2	0,31	265	297	221	154	233	230	194	141	293	281	258	228	276	273	294	211
chr14	64468545	64468905	SYNE2	0,37	279	302	241	153	234	244	195	155	293	276	274	246	289	276	285	228
chr14	64469382	64470102	SYNE2	0,38	279	301	249	161	252	245	198	151	318	301	285	241	294	292	299	218
chr14	64473670	64474030	SYNE2	0,36	228	237	182	140	200	192	171	122	263	231	242	196	216	246	243	175
chr14	64476578	64476938	SYNE2	0,36	263	270	206	153	213	216	179	124	265	260	253	247	255	269	278	202

chr14	64483089	64483449	SYNE2	0,26	246	275	184	139	175	200	183	126	277	245	247	195	253	236	248	189
chr14	64484213	64484549	SYNE2	0,31	272	292	203	142	222	231	183	139	296	290	281	221	274	275	285	214
chr14	64486630	64486846	SYNE2	0,29	230	268	197	137	195	205	171	127	237	253	232	216	233	229	249	166
chr14	64487952	64488312	SYNE2	0,42	243	242	206	138	214	218	165	124	277	245	262	219	263	260	248	198
chr14	64488480	64488840	SYNE2	0,32	298	312	238	171	215	256	197	154	318	294	306	254	287	306	312	214
chr14	64489404	64489644	SYNE2	0,32	211	241	183	127	173	179	151	123	248	201	208	170	205	198	212	165
chr14	64490895	64491255	SYNE2	0,43	153	147	98	94	140	122	110	81	175	145	152	127	156	151	149	118
chr14	64491522	64492194	SYNE2	0,36	269	279	224	158	221	238	194	137	302	282	289	228	261	281	277	211
chr14	64493192	64493552	SYNE2	0,43	169	168	135	115	177	158	119	90	189	169	182	147	190	186	172	142
chr14	64494183	64494615	SYNE2	0,36	252	244	204	149	215	232	171	125	268	265	264	211	272	249	265	199
chr14	64496514	64496874	SYNE2	0,35	245	264	190	141	225	218	181	127	273	256	247	212	255	247	258	191
chr14	64497664	64498144	SYNE2	0,38	222	237	183	140	197	200	158	114	255	226	224	202	231	215	231	172
chr14	64514615	64514975	SYNE2	0,31	296	331	255	157	225	258	214	137	331	310	305	262	286	311	324	236
chr14	64516221	64516701	SYNE2	0,37	281	305	236	179	269	240	216	146	338	308	300	257	308	299	331	239
chr14	64518173	64520405	SYNE2	0,36	303	329	242	175	248	257	221	159	337	312	307	264	312	308	325	235
chr14	64522582	64523062	SYNE2	0,38	283	278	209	157	231	233	183	137	298	258	276	226	269	273	269	209
chr14	64529360	64529720	SYNE2	0,36	204	211	148	122	178	177	129	118	205	187	211	168	200	204	200	158
chr14	64532078	64532438	SYNE2	0,37	225	254	181	138	198	196	158	129	256	222	219	196	245	230	224	171
chr14	64537255	64537735	SYNE2	0,36	225	255	182	141	222	198	167	127	257	229	244	210	245	231	239	175
chr14	64540589	64540949	SYNE2	0,36	276	297	205	150	210	213	185	135	288	266	273	213	275	256	266	187
chr14	64542560	64542920	SYNE2	0,37	282	287	222	141	229	234	191	133	303	292	283	234	303	289	284	213
chr14	64545073	64545433	SYNE2	0,36	305	306	266	173	250	262	233	162	338	324	322	269	298	309	332	235
chr14	64547084	64547444	SYNE2	0,40	252	262	208	151	215	211	158	123	278	243	239	220	258	244	266	189
chr14	64548109	64548349	SYNE2	0,46	162	178	150	102	151	143	115	89	213	161	170	143	169	163	169	144
chr14	64554297	64554633	SYNE2	0,33	263	295	238	170	224	228	206	149	314	298	297	262	304	277	302	226
chr14	64556232	64556472	SYNE2	0,26	233	226	187	133	209	198	159	113	258	218	240	205	228	219	216	174
chr14	64557518	64557854	SYNE2	0,35	256	291	219	156	221	221	183	130	285	259	266	229	266	244	292	194
chr14	64560012	64560252	SYNE2	0,34	293	299	245	167	244	246	208	154	321	300	296	254	299	302	336	210
chr14	64564585	64564945	SYNE2	0,48	156	156	123	90	153	138	100	92	169	147	164	144	151	150	164	127
chr14	64565381	64565621	SYNE2	0,43	106	109	83	85	103	85	82	63	134	120	123	104	112	98	103	87
chr14	64568584	64568824	SYNE2	0,51	97	86	72	55	90	81	68	55	112	78	93	68	95	89	72	74
chr14	64574146	64574386	SYNE2	0,46	166	190	126	111	166	148	120	105	202	183	165	144	167	155	181	136
chr14	64579993	64580353	SYNE2	0,55	43	45	26	30	47	40	34	28	48	32	38	36	47	43	42	35
chr14	64586055	64586415	SYNE2	0,40	193	206	163	120	197	175	147	107	218	200	218	172	208	203	190	155
chr14	64587590	64587830	SYNE2	0,38	225	238	197	145	203	213	167	118	258	216	242	207	228	237	221	168
chr14	64588670	64589254	SYNE2	0,37	288	299	239	162	248	249	206	144	297	293	277	249	284	282	313	219
chr14	64591742	64591982	SYNE2	0,39	221	239	209	143	221	191	169	126	265	238	255	202	238	225	227	181
chr14	64592964	64593600	SYNE2	0,46	223	240	190	146	218	205	162	131	261	233	243	204	251	233	242	182
chr14	64595100	64595340	SYNE2	0,34	243	274	209	142	230	232	179	144	296	270	271	218	241	257	278	193
chr14	64596439	64596679	SYNE2	0,44	163	153	124	100	155	158	111	88	194	156	173	136	177	157	165	135
chr14	64596685	64597045	SYNE2	0,45	176	174	146	116	163	176	128	104	218	171	204	157	205	200	190	145
chr14	64598889	64599249	SYNE2	0,35	276	286	212	147	221	237	192	128	282	263	265	242	290	267	270	184
chr14	64600732	64600972	SYNE2	0,37	246	237	188	142	189	202	167	121	243	234	230	188	240	242	261	194
chr14	64604422	64604782	SYNE2	0,42	182	178	131	123	157	150	109	95	211	159	174	151	166	179	176	133
chr14	64606604	64606844	SYNE2	0,35	219	239	191	155	217	205	154	106	247	237	242	185	246	208	232	171
chr14	64607969	64608329	SYNE2	0,34	279	295	247	162	245	249	205	150	311	287	271	253	288	298	320	205
chr14	64608552	64608912	SYNE2	0,36	273	324	237	155	241	244	202	151	318	294	310	246	287	283	324	223
chr14	64610397	64610757	SYNE2	0,43	196	181	146	120	169	156	119	97	206	185	198	171	200	178	199	145
chr14	64612683	64613043	SYNE2	0,39	195	200	152	115	161	171	134	98	209	181	199	146	184	179	180	141
chr14	64619177	64619537	SYNE2	0,35	311	304	263	176	232	252	218	159	311	321	316	266	298	310	308	241
chr14	64625247	64625607	SYNE2	0,42	225	226	173	149	195	201	167	124	242	235	243	206	235	231	261	183
chr14	64625974	64626214	SYNE2	0,39	191	201	166	137	187	187	141	108	213	202	206	184	192	208	218	158
chr14	64628711	64629071	SYNE2	0,52	94	98	73	75	98	82	62	57	117	90	99	90	99	92	100	85
chr14	64630029	64630389	SYNE2	0,47	177	182	146	119	171	150	120	103	210	177	182	174	181	179	200	158
chr14	64631945	64632185	SYNE2	0,32	231	242	173	146	191	208	149	145	240	226	236	199	232	221	231	164
chr14	64633871	64634464	SYNE2	0,43	224	234	181	137	200	195	160	130	241	226	233	187	220	222	235	166
chr14	64635521	64635761	SYNE2	0,42	223	242	183	130	189	207	169	114	260	228	237	189	230	222	241	181
chr14	64636882	64637242	SYNE2	0,48	149	148	113	100	135	124	112	88	174	147	159	137	156	155	153	107
chr14	64641578	64641938	SYNE2	0,40	242	267	201	148	211	210	184	137	296	260	242	218	260	260	251	191
chr14	64644018	64644258	SYNE2	0,37	201	236	170	129	217	190	136	115	242	212	202	194	207	213	203	166

chr14	64653039	64653399	SYNE2	0,47	198	195	140	121	186	160	147	109	212	184	196	165	189	195	194	164
chr14	64655166	64655526	SYNE2	0,43	175	168	130	109	163	154	127	97	181	164	177	146	171	165	159	136
chr14	64656691	64657051	SYNE2	0,39	186	199	156	123	192	159	126	105	212	186	200	170	196	188	206	151
chr14	64669419	64669779	SYNE2	0,45	195	212	145	121	183	176	123	100	220	189	203	158	204	193	188	151
chr14	64675389	64675749	SYNE2	0,52	104	102	83	73	108	103	73	59	133	91	119	93	110	101	92	90
chr14	64676040	64676400	SYNE2	0,53	110	98	93	78	111	98	83	68	121	93	114	95	106	101	94	97
chr14	64676570	64676930	SYNE2	0,61	31	35	28	26	41	29	27	29	44	31	32	35	36	37	34	36
chr14	64678627	64678867	SYNE2	0,52	122	133	106	93	130	131	94	79	153	120	127	119	134	130	114	116
chr14	64679445	64679805	SYNE2	0,50	110	111	97	75	128	104	83	61	136	112	119	115	126	125	133	100
chr14	64680833	64681289	SYNE2	0,54	72	72	55	61	76	67	57	54	88	75	78	64	72	74	84	66
chr14	64681917	64682157	SYNE2	0,46	226	234	175	152	195	207	165	136	243	233	247	206	230	217	231	182
chr14	64682915	64683155	SYNE2	0,50	196	196	159	122	173	160	137	110	225	188	191	161	183	197	191	152
chr14	64685052	64685292	SYNE2	0,50	66	60	56	54	67	56	49	41	79	64	73	55	65	58	60	63
chr14	64685846	64686206	SYNE2	0,44	171	177	147	108	169	158	116	83	195	164	174	137	161	165	161	131
chr14	64687074	64687434	SYNE2	0,54	101	103	84	73	109	91	66	65	128	84	118	91	97	103	84	87
chr14	64688156	64688516	SYNE2	0,54	89	78	53	63	93	77	48	48	94	68	84	65	82	82	73	77
chr14	64689822	64690182	SYNE2	0,44	181	170	138	115	161	146	122	98	174	160	177	138	164	163	165	137
chr14	64691101	64691341	SYNE2	0,51	68	64	49	50	80	71	44	48	77	61	69	57	68	64	53	63
chr14	64691618	64691858	SYNE2	0,44	103	103	80	88	116	104	76	69	118	108	120	102	112	110	110	85
chr14	64691983	64692103	SYNE2	0,66	25	14	11	12	19	21	16	8	22	19	21	13	24	20	17	11
chr14	64692148	64692340	SYNE2	0,58	38	47	38	29	43	38	35	28	67	37	50	45	49	49	41	33
chr14	68215074	68215434	ZFYVE26	0,58	38	36	31	38	49	37	27	25	53	38	49	38	49	38	31	36
chr14	68217670	68217910	ZFYVE26	0,54	137	124	106	81	123	115	90	70	140	113	130	105	111	125	113	112
chr14	68218971	68219331	ZFYVE26	0,51	89	78	68	61	82	74	61	47	101	75	85	76	89	87	77	77
chr14	68220333	68220573	ZFYVE26	0,39	217	236	180	147	200	204	161	129	230	230	252	195	212	228	210	191
chr14	68220739	68220979	ZFYVE26	0,50	132	138	95	80	127	105	82	79	150	114	125	106	110	122	120	110
chr14	68221687	68222047	ZFYVE26	0,48	216	216	161	128	204	196	146	109	232	224	203	193	220	220	217	188
chr14	68222583	68222943	ZFYVE26	0,49	191	200	150	118	186	183	135	109	219	205	192	185	192	194	190	150
chr14	68228011	68228371	ZFYVE26	0,51	90	72	71	69	79	71	56	54	103	81	88	76	99	90	100	84
chr14	68228844	68229204	ZFYVE26	0,53	130	133	114	87	124	122	84	84	151	131	153	111	138	127	142	114
chr14	68229282	68229618	ZFYVE26	0,49	221	244	195	145	220	202	154	139	258	221	243	206	232	218	240	194
chr14	68232873	68233233	ZFYVE26	0,58	55	47	51	47	61	52	35	36	52	56	59	48	65	55	56	54
chr14	68234368	68234608	ZFYVE26	0,44	298	321	219	154	245	235	205	130	306	289	268	232	285	287	321	210
chr14	68235129	68235369	ZFYVE26	0,40	166	192	140	123	180	164	125	103	218	205	181	162	204	180	184	148
chr14	68236258	68236498	ZFYVE26	0,53	91	88	72	62	91	87	63	56	98	86	88	79	83	94	97	81
chr14	68238659	68238971	ZFYVE26	0,53	86	92	67	73	105	77	68	53	85	89	84	76	80	92	90	82
chr14	68241661	68241901	ZFYVE26	0,43	87	87	58	61	91	74	70	45	109	72	83	62	88	80	86	73
chr14	68242519	68242879	ZFYVE26	0,50	148	160	121	92	157	129	110	81	173	159	174	142	155	141	161	123
chr14	68244183	68244543	ZFYVE26	0,50	211	217	175	124	186	191	158	122	239	212	229	187	224	235	230	171
chr14	68244783	68245023	ZFYVE26	0,45	142	153	124	101	149	118	91	76	171	147	151	137	153	157	152	139
chr14	68246889	68247129	ZFYVE26	0,50	99	79	47	62	83	63	48	42	87	80	94	79	83	89	81	76
chr14	68247967	68248327	ZFYVE26	0,51	168	176	135	115	159	144	117	94	190	152	160	146	156	161	168	122
chr14	68249437	68250325	ZFYVE26	0,55	114	111	82	78	112	89	78	64	124	104	124	98	111	116	119	106
chr14	68250981	68251221	ZFYVE26	0,49	119	117	92	65	116	107	73	61	143	121	116	90	117	127	106	97
chr14	68251704	68252064	ZFYVE26	0,54	166	153	123	92	145	136	102	89	183	140	155	146	149	154	165	131
chr14	68252522	68253002	ZFYVE26	0,46	212	224	160	120	191	174	152	109	220	199	210	178	207	215	210	168
chr14	68255943	68256423	ZFYVE26	0,48	207	210	160	120	191	172	159	110	231	197	215	182	213	219	211	172
chr14	68257209	68257569	ZFYVE26	0,54	92	100	74	78	101	92	65	57	128	98	91	89	96	105	96	87
chr14	68260220	68260580	ZFYVE26	0,53	75	68	53	51	82	72	54	55	91	68	77	69	82	74	79	58
chr14	68260801	68261041	ZFYVE26	0,46	222	251	188	146	214	202	148	135	273	237	236	206	223	224	248	160
chr14	68264310	68264550	ZFYVE26	0,51	56	50	45	40	58	55	34	29	63	58	57	49	59	59	51	54
chr14	68264674	68265394	ZFYVE26	0,51	211	210	156	127	182	180	136	106	220	193	203	175	214	197	214	158
chr14	68268717	68269077	ZFYVE26	0,51	83	83	69	54	96	84	67	59	118	82	89	82	92	94	87	76
chr14	68270719	68271079	ZFYVE26	0,47	163	166	137	111	169	138	115	88	186	159	165	147	189	171	188	138
chr14	68271857	68272432	ZFYVE26	0,53	115	106	97	80	111	99	78	70	127	107	113	102	122	103	104	93
chr14	68273206	68273446	ZFYVE26	0,48	107	123	85	80	118	114	88	72	128	102	117	117	117	112	110	105
chr14	68274015	68274735	ZFYVE26	0,57	85	78	67	57	79	76	59	45	94	82	82	75	84	80	79	73
chr14	68275841	68276081	ZFYVE26	0,40	171	211	132	122	190	167	120	106	222	164	196	147	184	178	166	148
chr14	68280621	68280861	ZFYVE26	0,41	218	238	191	117	199	177	164	113	235	234	218	195	243	211	220	181
chr14	68282403	68282763	ZFYVE26	0,49	189	217	168	131	185	182	158	122	218	207	212	167	213	213	212	176

chr14	76425428	76425788	TGFB3	0,58	33	31	24	29	34	29	22	16	33	31	39	25	29	28	33	26
chr14	76427162	76427522	TGFB3	0,47	164	148	124	97	147	143	96	88	179	152	163	134	155	162	159	131
chr14	76429564	76429924	TGFB3	0,59	57	48	45	45	46	52	37	34	71	44	61	41	54	59	49	51
chr14	76431864	76432104	TGFB3	0,45	273	300	234	158	230	230	190	142	305	288	269	234	279	277	298	200
chr14	76437413	76437653	TGFB3	0,54	111	94	71	74	104	81	72	61	106	87	102	86	114	100	99	88
chr14	76437823	76438159	TGFB3	0,55	112	119	86	82	106	100	92	71	132	116	132	99	124	119	121	111
chr14	76446820	76447300	TGFB3	0,58	115	110	89	86	109	107	85	66	134	98	121	98	118	104	108	93
chr14	77743651	77743891	POMT2	0,59	18	21	11	11	20	13	10	12	20	23	24	15	15	21	16	18
chr14	77744676	77744916	POMT2	0,62	9	13	5	7	12	8	8	4	15	3	9	4	8	9	9	6
chr14	77745009	77745249	POMT2	0,61	31	31	17	31	43	27	24	25	38	28	35	32	31	28	32	34
chr14	77746114	77746474	POMT2	0,61	57	51	44	51	64	54	43	33	77	48	66	49	62	59	60	55
chr14	77746650	77746890	POMT2	0,54	59	66	53	52	67	60	50	39	80	56	69	62	67	61	63	59
chr14	77750057	77750297	POMT2	0,48	110	117	96	69	115	97	81	62	121	113	116	97	105	108	102	103
chr14	77751218	77751458	POMT2	0,48	80	76	57	65	94	72	60	49	88	82	85	82	89	81	92	66
chr14	77751719	77752079	POMT2	0,46	217	221	189	128	194	183	165	118	240	231	224	188	215	220	237	162
chr14	77753005	77753245	POMT2	0,47	169	188	127	102	170	139	131	101	190	152	178	134	175	165	169	137
chr14	77755019	77755235	POMT2	0,39	255	269	208	167	207	220	188	132	279	277	253	196	250	274	293	205
chr14	77757569	77757809	POMT2	0,49	78	90	58	71	89	71	70	57	98	81	106	82	93	77	74	66
chr14	77762472	77762688	POMT2	0,62	18	17	13	19	23	16	11	11	26	14	18	15	18	21	11	14
chr14	77764952	77765192	POMT2	0,52	144	150	116	96	134	119	92	77	161	133	135	134	145	132	138	130
chr14	77765730	77765970	POMT2	0,52	79	85	65	60	93	74	64	53	100	67	92	72	82	81	85	70
chr14	77767332	77767692	POMT2	0,54	73	75	62	59	81	65	55	45	94	68	79	72	85	77	84	63
chr14	77769141	77769357	POMT2	0,55	59	54	39	35	51	45	37	33	56	45	53	47	38	51	48	55
chr14	77770968	77771208	POMT2	0,52	34	30	21	22	31	24	23	21	37	29	31	31	33	34	27	27
chr14	77772611	77772851	POMT2	0,45	215	192	156	119	189	173	156	104	242	192	202	192	217	212	246	159
chr14	77778213	77778453	POMT2	0,41	165	202	140	110	171	165	123	101	192	163	174	144	172	162	163	134
chr14	77786720	77787080	POMT2	0,73	3	3	3	1	6	1	1	2	2	3	4	2	3	3	3	2
chr14	90429377	90430097	TDP1	0,49	142	150	119	102	148	132	105	89	170	147	150	138	151	158	150	122
chr14	90432392	90432632	TDP1	0,34	234	228	179	144	193	197	142	116	252	232	223	173	214	207	238	172
chr14	90433618	90433858	TDP1	0,39	217	246	183	137	208	216	171	122	231	224	243	199	249	219	246	174
chr14	90437468	90437660	TDP1	0,49	64	79	54	64	93	75	54	43	80	70	75	67	72	76	81	76
chr14	90442021	90442261	TDP1	0,37	245	283	196	174	239	223	173	137	273	261	251	223	259	245	273	198
chr14	90446809	90447049	TDP1	0,42	207	228	178	131	178	175	150	112	216	193	215	174	210	201	209	166
chr14	90450763	90451123	TDP1	0,43	288	310	240	156	209	253	193	142	302	317	285	243	286	287	295	215
chr14	90451377	90451569	TDP1	0,30	153	186	144	104	146	151	117	90	187	151	180	135	158	154	158	128
chr14	90455161	90455521	TDP1	0,42	255	271	200	152	218	206	175	139	279	274	256	225	253	240	287	206
chr14	90455964	90456204	TDP1	0,33	251	279	198	155	207	225	184	126	281	257	256	211	247	249	265	216
chr14	90458258	90458378	TDP1	0,41	35	49	28	35	57	40	32	26	50	30	54	37	38	31	29	27
chr14	90459653	90459893	TDP1	0,36	243	271	247	145	221	221	199	137	274	304	283	228	243	265	294	201
chr14	90485590	90485830	TDP1	0,53	80	105	64	64	97	87	75	57	115	94	111	77	89	104	93	89
chr14	90499383	90499623	TDP1	0,44	61	64	57	51	73	60	48	38	70	61	71	59	66	66	66	55
chr14	90509330	90509570	TDP1	0,41	248	254	181	145	215	182	174	127	270	230	246	214	246	236	251	180
chr14	92530590	92530830	ATXN3	0,34	282	310	230	162	216	234	194	159	300	256	278	250	260	263	294	185
chr14	92537171	92537363	ATXN3	0,46	100	81	69	60	100	80	69	64	94	88	98	92	107	87	97	90
chr14	92547236	92547476	ATXN3	0,33	282	287	240	164	234	233	195	132	313	276	299	258	293	271	304	203
chr14	92548546	92548906	ATXN3	0,38	372	411	323	207	295	313	274	178	423	421	366	326	392	393	437	259
chr14	92549415	92549655	ATXN3	0,36	271	284	214	162	198	217	179	143	273	255	273	215	257	258	279	205
chr14	92554997	92555237	ATXN3	0,32	259	305	201	147	216	221	198	144	286	257	244	228	248	272	279	209
chr14	92559532	92559724	ATXN3	0,26	122	154	110	94	143	150	108	94	160	156	158	125	163	127	144	111
chr14	92560012	92560252	ATXN3	0,33	264	304	223	175	259	255	221	161	302	312	302	238	300	293	283	234
chr14	92562338	92562578	ATXN3	0,42	165	179	153	120	177	168	123	105	205	177	178	162	176	178	177	142
chr14	92562978	92563266	ATXN3	0,34	396	402	318	230	289	308	272	195	378	387	405	338	367	379	366	309
chr14	92572811	92572931	ATXN3	0,64	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
chr14	97299708	97300068	VRK1	0,37	250	276	206	142	209	216	175	135	294	257	256	203	267	236	276	199
chr14	97304060	97304252	VRK1	0,27	179	194	145	116	146	167	147	102	202	191	207	164	203	173	168	159
chr14	97312346	97312586	VRK1	0,32	287	326	259	179	220	262	207	165	308	319	304	257	293	303	310	220
chr14	97313517	97313757	VRK1	0,32	197	212	168	116	161	180	144	106	232	212	209	176	208	204	222	168
chr14	97319101	97319576	VRK1	0,34	273	322	229	158	195	252	214	146	319	302	278	260	283	289	292	229
chr14	97321506	97321746	VRK1	0,41	181	184	149	115	165	157	126	96	207	177	218	169	165	175	167	139
chr14	97322414	97322654	VRK1	0,33	249	287	230	148	216	222	184	136	280	254	267	230	259	258	249	218

chr14	97322773	97323013	VRK1	0,31	243	261	188	133	178	213	168	117	241	241	227	196	230	235	246	176
chr14	97326802	97327162	VRK1	0,29	230	270	184	141	151	195	159	132	250	235	251	192	244	243	254	174
chr14	97342291	97342531	VRK1	0,39	218	210	156	127	185	178	142	105	208	182	202	183	190	194	194	145
chr14	97347454	97347574	VRK1	0,33	145	164	103	95	127	134	98	94	131	116	146	132	147	138	114	103
chr15	23048724	23049444	NIPA1	0,57	140	135	119	86	131	111	98	74	154	147	140	121	141	142	142	112
chr15	23052494	23052854	NIPA1	0,43	219	246	183	137	204	207	166	126	250	211	249	205	215	220	231	176
chr15	23060739	23060979	NIPA1	0,46	129	133	99	81	119	123	85	78	157	115	152	120	116	123	114	115
chr15	23062175	23062415	NIPA1	0,44	152	149	113	101	147	133	103	80	194	147	161	121	159	145	149	119
chr15	23086223	23086367	NIPA1	0,74	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
chr15	34526007	34526247	SLC12A6	0,46	125	151	99	98	148	127	103	80	158	142	138	128	138	122	140	107
chr15	34527328	34527568	SLC12A6	0,45	173	200	143	116	157	146	119	103	205	191	186	162	178	177	185	153
chr15	34528127	34528487	SLC12A6	0,43	257	273	223	150	218	216	187	137	300	279	273	219	276	282	285	208
chr15	34528842	34529082	SLC12A6	0,45	174	144	118	103	144	131	106	87	192	151	162	143	159	147	154	136
chr15	34529565	34529805	SLC12A6	0,46	231	254	208	141	202	189	182	126	270	259	217	206	235	223	257	188
chr15	34530337	34530697	SLC12A6	0,43	186	208	147	122	184	170	141	103	230	179	203	170	190	200	199	156
chr15	34531083	34531443	SLC12A6	0,50	172	171	121	98	159	145	119	102	206	162	167	151	161	166	159	138
chr15	34532765	34533125	SLC12A6	0,46	202	207	176	118	167	170	150	102	219	215	194	187	205	209	216	171
chr15	34533365	34533605	SLC12A6	0,38	130	137	97	82	114	115	89	74	127	131	135	109	129	132	131	120
chr15	34534242	34534482	SLC12A6	0,36	246	259	199	139	196	209	179	124	254	223	211	201	235	234	244	172
chr15	34536103	34536343	SLC12A6	0,38	154	146	140	95	132	133	108	88	172	151	156	124	164	165	146	139
chr15	34537424	34537664	SLC12A6	0,43	150	152	111	100	145	138	110	68	166	133	158	121	149	139	153	119
chr15	34537800	34538160	SLC12A6	0,42	203	207	172	127	183	183	141	116	230	206	201	194	216	208	197	160
chr15	34542407	34542647	SLC12A6	0,38	272	281	228	158	217	215	205	134	317	300	277	247	288	298	301	211
chr15	34542760	34543358	SLC12A6	0,40	264	252	195	155	215	205	173	129	280	260	255	212	248	255	249	190
chr15	34544297	34544657	SLC12A6	0,39	216	220	175	120	174	187	152	105	211	210	229	195	225	214	227	165
chr15	34546489	34546849	SLC12A6	0,43	134	144	109	77	121	117	92	75	139	135	133	116	147	131	131	111
chr15	34547407	34547647	SLC12A6	0,41	145	141	121	95	129	120	101	77	154	137	153	125	148	144	135	108
chr15	34548336	34548576	SLC12A6	0,40	200	222	171	145	187	182	157	124	247	238	221	178	223	234	228	157
chr15	34549735	34550095	SLC12A6	0,42	174	175	137	109	148	153	113	92	194	153	182	141	174	173	165	136
chr15	34550959	34551199	SLC12A6	0,43	245	256	185	133	190	202	167	120	264	236	245	219	220	218	237	191
chr15	34553053	34553293	SLC12A6	0,35	288	302	212	165	218	219	205	139	280	270	291	250	269	255	277	193
chr15	34567447	34567687	SLC12A6	0,35	107	115	89	67	86	104	74	59	109	93	107	88	107	115	105	87
chr15	34610700	34610940	SLC12A6	0,42	239	269	192	130	203	228	189	142	279	277	265	234	263	241	258	204
chr15	34628505	34628985	SLC12A6	0,45	142	128	103	88	128	121	86	77	144	120	147	117	154	140	132	116
chr15	35082504	35082864	ACTC1	0,45	152	142	106	103	151	115	96	95	164	130	151	127	151	146	133	123
chr15	35083206	35083518	ACTC1	0,44	190	196	162	133	191	164	148	96	212	183	194	167	200	189	183	144
chr15	35084206	35084869	ACTC1	0,51	121	108	85	78	116	98	83	62	138	109	120	98	113	108	102	92
chr15	35085367	35085847	ACTC1	0,52	137	139	108	93	125	115	105	83	149	122	141	124	134	145	132	119
chr15	35086824	35087064	ACTC1	0,68	9	14	11	9	14	12	7	11	16	14	18	11	13	14	15	11
chr15	42646499	42646691	CAPN3	0,31	200	220	152	124	187	167	143	95	202	171	190	173	184	190	164	145
chr15	42651917	42652397	CAPN3	0,52	190	178	125	120	166	153	122	97	190	170	179	164	172	182	173	150
chr15	42676624	42676840	CAPN3	0,44	120	138	110	93	116	122	90	69	140	116	131	118	123	128	135	111
chr15	42678303	42678543	CAPN3	0,56	106	88	76	71	103	89	81	57	126	104	118	79	91	87	101	83
chr15	42679897	42680137	CAPN3	0,49	112	110	71	75	106	96	74	55	119	102	116	87	104	110	104	85
chr15	42681029	42681389	CAPN3	0,52	94	99	73	67	105	96	74	61	112	81	101	78	101	107	93	90
chr15	42682109	42682349	CAPN3	0,55	129	134	90	91	119	114	84	72	163	125	133	119	125	131	117	106
chr15	42684758	42684998	CAPN3	0,56	93	95	73	83	100	83	59	50	112	83	101	93	95	91	92	72
chr15	42686376	42686616	CAPN3	0,59	16	17	13	14	17	14	16	14	21	16	24	24	17	15	13	21
chr15	42688916	42689156	CAPN3	0,53	117	123	91	97	134	122	95	66	152	123	122	113	132	127	135	100
chr15	42691589	42691949	CAPN3	0,53	190	192	140	120	168	155	147	103	207	197	187	170	199	185	208	132
chr15	42693743	42694103	CAPN3	0,56	72	54	59	52	78	53	55	34	80	60	76	55	64	70	64	67
chr15	42694267	42694387	CAPN3	0,55	13	11	14	12	12	8	10	9	17	12	17	12	13	18	15	17
chr15	42694915	42695275	CAPN3	0,59	61	62	49	49	67	55	49	37	84	55	69	60	63	68	63	61
chr15	42695836	42696076	CAPN3	0,57	74	83	56	54	87	63	49	52	93	77	85	61	76	81	98	63
chr15	42698072	42698192	CAPN3	0,36	38	38	24	33	40	41	30	28	40	35	47	28	39	47	48	40
chr15	42700345	42700585	CAPN3	0,58	85	83	74	73	98	77	66	50	120	86	79	88	82	88	81	69
chr15	42701419	42701659	CAPN3	0,52	148	162	117	116	160	127	109	100	187	164	165	130	152	142	166	144
chr15	42701908	42702268	CAPN3	0,57	76	72	61	52	76	57	37	55	86	67	73	59	79	67	67	59
chr15	42702564	42702924	CAPN3	0,53	157	160	117	92	146	130	113	80	162	147	162	130	168	156	155	122
chr15	42703019	42703259	CAPN3	0,54	216	205	178	144	183	176	148	114	210	212	211	176	225	218	207	155

chr15	42703393	42703633	CAPN3	0,54	130	132	97	79	135	112	91	72	143	136	118	122	130	130	128	113
chr15	42703837	42704077	CAPN3	0,54	144	151	137	106	148	131	106	86	151	147	164	145	144	148	147	138
chr15	43037906	43038482	TTBK2	0,52	136	127	97	90	134	112	93	76	148	124	135	115	132	139	124	114
chr15	43044088	43045528	TTBK2	0,43	321	338	265	181	265	267	238	153	341	341	325	271	325	337	348	237
chr15	43067266	43067986	TTBK2	0,45	248	271	208	147	226	223	184	130	279	257	262	223	251	266	271	199
chr15	43069181	43069517	TTBK2	0,42	222	211	169	128	185	193	140	110	245	201	214	171	209	190	199	154
chr15	43075623	43075935	TTBK2	0,40	181	200	166	126	182	167	138	109	232	193	201	178	200	212	208	161
chr15	43086740	43087100	TTBK2	0,39	214	221	163	129	195	165	155	98	224	217	207	177	199	203	220	158
chr15	43102754	43102994	TTBK2	0,39	168	172	138	114	150	160	121	84	186	166	183	146	167	173	172	139
chr15	43103811	43104051	TTBK2	0,35	247	303	230	142	226	218	193	137	300	275	262	235	285	262	295	194
chr15	43109166	43109382	TTBK2	0,40	205	219	153	121	177	174	140	123	224	197	204	181	196	197	195	171
chr15	43120057	43120297	TTBK2	0,31	188	203	150	113	173	160	131	98	196	185	212	161	196	177	189	145
chr15	43122025	43122385	TTBK2	0,39	287	306	247	168	258	235	198	154	300	293	295	245	291	283	319	228
chr15	43132474	43132714	TTBK2	0,31	235	265	232	143	215	225	195	140	262	249	255	227	262	252	272	211
chr15	43164702	43165038	TTBK2	0,34	325	341	262	194	264	255	223	152	331	337	304	278	315	318	332	237
chr15	43170723	43170915	TTBK2	0,43	280	341	243	183	242	238	202	150	344	311	314	277	321	273	311	217
chr15	44855222	44855534	SPG11	0,36	321	349	267	179	261	278	231	165	368	347	324	289	333	336	356	250
chr15	44856640	44857000	SPG11	0,36	281	314	240	164	232	250	208	142	306	299	285	232	280	282	298	202
chr15	44857949	44858309	SPG11	0,53	84	73	59	62	83	74	57	55	95	71	81	74	79	74	77	65
chr15	44858338	44858578	SPG11	0,48	100	96	68	66	95	84	68	56	113	93	96	88	100	96	76	82
chr15	44859549	44859885	SPG11	0,49	126	124	105	84	141	109	91	80	144	107	132	122	121	117	126	101
chr15	44861529	44861769	SPG11	0,48	205	194	161	116	162	170	152	91	206	192	194	142	197	187	197	152
chr15	44862669	44862909	SPG11	0,57	30	34	22	24	31	30	20	21	42	23	37	28	31	27	33	30
chr15	44864829	44865069	SPG11	0,45	131	138	108	86	132	107	90	70	157	125	128	117	130	135	138	108
chr15	44865663	44866023	SPG11	0,52	145	133	90	97	133	114	104	73	159	127	142	119	150	148	137	122
chr15	44867049	44867289	SPG11	0,50	124	132	101	90	122	131	95	77	149	118	132	125	137	139	151	118
chr15	44875903	44876839	SPG11	0,46	274	267	220	160	222	222	182	129	292	256	248	218	273	254	271	206
chr15	44877803	44878139	SPG11	0,36	335	375	277	199	274	284	260	191	362	339	347	301	324	311	326	290
chr15	44881350	44881710	SPG11	0,40	169	178	138	103	155	162	135	92	184	165	178	144	184	178	175	136
chr15	44884462	44884702	SPG11	0,36	242	273	199	148	210	218	173	139	267	250	265	209	268	243	270	184
chr15	44887376	44887736	SPG11	0,41	241	247	197	153	210	215	190	128	277	268	261	221	252	245	259	195
chr15	44888176	44888656	SPG11	0,46	233	255	204	152	198	197	179	130	269	262	256	212	252	249	263	208
chr15	44888881	44889241	SPG11	0,38	268	284	216	182	231	240	185	142	292	248	253	238	256	252	267	226
chr15	44890396	44890636	SPG11	0,37	242	266	218	161	200	216	181	128	308	282	275	217	273	259	282	203
chr15	44890751	44891111	SPG11	0,41	291	290	237	169	227	233	184	142	298	292	282	247	287	296	315	214
chr15	44892627	44892939	SPG11	0,36	285	335	244	191	234	262	211	160	327	312	315	256	310	282	309	228
chr15	44898135	44898375	SPG11	0,35	195	222	177	121	162	173	156	109	216	213	216	173	203	190	208	163
chr15	44900598	44900910	SPG11	0,38	182	193	147	114	159	169	119	104	219	185	187	157	189	173	199	154
chr15	44902930	44903290	SPG11	0,36	254	264	213	141	205	217	190	125	268	254	270	214	248	245	256	203
chr15	44905560	44905800	SPG11	0,34	301	338	253	167	226	265	217	151	321	318	305	246	322	303	323	220
chr15	44907482	44907842	SPG11	0,40	245	296	225	145	210	223	190	132	284	263	251	214	251	269	264	202
chr15	44912298	44912634	SPG11	0,36	315	344	246	183	245	264	217	157	324	319	290	251	325	299	333	228
chr15	44913905	44914145	SPG11	0,38	277	308	235	161	228	251	219	153	296	289	299	241	290	295	302	213
chr15	44914361	44914601	SPG11	0,29	238	247	203	146	170	209	186	129	244	235	236	207	220	204	235	190
chr15	44914841	44915081	SPG11	0,31	328	357	266	180	215	251	225	180	347	328	310	257	306	301	339	235
chr15	44918436	44918796	SPG11	0,29	278	296	236	164	224	222	189	130	291	280	274	246	265	271	280	208
chr15	44920774	44921134	SPG11	0,33	360	399	291	195	258	299	254	179	380	360	347	299	356	355	385	261
chr15	44921328	44921688	SPG11	0,36	262	274	201	152	204	230	178	148	292	261	261	234	268	263	271	192
chr15	44925648	44925888	SPG11	0,29	240	269	210	146	208	220	192	139	241	256	253	210	259	226	248	200
chr15	44940956	44941316	SPG11	0,31	267	267	206	141	210	218	180	131	283	261	254	213	259	238	262	188
chr15	44943612	44944212	SPG11	0,43	283	310	235	153	238	246	191	135	307	282	281	245	299	274	310	213
chr15	44944275	44944515	SPG11	0,35	266	279	217	142	218	229	191	151	279	263	259	231	291	254	254	208
chr15	44949213	44949573	SPG11	0,36	317	356	278	188	275	277	224	180	352	330	319	262	348	314	348	242
chr15	44951208	44951568	SPG11	0,37	297	296	230	163	241	239	204	156	300	301	297	257	289	280	332	229
chr15	44952541	44952901	SPG11	0,36	290	319	234	169	225	252	217	153	322	302	280	251	289	268	306	214
chr15	44955536	44955896	SPG11	0,70	13	8	6	10	16	10	6	10	14	11	15	12	13	10	10	12
chr15	63334965	63335205	TPM1	0,72	4	4	1	3	6	1	2	5	7	3	3	7	4	3	4	4
chr15	63335887	63336079	TPM1	0,72	4	2	3	1	4	4	2	0	4	1	3	1	4	3	1	3
chr15	63336168	63336408	TPM1	0,59	59	75	44	47	66	50	39	38	78	52	61	53	61	54	59	49
chr15	63340720	63340960	TPM1	0,73	0	0	0	0	0	0	0	0	1	0	0	2	0	0	0	0

chr15	63349130	63349370	TPM1	0,52	75	100	68	62	90	77	54	45	97	79	98	73	88	102	90	69
chr15	63351700	63351940	TPM1	0,47	45	53	44	35	48	46	33	33	64	32	49	42	56	44	35	49
chr15	63352982	63353222	TPM1	0,58	82	73	64	57	81	64	53	46	91	76	83	70	80	79	76	73
chr15	63353314	63353554	TPM1	0,45	175	169	132	122	177	153	123	107	195	159	153	141	172	175	166	131
chr15	63353829	63354069	TPM1	0,48	212	223	168	130	182	183	134	105	223	210	204	170	219	203	234	172
chr15	63354324	63354564	TPM1	0,44	132	147	111	91	129	130	105	77	167	115	161	135	150	152	141	122
chr15	63354689	63354929	TPM1	0,37	252	289	207	168	219	215	185	141	279	285	258	207	280	269	262	214
chr15	63356183	63356423	TPM1	0,51	275	325	255	178	240	251	189	157	321	314	309	261	324	315	326	228
chr15	63358036	63358156	TPM1	0,42	213	239	160	140	211	205	161	137	265	234	229	188	231	226	242	198
chr15	63361985	63362225	TPM1	0,45	290	296	234	155	227	258	195	147	294	292	282	246	300	301	314	201
chr15	63363197	63363413	TPM1	0,39	236	271	189	147	210	209	179	120	267	252	251	219	233	233	240	195
chr15	65255884	65256100	SPG21	0,51	151	161	107	97	141	129	96	74	150	147	133	127	131	145	144	127
chr15	65257550	65257910	SPG21	0,39	290	294	235	159	242	236	218	158	292	301	289	253	300	290	321	214
chr15	65261525	65261765	SPG21	0,32	275	299	225	153	229	260	217	135	308	286	278	249	283	277	309	205
chr15	65262385	65262625	SPG21	0,39	262	255	183	154	211	217	175	131	275	239	248	211	233	241	241	189
chr15	65266832	65267192	SPG21	0,35	281	305	239	168	241	248	221	140	330	297	301	232	300	286	317	229
chr15	65268771	65268915	SPG21	0,30	164	170	114	111	126	128	108	84	177	151	159	127	147	153	145	129
chr15	65273102	65273462	SPG21	0,40	209	217	168	120	186	175	164	114	221	217	217	167	214	216	217	169
chr15	65275755	65275995	SPG21	0,33	126	152	119	88	117	134	104	85	159	142	143	121	136	140	140	127
chr15	73614797	73616691	HCN4	0,68	13	12	9	11	14	12	9	10	15	11	16	11	15	14	10	13
chr15	73617235	73617891	HCN4	0,64	36	35	25	27	35	34	22	23	47	31	35	30	29	35	27	30
chr15	73621842	73622202	HCN4	0,63	24	30	17	23	35	24	16	20	37	29	28	19	19	18	27	26
chr15	73624372	73624732	HCN4	0,61	38	29	35	31	31	28	28	22	42	29	32	27	41	33	38	38
chr15	73635637	73636237	HCN4	0,52	129	123	95	84	127	121	90	78	148	119	125	108	127	131	121	106
chr15	73659766	73660699	HCN4	0,74	11	11	11	12	14	12	9	11	18	13	16	15	15	12	15	14
chr15	76508798	76509038	ETFA	0,35	241	269	193	134	208	199	166	119	269	239	228	218	234	248	268	197
chr15	76518109	76518349	ETFA	0,34	290	338	232	188	243	245	229	173	328	324	288	276	324	301	312	212
chr15	76523570	76523762	ETFA	0,36	225	262	206	143	183	211	181	130	278	244	244	201	232	213	254	182
chr15	76566673	76566913	ETFA	0,37	287	296	233	147	230	231	208	123	279	293	318	248	260	257	299	208
chr15	76576011	76576251	ETFA	0,29	263	304	236	168	217	226	215	129	301	258	273	252	281	252	276	182
chr15	76577934	76578150	ETFA	0,32	316	337	267	174	256	292	236	155	329	313	328	259	281	308	310	228
chr15	76578646	76578886	ETFA	0,37	296	337	251	183	233	263	238	150	347	323	313	283	303	300	331	230
chr15	76580116	76580356	ETFA	0,35	166	181	134	121	152	157	132	97	182	145	191	129	172	158	174	127
chr15	76584692	76585120	ETFA	0,36	339	384	299	193	283	304	252	175	366	369	347	304	350	341	388	257
chr15	76587824	76588184	ETFA	0,37	263	257	182	147	211	221	159	133	268	239	244	202	239	241	242	182
chr15	76603589	76603829	ETFA	0,70	1	1	0	0	0	1	1	0	0	1	2	1	0	1	0	0
chr15	89859899	89860139	POLG	0,50	105	106	91	77	100	105	68	60	123	108	108	82	104	102	98	94
chr15	89860506	89860866	POLG	0,52	171	182	145	113	177	169	118	104	198	178	174	161	178	170	181	147
chr15	89861695	89862055	POLG	0,57	76	74	57	51	83	71	52	48	93	72	79	57	72	75	83	73
chr15	89862065	89862639	POLG	0,56	153	155	135	114	146	148	131	89	189	160	171	135	173	174	170	146
chr15	89863939	89864299	POLG	0,57	71	57	55	55	79	61	54	49	80	66	88	51	70	69	69	62
chr15	89864303	89864543	POLG	0,59	36	36	27	34	42	35	31	27	45	38	47	32	38	38	37	36
chr15	89864905	89865339	POLG	0,61	51	48	36	39	65	48	37	41	69	46	61	51	60	50	44	47
chr15	89865872	89866232	POLG	0,59	23	23	12	22	21	25	15	12	38	23	29	22	28	29	25	21
chr15	89866568	89866808	POLG	0,59	26	28	27	33	32	26	16	20	29	31	32	21	27	28	27	24
chr15	89866968	89867208	POLG	0,59	67	73	57	66	83	68	48	58	91	63	85	75	73	78	65	75
chr15	89867265	89867481	POLG	0,60	40	35	20	26	30	26	28	24	40	30	36	28	31	33	29	31
chr15	89868618	89868978	POLG	0,64	46	32	35	39	42	33	27	26	58	34	51	44	43	43	37	33
chr15	89869785	89870025	POLG	0,59	51	43	41	39	53	46	39	32	61	47	65	50	54	55	49	49
chr15	89870038	89870668	POLG	0,59	124	111	99	87	121	108	87	75	144	110	129	111	123	118	109	109
chr15	89871606	89872353	POLG	0,60	73	65	54	63	75	68	54	49	93	62	76	70	75	66	71	70
chr15	89873229	89873589	POLG	0,58	59	58	49	49	66	53	43	41	73	54	53	59	55	62	47	51
chr15	89876265	89876817	POLG	0,68	7	8	4	10	11	5	7	7	11	6	9	9	7	7	10	8
chr15	89876870	89876990	POLG	0,75	0	0	2	1	2	0	0	2	2	0	3	3	2	1	3	0
chr16	11643426	11643666	LITAF	0,59	14	14	13	13	15	11	13	10	16	12	17	12	11	10	15	18
chr16	11647310	11647622	LITAF	0,57	61	61	46	44	67	50	41	38	78	60	66	69	65	58	54	58
chr16	11650296	11650656	LITAF	0,54	41	43	35	36	49	43	29	28	54	41	49	35	46	39	42	43
chr16	28889931	28890171	ATP2A1	0,59	30	35	21	36	32	27	23	20	37	25	42	31	37	33	29	31
chr16	28890368	28890488	ATP2A1	0,50	35	45	28	34	54	38	31	30	51	40	46	36	37	34	31	37
chr16	28890742	28890982	ATP2A1	0,64	29	29	20	18	32	26	17	18	31	25	27	28	26	26	26	20

chr16	28892167	28892407	ATP2A1	0,54	239	257	209	167	230	214	182	134	290	264	274	221	258	265	269	211
chr16	28893720	28893960	ATP2A1	0,61	29	27	25	26	29	33	20	25	35	26	31	19	31	31	33	33
chr16	28895815	28896055	ATP2A1	0,56	110	97	86	83	101	101	68	57	128	100	112	103	105	107	94	80
chr16	28898432	28899110	ATP2A1	0,56	149	154	118	94	137	120	95	84	166	138	158	130	141	149	152	124
chr16	28900034	28900370	ATP2A1	0,59	67	63	57	56	82	50	46	53	83	55	75	64	63	67	60	65
chr16	28905399	28905639	ATP2A1	0,59	44	45	37	36	44	38	32	32	59	44	46	30	38	36	45	39
chr16	28905756	28905996	ATP2A1	0,59	80	79	67	61	83	68	67	61	93	73	89	80	78	84	75	77
chr16	28906088	28906328	ATP2A1	0,59	33	36	29	33	41	31	24	24	44	35	39	38	35	43	39	33
chr16	28909263	28909842	ATP2A1	0,58	76	72	65	60	75	66	51	46	90	69	77	71	76	79	80	61
chr16	28911829	28912309	ATP2A1	0,62	68	68	52	55	67	58	48	39	77	55	65	52	61	67	68	64
chr16	28913109	28913805	ATP2A1	0,61	30	22	23	21	34	31	24	19	35	27	30	25	25	28	25	26
chr16	28913823	28914285	ATP2A1	0,63	51	41	36	40	55	48	34	34	52	38	44	44	52	43	47	53
chr16	28914289	28914529	ATP2A1	0,66	112	100	94	97	119	105	88	76	156	107	114	101	122	124	115	108
chr16	28914582	28914822	ATP2A1	0,62	35	34	30	31	40	33	27	26	44	28	42	37	42	38	32	49
chr16	28914962	28915082	ATP2A1	0,58	111	100	80	92	121	100	77	79	145	90	118	122	130	112	115	104
chr16	28915393	28915633	ATP2A1	0,58	114	107	98	95	116	104	84	79	139	107	118	98	115	109	115	91
chr16	31191481	31191601	FUS	0,69	0	1	2	1	1	0	1	0	2	0	0	0	2	0	2	1
chr16	31193611	31194043	FUS	0,46	211	214	174	135	188	185	160	114	206	191	209	175	229	204	191	167
chr16	31195070	31195430	FUS	0,51	153	139	102	98	137	129	101	80	150	147	152	134	141	161	156	128
chr16	31195463	31195703	FUS	0,51	62	58	45	41	64	52	44	42	72	55	60	55	57	64	53	51
chr16	31196417	31196609	FUS	0,56	22	17	17	20	27	16	14	17	20	16	20	14	15	21	21	14
chr16	31198019	31198259	FUS	0,39	240	255	188	141	209	216	164	121	257	255	229	233	238	222	252	209
chr16	31199619	31199739	FUS	0,49	80	89	71	69	98	86	74	51	115	81	85	65	83	86	80	80
chr16	31200375	31200615	FUS	0,39	203	214	159	134	175	193	159	103	220	222	204	195	209	201	209	154
chr16	31200930	31201170	FUS	0,46	132	144	101	95	128	124	98	65	150	118	135	118	130	134	122	108
chr16	31201291	31201531	FUS	0,48	104	94	74	68	108	92	69	59	112	86	98	82	99	90	93	83
chr16	31201666	31201786	FUS	0,47	27	22	17	20	22	21	21	12	29	22	23	16	21	21	24	17
chr16	31201992	31202537	FUS	0,52	124	127	97	89	124	106	88	74	136	118	128	108	123	113	112	100
chr16	31202702	31202822	FUS	0,48	18	11	8	8	18	17	11	12	14	10	13	13	13	11	13	10
chr16	66545799	66546039	TK2	0,48	178	190	140	116	167	157	128	84	199	182	178	172	187	151	173	147
chr16	66547553	66547793	TK2	0,55	29	40	30	27	45	31	19	26	35	28	34	37	40	36	35	40
chr16	66550958	66551198	TK2	0,53	171	164	135	116	148	160	114	96	173	177	154	157	165	155	146	137
chr16	66551615	66551855	TK2	0,50	214	244	195	141	215	184	159	131	228	218	232	207	239	223	240	171
chr16	66562813	66563053	TK2	0,37	180	215	151	128	173	162	150	115	213	202	198	156	211	190	211	148
chr16	66565207	66565447	TK2	0,51	161	149	114	100	158	135	103	88	186	148	142	148	168	135	169	113
chr16	66570773	66571013	TK2	0,53	131	124	105	106	122	104	99	72	152	132	143	108	141	122	138	113
chr16	66575698	66575938	TK2	0,49	179	208	151	121	177	157	125	99	210	201	194	151	189	194	178	155
chr16	66582776	66583016	TK2	0,33	307	313	264	175	233	271	223	167	323	309	326	261	302	293	305	213
chr16	66583782	66584022	TK2	0,77	7	4	5	5	7	7	4	2	7	5	5	5	7	7	8	5
chr16	66584050	66584266	TK2	0,52	58	47	47	52	67	54	46	34	82	45	63	57	74	59	55	55
chr16	67313896	67314496	PLEKHG4	0,61	21	20	18	23	24	23	19	15	31	19	26	26	24	25	23	27
chr16	67314538	67315114	PLEKHG4	0,60	87	83	66	70	85	78	65	55	101	79	88	78	72	78	74	75
chr16	67315415	67316321	PLEKHG4	0,61	57	54	43	46	64	52	39	43	66	50	59	58	50	57	56	53
chr16	67316339	67316819	PLEKHG4	0,59	65	67	52	51	71	60	55	50	82	65	70	64	65	70	73	60
chr16	67318088	67318448	PLEKHG4	0,62	13	16	10	13	20	13	11	13	18	16	18	15	20	16	19	18
chr16	67318580	67319492	PLEKHG4	0,64	31	33	27	32	38	32	25	28	41	32	36	29	31	39	30	35
chr16	67320088	67321168	PLEKHG4	0,62	65	56	46	45	64	55	45	41	74	55	64	55	64	62	62	56
chr16	67321738	67321978	PLEKHG4	0,59	87	76	61	67	84	73	65	43	86	79	93	68	77	86	79	72
chr16	67321988	67322588	PLEKHG4	0,62	83	90	67	61	80	75	66	59	116	86	94	84	92	101	85	86
chr16	67322648	67322768	PLEKHG4	0,62	4	4	4	4	2	5	3	2	2	6	7	1	3	6	3	5
chr16	81348664	81348904	GAN	0,71	1	1	0	2	1	0	1	0	3	1	2	2	1	0	1	1
chr16	81385124	81385364	GAN	0,36	190	209	154	125	154	168	128	101	208	175	188	157	179	179	181	168
chr16	81387944	81388424	GAN	0,38	331	337	267	195	260	262	232	166	337	344	313	271	313	307	343	239
chr16	81390318	81390678	GAN	0,46	185	188	158	106	169	154	124	104	206	184	194	158	181	185	196	138
chr16	81391355	81391595	GAN	0,43	274	279	220	161	227	225	176	147	277	270	258	215	277	275	283	209
chr16	81396039	81396279	GAN	0,41	216	211	174	129	178	164	142	121	241	205	203	181	194	221	220	172
chr16	81397293	81397653	GAN	0,36	281	301	221	153	237	241	207	132	300	284	286	235	282	279	307	205
chr16	81398526	81398766	GAN	0,50	89	84	64	74	88	83	66	58	114	89	93	87	99	91	96	87
chr16	81398898	81399138	GAN	0,49	108	101	81	75	108	95	68	67	130	93	109	100	104	110	101	93
chr16	81410772	81411252	GAN	0,45	304	308	223	169	243	226	202	139	312	301	282	249	290	282	309	221

chr16	89574736	89575096	SPG7	0,78	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
chr16	89576828	89577068	SPG7	0,41	149	199	140	123	151	158	142	88	184	156	176	153	170	171	153	135
chr16	89579285	89579405	SPG7	0,39	83	88	77	75	104	85	63	70	117	85	95	101	92	99	81	77
chr16	89590354	89590714	SPG7	0,62	25	20	18	20	33	21	16	19	29	17	21	23	24	28	28	22
chr16	89592686	89592926	SPG7	0,48	160	174	139	109	165	133	123	102	188	148	176	145	153	160	169	125
chr16	89595815	89596055	SPG7	0,52	102	110	85	77	94	88	65	56	125	95	99	78	87	97	100	86
chr16	89597033	89597273	SPG7	0,54	79	55	58	56	74	59	52	46	78	67	66	57	76	66	67	55
chr16	89598263	89598575	SPG7	0,63	16	15	12	15	19	17	11	14	25	15	20	16	16	19	18	23
chr16	89598777	89599113	SPG7	0,58	36	44	34	42	36	39	29	32	52	37	42	39	38	42	32	39
chr16	89603089	89603425	SPG7	0,58	156	172	142	118	160	150	126	102	186	173	178	170	173	166	167	142
chr16	89610997	89611237	SPG7	0,61	36	42	19	37	36	41	30	28	48	38	39	35	42	35	39	38
chr16	89612996	89613236	SPG7	0,59	37	31	28	28	50	29	26	24	46	45	41	36	42	47	37	38
chr16	89614345	89614585	SPG7	0,62	26	28	27	28	38	24	23	19	39	29	31	33	29	31	24	31
chr16	89616875	89617091	SPG7	0,59	26	21	10	18	33	18	15	19	27	14	27	21	23	29	19	19
chr16	89619284	89619644	SPG7	0,58	96	95	82	78	111	95	80	57	115	97	98	84	106	91	114	88
chr16	89620104	89620464	SPG7	0,68	7	7	7	6	9	7	6	5	8	6	10	7	6	8	8	6
chr16	89620812	89621052	SPG7	0,62	5	7	5	4	8	7	8	8	13	6	9	13	7	8	7	9
chr16	89623217	89623577	SPG7	0,58	55	47	35	47	57	55	42	35	66	51	66	48	57	52	53	52
chr17	10293665	10294025	MYH8	0,42	277	293	229	164	234	248	192	152	305	293	279	246	274	294	300	216
chr17	10295126	10295366	MYH8	0,38	311	334	267	198	260	262	212	144	318	318	319	275	320	311	331	245
chr17	10295790	10296030	MYH8	0,43	298	305	238	180	252	245	202	143	329	318	305	241	309	282	310	208
chr17	10296097	10296577	MYH8	0,46	262	275	221	160	229	233	191	138	305	273	274	230	263	289	274	209
chr17	10297487	10297847	MYH8	0,51	96	116	87	76	116	92	74	68	132	100	102	107	118	120	120	99
chr17	10298363	10298843	MYH8	0,42	332	367	296	187	288	289	235	162	386	364	350	301	367	363	378	256
chr17	10299578	10300394	MYH8	0,44	275	268	217	165	247	226	205	145	303	287	280	249	295	284	311	213
chr17	10301678	10302263	MYH8	0,45	242	244	185	145	210	195	161	132	267	243	250	198	244	225	238	193
chr17	10302802	10303042	MYH8	0,46	147	156	114	99	134	132	96	90	177	147	155	116	145	153	145	128
chr17	10303624	10305184	MYH8	0,43	329	345	272	185	279	278	239	170	347	350	340	293	342	342	373	253
chr17	10307594	10307954	MYH8	0,43	272	263	205	145	222	216	180	128	273	252	267	214	252	274	277	190
chr17	10309295	10309775	MYH8	0,38	320	332	265	174	259	262	230	164	331	348	325	288	314	336	330	249
chr17	10309911	10310391	MYH8	0,37	297	330	242	178	256	256	214	152	329	309	295	261	322	298	326	231
chr17	10312327	10312990	MYH8	0,42	253	246	201	142	210	205	174	128	273	240	249	213	249	240	258	190
chr17	10313998	10314358	MYH8	0,39	261	292	244	155	241	234	200	138	309	304	280	229	297	311	330	205
chr17	10315625	10316105	MYH8	0,47	248	247	183	150	220	209	168	142	288	254	248	212	252	242	259	207
chr17	10317167	10317407	MYH8	0,42	294	291	225	169	249	231	197	136	326	321	308	260	321	295	318	240
chr17	10317414	10317894	MYH8	0,36	305	311	247	179	269	247	226	157	319	320	313	265	314	300	343	232
chr17	10318344	10319116	MYH8	0,41	235	252	192	138	206	205	173	124	258	246	246	212	237	238	264	185
chr17	10321902	10322382	MYH8	0,47	157	154	123	91	142	146	116	86	177	158	166	130	164	159	166	147
chr17	10323259	10323619	MYH8	0,45	238	231	192	144	197	201	152	112	243	242	229	205	241	251	245	180
chr17	10424492	10424852	MYH2	0,44	258	278	213	155	233	221	187	135	286	288	273	223	274	305	284	212
chr17	10426334	10427272	MYH2	0,43	308	320	252	181	259	261	220	159	340	315	315	278	307	308	341	239
chr17	10427722	10428442	MYH2	0,49	238	230	183	138	211	191	161	125	256	235	252	171	238	234	231	192
chr17	10428482	10429280	MYH2	0,43	279	278	226	176	238	246	197	145	301	289	295	236	279	284	310	228
chr17	10429833	10430433	MYH2	0,45	270	261	209	151	229	226	188	129	286	271	252	217	250	271	282	214
chr17	10431007	10431247	MYH2	0,46	165	125	119	102	143	146	93	95	169	145	150	120	151	149	168	131
chr17	10431947	10433483	MYH2	0,45	305	303	251	172	242	251	204	145	323	319	305	247	310	318	333	232
chr17	10434897	10435257	MYH2	0,42	315	330	250	193	251	254	219	152	345	343	328	266	315	314	368	230
chr17	10436535	10437015	MYH2	0,41	293	303	230	170	264	242	204	142	328	275	288	243	309	303	299	232
chr17	10438295	10438775	MYH2	0,41	270	280	220	168	245	233	183	144	307	296	284	233	283	282	296	203
chr17	10439788	10440004	MYH2	0,41	212	234	188	145	211	197	142	125	259	253	257	209	240	242	246	176
chr17	10440464	10441246	MYH2	0,43	275	283	221	152	240	240	194	134	299	275	278	233	271	286	287	209
chr17	10442460	10442940	MYH2	0,45	278	296	221	173	243	234	191	138	305	282	281	240	275	290	302	220
chr17	10443193	10443433	MYH2	0,41	289	292	226	167	240	239	194	135	304	300	289	235	291	289	317	200
chr17	10443842	10444082	MYH2	0,37	236	257	207	154	219	211	159	135	252	244	235	219	231	232	259	184
chr17	10446120	10446506	MYH2	0,33	292	301	253	176	212	263	202	142	301	289	275	247	288	284	329	223
chr17	10446935	10447535	MYH2	0,41	238	251	183	131	209	206	167	110	266	234	232	190	239	235	238	170
chr17	10448560	10448896	MYH2	0,46	143	134	109	97	134	133	92	64	152	148	142	133	144	139	161	127
chr17	10450714	10451314	MYH2	0,46	263	268	204	148	230	225	176	126	295	268	270	233	273	278	311	197
chr17	10531862	10532102	MYH3	0,48	108	110	83	68	100	99	73	60	124	102	116	98	104	123	109	85
chr17	10532862	10533326	MYH3	0,54	199	193	168	119	175	168	143	111	218	194	209	177	204	205	201	157

chr17	10533351	10533831	MYH3	0,57	205	205	178	132	189	172	141	114	241	209	208	191	224	217	232	173
chr17	10534830	10535406	MYH3	0,59	67	59	53	50	70	65	46	42	77	60	66	56	70	60	58	60
chr17	10535730	10536186	MYH3	0,51	250	230	193	152	208	207	169	134	255	243	249	228	251	247	257	184
chr17	10536849	10537089	MYH3	0,51	238	269	187	160	207	205	165	136	266	262	253	218	233	251	267	208
chr17	10537236	10537596	MYH3	0,47	219	233	190	143	209	205	157	120	241	210	235	200	240	252	235	175
chr17	10538092	10538428	MYH3	0,47	241	268	213	162	219	227	179	134	310	270	273	234	268	261	281	216
chr17	10538601	10538961	MYH3	0,52	194	175	147	112	163	170	123	102	215	180	184	142	193	193	188	152
chr17	10538978	10539194	MYH3	0,43	273	270	217	159	244	231	184	153	300	285	264	226	272	251	265	216
chr17	10541068	10541854	MYH3	0,58	149	128	108	95	137	122	94	80	160	134	143	124	137	146	150	120
chr17	10542103	10542559	MYH3	0,44	261	271	235	174	225	222	192	142	295	275	290	228	274	275	294	230
chr17	10542580	10543180	MYH3	0,43	327	328	269	177	252	274	217	163	331	332	307	275	324	321	343	247
chr17	10543259	10543979	MYH3	0,47	288	301	239	173	254	256	206	149	312	291	281	239	292	286	306	221
chr17	10544305	10544785	MYH3	0,41	312	328	270	195	260	267	234	168	334	340	316	266	300	310	345	254
chr17	10545471	10546407	MYH3	0,47	244	245	189	151	210	214	175	130	272	240	253	204	246	248	262	190
chr17	10547603	10548083	MYH3	0,43	258	253	194	155	229	224	182	132	286	255	263	213	254	241	268	192
chr17	10548946	10549426	MYH3	0,54	154	169	137	112	147	133	118	87	169	134	151	146	164	163	163	135
chr17	10550440	10550800	MYH3	0,47	226	226	179	139	209	196	168	122	238	234	217	203	241	230	245	178
chr17	10551819	10552011	MYH3	0,44	111	105	99	84	138	95	77	66	133	106	127	94	125	123	105	112
chr17	10552827	10553067	MYH3	0,46	243	232	171	142	211	201	159	122	267	223	220	184	217	231	244	180
chr17	10553584	10553824	MYH3	0,41	163	196	147	107	145	159	128	95	190	175	193	152	195	178	177	126
chr17	10554726	10555062	MYH3	0,53	103	104	74	79	115	102	72	55	130	101	115	101	102	98	106	97
chr17	10555686	10555926	MYH3	0,54	127	124	92	95	130	122	93	86	133	129	144	113	129	110	115	121
chr17	10558099	10558459	MYH3	0,55	211	209	193	127	179	190	150	121	245	224	237	185	202	221	233	167
chr17	15134135	15134495	PMP22	0,58	71	71	59	54	72	63	48	46	82	74	76	62	87	76	70	60
chr17	15142677	15143037	PMP22	0,53	193	189	151	106	157	168	136	112	233	205	194	166	200	205	207	157
chr17	15162330	15162546	PMP22	0,50	111	111	90	69	103	104	77	64	119	104	130	88	111	95	106	94
chr17	15163885	15164125	PMP22	0,66	7	8	8	8	7	7	4	6	8	3	8	7	14	6	6	5
chr17	19552180	19552540	ALDH3A2	0,67	8	5	8	8	9	8	5	5	13	9	11	7	10	7	6	10
chr17	19554795	19555155	ALDH3A2	0,43	247	262	204	158	204	225	184	128	273	236	236	213	263	244	259	191
chr17	19555782	19556022	ALDH3A2	0,34	265	278	221	151	225	248	183	138	298	269	290	216	279	265	267	201
chr17	19559602	19559962	ALDH3A2	0,42	240	235	181	146	199	209	162	129	263	222	262	177	253	231	246	179
chr17	19560996	19561236	ALDH3A2	0,35	228	247	175	144	209	198	151	141	244	239	246	211	228	237	234	189
chr17	19564330	19564690	ALDH3A2	0,34	237	282	197	144	202	217	172	141	273	269	243	200	241	254	252	202
chr17	19566548	19566908	ALDH3A2	0,35	278	315	225	162	224	238	201	129	277	270	287	228	263	274	294	200
chr17	19568190	19568430	ALDH3A2	0,47	142	146	100	76	123	106	88	77	153	128	140	117	136	131	127	110
chr17	19574971	19575331	ALDH3A2	0,44	221	238	175	131	210	200	149	139	248	233	231	193	217	231	246	178
chr17	19576385	19576625	ALDH3A2	0,42	168	179	152	110	169	144	131	99	190	184	186	148	162	165	174	144
chr17	19578817	19578937	ALDH3A2	0,39	222	232	172	152	175	205	157	136	243	220	243	196	200	208	215	182
chr17	37821547	37821787	TCAP	0,62	15	16	9	16	17	22	10	11	17	16	18	16	18	18	16	13
chr17	37821865	37822465	TCAP	0,67	22	14	16	17	26	19	19	14	23	18	23	15	23	23	17	22
chr17	39911891	39912251	JUP	0,62	37	34	26	29	41	31	29	20	45	30	30	39	45	37	37	38
chr17	39912326	39912566	JUP	0,56	117	133	101	92	112	104	97	75	139	117	137	108	120	111	116	101
chr17	39913620	39914100	JUP	0,64	25	22	21	19	29	20	17	14	34	26	29	25	21	28	25	21
chr17	39914590	39914830	JUP	0,61	57	41	32	34	53	41	35	31	52	49	47	41	52	50	54	41
chr17	39914864	39915224	JUP	0,60	130	128	111	108	147	117	99	82	152	129	152	129	137	125	137	112
chr17	39919187	39919643	JUP	0,60	53	41	36	35	56	43	32	29	58	46	44	42	56	48	44	51
chr17	39920896	39921426	JUP	0,60	89	98	70	65	101	85	69	57	103	96	97	81	96	94	102	77
chr17	39923551	39923911	JUP	0,64	20	15	12	14	20	15	9	9	19	12	20	13	22	15	16	16
chr17	39925159	39925519	JUP	0,66	15	14	10	13	20	11	12	14	19	15	13	16	11	13	14	12
chr17	39925619	39925979	JUP	0,66	18	15	11	13	24	18	15	14	22	15	23	18	17	17	17	18
chr17	39927822	39928182	JUP	0,61	37	28	35	31	40	33	24	27	49	36	41	31	37	34	34	34
chr17	40556635	40557475	PTRF	0,65	43	41	36	40	47	37	29	30	52	39	48	38	42	42	44	42
chr17	40574579	40575179	PTRF	0,68	30	27	28	27	43	34	25	23	42	31	36	32	37	34	28	40
chr17	4801928	4802612	CHRNE	0,65	26	24	15	18	22	20	18	14	21	19	23	20	20	17	21	21
chr17	4802630	4802966	CHRNE	0,67	12	11	9	12	16	11	6	5	14	8	11	11	12	14	7	10
chr17	4803986	4804586	CHRNE	0,67	15	14	11	14	23	17	15	11	21	14	20	11	20	15	15	18
chr17	4804749	4804989	CHRNE	0,61	33	34	28	21	33	27	24	18	37	23	33	31	27	36	24	27
chr17	4805124	4806166	CHRNE	0,59	110	113	88	74	110	97	83	65	123	95	118	92	116	107	107	98
chr17	4806215	4806455	CHRNE	0,59	98	92	85	73	96	85	73	69	128	92	107	95	103	97	87	84
chr17	48243372	48243492	SGCA	0,66	0	0	0	2	2	0	0	0	1	1	1	1	1	2	0	0

chr17	48244672	48245152	SGCA	0,65	46	39	35	34	51	44	31	39	48	37	45	34	49	45	50	42
chr17	48245223	48245463	SGCA	0,61	125	126	110	91	136	122	92	88	137	119	145	117	137	121	123	116
chr17	48245653	48246013	SGCA	0,63	50	50	35	41	56	42	34	31	63	41	51	44	56	37	41	50
chr17	48246353	48246713	SGCA	0,59	105	105	92	85	109	101	75	72	125	97	117	103	105	113	109	104
chr17	48247427	48247787	SGCA	0,63	102	96	93	82	116	82	71	70	131	102	101	97	119	108	94	103
chr17	48247893	48248133	SGCA	0,55	36	23	26	19	36	27	21	22	41	27	35	25	31	28	25	29
chr17	48252527	48252887	SGCA	0,63	13	11	7	11	10	8	5	8	17	10	11	14	11	10	8	10
chr17	4855046	4855286	ENO3	0,56	79	84	67	68	87	76	70	55	93	71	87	78	85	76	75	77
chr17	4856049	4856721	ENO3	0,56	143	132	112	90	124	121	102	70	155	134	142	126	146	129	141	109
chr17	4856953	4857193	ENO3	0,62	23	23	19	16	30	24	25	23	25	25	20	19	23	27	23	19
chr17	4858300	4858980	ENO3	0,58	84	77	58	63	82	70	59	47	92	70	82	73	77	77	76	69
chr17	4859222	4859534	ENO3	0,58	49	52	41	33	54	41	33	26	59	48	53	50	47	44	47	53
chr17	4859801	4860041	ENO3	0,56	93	96	94	71	104	90	69	66	115	99	99	95	90	96	90	95
chr17	4860053	4860413	ENO3	0,57	117	116	92	83	114	104	92	62	138	105	123	103	136	127	114	104
chr17	61600692	61600932	KCNH6	0,65	25	26	18	26	26	21	16	16	28	21	29	26	20	22	26	24
chr17	61601434	61601794	KCNH6	0,61	141	147	120	92	154	137	119	83	168	143	164	130	151	150	167	133
chr17	61607377	61607977	KCNH6	0,64	40	34	33	36	51	40	33	25	51	41	43	40	40	38	39	36
chr17	61611159	61611759	KCNH6	0,63	60	65	45	47	68	54	38	44	69	60	75	57	64	62	61	61
chr17	61612929	61613505	KCNH6	0,58	103	104	85	79	106	102	80	65	124	102	116	97	106	104	107	104
chr17	61615345	61615705	KCNH6	0,67	32	33	30	40	37	29	29	24	45	32	39	34	39	41	35	32
chr17	61615716	61616076	KCNH6	0,69	12	10	7	9	13	9	8	8	12	9	13	6	10	11	8	11
chr17	61619518	61619878	KCNH6	0,62	58	49	43	43	60	47	38	36	63	43	53	49	57	55	45	44
chr17	61620858	61621098	KCNH6	0,61	66	69	45	67	76	56	52	51	86	58	82	68	69	74	68	72
chr17	61621306	61621870	KCNH6	0,63	26	24	21	21	26	22	15	15	28	23	27	23	23	24	25	25
chr17	61622351	61622711	KCNH6	0,61	35	29	30	27	37	31	23	26	34	32	32	33	32	37	39	31
chr17	61622876	61623332	KCNH6	0,56	161	156	135	119	166	136	137	95	205	164	183	159	190	164	183	144
chr17	62018021	62019461	SCN4A	0,61	84	89	67	60	86	76	59	54	101	79	86	75	90	86	80	79
chr17	62020080	62020560	SCN4A	0,58	86	84	70	62	88	81	54	50	110	81	92	78	82	92	92	75
chr17	62021073	62021289	SCN4A	0,56	75	86	68	63	99	75	64	55	104	72	104	77	98	85	85	78
chr17	62021981	62022221	SCN4A	0,57	84	88	77	81	98	81	65	62	106	77	100	84	98	86	92	79
chr17	62022273	62022513	SCN4A	0,61	97	92	81	81	105	82	71	69	98	78	103	91	98	96	110	88
chr17	62022618	62023098	SCN4A	0,60	64	57	47	44	67	59	38	37	66	54	63	51	57	58	61	57
chr17	62024345	62024585	SCN4A	0,68	9	6	5	6	6	6	5	5	7	6	7	6	6	7	6	7
chr17	62025156	62025516	SCN4A	0,61	68	73	57	53	77	63	48	47	88	64	73	65	76	75	69	70
chr17	62025867	62026227	SCN4A	0,64	32	32	22	30	36	34	28	25	43	29	36	29	37	31	27	39
chr17	62026700	62026940	SCN4A	0,65	18	17	14	20	27	24	17	13	22	17	20	20	14	24	15	23
chr17	62028769	62029345	SCN4A	0,63	28	30	18	25	36	30	19	18	34	28	29	31	30	28	25	30
chr17	62034459	62034939	SCN4A	0,59	124	124	100	81	118	113	90	77	154	117	137	113	131	136	128	105
chr17	62036531	62036891	SCN4A	0,60	76	65	53	61	79	71	52	50	89	67	77	82	75	73	81	74
chr17	62038491	62038851	SCN4A	0,59	88	80	67	60	86	78	57	51	97	74	85	68	83	83	74	76
chr17	62040928	62041288	SCN4A	0,62	42	41	33	40	52	41	33	30	50	32	58	42	52	39	38	38
chr17	62041752	62042112	SCN4A	0,57	175	178	153	117	174	164	132	104	218	190	199	161	197	208	199	163
chr17	62043352	62043712	SCN4A	0,60	26	26	18	25	35	29	25	21	35	29	28	33	35	30	27	34
chr17	62043752	62043992	SCN4A	0,58	73	76	70	67	104	76	57	56	104	78	76	82	93	81	88	80
chr17	62045308	62045788	SCN4A	0,57	140	135	107	94	137	126	96	86	162	130	150	130	136	134	145	123
chr17	62048447	62048687	SCN4A	0,63	10	11	8	8	10	7	6	9	11	11	9	9	11	8	8	5
chr17	62049025	62049265	SCN4A	0,63	30	25	24	18	30	24	18	21	32	22	28	29	23	27	25	27
chr17	62049420	62049857	SCN4A	0,58	56	49	42	46	64	53	41	38	69	52	55	55	60	57	50	52
chr17	62049898	62050258	SCN4A	0,63	64	62	53	48	72	48	50	36	65	54	67	53	59	56	62	58
chr17	62473842	62474202	POLG2	0,32	301	335	242	175	241	254	221	154	318	307	284	239	291	283	315	232
chr17	62476335	62476575	POLG2	0,29	261	296	215	165	203	223	204	125	263	254	248	224	252	251	258	196
chr17	62478987	62479203	POLG2	0,35	154	163	117	107	145	133	105	72	167	139	151	135	149	131	139	138
chr17	62481790	62482030	POLG2	0,39	239	270	208	163	221	231	191	135	284	258	276	235	261	255	288	198
chr17	62486843	62487179	POLG2	0,36	292	294	250	160	229	236	205	137	311	278	272	235	286	275	279	223
chr17	62488716	62489194	POLG2	0,35	275	289	224	167	225	233	208	146	302	290	276	226	280	269	283	218
chr17	62492445	62493165	POLG2	0,56	201	199	156	127	175	170	145	107	210	180	192	175	186	191	178	159
chr17	68171102	68172542	KCNJ2	0,48	230	227	185	135	193	192	153	109	242	211	230	190	219	228	234	181
chr17	7123240	7123480	ACADVL	0,74	8	10	8	5	8	6	5	6	10	6	7	8	6	7	5	6
chr17	7123719	7124439	ACADVL	0,58	105	103	83	80	110	92	78	66	127	95	120	95	101	103	97	95
chr17	7124748	7125108	ACADVL	0,57	123	114	98	72	110	106	84	69	119	107	122	111	120	111	110	91

chr17	7125205	7125685	ACADVL	0,57	213	210	166	131	179	177	153	112	228	199	206	182	208	210	215	166
chr17	7125904	7126264	ACADVL	0,55	72	70	60	56	75	71	50	46	95	69	79	62	80	70	66	75
chr17	7126383	7126623	ACADVL	0,56	61	66	48	45	66	56	37	44	60	57	62	52	61	68	57	57
chr17	7126878	7128506	ACADVL	0,59	72	71	54	55	78	65	53	46	83	62	70	63	75	67	68	63
chr17	7348347	7348827	CHRNB1	0,70	8	7	6	5	12	8	6	6	10	6	9	8	7	11	9	7
chr17	7349289	7349505	CHRNB1	0,53	96	98	73	73	99	91	63	62	130	101	112	100	106	86	93	93
chr17	7350075	7350291	CHRNB1	0,62	20	18	12	12	21	13	13	12	20	14	20	16	18	20	16	19
chr17	7350330	7350546	CHRNB1	0,57	107	109	92	90	112	91	74	69	125	88	101	96	99	106	102	95
chr17	7350769	7351009	CHRNB1	0,55	121	124	101	87	117	93	94	68	130	105	116	104	103	103	117	101
chr17	7351822	7352182	CHRNB1	0,55	228	218	184	136	200	204	151	109	251	216	213	190	222	229	229	178
chr17	7357547	7357907	CHRNB1	0,50	325	327	270	192	267	266	232	172	332	334	324	286	330	314	345	249
chr17	7358508	7358868	CHRNB1	0,54	169	165	133	115	157	137	106	96	188	157	158	143	158	165	150	146
chr17	7359006	7359366	CHRNB1	0,59	64	82	57	51	84	67	56	47	75	70	73	72	75	70	64	62
chr17	7359791	7360151	CHRNB1	0,58	169	172	137	109	162	147	123	95	182	160	177	139	164	165	167	132
chr17	78078298	78079018	GAA	0,67	31	27	21	24	34	27	19	27	38	25	30	30	30	30	27	30
chr17	78079464	78079800	GAA	0,62	22	20	14	21	33	20	19	15	29	17	24	22	23	24	23	29
chr17	78081295	78081535	GAA	0,66	7	10	6	10	14	10	6	7	10	7	13	12	8	13	9	9
chr17	78081550	78081766	GAA	0,69	5	2	2	4	4	4	3	3	5	3	2	3	4	4	2	3
chr17	78081997	78082717	GAA	0,65	21	26	20	20	28	21	20	19	33	24	26	25	27	22	23	21
chr17	78083678	78083918	GAA	0,67	19	19	18	15	19	15	12	12	27	13	14	17	15	19	19	18
chr17	78084434	78084914	GAA	0,62	62	53	46	40	60	55	39	44	74	45	69	52	58	54	57	58
chr17	78085720	78085960	GAA	0,66	68	61	43	59	74	45	41	44	76	52	63	57	67	63	62	49
chr17	78086323	78086563	GAA	0,68	2	1	7	4	6	2	3	4	8	4	4	2	5	4	3	3
chr17	78086570	78087270	GAA	0,68	8	10	9	9	14	10	8	9	9	10	8	12	10	10	11	11
chr17	78090657	78091017	GAA	0,63	55	50	37	45	60	51	38	35	64	58	55	49	57	55	57	56
chr17	78091293	78091653	GAA	0,68	13	9	8	10	12	9	8	10	13	7	13	10	9	9	10	8
chr17	78091893	78092253	GAA	0,66	21	18	23	20	22	24	15	16	30	22	26	22	24	26	24	23
chr17	78092347	78092707	GAA	0,62	112	93	81	77	96	81	74	64	116	87	108	82	102	99	102	77
chr17	78092980	78093220	GAA	0,62	27	26	29	24	38	25	21	18	35	24	36	23	36	32	30	29
chr18	28647885	28648197	DSC2	0,39	323	352	259	179	254	259	213	150	334	321	315	248	298	322	339	243
chr18	28648219	28648411	DSC2	0,30	261	277	218	142	222	215	192	121	311	259	263	223	261	253	276	203
chr18	28648808	28649168	DSC2	0,48	119	114	95	75	117	104	80	69	134	106	103	100	112	115	115	94
chr18	28650633	28650873	DSC2	0,37	262	254	185	160	206	206	176	128	282	245	236	222	251	267	244	214
chr18	28651508	28651868	DSC2	0,38	250	260	220	155	215	226	178	139	281	274	278	224	275	274	280	209
chr18	28654580	28654940	DSC2	0,38	263	272	220	149	223	234	185	147	288	259	269	220	262	264	264	209
chr18	28659746	28659986	DSC2	0,36	300	298	258	154	236	263	216	139	329	296	312	276	295	306	316	228
chr18	28660009	28660369	DSC2	0,37	352	362	286	195	272	289	248	162	373	363	340	282	324	365	365	271
chr18	28662116	28662476	DSC2	0,30	326	345	244	172	227	257	208	145	329	304	309	267	315	328	340	235
chr18	28662838	28663078	DSC2	0,29	264	279	216	154	232	209	183	144	295	273	253	223	249	271	253	210
chr18	28666441	28666801	DSC2	0,41	314	303	227	158	250	260	196	150	322	304	316	248	301	302	304	218
chr18	28667547	28667883	DSC2	0,33	262	282	211	144	214	229	180	137	293	270	269	219	258	275	278	203
chr18	28669299	28669659	DSC2	0,35	308	325	254	157	252	252	210	155	298	320	298	251	298	300	306	243
chr18	28670930	28671170	DSC2	0,37	256	275	183	141	218	219	178	144	281	260	248	208	251	260	268	197
chr18	28671983	28672343	DSC2	0,34	305	334	232	171	228	248	205	148	331	314	295	270	316	312	337	221
chr18	28673473	28673665	DSC2	0,31	211	228	166	140	191	195	149	134	238	209	235	193	206	227	217	166
chr18	28681779	28682019	DSC2	0,75	2	2	0	1	3	1	0	1	1	0	2	1	1	1	0	2
chr18	29078200	29078320	DSG2	0,74	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0
chr18	29098099	29098339	DSG2	0,35	210	234	189	142	207	201	153	117	263	240	235	199	255	254	253	186
chr18	29099712	29099952	DSG2	0,41	139	146	109	97	149	106	99	78	181	139	164	111	131	140	135	115
chr18	29100715	29101233	DSG2	0,32	279	306	230	179	217	248	215	144	301	280	297	243	278	260	308	230
chr18	29101972	29102308	DSG2	0,33	298	331	242	157	226	239	194	155	316	315	279	257	302	299	301	215
chr18	29104359	29104938	DSG2	0,35	241	263	188	143	207	208	179	129	283	245	246	222	251	255	247	188
chr18	29110842	29111322	DSG2	0,34	302	334	245	174	253	268	220	158	353	330	322	283	333	323	356	238
chr18	29115123	29115483	DSG2	0,29	265	282	212	152	214	227	180	132	275	251	273	210	268	273	266	200
chr18	29116098	29116458	DSG2	0,39	250	246	194	157	206	222	166	126	291	251	260	213	247	263	240	206
chr18	29118647	29119007	DSG2	0,47	149	131	111	98	144	139	87	80	169	132	152	128	147	147	137	123
chr18	29121096	29121336	DSG2	0,43	143	157	104	98	145	132	112	94	166	140	157	128	158	150	144	125
chr18	29122408	29122888	DSG2	0,50	120	112	85	79	121	103	86	63	148	108	120	109	129	126	123	104
chr18	29125663	29126815	DSG2	0,44	281	306	248	174	240	252	207	152	320	302	291	253	297	303	327	231
chr18	29171779	29172019	TTR	0,51	140	152	108	95	117	143	101	86	174	135	154	144	149	152	149	115

chr18	29172803	29173043	TTR	0,50	125	110	101	77	109	105	70	69	150	97	116	90	109	120	110	101
chr18	29175030	29175270	TTR	0,44	173	191	140	122	159	157	110	81	203	177	192	162	194	184	190	127
chr18	29178464	29178704	TTR	0,54	134	138	96	95	129	113	93	73	159	127	148	119	135	134	132	107
chr18	32335853	32336093	DTNA	0,36	203	240	167	128	170	179	147	107	239	217	219	184	217	238	211	170
chr18	32345844	32346084	DTNA	0,38	257	279	202	141	216	211	180	132	284	255	262	214	276	257	262	195
chr18	32373927	32374287	DTNA	0,46	283	326	236	170	240	262	198	150	312	314	282	253	309	293	329	224
chr18	32386144	32386360	DTNA	0,38	243	249	189	129	197	215	172	135	256	232	249	221	238	256	247	181
chr18	32391819	32392179	DTNA	0,38	238	270	206	147	230	214	179	138	293	245	262	193	250	255	287	186
chr18	32395805	32396045	DTNA	0,39	200	210	150	130	182	197	132	105	215	180	197	161	194	213	187	155
chr18	32398030	32398390	DTNA	0,46	252	248	217	141	223	226	166	128	267	265	256	202	263	246	252	198
chr18	32400696	32400936	DTNA	0,45	248	265	217	147	248	218	171	139	301	262	280	232	250	254	283	192
chr18	32405174	32405294	DTNA	0,34	100	95	80	60	105	78	55	54	121	88	105	86	111	100	94	88
chr18	32407478	32407718	DTNA	0,41	332	374	250	169	277	275	212	146	362	346	327	297	340	322	370	250
chr18	32408899	32409139	DTNA	0,39	249	274	212	158	228	209	183	138	286	276	276	228	271	266	253	208
chr18	32417976	32418216	DTNA	0,44	162	149	136	103	165	152	112	85	199	157	162	133	156	152	169	124
chr18	32418634	32418874	DTNA	0,50	225	202	166	129	194	198	151	108	234	209	222	196	228	210	236	142
chr18	32428183	32428423	DTNA	0,43	137	156	96	91	132	129	95	74	161	137	159	113	142	153	135	110
chr18	32431723	32431963	DTNA	0,42	266	297	234	163	252	241	186	155	331	300	286	235	295	303	314	199
chr18	32438185	32438425	DTNA	0,49	122	122	100	71	117	121	82	70	135	118	122	113	121	132	108	107
chr18	32443857	32444097	DTNA	0,44	105	109	82	77	110	90	59	69	122	97	112	96	102	114	107	77
chr18	32445979	32446219	DTNA	0,43	172	161	124	99	135	140	107	94	181	148	167	134	160	169	161	138
chr18	32455102	32455462	DTNA	0,50	97	91	68	60	101	83	54	54	115	92	103	69	96	92	90	85
chr18	32457607	32457847	DTNA	0,39	210	239	177	138	191	195	163	124	241	203	225	184	231	219	214	164
chr18	32459418	32459778	DTNA	0,40	249	282	211	165	235	210	184	130	310	284	257	230	266	259	289	204
chr18	32461978	32462218	DTNA	0,47	164	149	132	99	146	137	114	100	187	172	174	129	158	153	176	134
chr18	32464640	32464760	DTNA	0,42	244	269	195	152	194	212	153	114	249	241	237	209	236	248	281	183
chr18	77439864	77440344	CTDP1	0,79	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
chr18	77455175	77455391	CTDP1	0,53	56	53	42	46	60	51	40	41	67	54	60	50	66	56	59	48
chr18	77455903	77456143	CTDP1	0,63	16	18	12	11	21	9	10	13	20	10	17	16	15	11	10	12
chr18	77457803	77458043	CTDP1	0,48	72	71	68	59	83	70	56	44	96	62	72	80	74	67	70	61
chr18	77464661	77465021	CTDP1	0,57	49	46	39	43	55	56	40	35	61	55	65	61	47	57	55	46
chr18	77470270	77470510	CTDP1	0,42	250	306	218	173	229	260	196	158	294	259	264	241	267	267	284	219
chr18	77472874	77473234	CTDP1	0,38	223	244	190	154	200	200	169	114	254	220	227	199	230	228	249	177
chr18	77474393	77475185	CTDP1	0,65	55	54	37	43	53	43	36	34	55	47	55	45	53	53	52	49
chr18	77475201	77475633	CTDP1	0,59	43	46	29	40	45	45	30	32	53	40	50	42	52	52	50	45
chr18	77477425	77478092	CTDP1	0,67	14	17	14	13	21	16	10	11	22	15	21	13	19	17	15	18
chr18	77488807	77489167	CTDP1	0,58	76	76	57	64	81	69	58	48	92	74	73	68	69	79	69	64
chr18	77496257	77496617	CTDP1	0,68	6	6	3	4	4	4	3	5	7	4	5	3	5	4	5	6
chr18	77513600	77513840	CTDP1	0,63	11	10	3	8	12	7	8	9	11	9	12	7	7	10	5	7
chr19	10828903	10829143	DNM2	0,72	3	3	1	2	2	3	2	4	4	2	2	4	1	3	2	2
chr19	10870330	10870570	DNM2	0,59	114	136	96	83	128	93	77	69	138	108	115	102	110	113	104	97
chr19	10883049	10883409	DNM2	0,52	209	209	160	127	166	167	149	100	229	211	200	178	194	205	203	151
chr19	10886300	10886660	DNM2	0,60	86	91	70	62	100	84	59	65	108	78	95	82	82	91	84	78
chr19	10887751	10887967	DNM2	0,59	14	18	9	11	20	14	9	6	16	10	14	11	15	12	11	10
chr19	10893535	10893895	DNM2	0,57	63	68	46	51	71	56	47	44	80	57	71	63	68	65	47	66
chr19	10897130	10897490	DNM2	0,61	78	67	59	61	76	59	49	42	82	58	77	70	67	70	70	60
chr19	10904343	10904583	DNM2	0,58	34	43	27	29	54	33	25	30	47	40	39	32	45	34	34	42
chr19	10905961	10906201	DNM2	0,58	64	73	60	51	79	55	49	45	68	68	65	64	59	65	61	57
chr19	10906685	10906925	DNM2	0,54	43	39	37	53	48	46	41	28	53	47	52	46	44	47	46	39
chr19	10908004	10908244	DNM2	0,53	93	98	68	72	100	76	67	49	111	74	91	78	85	80	93	77
chr19	10909068	10909284	DNM2	0,56	98	104	88	79	110	93	65	67	125	97	111	97	107	106	101	88
chr19	10912878	10913118	DNM2	0,56	115	132	93	85	137	127	80	78	146	119	130	113	119	119	115	100
chr19	10916497	10916737	DNM2	0,60	68	71	47	61	80	63	53	48	89	65	72	75	68	62	68	73
chr19	10919197	10919317	DNM2	0,47	29	29	21	24	42	34	23	24	30	31	31	34	32	28	29	25
chr19	10922876	10923116	DNM2	0,64	34	29	25	26	35	33	21	20	33	25	34	25	37	33	30	41
chr19	10930590	10930830	DNM2	0,58	62	59	43	51	65	50	36	44	79	51	66	55	65	63	62	44
chr19	10934399	10934639	DNM2	0,66	23	25	16	17	37	20	16	18	34	16	29	28	24	32	23	26
chr19	10935625	10935937	DNM2	0,56	73	63	62	57	75	69	55	47	81	75	71	60	73	61	63	62
chr19	10939647	10940007	DNM2	0,63	15	16	13	17	20	15	15	14	26	15	20	20	16	18	19	16
chr19	10940748	10941108	DNM2	0,68	57	61	43	47	75	49	49	44	74	50	71	52	65	62	61	52

chr19	10941568	10941808	DNM2	0,70	6	5	6	7	10	8	3	5	8	7	6	7	3	5	7	7
chr19	13318074	13318674	CACNA1A	0,78	2	1	1	1	2	1	1	1	2	1	2	2	2	1	1	2
chr19	13318694	13318934	CACNA1A	0,60	15	14	14	15	16	10	10	11	15	18	20	13	19	12	12	15
chr19	13319486	13319702	CACNA1A	0,73	1	1	1	0	2	0	0	3	3	1	0	0	0	1	1	1
chr19	13319725	13319917	CACNA1A	0,67	6	5	3	5	10	4	7	11	9	6	5	6	4	7	6	9
chr19	13320093	13320405	CACNA1A	0,70	3	3	3	3	3	3	3	2	4	1	5	2	2	4	3	3
chr19	13321328	13321568	CACNA1A	0,59	18	13	9	12	25	17	12	9	19	12	17	15	11	11	10	13
chr19	13322853	13323093	CACNA1A	0,66	18	14	10	12	14	12	11	8	13	10	11	12	13	10	10	11
chr19	13323146	13323619	CACNA1A	0,66	13	11	10	13	12	9	8	11	18	9	14	13	11	11	12	11
chr19	13324976	13325216	CACNA1A	0,63	29	27	23	25	32	27	24	26	38	30	36	22	37	29	31	26
chr19	13325248	13325488	CACNA1A	0,60	53	59	49	45	70	59	36	50	90	66	63	56	67	66	59	62
chr19	13335413	13335653	CACNA1A	0,62	28	26	19	19	31	24	21	20	28	22	27	21	26	28	22	23
chr19	13338206	13338350	CACNA1A	0,61	3	3	1	2	4	4	2	5	2	2	2	6	3	3	2	3
chr19	13339440	13339680	CACNA1A	0,47	142	145	103	101	146	121	118	96	150	138	150	135	140	119	120	114
chr19	13340839	13341079	CACNA1A	0,58	35	32	30	26	38	31	27	23	43	34	34	31	30	30	36	28
chr19	13342418	13342778	CACNA1A	0,55	103	106	82	84	108	80	82	64	119	100	118	93	113	114	104	98
chr19	13345672	13345912	CACNA1A	0,54	133	133	112	97	137	123	89	81	163	131	136	121	128	125	123	123
chr19	13345935	13346175	CACNA1A	0,57	71	66	47	51	73	67	49	42	76	66	75	63	64	59	56	60
chr19	13346366	13346606	CACNA1A	0,57	74	63	52	57	58	51	52	48	75	49	68	55	58	63	62	60
chr19	13355917	13356157	CACNA1A	0,60	78	78	63	56	74	66	52	52	92	70	87	68	81	75	73	74
chr19	13363739	13363979	CACNA1A	0,54	96	89	75	65	97	82	73	50	103	91	102	82	85	87	84	72
chr19	13365886	13366126	CACNA1A	0,60	20	20	21	16	30	20	13	13	23	22	25	22	23	22	17	19
chr19	13368061	13368373	CACNA1A	0,51	128	136	109	91	140	110	101	76	158	141	151	124	147	141	141	113
chr19	13370326	13370566	CACNA1A	0,56	88	98	76	61	93	87	63	58	101	90	90	75	99	95	99	78
chr19	13372163	13372523	CACNA1A	0,52	104	103	71	74	118	88	77	60	127	91	113	92	110	97	107	81
chr19	13373506	13373722	CACNA1A	0,55	140	142	105	91	116	120	102	80	150	126	127	124	142	128	134	99
chr19	13386596	13386836	CACNA1A	0,60	24	28	19	17	28	22	20	22	34	29	34	23	30	29	29	32
chr19	13387816	13388032	CACNA1A	0,47	175	168	133	104	136	129	108	73	160	151	170	142	132	158	140	137
chr19	13394025	13394265	CACNA1A	0,60	34	34	23	24	39	26	25	29	44	36	41	31	31	37	32	31
chr19	13395830	13395950	CACNA1A	0,65	4	9	7	5	6	8	8	3	8	5	10	8	6	11	11	7
chr19	13395967	13396087	CACNA1A	0,50	8	15	12	7	14	16	11	9	26	13	18	14	17	17	21	13
chr19	13397285	13397861	CACNA1A	0,62	27	22	20	24	31	31	19	23	36	25	36	24	29	32	31	26
chr19	13409276	13409444	CACNA1A	0,57	121	129	116	108	153	134	111	92	180	132	152	142	158	144	164	120
chr19	13409550	13410246	CACNA1A	0,69	38	36	28	28	48	38	32	29	46	35	43	32	37	42	38	41
chr19	13411296	13411536	CACNA1A	0,52	213	202	156	116	190	178	147	111	232	210	193	166	188	202	220	164
chr19	13414292	13414508	CACNA1A	0,57	70	70	63	44	69	74	64	42	83	68	70	68	77	90	85	67
chr19	13414519	13414759	CACNA1A	0,57	174	181	145	122	162	157	127	112	199	161	173	165	179	177	189	136
chr19	13418511	13418751	CACNA1A	0,55	109	108	80	81	109	83	83	80	121	98	108	88	116	116	112	82
chr19	13418879	13419119	CACNA1A	0,58	62	56	50	46	68	50	51	39	66	54	65	47	63	62	65	52
chr19	13419165	13419405	CACNA1A	0,53	84	83	62	64	98	70	60	58	97	81	108	72	93	80	78	74
chr19	13423418	13423658	CACNA1A	0,46	127	137	95	90	133	115	88	63	137	139	132	110	131	121	116	111
chr19	13427850	13428210	CACNA1A	0,52	85	95	63	60	95	71	63	47	95	75	87	74	92	96	74	73
chr19	13441007	13441223	CACNA1A	0,49	82	79	64	76	92	81	67	53	100	87	101	81	84	78	82	70
chr19	13443590	13443830	CACNA1A	0,58	48	47	36	28	61	49	29	24	62	38	47	47	48	44	48	37
chr19	13445129	13445369	CACNA1A	0,51	157	165	123	121	153	122	114	107	171	155	162	134	160	162	149	131
chr19	13446551	13446791	CACNA1A	0,52	34	44	35	29	45	39	32	28	37	39	44	36	46	42	47	38
chr19	13470336	13470696	CACNA1A	0,54	124	136	101	76	115	113	84	76	131	124	148	107	127	138	125	106
chr19	13476026	13476386	CACNA1A	0,51	158	156	123	91	139	131	110	84	169	150	155	144	145	151	150	124
chr19	13482414	13482630	CACNA1A	0,59	16	14	12	18	22	9	9	12	15	15	17	12	16	17	23	12
chr19	13563639	13563879	CACNA1A	0,48	197	201	170	130	168	180	143	110	215	197	200	169	203	194	209	152
chr19	13565853	13566093	CACNA1A	0,47	105	105	88	67	107	92	81	71	121	93	98	105	104	99	109	89
chr19	13616651	13617107	CACNA1A	0,67	60	57	44	40	61	56	43	37	70	60	63	53	55	71	62	58
chr19	3633105	3633225	PIP5K1C	0,68	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
chr19	3633356	3633596	PIP5K1C	0,68	4	4	4	5	7	4	2	6	3	3	4	3	4	6	5	2
chr19	3638827	3639067	PIP5K1C	0,66	4	6	2	6	7	4	2	3	6	6	5	7	4	7	3	2
chr19	3641634	3641874	PIP5K1C	0,67	8	6	4	7	10	7	5	5	15	6	11	6	7	6	9	8
chr19	3642796	3642988	PIP5K1C	0,64	6	7	6	6	6	13	4	9	11	8	10	12	13	4	7	12
chr19	3643189	3643429	PIP5K1C	0,67	14	16	10	10	19	15	14	10	19	11	16	12	13	12	11	18
chr19	3643986	3644346	PIP5K1C	0,69	4	8	5	7	6	4	5	4	9	5	7	5	6	5	6	5
chr19	3645893	3646133	PIP5K1C	0,65	29	24	12	29	30	20	19	18	31	22	24	23	23	27	18	18

chr19	3647318	3647438	PIP5K1C	0,63	2	0	1	0	2	2	1	1	2	0	1	2	0	0	1	3
chr19	3648544	3648784	PIP5K1C	0,70	5	5	4	3	6	4	6	3	6	4	4	5	4	5	6	5
chr19	3651746	3652106	PIP5K1C	0,68	14	10	10	7	17	11	8	7	14	7	11	10	9	9	9	12
chr19	3653197	3653677	PIP5K1C	0,67	27	26	23	27	37	27	24	22	41	25	31	29	30	33	31	25
chr19	3656298	3656658	PIP5K1C	0,64	34	22	21	26	35	27	17	19	34	23	35	27	28	29	30	32
chr19	3660936	3661152	PIP5K1C	0,61	43	36	27	29	44	42	38	20	46	34	41	41	36	41	45	33
chr19	3661813	3662053	PIP5K1C	0,68	7	10	7	9	12	7	8	4	11	7	10	8	15	10	10	6
chr19	3664745	3664985	PIP5K1C	0,63	14	12	13	14	17	15	10	13	24	20	19	17	16	11	12	15
chr19	3667296	3667416	PIP5K1C	0,60	7	5	4	8	7	9	3	6	6	6	5	4	4	4	9	5
chr19	3700220	3700388	PIP5K1C	0,80	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chr19	38924431	38924551	RYR1	0,62	26	37	24	30	33	23	29	24	35	23	29	24	33	26	32	27
chr19	38931324	38931564	RYR1	0,66	5	6	4	7	9	5	5	7	7	5	9	7	7	6	7	9
chr19	38932980	38933100	RYR1	0,64	14	19	14	9	20	14	8	14	20	15	12	13	18	16	11	13
chr19	38934174	38934294	RYR1	0,66	22	26	30	23	32	15	17	13	32	22	30	16	26	33	30	25
chr19	38934336	38934456	RYR1	0,6	24	36	28	28	36	18	22	19	45	26	38	27	32	38	40	35
chr19	38934784	38934904	RYR1	0,58	63	50	49	50	63	57	41	39	67	44	60	60	61	52	57	60
chr19	38935210	38935330	RYR1	0,64	37	41	44	40	54	39	29	41	51	34	51	33	44	36	40	43
chr19	38937098	38937218	RYR1	0,58	124	137	100	97	125	112	101	80	154	123	128	124	128	124	125	105
chr19	38937310	38937430	RYR1	0,6	43	50	31	36	37	45	28	27	56	33	35	37	48	38	46	36
chr19	38938952	38939192	RYR1	0,66	34	26	18	25	35	28	31	25	37	27	30	32	33	35	25	26
chr19	38939250	38939457	RYR1	0,64	26	28	16	26	29	29	20	25	32	24	40	32	32	30	31	35
chr19	38942344	38942584	RYR1	0,65	16	14	10	14	21	20	19	17	30	13	17	17	15	21	19	12
chr19	38943436	38943676	RYR1	0,66	7	7	6	6	7	5	5	8	12	6	9	7	8	7	7	5
chr19	38945822	38946062	RYR1	0,54	207	215	162	125	212	187	168	121	253	206	230	190	216	205	215	176
chr19	38946078	38946198	RYR1	0,58	235	223	181	132	204	200	180	131	261	239	243	216	236	214	230	177
chr19	38946211	38946451	RYR1	0,58	148	154	118	110	166	135	113	87	186	143	174	149	144	168	158	131
chr19	38948083	38948323	RYR1	0,53	251	244	213	151	221	206	175	119	280	263	266	208	257	234	275	183
chr19	38948631	38948991	RYR1	0,62	103	96	74	70	95	79	70	57	122	88	93	86	88	92	97	82
chr19	38949761	38950001	RYR1	0,62	66	52	50	51	84	64	43	38	82	57	74	62	79	72	78	57
chr19	38951002	38951242	RYR1	0,63	99	98	85	77	109	96	76	62	118	105	103	88	106	98	107	83
chr19	38954054	38954174	RYR1	0,65	20	21	16	22	27	19	16	15	26	17	25	23	17	22	18	22
chr19	38954378	38954498	RYR1	0,56	18	25	18	20	26	14	17	16	30	14	21	18	26	23	18	22
chr19	38955260	38955380	RYR1	0,63	12	7	7	11	15	8	6	9	14	5	14	13	11	9	10	13
chr19	38956704	38957044	RYR1	0,66	19	14	12	15	18	10	9	8	19	16	16	15	15	14	16	17
chr19	38958230	38958470	RYR1	0,61	64	64	47	53	68	66	50	50	87	61	67	54	71	70	67	60
chr19	38959572	38959793	RYR1	0,59	49	52	42	45	59	41	35	38	49	39	50	39	48	52	52	45
chr19	38959939	38960168	RYR1	0,6	187	165	150	111	154	161	138	91	199	171	184	160	183	190	188	133
chr19	38963973	38964453	RYR1	0,69	35	30	24	25	37	25	24	25	35	31	35	30	33	34	32	31
chr19	38965903	38966143	RYR1	0,61	15	13	8	15	13	12	8	11	19	10	19	14	12	12	15	12
chr19	38968309	38968549	RYR1	0,62	57	60	40	51	61	52	40	33	73	59	63	60	55	64	62	51
chr19	38969037	38969277	RYR1	0,56	50	49	33	44	50	46	41	34	58	50	50	41	43	50	44	39
chr19	38973649	38973769	RYR1	0,55	226	245	185	158	219	194	146	135	297	229	266	219	245	262	268	192
chr19	38973922	38974162	RYR1	0,67	34	34	30	28	40	31	23	25	47	24	38	33	30	37	31	35
chr19	38976175	38976895	RYR1	0,65	55	48	39	41	61	45	47	38	60	44	60	52	51	55	57	49
chr19	38979764	38979884	RYR1	0,5	47	38	32	33	50	52	33	29	47	45	59	53	47	49	37	38
chr19	38980006	38980126	RYR1	0,56	44	54	40	49	58	45	34	28	56	46	58	45	52	52	50	45
chr19	38980695	38980935	RYR1	0,61	116	109	89	86	130	103	83	64	135	104	121	107	112	118	116	98
chr19	38981256	38981376	RYR1	0,44	240	296	201	181	243	230	189	143	304	261	281	230	276	250	281	199
chr19	38983101	38983322	RYR1	0,59	45	44	30	39	47	36	31	36	47	38	45	44	44	41	41	45
chr19	38984948	38985308	RYR1	0,65	91	77	66	57	85	74	64	51	103	90	83	79	81	86	85	77
chr19	38986851	38986971	RYR1	0,57	60	51	47	49	61	52	37	32	78	64	63	62	65	64	62	52
chr19	38986994	38987234	RYR1	0,64	59	58	44	49	63	47	39	32	72	55	58	52	59	62	57	53
chr19	38987486	38987606	RYR1	0,63	23	24	19	24	29	21	17	21	28	19	25	22	31	28	25	23
chr19	38989695	38989935	RYR1	0,61	15	12	18	11	15	16	10	10	19	13	15	13	18	10	17	12
chr19	38990247	38990487	RYR1	0,69	32	28	23	31	36	35	29	22	35	22	38	30	34	28	34	38
chr19	38990541	38990661	RYR1	0,59	81	72	51	62	84	62	47	56	92	62	78	67	79	73	72	76
chr19	38991185	38991665	RYR1	0,65	31	33	22	27	36	30	22	17	40	27	35	28	30	29	32	34
chr19	38993136	38993376	RYR1	0,64	10	12	10	17	19	16	9	10	17	17	15	20	15	11	17	15
chr19	38993504	38993624	RYR1	0,63	33	33	33	29	38	29	24	27	38	30	37	39	41	35	33	41
chr19	38994809	38995049	RYR1	0,59	136	131	116	96	142	126	96	92	159	126	137	122	138	150	142	116

chr19	38995349	38995589	RYR1	0,58	91	82	67	71	94	81	59	64	116	78	98	81	100	89	103	84
chr19	38995621	38995741	RYR1	0,56	117	131	92	95	144	115	96	103	164	137	137	123	134	131	145	120
chr19	38995933	38996053	RYR1	0,54	55	47	47	47	70	48	40	38	68	52	56	46	61	50	48	52
chr19	38996395	38996635	RYR1	0,59	59	52	40	39	62	43	41	43	69	56	64	48	56	58	50	52
chr19	38996919	38997039	RYR1	0,59	106	115	96	84	114	98	75	65	155	97	124	110	129	106	103	107
chr19	38997088	38997208	RYR1	0,59	33	26	29	26	32	28	22	18	38	28	37	31	37	32	33	31
chr19	38997410	38997650	RYR1	0,63	22	24	18	19	28	21	19	13	35	25	26	23	25	20	26	20
chr19	38998349	38998469	RYR1	0,54	98	96	85	74	104	87	66	59	129	93	115	92	103	105	84	93
chr19	39001111	39001231	RYR1	0,52	212	205	160	132	174	179	157	108	235	194	188	171	204	202	216	158
chr19	39001240	39001480	RYR1	0,57	263	278	233	164	200	231	185	142	287	266	251	213	256	268	265	200
chr19	39002165	39002285	RYR1	0,53	423	415	357	226	358	370	282	219	441	428	435	372	426	425	467	324
chr19	39002681	39002801	RYR1	0,67	48	45	36	40	46	38	43	29	81	43	49	48	55	47	43	53
chr19	39002823	39003183	RYR1	0,62	79	81	63	66	80	73	63	53	94	74	98	72	84	71	74	74
chr19	39005646	39005766	RYR1	0,55	33	38	23	28	35	34	26	28	41	31	35	29	25	38	35	36
chr19	39006671	39006911	RYR1	0,66	2	3	1	1	1	1	1	1	4	1	1	1	0	0	4	1
chr19	39007984	39008344	RYR1	0,66	38	32	23	25	42	31	22	24	36	26	38	33	33	31	32	23
chr19	39009793	39010153	RYR1	0,68	12	8	9	8	14	9	9	11	14	6	10	11	12	9	12	10
chr19	39013651	39013771	RYR1	0,65	31	27	23	20	31	28	17	15	36	17	34	32	23	30	33	30
chr19	39013842	39013962	RYR1	0,5	65	63	53	49	73	74	46	48	86	66	90	65	75	67	69	55
chr19	39014501	39014621	RYR1	0,6	3	6	3	4	6	2	4	4	7	6	3	5	6	3	5	6
chr19	39015953	39016176	RYR1	0,64	18	19	10	18	22	22	14	13	27	15	22	17	22	19	21	18
chr19	39017602	39017722	RYR1	0,52	88	88	72	73	107	83	76	62	107	78	104	75	91	81	87	76
chr19	39018235	39018475	RYR1	0,66	6	8	5	5	10	5	4	5	12	5	7	6	6	5	6	7
chr19	39018941	39019061	RYR1	0,6	26	25	15	17	25	23	21	18	34	21	33	18	26	33	23	25
chr19	39019226	39019346	RYR1	0,54	68	64	40	44	76	56	52	51	71	54	81	65	67	69	62	61
chr19	39019477	39019597	RYR1	0,55																
chr19	39019638	39019758	RYR1	0,65	21	15	14	18	25	15	9	21	25	17	17	21	20	23	21	20
chr19	39023095	39023215	RYR1	0,55	95	101	70	65	103	92	84	67	105	92	97	81	94	88	98	97
chr19	39023283	39023403	RYR1	0,61	51	40	31	31	42	43	41	22	44	37	47	44	36	38	38	49
chr19	39025349	39025469	RYR1	0,61	16	19	14	19	25	11	14	18	20	14	16	14	20	16	11	11
chr19	39025760	39025880	RYR1	0,56	71	69	46	49	61	63	53	45	92	62	72	63	61	68	61	60
chr19	39025933	39026053	RYR1	0,5	118	125	101	83	127	124	74	76	161	115	117	119	123	129	108	109
chr19	39026613	39026733	RYR1	0,5	43	60	45	41	61	44	33	34	67	44	50	49	53	48	43	49
chr19	39027355	39027475	RYR1	0,64	51	55	50	50	64	48	41	44	70	55	56	54	61	65	59	50
chr19	39028499	39028619	RYR1	0,53	92	87	70	73	84	75	64	56	106	73	88	94	86	81	93	82
chr19	39033970	39034090	RYR1	0,5	214	205	181	139	220	173	161	118	244	188	228	196	230	221	215	176
chr19	39034115	39034355	RYR1	0,62	134	129	101	94	146	127	100	85	168	124	143	120	132	128	126	109
chr19	39034402	39034522	RYR1	0,64	58	57	39	43	74	59	40	34	73	60	53	51	55	56	57	61
chr19	39037065	39037185	RYR1	0,55	45	36	33	42	44	44	23	26	61	34	49	38	44	37	35	36
chr19	39038846	39039086	RYR2	0,57	75	75	62	64	85	76	56	57	97	72	82	64	78	74	68	75
chr19	39051743	39052103	RYR3	0,61	49	38	34	47	46	42	29	32	60	43	55	47	47	47	41	40
chr19	39055553	39055750	RYR4	0,64	7	6	3	3	9	7	5	5	5	3	8	3	8	6	8	6
chr19	39056031	39056462	RYR5	0,73	8	8	6	8	11	7	6	7	10	6	9	8	9	9	9	9
chr19	39057528	39057648	RYR6	0,66	4	2	3	3	4	5	4	5	8	5	5	6	5	3	5	3
chr19	39058364	39058604	RYR7	0,61	87	75	71	70	99	68	57	54	98	78	89	77	84	78	86	83
chr19	39061229	39061349	RYR8	0,47	244	264	187	149	230	227	169	147	273	268	276	217	251	213	267	206
chr19	39062604	39062929	RYR9	0,59	45	54	39	43	56	39	33	28	62	48	57	43	49	42	51	43
chr19	39063761	39064001	RYR10	0,62	16	23	7	18	17	12	14	17	22	14	20	15	19	13	15	18
chr19	39066519	39066639	RYR11	0,5	184	188	176	117	186	168	139	110	220	189	200	177	188	216	208	156
chr19	39068502	39068742	RYR12	0,62	49	50	42	50	54	47	39	34	73	53	57	48	61	55	54	55
chr19	39068753	39068873	RYR13	0,6	37	30	29	32	38	37	23	16	48	32	44	30	38	31	36	33
chr19	39070574	39070814	RYR14	0,61	73	65	47	51	87	54	50	52	88	59	84	63	70	72	76	67
chr19	39070956	39071162	RYR15	0,61	79	82	63	68	85	63	58	49	107	65	86	70	77	76	86	51
chr19	39075560	39075773	RYR16	0,6	33	31	22	26	36	27	20	25	36	30	34	24	35	39	29	26
chr19	39076549	39076669	RYR17	0,56	96	83	67	66	87	85	57	60	106	86	91	89	98	94	90	81
chr19	39076720	39076840	RYR18	0,53	86	95	69	63	89	82	54	54	98	86	86	83	100	87	84	70
chr19	39077130	39077250	RYR19	0,5	65	67	59	61	65	61	40	40	81	66	71	66	67	66	68	59
chr19	39077952	39078072	RYR20	0,5	184	201	157	145	195	162	138	114	225	177	198	183	214	188	186	169
chr19	40899818	40900178	PRX	0,68	11	10	5	9	14	5	7	6	10	6	11	5	6	9	10	7
chr19	40900192	40903984	PRX	0,63	117	111	92	76	111	98	81	67	132	109	120	102	117	113	119	97

chr19	40904413	40904773	PRX	0,67	13	13	9	10	15	12	10	7	14	11	16	16	11	15	12	17
chr19	40909510	40909870	PRX	0,66	12	13	9	11	14	14	9	9	14	12	15	12	15	12	11	14
chr19	40913729	40913945	PRX	0,63	13	13	11	14	21	13	14	16	19	14	18	20	20	13	15	17
chr19	46273638	46273998	DMPK	0,70	8	6	9	9	10	6	5	7	9	8	10	10	10	7	11	9
chr19	46274153	46274393	DMPK	0,75	1	0	0	0	1	0	1	1	2	1	1	0	0	0	1	1
chr19	46274510	46274750	DMPK	0,66	9	10	8	12	13	6	11	8	16	8	13	7	7	10	10	8
chr19	46274754	46274994	DMPK	0,68	15	18	19	17	20	18	13	19	26	19	16	17	25	19	18	20
chr19	46275008	46275248	DMPK	0,67	10	5	5	6	10	5	5	6	7	4	5	3	4	9	4	6
chr19	46275834	46276074	DMPK	0,62	23	22	15	17	30	18	16	12	25	21	17	23	21	23	28	16
chr19	46278129	46278369	DMPK	0,61	23	20	16	17	23	28	16	19	25	28	22	18	23	20	24	21
chr19	46280497	46281217	DMPK	0,64	27	25	21	25	34	28	18	22	30	26	31	28	31	27	30	25
chr19	46281311	46281551	DMPK	0,63	39	29	22	36	37	25	22	27	33	29	31	29	27	37	34	30
chr19	46281644	46282004	DMPK	0,61	42	47	36	32	47	38	31	28	51	39	36	40	39	36	39	35
chr19	46282470	46282830	DMPK	0,62	118	112	93	79	131	104	83	75	134	112	104	105	111	128	109	106
chr19	46282936	46283416	DMPK	0,60	72	65	49	57	78	64	59	53	83	64	79	75	74	71	68	69
chr19	46285350	46285710	DMPK	0,70	8	13	5	9	7	6	3	7	10	5	8	10	7	8	8	6
chr19	47258611	47260291	FKRP	0,70	17	13	12	15	18	15	14	12	19	13	16	14	17	15	14	16
chr19	49472466	49472946	GYS1	0,68	5	3	2	4	5	5	6	3	5	3	5	4	3	4	6	6
chr19	49472951	49473191	GYS1	0,63	11	12	12	17	14	15	13	10	18	14	16	9	14	12	16	13
chr19	49473704	49474064	GYS1	0,59	38	40	29	34	52	34	33	31	51	34	45	36	41	36	34	37
chr19	49474112	49474352	GYS1	0,58	46	44	39	45	64	46	35	41	65	49	50	46	47	54	40	53
chr19	49477414	49477654	GYS1	0,55	21	20	15	22	25	20	12	12	32	19	29	25	26	25	17	24
chr19	49477813	49478053	GYS1	0,60	21	25	20	21	28	23	16	16	27	15	28	19	23	21	18	21
chr19	49481117	49481477	GYS1	0,54	121	136	91	84	124	103	88	71	140	119	121	110	132	136	117	107
chr19	49484719	49484959	GYS1	0,59	63	59	46	58	70	51	45	46	79	57	68	55	65	71	65	59
chr19	49485451	49485691	GYS1	0,54	117	134	105	95	132	118	91	73	149	132	129	114	132	110	111	115
chr19	49485956	49486172	GYS1	0,46	125	154	109	96	139	130	111	90	188	142	163	134	160	151	162	123
chr19	49488663	49488903	GYS1	0,59	59	56	43	46	66	53	45	38	77	54	58	51	56	53	52	55
chr19	49489019	49489379	GYS1	0,61	49	55	45	49	60	53	47	38	80	49	61	46	66	58	48	53
chr19	49490366	49490726	GYS1	0,61	36	42	32	32	45	34	27	31	46	33	47	32	43	39	36	43
chr19	49494469	49494829	GYS1	0,68	43	46	35	38	47	39	33	32	54	41	43	39	55	39	44	39
chr19	49496190	49496430	GYS1	0,63	36	39	28	43	40	39	35	23	51	40	51	43	50	36	39	37
chr19	50823434	50823674	KCNC3	0,66	3	1	0	1	3	2	0	1	1	1	4	3	1	1	2	1
chr19	50823765	50824125	KCNC3	0,65	12	14	13	13	15	18	11	10	15	16	17	14	15	18	17	15
chr19	50826125	50827445	KCNC3	0,64	34	28	22	24	36	31	24	21	37	25	29	28	34	27	28	30
chr19	50831360	50831552	KCNC3	0,75	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0
chr19	50831651	50832083	KCNC3	0,71	5	5	5	8	8	7	3	6	8	6	7	7	6	4	8	5
chr19	50936159	50936279	MYBPC2	0,68	1	2	0	2	1	0	0	0	1	1	2	0	1	0	1	0
chr19	50938366	50938582	MYBPC2	0,62	11	10	9	13	13	13	9	10	19	13	14	16	12	9	10	11
chr19	50938936	50939128	MYBPC2	0,62	125	124	95	89	128	105	76	88	139	109	142	124	124	134	119	105
chr19	50939186	50939522	MYBPC2	0,61	26	24	21	19	20	19	18	13	26	17	30	20	20	27	22	22
chr19	50939812	50940052	MYBPC2	0,60	34	30	21	25	29	25	24	15	35	27	31	29	34	30	31	25
chr19	50940633	50941065	MYBPC2	0,58	86	90	72	69	105	83	72	62	105	88	100	84	85	80	93	78
chr19	50944054	50944414	MYBPC2	0,58	48	51	43	48	56	53	43	36	71	58	58	47	56	57	65	56
chr19	50945383	50945623	MYBPC2	0,55	72	67	66	53	82	71	51	54	88	66	78	69	72	78	73	55
chr19	50946671	50947151	MYBPC2	0,53	54	60	45	45	62	47	45	37	77	56	61	47	61	56	52	49
chr19	50949042	50949378	MYBPC2	0,59	58	58	50	48	59	54	36	39	70	59	66	51	58	62	57	57
chr19	50951435	50951699	MYBPC2	0,55	62	67	50	46	70	59	42	39	80	56	79	58	61	56	67	68
chr19	50954018	50954258	MYBPC2	0,64	55	40	46	41	54	52	39	35	64	57	62	56	60	51	48	51
chr19	50954482	50954602	MYBPC2	0,57	10	6	2	5	6	5	4	4	12	4	6	4	7	5	10	4
chr19	50955045	50955261	MYBPC2	0,58	53	59	49	56	74	43	32	41	83	55	63	50	52	62	52	56
chr19	50957209	50957689	MYBPC2	0,61	42	39	35	28	44	33	30	23	45	38	46	42	35	45	38	36
chr19	50958278	50958638	MYBPC2	0,54	201	170	147	127	186	159	134	102	216	200	181	173	210	194	216	154
chr19	50958713	50958953	MYBPC2	0,60	51	61	50	47	58	54	45	36	69	55	65	52	61	68	62	51
chr19	50961747	50962645	MYBPC2	0,69	13	13	11	13	16	13	10	10	14	15	16	11	14	14	15	14
chr19	50963246	50963486	MYBPC2	0,56	12	10	8	13	16	12	9	8	11	14	16	12	13	11	13	13
chr19	50964698	50965058	MYBPC2	0,58	119	124	110	103	130	109	88	85	161	123	143	117	133	130	125	112
chr19	50965104	50965344	MYBPC2	0,58	67	73	55	53	78	52	44	44	70	56	74	60	66	65	61	62
chr19	50967572	50967884	MYBPC2	0,62	40	55	40	38	59	46	32	25	58	49	47	47	48	48	44	51
chr19	50969396	50969516	MYBPC2	0,63	0	1	0	0	2	0	2	0	4	1	0	0	2	2	3	2

chr19	51848393	51848729	ETFB	0,57	66	75	58	55	74	63	45	50	84	62	72	55	68	66	60	57
chr19	51850052	51850412	ETFB	0,64	16	18	14	11	22	13	12	10	22	13	20	15	17	18	15	19
chr19	51856281	51856593	ETFB	0,64	20	21	22	28	31	24	18	15	37	22	35	25	28	35	24	24
chr19	51857373	51857637	ETFB	0,62	95	81	62	69	91	72	61	56	104	72	88	63	79	79	77	84
chr19	51857719	51857959	ETFB	0,42	243	236	189	130	200	180	162	137	254	218	250	192	225	227	216	168
chr19	51869431	51869671	ETFB	0,69	8	8	6	7	8	6	3	5	9	5	7	9	7	8	6	5
chr19	54385653	54386013	PRKCG	0,63	28	25	22	19	35	25	21	24	38	27	26	30	24	29	24	31
chr19	54386312	54386552	PRKCG	0,65	12	6	8	9	11	9	5	8	12	7	12	13	16	12	7	10
chr19	54387335	54387575	PRKCG	0,58	75	76	60	57	85	61	46	45	95	68	77	67	67	68	75	71
chr19	54392827	54393067	PRKCG	0,64	114	103	89	80	112	102	95	71	134	101	110	96	125	115	98	111
chr19	54393085	54393325	PRKCG	0,71	13	7	10	7	11	9	10	6	17	10	12	11	11	13	10	12
chr19	54394825	54395185	PRKCG	0,55	146	170	139	107	139	137	106	74	201	156	145	151	156	164	167	136
chr19	54395709	54395949	PRKCG	0,66	5	7	3	8	8	8	6	8	11	3	7	7	8	8	7	9
chr19	54396165	54396405	PRKCG	0,60	45	36	31	38	38	44	36	30	50	37	48	31	43	44	32	42
chr19	54396510	54396750	PRKCG	0,51	181	214	170	128	172	163	153	114	217	200	183	187	196	196	199	171
chr19	54401241	54401457	PRKCG	0,58	116	117	85	84	104	107	81	72	137	103	103	92	115	108	110	93
chr19	54401607	54401967	PRKCG	0,62	20	21	14	20	20	20	14	12	22	15	19	14	19	19	18	20
chr19	54403430	54403790	PRKCG	0,57	119	105	78	76	112	98	69	63	123	101	114	88	98	113	107	100
chr19	54403813	54404053	PRKCG	0,57	116	107	88	75	122	110	74	59	130	117	131	86	106	109	104	106
chr19	54406246	54406486	PRKCG	0,56	114	96	80	74	106	88	69	64	112	82	90	97	99	97	87	78
chr19	54407822	54408062	PRKCG	0,59	66	72	58	53	94	70	54	46	79	76	76	72	71	69	67	59
chr19	54409538	54409778	PRKCG	0,59	30	35	30	30	45	28	24	30	43	32	35	36	31	35	35	34
chr19	54409874	54410234	PRKCG	0,66	27	16	18	24	28	19	10	16	24	18	24	22	23	26	21	26
chr19	55644185	55644425	TNNT1	0,59	16	24	17	19	22	21	12	18	28	12	23	22	17	21	16	21
chr19	55645154	55645603	TNNT1	0,64	35	42	26	34	44	29	35	34	40	36	43	35	43	41	33	35
chr19	55648405	55648645	TNNT1	0,63	71	69	57	62	73	69	48	52	91	61	75	71	66	76	73	66
chr19	55649306	55649522	TNNT1	0,58	187	193	144	131	162	145	115	98	197	176	189	169	200	182	179	154
chr19	55652193	55652409	TNNT1	0,59	27	30	20	19	29	17	16	16	28	21	28	23	26	21	34	18
chr19	55652491	55652731	TNNT1	0,61	106	91	81	72	111	85	70	67	115	93	104	91	101	111	104	80
chr19	55653197	55653365	TNNT1	0,63	19	15	16	13	18	13	13	10	17	14	17	13	12	15	15	11
chr19	55656802	55657042	TNNT1	0,55	83	74	48	50	87	67	60	45	91	70	83	72	76	75	75	65
chr19	55657697	55657937	TNNT1	0,72	0	0	0	1	1	0	0	1	2	1	1	0	1	1	1	0
chr19	55657941	55658181	TNNT1	0,73	1	2	2	0	0	0	1	0	1	1	1	0	2	2	0	0
chr19	55658322	55658581	TNNT1	0,66	5	8	4	7	10	10	6	6	7	9	6	11	9	5	7	7
chr19	55663123	55663363	TNNI3	0,55	104	104	84	76	101	99	76	64	108	94	93	91	92	101	97	91
chr19	55665305	55665665	TNNI3	0,58	139	134	102	104	146	129	104	79	153	113	134	133	132	130	122	113
chr19	55666030	55666246	TNNI3	0,58	177	182	149	113	179	155	111	106	221	159	178	147	181	190	186	154
chr19	55667514	55667754	TNNI3	0,66	6	5	5	5	8	5	3	3	7	4	4	3	7	6	2	6
chr19	55667871	55668111	TNNI3	0,61	65	62	42	52	78	58	43	40	80	60	62	58	62	66	56	59
chr19	55668339	55668579	TNNI3	0,68	9	9	5	12	7	5	7	6	10	4	11	12	9	5	8	3
chr19	55668826	55669066	TNNI3	0,67	14	13	9	9	15	10	5	11	13	9	16	9	10	10	14	10
chr19	7600329	7600569	PNPLA6	0,63	32	26	17	23	34	21	19	19	24	32	28	28	23	27	22	32
chr19	7600610	7600970	PNPLA6	0,70	12	11	9	12	16	9	12	9	12	10	18	9	14	13	12	15
chr19	7600997	7601237	PNPLA6	0,63	83	69	64	54	76	67	59	48	88	67	71	65	72	73	78	74
chr19	7601263	7601503	PNPLA6	0,54	214	220	188	149	199	188	142	125	255	211	206	192	219	215	212	181
chr19	7604698	7605298	PNPLA6	0,65	16	11	11	12	19	17	14	12	17	13	17	9	15	14	18	14
chr19	7605435	7605675	PNPLA6	0,59	33	28	27	33	36	23	22	22	38	25	28	25	32	33	36	24
chr19	7605752	7605992	PNPLA6	0,71	7	6	5	6	9	6	3	6	10	6	6	8	5	6	8	6
chr19	7606164	7606644	PNPLA6	0,67	34	31	28	32	41	29	28	30	42	29	38	35	36	37	34	41
chr19	7606791	7607031	PNPLA6	0,61	22	14	12	21	20	18	11	15	30	18	22	26	17	18	20	20
chr19	7607372	7608068	PNPLA6	0,63	46	45	33	39	47	45	31	34	55	39	48	41	47	42	41	39
chr19	7614715	7615075	PNPLA6	0,63	58	54	42	42	61	45	35	37	63	44	53	46	50	62	50	56
chr19	7615114	7615594	PNPLA6	0,66	16	19	12	16	16	15	11	11	21	14	20	17	16	19	11	17
chr19	7615798	7616014	PNPLA6	0,69	8	6	1	2	7	5	5	5	10	6	6	6	4	4	8	6
chr19	7616165	7616405	PNPLA6	0,62	22	27	15	22	28	21	17	18	25	19	28	26	26	24	29	25
chr19	7618697	7619246	PNPLA6	0,60	22	22	17	19	22	22	13	18	29	22	25	20	21	19	23	22
chr19	7619344	7620049	PNPLA6	0,68	19	17	18	21	23	20	18	19	25	20	22	17	25	20	23	20
chr19	7620073	7620313	PNPLA6	0,69	9	10	6	15	14	14	8	8	16	13	11	9	12	13	13	15
chr19	7620390	7620750	PNPLA6	0,65	27	22	19	15	29	16	16	16	25	21	21	15	18	18	20	22
chr19	7621234	7621714	PNPLA6	0,60	140	146	117	98	138	121	103	84	159	140	150	130	152	132	140	111

chr19	7621991	7622231	PNPLA6	0,67	2	4	1	2	4	3	4	3	1	2	4	7	3	2	3	5
chr19	7623646	7624126	PNPLA6	0,60	73	66	55	64	71	66	50	40	79	68	76	59	71	69	65	68
chr19	7625471	7625711	PNPLA6	0,58	20	20	15	27	26	23	18	14	25	20	23	18	18	21	19	21
chr19	7625811	7626530	PNPLA6	0,66	27	21	18	20	24	22	15	17	28	16	25	22	25	23	21	23
chr2	127806035	127806275	BIN1	0,62	13	10	8	8	18	11	12	6	19	8	11	9	13	10	9	11
chr2	127807927	127808167	BIN1	0,66	21	23	24	27	24	23	17	13	29	19	30	29	23	31	24	25
chr2	127808313	127808553	BIN1	0,65	7	5	7	5	9	6	4	4	10	6	6	7	4	8	5	4
chr2	127808654	127808894	BIN1	0,67	3	2	1	3	8	2	2	2	6	5	3	4	4	3	5	2
chr2	127809764	127810004	BIN1	0,68	1	0	0	0	1	0	0	1	2	0	1	0	0	2	2	1
chr2	127810889	127811129	BIN1	0,63	18	17	14	13	27	20	14	14	28	20	25	23	24	18	20	19
chr2	127811414	127811654	BIN1	0,65	9	11	6	11	15	8	7	7	9	5	9	8	8	6	10	7
chr2	127814992	127815232	BIN1	0,68	8	4	5	6	8	4	3	6	10	6	9	5	6	6	9	6
chr2	127816637	127816829	BIN1	0,61	17	10	10	16	16	19	10	16	21	8	20	19	16	14	14	18
chr2	127818073	127818313	BIN1	0,62	44	40	32	33	52	37	25	38	59	36	37	35	40	38	29	39
chr2	127819611	127819851	BIN1	0,65	11	11	7	12	12	9	7	7	14	6	9	11	7	8	13	9
chr2	127821064	127821304	BIN1	0,61	42	41	30	34	46	29	27	29	44	30	45	38	38	39	38	41
chr2	127821431	127821671	BIN1	0,66	50	43	33	36	48	45	32	30	61	34	54	45	53	39	50	49
chr2	127825664	127825904	BIN1	0,59	26	29	25	22	32	24	21	19	33	22	30	31	25	24	26	28
chr2	127826433	127826673	BIN1	0,63	25	22	16	21	28	16	15	20	26	21	23	20	27	24	27	18
chr2	127827498	127827738	BIN1	0,63	21	32	18	20	23	32	17	13	25	24	28	19	23	29	19	18
chr2	127828055	127828484	BIN1	0,62	81	80	61	65	90	70	59	53	99	74	86	78	85	87	93	75
chr2	127834121	127834361	BIN1	0,52	180	169	146	119	174	151	126	104	212	167	193	146	183	166	177	136
chr2	127864357	127864597	BIN1	0,73	7	3	6	4	7	4	2	5	5	4	6	7	7	7	6	6
chr2	152342180	152342540	NEB	0,44	230	226	174	147	208	190	155	128	256	252	243	199	237	242	232	168
chr2	152346417	152346657	NEB	0,35	181	180	141	114	170	149	140	92	211	187	189	158	187	181	200	136
chr2	152346778	152347090	NEB	0,49	113	106	89	69	115	101	66	66	125	103	114	91	117	116	98	106
chr2	152348122	152348362	NEB	0,40	214	233	168	113	185	196	142	111	222	219	212	197	208	216	230	194
chr2	152348523	152349074	NEB	0,43	301	307	251	165	267	260	216	147	335	320	302	261	296	311	317	234
chr2	152349792	152350032	NEB	0,40	203	207	152	122	184	182	131	102	229	197	209	175	206	208	197	149
chr2	152350214	152350454	NEB	0,39	215	231	159	127	198	201	151	117	239	210	225	178	223	230	230	179
chr2	152350600	152350840	NEB	0,40	165	185	147	104	152	160	109	102	190	166	189	137	180	173	174	140
chr2	152352715	152352955	NEB	0,35	231	251	190	147	195	222	162	120	271	254	242	223	228	232	252	180
chr2	152353380	152353620	NEB	0,34	268	296	217	162	220	227	188	156	318	274	296	212	272	272	292	195
chr2	152354065	152354305	NEB	0,34	237	269	188	154	205	218	168	125	255	289	251	241	269	270	278	197
chr2	152354699	152354939	NEB	0,38	233	255	189	143	202	203	168	113	258	267	246	205	230	234	276	190
chr2	152355810	152356002	NEB	0,41	156	158	122	110	160	153	112	92	184	169	155	138	163	172	169	134
chr2	152357830	152358070	NEB	0,37	241	229	177	132	200	198	164	119	245	219	222	183	217	228	231	177
chr2	152359264	152359480	NEB	0,41	197	213	176	133	179	165	158	96	227	193	206	182	215	199	202	162
chr2	152359788	152360028	NEB	0,35	188	222	157	117	181	176	138	116	230	216	200	180	189	221	200	150
chr2	152361917	152362157	NEB	0,44	190	189	152	124	179	163	130	101	208	179	201	166	201	189	199	165
chr2	152362605	152362845	NEB	0,42	250	274	210	153	213	217	167	137	284	276	258	229	279	250	277	179
chr2	152363354	152363594	NEB	0,31	221	238	162	133	171	190	161	119	239	230	249	188	225	232	222	170
chr2	152364450	152364690	NEB	0,45	146	146	109	81	137	117	91	71	172	130	142	131	149	150	144	115
chr2	152369178	152369418	NEB	0,39	249	286	211	163	218	216	178	137	268	270	260	227	255	276	273	194
chr2	152370030	152370270	NEB	0,39	269	285	211	154	217	239	198	131	309	303	267	226	267	296	307	213
chr2	152370766	152371006	NEB	0,43	227	236	195	136	190	211	174	130	265	220	230	196	232	236	248	204
chr2	152371266	152371506	NEB	0,36	186	190	136	130	166	150	124	86	201	175	191	152	173	169	179	132
chr2	152372904	152373144	NEB	0,43	213	219	181	148	197	195	168	124	239	224	243	227	233	234	244	171
chr2	152374763	152375003	NEB	0,43	213	215	161	132	218	173	137	111	231	199	212	172	215	212	199	174
chr2	152375410	152375650	NEB	0,41	164	203	146	104	166	141	121	105	196	191	181	138	184	178	180	155
chr2	152376149	152376365	NEB	0,35	297	322	235	154	212	254	222	168	314	319	291	250	276	282	313	226
chr2	152380731	152381187	NEB	0,48	223	226	169	152	221	201	164	125	260	215	230	196	218	254	226	197
chr2	152381611	152381851	NEB	0,43	190	233	154	139	184	200	149	122	242	196	217	193	201	215	224	179
chr2	152382383	152382863	NEB	0,50	194	189	164	130	183	168	141	107	225	195	203	185	197	209	193	164
chr2	152383363	152383603	NEB	0,47	163	147	116	92	138	129	106	86	172	145	143	132	151	144	155	119
chr2	152383926	152384166	NEB	0,44	91	116	87	78	107	95	70	63	122	101	112	96	95	98	97	91
chr2	152385655	152385895	NEB	0,41	198	203	152	115	163	161	134	109	214	198	205	157	198	199	186	161
chr2	152387441	152387681	NEB	0,39	220	228	173	138	193	193	161	124	238	223	222	201	208	222	243	175
chr2	152388268	152388484	NEB	0,36	201	220	164	121	184	174	143	104	238	195	208	174	210	209	217	160
chr2	152389887	152390127	NEB	0,41	279	301	225	169	239	255	188	156	332	342	311	241	301	299	324	221

chr2	152390660	152390900	NEB	0,49	187	205	145	125	169	169	124	112	238	201	219	168	196	199	211	163
chr2	152392136	152392376	NEB	0,34	262	298	227	159	223	234	196	149	291	300	287	248	263	302	307	224
chr2	152393577	152393817	NEB	0,39	208	238	167	130	177	208	174	122	259	211	239	200	211	223	257	170
chr2	152394317	152394557	NEB	0,38	167	179	137	99	143	166	126	101	219	170	188	149	184	179	176	159
chr2	152394585	152394825	NEB	0,34	210	224	183	140	180	206	160	114	255	227	241	192	225	223	230	169
chr2	152396789	152397029	NEB	0,42	172	169	142	116	174	152	115	87	183	167	181	161	180	174	151	137
chr2	152397142	152397382	NEB	0,42	176	196	133	112	174	144	127	88	200	162	186	150	185	180	167	130
chr2	152397892	152398132	NEB	0,47	181	200	146	112	175	152	130	115	197	193	220	159	196	182	190	138
chr2	152402336	152402576	NEB	0,34	272	285	211	159	218	218	193	142	277	251	255	229	263	268	264	209
chr2	152402787	152403027	NEB	0,51	84	80	58	56	98	86	58	50	101	88	96	71	86	90	82	69
chr2	152403857	152404337	NEB	0,43	268	265	204	161	204	216	185	131	271	271	258	230	280	277	266	204
chr2	152404755	152404995	NEB	0,42	179	182	134	102	189	182	110	104	194	174	168	157	181	173	190	131
chr2	152406081	152406321	NEB	0,46	93	97	67	59	103	80	68	56	123	86	105	83	95	102	99	89
chr2	152408185	152408425	NEB	0,49	114	99	74	65	112	99	76	56	118	91	103	90	118	106	91	96
chr2	152409117	152409357	NEB	0,45	255	266	195	141	197	212	152	138	317	257	270	216	264	263	277	186
chr2	152409843	152410083	NEB	0,37	220	204	161	128	200	180	136	115	229	190	207	167	202	195	221	147
chr2	152410260	152410620	NEB	0,49	182	204	146	105	177	160	126	95	207	194	189	169	168	182	193	147
chr2	152411426	152411642	NEB	0,38	206	235	178	140	195	193	158	101	254	230	229	184	228	221	230	208
chr2	152417039	152417279	NEB	0,51	76	73	76	63	92	95	58	64	113	74	94	71	89	87	88	63
chr2	152417431	152417911	NEB	0,39	249	247	188	140	197	211	168	130	262	235	239	196	248	247	244	198
chr2	152418555	152418795	NEB	0,43	214	235	183	135	213	189	158	125	244	222	228	205	200	231	238	171
chr2	152419043	152419403	NEB	0,43	178	181	133	110	141	158	127	97	190	174	175	144	178	177	182	143
chr2	152420056	152420510	NEB	0,45	237	255	200	138	219	225	170	127	277	252	245	217	248	249	264	194
chr2	152421475	152421691	NEB	0,32	222	236	181	138	180	182	157	129	254	219	248	203	206	199	213	173
chr2	152421946	152422401	NEB	0,44	248	252	181	136	212	200	159	113	280	229	243	211	247	244	252	188
chr2	152423597	152424077	NEB	0,43	300	340	254	163	249	249	199	148	330	311	319	260	310	304	340	227
chr2	152424518	152424758	NEB	0,47	167	169	131	115	176	157	117	97	197	167	184	146	178	195	179	155
chr2	152424760	152425000	NEB	0,46	158	165	128	99	149	142	123	90	179	152	169	120	154	162	148	141
chr2	152425060	152425300	NEB	0,37	272	298	234	168	220	234	196	148	324	309	300	245	303	291	334	217
chr2	152425713	152425953	NEB	0,45	147	152	112	87	126	124	98	74	148	140	136	127	157	129	126	113
chr2	152426510	152427182	NEB	0,50	140	137	97	91	139	118	90	82	154	133	147	121	141	148	136	121
chr2	152432140	152432380	NEB	0,37	217	267	200	141	194	208	176	125	260	244	251	205	239	252	256	184
chr2	152432586	152432946	NEB	0,39	217	225	183	148	193	205	160	119	238	213	225	209	227	234	240	174
chr2	152435767	152436247	NEB	0,48	78	68	45	48	75	74	76	44	81	110	81	88	76	115	72	91
chr2	152437244	152437484	NEB	0,47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chr2	152437928	152438168	NEB	0,41	54	54	39	31	47	51	40	26	55	60	68	45	47	61	69	43
chr2	152438899	152439259	NEB	0,44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chr2	152439884	152440364	NEB	0,42	8	0	12	8	5	21	10	7	7	18	15	12	15	22	10	13
chr2	152441954	152442194	NEB	0,39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chr2	152442850	152443090	NEB	0,41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chr2	152443800	152444136	NEB	0,45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chr2	152446318	152446798	NEB	0,48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chr2	152447795	152448035	NEB	0,47	187	256	217	161	200	203	169	136	259	226	236	223	237	246	225	190
chr2	152448479	152448719	NEB	0,41	346	317	314	256	343	324	264	226	379	383	383	350	346	365	361	306
chr2	152449451	152449811	NEB	0,44	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0
chr2	152450436	152450916	NEB	0,42	35	14	82	42	91	69	35	34	59	55	108	40	62	72	68	40
chr2	152452507	152452747	NEB	0,39	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chr2	152453403	152453643	NEB	0,41	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chr2	152454353	152454689	NEB	0,45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chr2	152456871	152457351	NEB	0,48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chr2	152458348	152458588	NEB	0,47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chr2	152459032	152459272	NEB	0,41	2	3	0	0	0	0	0	0	1	0	0	0	2	2	1	0
chr2	152460004	152460364	NEB	0,44	401	425	268	152	261	232	166	135	328	283	262	237	276	323	355	178
chr2	152460989	152461469	NEB	0,42	20	29	0	4	6	0	9	2	14	11	7	12	11	15	13	12
chr2	152463053	152463293	NEB	0,39	98	14	0	61	60	75	133	57	15	204	88	145	95	105	11	131
chr2	152463949	152464189	NEB	0,42	68	0	0	30	53	50	91	23	4	129	68	103	69	72	0	81
chr2	152464899	152465235	NEB	0,45	0	0	0	0	0	0	0	0	1	0	1	0	1	0	0	0
chr2	152466238	152466718	NEB	0,43	332	354	262	190	276	271	206	161	350	349	316	287	335	339	368	248
chr2	152466963	152467203	NEB	0,47	219	234	178	137	200	193	152	116	242	217	238	185	231	231	242	176
chr2	152467207	152467447	NEB	0,45	161	172	131	104	153	151	118	86	182	163	167	151	157	170	151	141

chr2	152468621	152468981	NEB	0,44	252	250	195	149	216	225	176	121	279	263	281	228	276	277	270	193
chr2	152470730	152471162	NEB	0,46	248	275	219	147	222	230	187	123	312	294	275	221	292	260	262	200
chr2	152472449	152472689	NEB	0,37	288	289	228	163	247	235	182	134	315	307	275	244	296	296	300	202
chr2	152473809	152474049	NEB	0,34	194	234	157	118	175	164	147	101	230	173	206	180	186	191	199	162
chr2	152474710	152475070	NEB	0,37	308	319	243	148	240	245	201	133	327	310	307	244	302	287	335	230
chr2	152475880	152476360	NEB	0,46	228	245	186	133	216	201	160	113	256	241	237	198	237	269	249	194
chr2	152477368	152477608	NEB	0,44	146	155	120	102	164	137	96	78	191	151	161	139	171	154	162	131
chr2	152481979	152482219	NEB	0,35	246	274	185	131	211	197	175	127	281	231	236	214	236	243	254	176
chr2	152483437	152483797	NEB	0,41	274	262	209	157	218	215	187	136	261	258	250	215	267	264	266	192
chr2	152483952	152484408	NEB	0,47	283	291	247	152	237	230	194	141	308	287	277	232	274	289	305	218
chr2	152485986	152486226	NEB	0,41	193	234	171	124	177	163	144	97	227	220	193	179	202	205	215	151
chr2	152487160	152487400	NEB	0,36	299	291	230	153	228	232	202	133	305	300	280	245	278	284	283	200
chr2	152487578	152487938	NEB	0,42	263	285	215	169	249	243	199	146	298	294	264	237	280	279	301	219
chr2	152490083	152490563	NEB	0,45	279	307	237	171	246	248	194	142	303	299	288	256	275	281	308	229
chr2	152492674	152492914	NEB	0,42	221	227	187	126	189	199	150	107	247	222	217	207	220	232	223	183
chr2	152495725	152495965	NEB	0,37	238	271	217	152	187	206	181	137	255	229	249	207	262	245	253	200
chr2	152496292	152496652	NEB	0,38	261	291	209	151	211	229	191	137	289	287	262	239	272	275	280	211
chr2	152496784	152497264	NEB	0,48	270	256	193	151	206	216	188	128	267	270	258	216	255	263	269	194
chr2	152498995	152499475	NEB	0,37	298	312	249	174	250	251	205	138	307	301	321	263	317	321	311	230
chr2	152499585	152499945	NEB	0,34	267	301	212	162	234	226	184	125	276	266	283	226	280	273	294	215
chr2	152500247	152500727	NEB	0,48	293	304	244	160	241	256	196	162	312	301	290	256	285	297	319	221
chr2	152500915	152501155	NEB	0,37	279	287	241	164	226	233	183	145	308	285	277	222	295	302	280	224
chr2	152502575	152502815	NEB	0,33	280	310	215	163	217	227	178	141	289	283	273	231	284	283	282	196
chr2	152506648	152506984	NEB	0,42	316	347	277	168	277	270	221	166	338	344	328	288	317	320	330	243
chr2	152507003	152507483	NEB	0,39	389	381	297	196	291	314	254	184	383	379	365	318	377	374	401	278
chr2	152510439	152510679	NEB	0,34	250	274	208	154	196	222	202	130	264	270	259	222	260	281	278	187
chr2	152511764	152511980	NEB	0,30	226	252	192	133	168	200	166	136	267	230	244	184	227	213	229	179
chr2	152512253	152513062	NEB	0,43	329	343	276	177	269	278	224	165	346	341	322	277	341	332	360	246
chr2	152514430	152514670	NEB	0,41	238	222	195	139	204	203	161	122	255	221	229	229	229	247	235	201
chr2	152515510	152515750	NEB	0,36	243	265	230	139	213	208	167	124	280	233	246	224	254	239	254	186
chr2	152518571	152518931	NEB	0,39	318	343	252	171	278	257	218	162	343	338	332	287	331	329	331	226
chr2	152519977	152520457	NEB	0,45	236	235	192	144	204	185	163	121	250	243	237	196	246	230	250	189
chr2	152520948	152521188	NEB	0,43	292	326	235	180	248	238	213	160	311	300	285	257	290	307	342	232
chr2	152521204	152521444	NEB	0,43	227	238	183	126	194	198	162	116	254	253	224	199	228	232	263	180
chr2	152521769	152522129	NEB	0,47	288	307	249	174	237	239	204	143	319	319	268	240	308	296	323	225
chr2	152522519	152522999	NEB	0,44	236	250	182	137	194	199	179	117	258	236	238	212	238	225	251	173
chr2	152524254	152524494	NEB	0,35	303	320	258	162	224	259	206	158	312	314	290	259	308	302	318	220
chr2	152525472	152525712	NEB	0,41	167	166	135	107	161	167	124	90	193	160	179	159	180	161	164	134
chr2	152527459	152527819	NEB	0,45	269	283	222	149	221	228	186	131	302	276	284	236	282	283	295	208
chr2	152528798	152529278	NEB	0,43	268	304	235	160	240	235	204	149	308	291	281	246	293	280	296	221
chr2	152530924	152531164	NEB	0,41	184	198	174	137	207	187	159	114	228	203	214	176	210	215	212	152
chr2	152531760	152531976	NEB	0,36	269	290	206	143	224	229	189	130	303	278	304	246	270	264	272	221
chr2	152534001	152534785	NEB	0,42	349	373	290	192	276	290	229	169	363	369	355	303	359	351	377	258
chr2	152536145	152536625	NEB	0,43	226	226	177	154	195	178	144	123	245	227	224	199	219	225	239	168
chr2	152537172	152537412	NEB	0,33	233	270	182	132	195	226	178	132	263	256	259	213	235	271	268	192
chr2	152539109	152539349	NEB	0,43	278	306	241	154	228	228	200	155	319	279	299	246	297	287	305	217
chr2	152541210	152541570	NEB	0,40	219	232	185	130	208	189	167	125	280	232	234	201	222	249	234	180
chr2	152543849	152544329	NEB	0,44	250	271	202	147	205	232	177	129	279	252	251	215	263	251	271	192
chr2	152544737	152544977	NEB	0,36	304	343	231	164	226	254	216	163	324	302	298	253	285	306	305	219
chr2	152547169	152547409	NEB	0,37	205	204	175	135	190	191	147	102	251	209	224	191	219	199	226	172
chr2	152548326	152548926	NEB	0,35	365	392	297	196	282	286	248	171	387	384	349	302	344	360	393	268
chr2	152550749	152551229	NEB	0,44	248	283	204	155	214	223	187	136	289	269	253	217	270	247	266	202
chr2	152552023	152552263	NEB	0,37	228	243	192	132	198	198	156	121	279	243	247	204	239	243	255	168
chr2	152553079	152553319	NEB	0,44	172	163	132	101	146	141	120	91	177	159	159	136	166	147	167	126
chr2	152553551	152554271	NEB	0,36	332	365	289	188	266	295	246	171	375	358	336	291	352	358	365	256
chr2	152563332	152563572	NEB	0,37	196	216	165	130	172	173	148	116	220	210	207	180	205	216	197	165
chr2	152566103	152566343	NEB	0,38	240	252	190	137	208	207	162	123	292	254	246	215	238	242	262	217
chr2	152566879	152567119	NEB	0,37	277	281	232	154	222	244	203	143	307	278	272	231	283	286	288	224
chr2	152573861	152574101	NEB	0,39	229	225	163	134	192	193	155	104	241	228	230	177	213	222	206	174
chr2	152579827	152580067	NEB	0,43	223	226	163	137	202	187	136	108	231	215	229	184	236	214	213	160

chr2	152580705	152580945	NEB	0,46	120	119	74	76	114	98	80	63	128	102	123	96	105	104	107	107
chr2	152581302	152581542	NEB	0,40	345	361	279	189	292	295	243	168	363	376	355	274	356	365	363	269
chr2	152581900	152582140	NEB	0,41	280	288	218	166	222	218	175	139	288	278	258	233	252	251	295	177
chr2	152584132	152584492	NEB	0,40	238	252	184	139	216	217	173	118	268	240	274	201	240	241	260	188
chr2	152586029	152586269	NEB	0,49	151	146	110	94	142	128	99	84	167	154	147	142	162	141	144	123
chr2	152589532	152589772	NEB	0,32	308	375	280	174	265	277	218	168	356	318	329	281	321	339	339	259
chr2	152695522	152696002	CACNB4	0,40	250	233	198	145	217	215	177	117	265	240	247	219	245	240	259	184
chr2	152698329	152698689	CACNB4	0,43	227	240	165	142	204	180	151	129	234	201	245	181	222	206	203	175
chr2	152709889	152710129	CACNB4	0,36	256	274	204	161	219	228	168	134	278	262	248	225	256	258	267	193
chr2	152711633	152711993	CACNB4	0,34	254	286	212	160	218	229	186	124	298	271	264	242	270	277	279	210
chr2	152717159	152717399	CACNB4	0,43	144	167	131	99	149	138	114	97	175	172	165	154	162	150	177	132
chr2	152725599	152725839	CACNB4	0,39	207	216	157	128	184	164	154	109	255	233	224	180	234	208	233	177
chr2	152726964	152727204	CACNB4	0,49	134	123	101	92	142	129	93	76	166	124	138	113	128	124	129	109
chr2	152727305	152727425	CACNB4	0,31	177	173	129	114	160	148	120	96	186	171	177	141	182	150	168	126
chr2	152728848	152729088	CACNB4	0,37	243	269	202	152	209	204	155	131	278	251	254	215	255	234	247	186
chr2	152732884	152733124	CACNB4	0,38	287	304	220	163	236	241	198	148	296	283	276	247	260	286	297	212
chr2	152737254	152737494	CACNB4	0,46	41	49	42	33	55	49	32	27	54	45	60	40	42	51	54	39
chr2	152739704	152739944	CACNB4	0,48	150	141	120	99	134	130	101	81	169	134	155	135	159	163	164	101
chr2	152830157	152830277	CACNB4	0,38	93	96	77	61	112	96	64	55	105	89	105	81	97	79	83	93
chr2	152954765	152955005	CACNB4	0,69	37	43	23	33	41	36	29	20	53	30	36	28	36	43	38	33
chr2	152955098	152955218	CACNB4	0,61	0	0	1	2	1	1	0	0	1	0	3	2	1	0	1	1
chr2	152955455	152955575	CACNB4	0,73	1	1	0	0	1	0	0	2	0	0	2	0	3	1	0	0
chr2	158593989	158594229	ACVR1	0,40	194	197	147	102	176	159	127	96	204	187	197	142	187	171	182	142
chr2	158594896	158595136	ACVR1	0,44	249	241	191	142	220	225	156	117	249	242	246	209	241	238	250	174
chr2	158617310	158617670	ACVR1	0,45	206	215	163	118	194	177	136	107	218	194	208	181	223	209	232	155
chr2	158622354	158622810	ACVR1	0,38	261	280	213	144	219	215	179	129	282	252	273	227	273	282	272	197
chr2	158626772	158627132	ACVR1	0,44	271	265	219	152	225	248	177	148	292	280	278	253	280	278	284	206
chr2	158630529	158630769	ACVR1	0,39	200	222	171	129	183	185	131	117	219	229	198	168	218	215	205	160
chr2	158634568	158634928	ACVR1	0,46	187	195	143	123	176	171	124	102	227	187	178	159	193	205	200	153
chr2	158636740	158637220	ACVR1	0,49	135	130	99	92	137	113	95	79	165	121	141	125	146	136	146	111
chr2	158655851	158656091	ACVR1	0,36	235	238	211	148	191	183	160	119	260	242	241	214	234	237	251	183
chr2	175612797	175613037	CHRNA1	0,51	112	113	96	85	142	88	80	78	134	116	131	106	128	109	136	106
chr2	175613287	175613599	CHRNA1	0,43	183	197	146	119	154	148	119	96	190	180	182	177	180	179	192	159
chr2	175614605	175614965	CHRNA1	0,52	98	91	73	70	87	77	73	50	112	79	101	80	103	101	87	84
chr2	175618169	175618529	CHRNA1	0,58	40	39	30	23	38	31	24	21	42	31	38	28	40	45	36	32
chr2	175618864	175619224	CHRNA1	0,49	136	139	106	79	122	114	94	73	142	124	139	120	120	125	133	108
chr2	175622228	175622468	CHRNA1	0,41	195	200	143	121	180	171	137	121	229	192	195	193	201	194	214	163
chr2	175623960	175624468	CHRNA1	0,51	145	148	117	99	130	142	111	81	168	143	149	127	149	148	154	117
chr2	175628980	175629220	CHRNA1	0,58	47	43	40	43	53	40	34	26	55	46	43	46	51	55	47	47
chr2	176981453	176982413	HOXD10	0,55	199	192	167	125	188	165	139	111	222	200	199	179	209	202	215	159
chr2	176983643	176983955	HOXD10	0,46	121	133	93	96	139	110	91	77	138	120	129	114	132	122	130	103
chr2	179391646	179392563	TTN	0,42	292	313	245	170	244	259	220	145	332	318	283	250	294	308	323	240
chr2	179392933	179394013	TTN	0,40	325	332	262	187	264	278	239	171	345	331	324	283	340	321	350	245
chr2	179394584	179401365	TTN	0,43	340	359	281	188	276	287	243	172	364	358	340	294	354	356	374	261
chr2	179401614	179402694	TTN	0,42	308	325	257	176	250	262	218	157	358	315	313	260	312	308	330	230
chr2	179403176	179405185	TTN	0,43	328	337	263	180	267	275	232	159	340	343	328	285	328	339	355	246
chr2	179405983	179406415	TTN	0,44	190	207	135	111	173	146	129	92	219	189	190	172	196	183	194	150
chr2	179406913	179408452	TTN	0,42	304	333	252	178	249	266	219	159	328	320	310	257	309	321	330	236
chr2	179408476	179409316	TTN	0,41	300	323	245	171	244	260	222	156	341	324	304	266	317	309	328	237
chr2	179410027	179415096	TTN	0,43	342	351	276	189	270	284	233	163	356	352	335	286	342	349	360	253
chr2	179415600	179416080	TTN	0,40	284	300	227	164	247	231	181	139	305	291	284	240	268	279	297	208
chr2	179416279	179419031	TTN	0,41	344	366	280	197	287	284	247	165	369	360	345	298	354	357	378	256
chr2	179419089	179419975	TTN	0,40	299	318	237	171	252	249	213	145	327	290	300	253	305	290	320	215
chr2	179421482	179423455	TTN	0,40	330	351	268	181	270	274	229	165	351	338	330	280	340	347	352	249
chr2	179423950	179442983	TTN	0,43	356	370	294	195	291	296	249	170	378	374	352	304	357	364	386	268
chr2	179443248	179444208	TTN	0,42	331	344	253	180	279	271	231	158	361	335	325	276	341	331	351	242
chr2	179444211	179445411	TTN	0,40	335	364	280	190	286	294	248	164	367	366	353	292	358	358	369	252
chr2	179446138	179447366	TTN	0,44	264	268	208	154	240	223	190	136	292	269	260	230	265	271	279	210
chr2	179447570	179448050	TTN	0,38	279	297	232	159	249	234	187	148	301	303	284	241	299	284	298	223
chr2	179448243	179448723	TTN	0,41	254	248	198	154	218	211	173	140	281	236	245	219	258	253	256	188

chr2	179448928	179450176	TTN	0,43	309	324	258	183	253	268	219	149	325	323	306	258	322	313	338	238
chr2	179451142	179451622	TTN	0,41	296	298	235	163	238	246	208	145	315	291	291	260	300	303	316	217
chr2	179451735	179453055	TTN	0,42	284	299	235	173	244	245	206	151	314	292	294	247	287	277	307	224
chr2	179453214	179459461	TTN	0,41	362	383	303	202	295	305	251	175	389	389	366	310	371	380	398	272
chr2	179460144	179460624	TTN	0,38	298	318	245	165	258	241	201	150	310	297	292	244	279	281	303	231
chr2	179462164	179462884	TTN	0,39	365	395	298	196	285	293	258	169	383	394	365	314	367	379	385	274
chr2	179463162	179464668	TTN	0,40	309	325	265	179	261	269	223	154	338	316	311	271	330	325	331	246
chr2	179465516	179466596	TTN	0,41	325	349	267	187	272	279	229	160	345	343	321	281	338	337	358	258
chr2	179466611	179467402	TTN	0,39	316	326	252	171	259	255	221	146	328	313	311	277	305	316	331	235
chr2	179468517	179469117	TTN	0,41	300	350	261	187	252	265	226	155	354	336	319	278	334	316	353	244
chr2	179469368	179470530	TTN	0,40	319	331	251	176	268	268	228	164	351	329	301	267	319	329	345	240
chr2	179471695	179473723	TTN	0,40	349	367	286	197	285	289	249	168	374	374	348	289	366	367	383	266
chr2	179473875	179475206	TTN	0,40	344	361	282	199	273	288	230	165	370	373	345	293	348	348	372	265
chr2	179475660	179478111	TTN	0,41	350	368	290	188	294	300	245	166	382	371	350	299	359	368	386	270
chr2	179478390	179479681	TTN	0,41	316	328	266	173	257	270	218	160	332	337	315	276	318	332	347	239
chr2	179479942	179480621	TTN	0,40	356	381	278	187	287	296	259	188	388	378	351	292	365	371	386	269
chr2	179481112	179482342	TTN	0,38	357	373	295	194	280	294	244	165	374	372	338	298	352	357	376	253
chr2	179482416	179483667	TTN	0,40	358	396	313	200	286	304	268	179	389	395	359	308	366	381	402	278
chr2	179484293	179486828	TTN	0,37	400	422	334	207	299	326	266	191	410	415	395	337	401	406	434	290
chr2	179487325	179487565	TTN	0,37	205	225	175	123	182	196	140	107	235	213	226	193	221	238	217	150
chr2	179489084	179489564	TTN	0,42	303	322	246	173	260	250	217	148	334	325	299	271	307	320	320	225
chr2	179489941	179490181	TTN	0,37	290	300	241	146	233	242	213	155	316	309	306	253	283	295	316	221
chr2	179493918	179494278	TTN	0,43	184	182	140	113	167	167	130	95	222	192	199	177	212	198	210	158
chr2	179494910	179495150	TTN	0,36	169	182	143	107	154	147	121	82	210	170	173	152	162	156	160	137
chr2	179495478	179496078	TTN	0,42	325	350	269	189	261	273	224	162	365	350	325	273	332	351	361	240
chr2	179496766	179497827	TTN	0,39	315	327	252	179	252	274	212	157	333	332	309	266	322	323	339	243
chr2	179497858	179498916	TTN	0,39	355	358	293	189	289	301	248	176	383	365	350	305	354	375	397	270
chr2	179499034	179499634	TTN	0,40	267	280	210	159	222	230	182	135	295	261	265	225	267	276	280	207
chr2	179499841	179500544	TTN	0,36	336	343	268	178	271	283	241	157	359	350	339	288	344	340	367	246
chr2	179500588	179501625	TTN	0,41	302	305	239	165	270	268	211	150	329	319	303	258	311	313	310	237
chr2	179502000	179502240	TTN	0,34	213	243	190	144	201	202	159	136	258	238	250	223	261	255	257	186
chr2	179504350	179504590	TTN	0,39	176	180	133	122	179	153	137	88	211	166	193	155	200	178	174	135
chr2	179504685	179504925	TTN	0,38	174	175	131	103	151	145	122	99	193	155	181	140	183	186	174	131
chr2	179505192	179505432	TTN	0,31	242	267	211	125	178	226	186	126	280	231	254	223	241	238	248	192
chr2	179505884	179506124	TTN	0,28	209	261	197	138	148	200	179	121	251	241	239	207	245	247	236	174
chr2	179506883	179507123	TTN	0,32	265	282	220	151	195	220	196	136	284	286	281	231	265	276	287	197
chr2	179509188	179509428	TTN	0,35	214	225	178	130	208	190	155	110	238	223	241	191	240	222	227	173
chr2	179510581	179510821	TTN	0,38	136	140	96	89	138	125	88	81	144	146	140	105	137	135	132	98
chr2	179514462	179514702	TTN	0,33	225	266	197	134	201	222	168	135	280	246	261	208	252	245	272	192
chr2	179514824	179515064	TTN	0,37	257	271	189	149	222	229	168	118	253	258	244	211	222	248	248	191
chr2	179515399	179515639	TTN	0,39	234	239	202	139	191	203	162	118	249	243	247	212	248	247	233	191
chr2	179515888	179517346	TTN	0,41	271	287	220	156	235	228	189	135	290	257	277	236	267	281	277	211
chr2	179523676	179524036	TTN	0,40	8	17	12	11	21	21	16	10	19	22	16	12	14	39	18	19
chr2	179527617	179527857	TTN	0,47	89	111	83	68	121	85	69	66	135	113	113	88	114	115	103	108
chr2	179534845	179535037	TTN	0,37	210	238	206	144	165	193	174	118	260	219	256	215	230	215	227	163
chr2	179535736	179535976	TTN	0,36	177	201	145	108	151	158	142	97	201	180	213	168	185	194	187	145
chr2	179536641	179536881	TTN	0,33	263	329	267	178	237	256	217	159	351	338	324	268	326	314	341	248
chr2	179536938	179537515	TTN	0,35	321	344	260	172	243	273	240	150	341	351	329	274	332	344	358	247
chr2	179538278	179538518	TTN	0,34	192	218	160	130	172	156	145	103	215	204	207	177	207	204	210	144
chr2	179538943	179539135	TTN	0,40	209	244	162	141	182	189	148	109	224	214	223	186	222	220	200	162
chr2	179539682	179539922	TTN	0,33	243	289	202	148	200	240	183	129	273	270	280	214	276	246	282	207
chr2	179540564	179540804	TTN	0,38	277	314	239	154	242	247	197	150	310	289	282	251	308	280	300	222
chr2	179542255	179542735	TTN	0,40	382	394	336	209	300	326	261	172	413	419	407	333	409	417	420	307
chr2	179542773	179543013	TTN	0,34	304	314	242	167	252	257	219	140	317	301	315	253	278	306	296	245
chr2	179543062	179543302	TTN	0,38	297	300	234	161	227	225	206	138	288	289	284	235	270	267	285	205
chr2	179543395	179543635	TTN	0,37	277	293	226	159	232	221	186	142	281	308	261	225	280	261	277	214
chr2	179543984	179544224	TTN	0,38	252	266	207	148	220	220	175	139	283	265	252	213	257	272	253	193
chr2	179544247	179544487	TTN	0,39	298	289	229	161	253	234	219	142	302	295	296	254	280	306	307	232
chr2	179544521	179544881	TTN	0,41	374	398	311	205	296	300	274	182	398	395	388	325	379	403	424	302
chr2	179544899	179545139	TTN	0,40	311	314	252	165	248	254	224	159	352	325	309	253	327	314	316	237

chr2	179545731	179545971	TTN	0,31	292	309	240	165	229	271	222	152	317	292	316	249	305	291	320	228
chr2	179546019	179546259	TTN	0,36	298	309	241	174	239	270	205	170	330	315	311	251	302	319	334	231
chr2	179546306	179546546	TTN	0,34	251	267	191	129	202	221	179	118	259	232	239	213	230	246	264	192
chr2	179547371	179547707	TTN	0,36	346	399	294	195	254	293	264	188	400	395	366	304	381	386	402	269
chr2	179547857	179548097	TTN	0,33	214	231	194	142	166	188	165	123	248	244	230	203	233	244	235	197
chr2	179548647	179548887	TTN	0,37	237	258	187	132	187	201	162	139	253	247	262	209	250	227	235	173
chr2	179548978	179549218	TTN	0,39	255	240	186	140	199	202	164	123	261	244	223	182	239	244	213	183
chr2	179549314	179549794	TTN	0,37	252	288	222	154	216	242	194	141	295	285	284	232	260	282	277	208
chr2	179549898	179550138	TTN	0,31	244	283	217	167	192	223	197	136	311	288	273	244	276	272	285	203
chr2	179550164	179550404	TTN	0,34	311	342	256	175	224	266	212	152	342	328	320	260	347	313	358	242
chr2	179552774	179553014	TTN	0,38	366	395	320	200	270	301	266	186	405	393	349	331	404	398	445	267
chr2	179553334	179553574	TTN	0,36	220	256	189	141	187	200	166	89	272	236	208	190	226	233	246	166
chr2	179553737	179554401	TTN	0,38	255	265	204	154	213	229	179	135	290	270	253	226	259	262	282	195
chr2	179554461	179554701	TTN	0,37	258	263	208	152	222	230	191	145	298	270	275	216	255	270	265	199
chr2	179556664	179556904	TTN	0,43	159	171	131	109	163	157	113	93	177	163	170	147	176	183	165	142
chr2	179557145	179557385	TTN	0,36	168	173	129	107	169	151	111	94	219	178	190	135	181	166	177	149
chr2	179558255	179558495	TTN	0,28	307	331	248	174	275	275	221	149	331	303	307	264	307	294	326	228
chr2	179558571	179558811	TTN	0,37	333	380	293	181	270	288	233	169	387	377	361	293	327	345	404	263
chr2	179559244	179559690	TTN	0,29	298	305	229	159	184	252	184	153	310	289	305	235	285	281	301	225
chr2	179559985	179560225	TTN	0,34	269	307	213	141	173	233	205	146	307	296	269	258	282	288	284	197
chr2	179560530	179560650	TTN	0,36	163	206	137	106	148	186	115	94	209	199	190	163	191	199	167	141
chr2	179560783	179561095	TTN	0,34	367	419	299	198	279	309	262	163	382	373	345	308	378	366	395	288
chr2	179561751	179561991	TTN	0,29	191	209	148	104	157	190	143	102	228	190	186	166	198	198	189	163
chr2	179563461	179563653	TTN	0,34	282	282	225	160	231	241	210	164	314	302	297	246	265	300	274	223
chr2	179565768	179566008	TTN	0,37	132	135	109	81	144	117	98	64	149	125	145	122	133	140	122	123
chr2	179566164	179566404	TTN	0,42	165	172	118	110	127	142	117	85	173	146	153	128	162	152	149	130
chr2	179566659	179567053	TTN	0,38	277	299	224	155	226	216	194	137	288	268	255	231	277	284	303	205
chr2	179567105	179567465	TTN	0,41	263	305	222	155	230	213	194	151	307	273	266	243	277	273	276	224
chr2	179568763	179569764	TTN	0,38	365	390	297	194	280	306	243	178	389	392	352	306	371	375	395	260
chr2	179569812	179570172	TTN	0,33	325	350	282	182	264	264	243	168	358	335	329	293	327	321	370	242
chr2	179571142	179571754	TTN	0,38	297	319	259	167	246	264	205	152	333	320	315	269	310	308	338	229
chr2	179572156	179572636	TTN	0,41	286	310	242	161	249	260	192	148	312	313	296	260	309	311	320	225
chr2	179574197	179574677	TTN	0,40	257	266	215	150	208	223	185	136	271	270	251	216	269	275	275	201
chr2	179575265	179576172	TTN	0,40	280	294	235	161	253	234	197	141	318	284	285	243	293	287	303	213
chr2	179576575	179578114	TTN	0,39	330	350	278	178	263	287	239	160	355	349	345	291	342	352	371	251
chr2	179578522	179579399	TTN	0,40	341	348	276	188	276	270	231	164	366	342	319	293	335	345	361	235
chr2	179579611	179580091	TTN	0,43	233	253	188	137	208	205	178	128	257	236	222	209	252	257	247	193
chr2	179580120	179580600	TTN	0,42	257	295	215	153	224	243	179	132	296	289	268	242	301	307	313	221
chr2	179581725	179583784	TTN	0,42	313	332	260	176	267	273	221	159	334	337	314	266	330	334	345	237
chr2	179583794	179585490	TTN	0,42	297	321	242	173	248	262	210	150	317	309	300	257	305	313	320	225
chr2	179585548	179586028	TTN	0,37	302	316	255	168	244	270	202	161	320	328	311	278	305	308	329	233
chr2	179586477	179589365	TTN	0,40	352	372	281	181	280	287	243	169	374	374	349	299	359	366	384	263
chr2	179590053	179590873	TTN	0,40	329	368	277	183	273	271	231	166	352	361	325	275	339	347	343	248
chr2	179591717	179592197	TTN	0,39	304	298	213	151	228	239	193	152	304	285	276	236	299	289	296	217
chr2	179592210	179592690	TTN	0,37	318	347	257	181	261	274	230	154	339	339	318	271	330	357	362	257
chr2	179592740	179598712	TTN	0,41	334	343	272	187	277	280	236	164	358	348	336	290	347	347	363	252
chr2	179598964	179599804	TTN	0,38	317	327	257	172	267	257	231	153	333	333	297	264	323	326	347	234
chr2	179600159	179600879	TTN	0,42	286	305	242	163	252	239	198	144	321	315	296	251	289	298	308	216
chr2	179602707	179603187	TTN	0,44	205	209	160	135	191	184	146	116	237	213	209	182	212	208	213	164
chr2	179603801	179606681	TTN	0,42	347	358	281	184	284	293	247	173	370	371	351	297	355	362	381	263
chr2	179610238	179616838	TTN	0,38	383	412	321	202	298	325	270	183	409	415	380	327	401	402	430	287
chr2	179617758	179617998	TTN	0,28	254	285	204	159	184	225	171	133	295	279	278	216	285	256	283	202
chr2	179620876	179621596	TTN	0,41	285	314	243	173	246	252	199	148	307	300	294	256	313	285	314	244
chr2	179622215	179622695	TTN	0,44	258	263	205	144	203	216	162	135	272	255	261	213	260	260	256	204
chr2	179623624	179623984	TTN	0,36	291	306	270	180	239	246	221	151	326	312	320	251	318	313	327	244
chr2	179628846	179629086	TTN	0,44	158	166	134	110	163	146	104	80	190	161	166	143	163	164	164	121
chr2	179629155	179629635	TTN	0,45	171	170	134	113	166	147	114	100	195	165	165	148	174	179	173	138
chr2	179631043	179631403	TTN	0,40	289	314	236	164	242	240	190	137	313	302	282	251	299	301	337	224
chr2	179632433	179632913	TTN	0,40	297	322	254	167	245	246	209	155	336	330	313	254	310	323	343	239
chr2	179633337	179633769	TTN	0,38	280	289	236	175	237	238	206	149	289	297	274	240	288	292	296	225

chr2	179634374	179635454	TTN	0,42	318	321	252	168	258	266	216	143	340	326	308	278	320	316	345	234
chr2	179635827	179636307	TTN	0,38	256	285	221	157	233	238	190	146	290	278	276	239	287	283	292	211
chr2	179637783	179639302	TTN	0,38	357	369	292	195	282	294	246	164	380	374	350	304	364	366	387	259
chr2	179639548	179642806	TTN	0,41	336	351	272	189	277	291	239	166	361	353	331	291	349	341	363	254
chr2	179643542	179644252	TTN	0,34	301	309	247	177	246	252	222	152	335	311	311	263	326	317	313	227
chr2	179644674	179644794	TTN	0,32	263	258	212	158	224	216	204	125	284	266	251	231	264	281	256	168
chr2	179644804	179645020	TTN	0,36	295	324	238	181	221	241	216	151	335	298	281	263	303	298	308	227
chr2	179645738	179646098	TTN	0,37	298	324	241	166	222	267	209	158	328	305	314	272	301	312	329	239
chr2	179646866	179647417	TTN	0,43	266	279	202	153	235	237	188	155	318	281	273	247	284	287	269	214
chr2	179647481	179647841	TTN	0,46	202	197	165	117	174	178	131	110	220	198	187	169	209	204	188	155
chr2	179648359	179648599	TTN	0,33	199	227	176	127	198	205	155	116	242	210	199	176	215	209	210	166
chr2	179648697	179649177	TTN	0,50	202	195	136	120	180	161	125	101	221	185	200	175	176	195	202	150
chr2	179650287	179650961	TTN	0,45	207	206	171	133	190	185	144	112	229	195	198	181	224	200	220	163
chr2	179654035	179654275	TTN	0,37	232	271	197	144	209	221	183	141	285	273	252	207	255	261	267	216
chr2	179654653	179654893	TTN	0,32	318	314	284	167	213	257	213	153	346	326	314	264	316	312	335	249
chr2	179655383	179655623	TTN	0,39	236	251	175	131	213	210	169	128	266	247	238	196	249	238	268	197
chr2	179656741	179656981	TTN	0,30	214	239	175	128	181	199	162	117	248	231	219	175	242	207	210	181
chr2	179658079	179658319	TTN	0,39	294	324	226	168	225	250	200	141	317	285	301	250	297	293	324	221
chr2	179659021	179659381	TTN	0,48	178	179	141	106	174	159	125	92	197	156	188	145	192	178	180	148
chr2	179659573	179660053	TTN	0,52	158	157	116	106	146	133	97	85	167	148	155	132	154	161	148	128
chr2	179664149	179664725	TTN	0,47	183	195	146	119	173	154	121	98	195	171	178	151	180	183	184	153
chr2	179665025	179665505	TTN	0,51	239	223	187	137	195	194	149	118	247	227	224	180	235	230	242	170
chr2	179666786	179667146	TTN	0,52	135	132	101	82	123	114	88	83	149	135	129	114	127	135	133	109
chr2	179669203	179669443	TTN	0,45	85	85	68	65	96	68	65	41	101	72	84	66	92	89	82	76
chr2	190921873	190922473	MSTN	0,38	305	329	256	173	253	260	229	145	366	327	343	268	338	331	361	231
chr2	190924734	190925214	MSTN	0,36	336	375	267	184	264	287	251	173	355	366	322	277	358	336	390	246
chr2	190926895	190927375	MSTN	0,38	333	339	264	190	256	279	244	153	340	360	326	290	343	327	370	238
chr2	198351665	198352001	HSPD1	0,41	206	200	160	110	165	168	153	110	207	215	189	158	203	212	200	147
chr2	198352490	198352850	HSPD1	0,31	306	319	263	173	244	261	219	152	318	313	311	258	307	297	336	228
chr2	198352947	198353307	HSPD1	0,38	336	371	291	193	281	286	258	168	347	334	329	292	354	338	369	256
chr2	198353668	198354028	HSPD1	0,39	325	336	260	171	272	265	234	149	351	328	320	278	329	336	356	233
chr2	198354850	198355090	HSPD1	0,35	323	368	288	182	298	268	252	165	369	339	315	294	352	336	362	248
chr2	198357951	198358311	HSPD1	0,35	310	355	275	184	257	270	237	162	327	353	317	295	339	319	354	244
chr2	198358840	198359056	HSPD1	0,27	231	244	199	158	204	200	175	126	220	254	234	190	239	200	243	179
chr2	198359308	198359548	HSPD1	0,35	322	357	280	181	260	272	257	148	330	345	330	300	375	341	375	240
chr2	198359938	198360178	HSPD1	0,34	273	296	213	161	228	233	208	133	293	307	301	232	283	282	281	229
chr2	198361809	198362169	HSPD1	0,40	302	320	263	179	248	261	214	147	337	313	300	257	306	278	322	239
chr2	198363305	198363665	HSPD1	0,45	82	82	64	60	89	74	70	50	114	90	100	81	88	86	77	66
chr2	202566472	202566712	ALS2	0,35	173	198	131	123	173	159	148	102	183	185	196	157	182	168	190	140
chr2	202568772	202569012	ALS2	0,37	241	244	221	135	186	222	171	123	271	268	249	205	269	248	263	195
chr2	202569071	202569431	ALS2	0,40	263	273	224	159	222	238	199	141	303	271	273	240	280	280	301	226
chr2	202569772	202570012	ALS2	0,37	299	258	214	132	210	233	179	130	271	274	259	211	284	257	264	188
chr2	202570041	202570281	ALS2	0,32	269	299	214	160	220	233	194	140	302	271	261	227	285	253	270	207
chr2	202571476	202571836	ALS2	0,38	256	276	224	146	208	209	162	125	274	259	242	205	245	255	272	217
chr2	202572532	202572772	ALS2	0,44	142	139	100	100	123	126	91	86	137	135	148	114	129	127	132	115
chr2	202574502	202574862	ALS2	0,43	200	208	161	122	207	167	149	111	238	206	218	184	215	204	221	166
chr2	202575652	202575892	ALS2	0,34	221	266	184	130	194	198	153	119	245	233	223	198	221	229	256	185
chr2	202580298	202580658	ALS2	0,44	250	279	216	148	231	220	188	137	274	237	261	225	263	250	269	197
chr2	202582746	202582986	ALS2	0,35	280	277	248	149	221	245	199	154	307	274	286	246	281	274	309	218
chr2	202587684	202587924	ALS2	0,32	235	267	213	148	184	220	185	126	261	252	248	217	230	254	261	184
chr2	202587988	202588228	ALS2	0,36	338	340	272	167	248	268	227	154	355	349	323	274	322	335	352	231
chr2	202588919	202589279	ALS2	0,37	311	360	269	179	279	277	226	161	358	328	341	290	316	329	366	254
chr2	202590007	202590247	ALS2	0,37	308	332	284	178	262	251	216	159	361	334	317	266	327	328	342	234
chr2	202591119	202591667	ALS2	0,41	329	367	271	177	273	280	234	157	353	342	327	264	315	326	351	253
chr2	202591847	202592087	ALS2	0,37	265	288	204	162	222	218	175	153	286	266	255	229	247	266	267	211
chr2	202592342	202592582	ALS2	0,54	39	48	27	34	37	34	27	33	50	39	47	29	33	38	36	34
chr2	202593178	202593418	ALS2	0,43	179	180	141	111	167	141	128	98	189	169	161	166	185	183	193	147
chr2	202593720	202593960	ALS2	0,41	154	164	129	118	156	149	124	105	190	167	165	141	172	155	158	141
chr2	202597899	202598259	ALS2	0,34	265	316	229	168	206	230	206	136	302	280	287	249	278	284	295	206
chr2	202603305	202603545	ALS2	0,39	206	211	166	131	179	166	157	117	218	204	204	181	207	215	209	167

chr2	202606306	202606666	ALS2	0,42	197	199	155	133	180	170	149	112	228	207	201	186	207	196	209	167
chr2	202608886	202609222	ALS2	0,36	275	291	229	147	223	236	217	134	299	290	281	241	296	273	308	208
chr2	202611199	202611559	ALS2	0,42	210	216	174	125	192	188	146	108	239	218	222	182	222	221	221	185
chr2	202614353	202614593	ALS2	0,35	215	243	185	126	180	193	167	124	229	220	237	194	232	206	231	178
chr2	202617796	202618036	ALS2	0,41	228	248	200	142	229	212	166	129	240	230	244	209	227	229	236	179
chr2	202619129	202619489	ALS2	0,49	182	184	153	128	178	165	135	105	229	177	207	165	196	196	196	170
chr2	202622045	202622501	ALS2	0,45	255	264	222	156	237	216	178	145	290	264	273	223	282	273	282	206
chr2	202625472	202626552	ALS2	0,46	299	311	259	169	251	254	223	158	324	313	309	258	315	309	334	227
chr2	202631848	202632208	ALS2	0,43	297	310	242	173	233	266	209	156	325	302	305	245	289	301	307	227
chr2	202633538	202633658	ALS2	0,39	123	123	92	93	117	128	90	86	173	119	124	124	143	125	121	112
chr2	220283157	220283853	DES	0,71	6	5	4	7	8	7	3	5	9	4	7	5	9	5	5	7
chr2	220284762	220285477	DES	0,58	55	49	45	49	70	52	44	38	73	49	63	57	64	58	53	56
chr2	220285484	220285724	DES	0,60	47	45	43	38	54	44	33	37	72	49	57	39	52	49	40	49
chr2	220285991	220286351	DES	0,61	32	27	25	28	40	29	23	21	44	35	41	33	38	32	30	23
chr2	220288424	220288640	DES	0,60	35	32	22	25	36	31	22	19	41	28	40	36	28	32	35	33
chr2	220290305	220290545	DES	0,59	48	51	35	40	54	48	32	33	61	38	52	45	50	52	49	49
chr2	220290571	220290811	DES	0,61	55	58	44	54	72	60	43	45	75	59	63	51	65	67	76	55
chr2	233390831	233391071	CHRND	0,66	7	3	3	5	4	2	4	4	5	3	5	3	5	4	2	5
chr2	233391131	233391491	CHRND	0,63	16	14	13	11	20	15	18	8	21	13	16	13	15	12	13	18
chr2	233392056	233392176	CHRND	0,50	17	17	12	18	24	19	16	13	22	18	20	13	15	16	16	12
chr2	233392906	233393468	CHRND	0,59	259	245	203	155	217	208	185	143	272	273	262	222	251	259	275	202
chr2	233393499	233393715	CHRND	0,60	151	145	123	105	133	130	113	97	171	144	167	131	142	161	164	134
chr2	233394568	233394928	CHRND	0,65	145	147	104	102	142	125	105	96	165	130	142	133	156	126	149	124
chr2	233395953	233396385	CHRND	0,61	121	117	97	86	115	115	81	68	150	121	119	109	125	129	121	108
chr2	233398602	233399154	CHRND	0,59	67	70	60	50	74	72	54	39	79	71	74	66	70	72	71	60
chr2	233399750	233400110	CHRND	0,63	60	63	47	39	71	58	38	47	71	52	61	58	54	57	62	51
chr2	233404364	233404604	CHRNG	0,63	16	16	15	14	25	16	19	14	21	12	25	18	17	20	19	18
chr2	233404651	233404891	CHRNG	0,64	44	30	24	28	37	30	25	21	46	28	33	29	44	36	33	32
chr2	233404992	233405232	CHRNG	0,62	58	55	47	50	65	51	48	45	73	52	70	59	58	62	52	53
chr2	233405246	233405486	CHRNG	0,65	29	25	21	25	32	34	20	16	32	22	26	27	28	30	27	30
chr2	233405981	233406341	CHRNG	0,62	145	143	128	101	144	133	108	83	173	154	163	136	152	151	153	123
chr2	233407061	233407301	CHRNG	0,58	76	75	63	64	96	65	55	58	101	79	86	74	80	80	81	79
chr2	233407511	233407871	CHRNG	0,63	162	149	127	115	147	147	136	95	191	172	163	152	167	171	195	138
chr2	233407921	233408161	CHRNG	0,59	50	46	38	40	62	54	42	35	58	47	66	55	56	68	60	54
chr2	233408231	233408471	CHRNG	0,62	72	56	52	59	72	64	53	51	83	56	71	61	70	63	72	50
chr2	233409003	233409363	CHRNG	0,65	28	25	23	25	30	27	22	23	37	25	35	30	24	30	30	27
chr2	233409426	233409666	CHRNG	0,57	30	29	27	22	34	24	20	21	35	23	30	26	31	31	24	24
chr2	233410159	233410519	CHRNG	0,61	48	37	36	30	47	32	30	29	57	39	41	37	36	36	38	38
chr2	238233316	238233556	COL6A3	0,53	32	27	26	23	32	28	26	19	35	26	32	28	31	21	26	32
chr2	238234104	238234464	COL6A3	0,39	298	324	257	174	254	254	225	148	321	319	305	263	320	310	330	231
chr2	238242021	238242261	COL6A3	0,41	130	152	101	105	133	119	117	88	167	139	153	128	157	142	151	120
chr2	238243160	238243640	COL6A3	0,47	134	138	110	102	134	117	98	81	149	129	134	114	128	138	128	110
chr2	238244676	238245276	COL6A3	0,51	164	177	128	106	144	142	122	92	169	171	169	135	168	167	167	128
chr2	238247631	238247847	COL6A3	0,32	131	137	96	81	124	113	91	73	146	107	141	118	114	142	128	105
chr2	238249023	238249863	COL6A3	0,55	139	130	106	93	121	113	91	72	149	125	131	119	130	135	125	115
chr2	238250635	238250875	COL6A3	0,45	128	140	103	100	124	133	90	82	164	134	135	132	117	145	130	105
chr2	238252933	238253958	COL6A3	0,49	165	168	123	112	149	145	121	97	174	159	166	139	161	165	161	133
chr2	238255054	238255270	COL6A3	0,51	53	59	51	40	79	60	45	34	68	53	66	49	66	65	55	51
chr2	238256360	238256600	COL6A3	0,51	146	135	96	96	142	133	97	83	160	131	144	125	142	146	160	107
chr2	238256913	238257129	COL6A3	0,36	248	228	196	154	209	206	179	110	255	233	239	197	222	236	268	180
chr2	238257160	238257400	COL6A3	0,51	146	142	139	108	152	123	110	70	162	124	162	133	145	133	161	122
chr2	238258700	238258940	COL6A3	0,58	52	44	32	40	57	47	31	32	58	43	46	47	53	40	40	47
chr2	238259683	238259923	COL6A3	0,57	48	44	38	45	56	44	39	39	54	47	60	53	44	47	50	47
chr2	238261075	238261315	COL6A3	0,55	58	52	43	33	55	41	37	28	66	44	55	53	55	57	55	44
chr2	238261918	238262134	COL6A3	0,52	54	54	41	42	59	41	33	31	71	47	52	51	47	49	54	51
chr2	238263439	238263679	COL6A3	0,58	30	24	22	18	31	24	15	17	36	26	32	23	29	21	27	26
chr2	238265887	238266127	COL6A3	0,51	126	132	98	99	139	130	87	79	154	130	131	121	152	127	124	110
chr2	238266372	238266612	COL6A3	0,54	129	133	117	97	118	135	100	98	149	118	146	122	141	150	137	114
chr2	238267074	238267314	COL6A3	0,41	343	364	288	193	276	303	273	172	363	373	340	308	360	373	390	258
chr2	238267584	238268137	COL6A3	0,45	274	283	221	156	241	232	201	138	290	302	283	238	289	294	301	202

chr2	238268646	238268886	COL6A3	0,61	117	116	113	77	118	101	83	74	142	100	122	97	119	107	113	115
chr2	238269670	238269910	COL6A3	0,46	178	174	133	124	171	131	122	85	194	173	186	143	172	171	179	124
chr2	238270307	238270547	COL6A3	0,53	69	75	55	53	77	66	45	43	92	65	75	58	74	69	65	57
chr2	238271788	238272148	COL6A3	0,44	269	295	216	160	239	245	188	151	306	292	269	237	291	278	296	218
chr2	238272907	238273123	COL6A3	0,47	108	124	95	79	115	97	79	61	127	106	111	104	123	133	109	87
chr2	238274269	238274749	COL6A3	0,56	66	80	47	45	73	63	51	47	80	69	67	66	68	67	71	53
chr2	238275269	238275989	COL6A3	0,50	202	205	157	131	180	172	148	107	243	200	212	176	214	203	209	167
chr2	238277152	238277872	COL6A3	0,52	147	148	111	99	149	128	110	88	165	139	153	123	152	147	143	133
chr2	238280291	238281083	COL6A3	0,57	112	106	81	76	106	96	75	63	125	98	105	95	101	105	112	91
chr2	238282998	238283718	COL6A3	0,60	68	58	47	49	67	60	44	43	70	55	75	59	61	68	60	58
chr2	238285340	238286060	COL6A3	0,51	176	182	145	129	172	159	137	99	218	184	180	163	198	181	192	155
chr2	238287218	238287938	COL6A3	0,50	143	140	108	93	127	128	106	74	160	131	143	125	150	146	151	115
chr2	238289489	238290209	COL6A3	0,52	143	126	118	91	133	129	104	88	160	136	149	122	138	155	145	129
chr2	238296165	238296885	COL6A3	0,52	124	115	104	82	118	107	88	78	162	124	143	111	134	141	129	106
chr2	238303178	238303898	COL6A3	0,43	253	274	216	159	226	227	191	141	294	272	275	231	265	259	274	206
chr2	238305294	238305534	COL6A3	0,41	169	158	116	90	138	163	105	94	184	147	153	140	155	162	160	117
chr2	32288798	32288990	SPAST	0,71	6	5	3	8	9	7	5	7	5	6	8	6	12	8	7	8
chr2	32289016	32289376	SPAST	0,69	19	19	17	18	24	17	13	19	21	15	21	23	27	19	18	19
chr2	32312483	32312723	SPAST	0,32	244	268	207	183	208	206	164	136	276	271	270	209	258	246	255	192
chr2	32314512	32314752	SPAST	0,31	187	231	157	121	167	172	137	97	230	190	190	174	189	179	196	173
chr2	32323832	32324048	SPAST	0,36	156	189	153	104	171	149	112	98	198	164	182	139	183	172	160	129
chr2	32339620	32339980	SPAST	0,37	249	266	217	146	219	235	177	129	270	254	249	214	251	243	251	210
chr2	32340702	32340942	SPAST	0,30	256	298	210	163	213	215	202	149	301	251	256	233	235	250	267	200
chr2	32341114	32341354	SPAST	0,36	257	270	204	157	228	230	200	136	273	252	270	230	260	262	274	189
chr2	32351933	32352173	SPAST	0,36	162	164	135	122	156	149	130	93	190	155	174	160	164	180	157	136
chr2	32353392	32353632	SPAST	0,29	241	242	202	139	188	201	170	143	248	230	222	201	254	200	224	176
chr2	32361549	32361789	SPAST	0,32	247	262	213	156	180	228	193	136	280	248	255	207	251	270	248	191
chr2	32361871	32362337	SPAST	0,32	242	260	195	153	212	222	185	125	269	239	232	189	247	231	235	197
chr2	32366870	32367062	SPAST	0,30	163	186	134	113	136	156	131	90	183	184	161	153	175	174	169	131
chr2	32368339	32368531	SPAST	0,26	186	232	167	137	190	183	147	105	212	183	199	188	193	199	159	170
chr2	32369947	32370163	SPAST	0,39	110	109	85	74	125	111	77	71	140	118	129	118	124	115	107	95
chr2	32372186	32372426	SPAST	0,30	313	324	234	187	266	253	223	183	302	279	282	266	296	282	277	255
chr2	32379383	32379623	SPAST	0,35	250	272	213	145	231	222	180	122	293	253	261	207	261	268	263	199
chr2	71681052	71681292	DYSF	0,68	42	34	33	34	49	39	26	31	44	36	60	50	45	44	38	49
chr2	71693897	71694137	DYSF	0,72	6	3	2	3	4	7	2	1	3	4	3	6	2	4	3	2
chr2	71707920	71708160	DYSF	0,50	151	158	116	105	151	128	117	87	154	151	153	143	163	153	157	132
chr2	71708970	71709186	DYSF	0,55	35	29	18	19	31	22	21	20	39	21	25	22	31	28	26	22
chr2	71730276	71730516	DYSF	0,59	75	74	57	54	71	69	47	42	94	69	80	68	72	69	76	78
chr2	71738873	71739113	DYSF	0,66	36	31	33	27	31	32	21	28	37	26	27	39	30	43	27	33
chr2	71740295	71740535	DYSF	0,62	14	15	10	11	16	12	10	11	13	14	14	12	14	18	17	16
chr2	71740768	71741128	DYSF	0,61	53	44	38	45	52	50	43	38	70	49	61	48	55	59	47	48
chr2	71742696	71742936	DYSF	0,59	22	24	21	20	27	21	18	21	33	28	25	22	31	31	23	21
chr2	71743220	71743436	DYSF	0,52	190	196	165	122	170	165	137	116	217	191	201	177	178	207	195	157
chr2	71744023	71744263	DYSF	0,52	123	145	99	86	145	117	97	76	146	126	143	128	128	125	127	115
chr2	71747252	71747444	DYSF	0,49	88	87	72	63	92	72	76	57	109	83	80	78	89	94	78	79
chr2	71747856	71748096	DYSF	0,59	62	62	46	42	65	46	35	32	69	51	56	49	66	50	50	53
chr2	71753292	71753532	DYSF	0,59	26	31	19	21	25	25	20	18	25	19	29	22	22	23	24	25
chr2	71755359	71755599	DYSF	0,56	75	70	59	58	89	59	56	41	91	75	83	79	82	69	83	70
chr2	71762064	71762539	DYSF	0,58	105	120	85	86	115	96	85	68	132	102	117	113	124	113	119	91
chr2	71766207	71766447	DYSF	0,56	211	223	168	143	187	188	146	112	245	214	205	160	200	206	219	175
chr2	71776380	71776620	DYSF	0,57	54	63	35	42	64	65	41	47	61	71	56	54	57	54	58	61
chr2	71778108	71778348	DYSF	0,61	68	65	49	49	66	54	44	47	78	53	67	64	58	59	59	60
chr2	71778673	71778913	DYSF	0,66	29	29	23	19	25	23	21	13	22	17	26	29	26	21	27	29
chr2	71780049	71780409	DYSF	0,60	92	97	65	77	94	86	58	60	118	88	92	83	100	92	93	80
chr2	71780878	71781118	DYSF	0,59	53	63	45	37	65	48	45	43	64	45	52	49	50	48	57	47
chr2	71783027	71783267	DYSF	0,66	3	2	2	5	3	3	3	4	8	3	4	4	3	3	3	6
chr2	71788797	71789157	DYSF	0,61	61	61	56	60	70	58	47	41	78	57	74	60	73	68	63	54
chr2	71791085	71791445	DYSF	0,56	199	190	154	112	167	153	142	96	216	198	188	160	201	184	199	162
chr2	71794974	71795574	DYSF	0,61	77	74	64	62	91	74	65	51	102	83	93	80	83	85	83	73
chr2	71796886	71797126	DYSF	0,59	32	27	22	23	37	31	17	23	32	30	34	22	25	27	32	28

chr2	71797291	71797531	DYSF	0,58	72	59	51	49	79	65	52	48	70	60	67	60	66	61	70	62
chr2	71797619	71797979	DYSF	0,60	31	25	25	22	27	25	18	17	39	27	36	28	29	31	22	25
chr2	71801234	71801594	DYSF	0,63	22	20	20	17	26	18	16	14	28	22	31	22	23	16	16	20
chr2	71816649	71816889	DYSF	0,54	120	125	106	73	106	96	88	58	137	110	126	99	119	121	122	101
chr2	71817259	71817499	DYSF	0,57	61	51	36	48	55	57	38	34	71	56	69	47	50	53	56	59
chr2	71825604	71825964	DYSF	0,60	91	88	83	76	103	85	71	57	116	92	102	75	103	93	90	94
chr2	71827721	71828081	DYSF	0,59	45	35	28	35	46	37	26	20	40	32	39	36	36	34	39	37
chr2	71828523	71828763	DYSF	0,51	180	177	147	111	155	159	123	79	201	181	179	148	192	189	191	164
chr2	71829800	71830040	DYSF	0,59	21	23	15	17	24	17	16	11	27	16	21	21	18	21	21	16
chr2	71838336	71838855	DYSF	0,61	91	94	77	73	102	81	71	57	109	80	92	91	85	100	92	85
chr2	71839673	71840033	DYSF	0,59	29	25	20	21	36	25	24	23	39	24	33	29	29	29	25	25
chr2	71840381	71840621	DYSF	0,61	52	45	48	48	59	51	38	44	64	50	63	59	65	57	48	51
chr2	71847587	71847803	DYSF	0,53	220	220	173	135	190	168	152	117	251	219	224	202	221	208	220	165
chr2	71871023	71871263	DYSF	0,50	126	127	82	81	121	105	82	70	128	116	118	111	122	123	114	101
chr2	71883275	71883491	DYSF	0,51	83	74	66	66	90	76	59	60	106	78	98	77	82	83	79	73
chr2	71885956	71886244	DYSF	0,52	209	189	165	132	180	165	146	103	200	197	196	164	205	201	198	155
chr2	71887615	71887855	DYSF	0,49	96	111	73	67	91	96	76	63	125	95	96	90	96	107	92	84
chr2	71891302	71891638	DYSF	0,58	82	75	63	62	95	64	55	56	89	70	84	72	80	80	80	67
chr2	71892182	71892542	DYSF	0,55	222	223	168	139	201	182	140	119	243	195	215	189	220	217	204	168
chr2	71894455	71894695	DYSF	0,62	13	13	10	11	18	12	6	14	20	11	17	9	19	14	18	14
chr2	71895839	71896055	DYSF	0,64	26	24	23	24	30	23	23	17	32	22	25	26	28	22	30	32
chr2	71896169	71896409	DYSF	0,52	124	110	100	90	128	114	83	74	150	107	124	119	110	118	125	107
chr2	71896625	71896985	DYSF	0,47	220	241	200	145	218	218	173	132	264	243	216	195	230	231	236	176
chr2	71901256	71901496	DYSF	0,52	91	87	69	66	92	85	58	52	97	84	102	77	92	82	88	68
chr2	71906095	71906455	DYSF	0,52	136	121	110	96	134	110	90	82	159	126	140	118	146	127	137	111
chr2	71908065	71908305	DYSF	0,61	11	11	7	7	9	9	8	7	7	7	10	7	8	5	8	9
chr2	71909554	71909746	DYSF	0,58	98	117	91	87	109	108	77	69	142	98	113	101	114	121	109	96
chr2	71909752	71909872	DYSF	0,55	81	99	75	81	101	103	72	56	115	85	113	92	93	95	92	87
chr2	71913482	71913722	DYSF	0,65	43	41	30	41	46	45	37	31	58	41	47	31	46	37	42	41
chr2	74588574	74588814	DCTN1	0,59	31	39	35	38	45	39	29	31	46	36	38	28	42	40	32	34
chr2	74589103	74589343	DCTN1	0,59	45	44	43	39	55	37	33	36	66	43	48	49	56	56	51	46
chr2	74589696	74589936	DCTN1	0,57	34	26	25	26	32	31	24	15	37	31	34	22	33	30	32	31
chr2	74590032	74590607	DCTN1	0,56	45	42	37	35	53	36	33	29	52	45	50	40	48	49	41	45
chr2	74590687	74590807	DCTN1	0,60	3	2	2	4	4	3	3	5	1	6	7	3	5	4	6	6
chr2	74592104	74592464	DCTN1	0,55	98	101	72	68	102	83	72	57	108	94	105	78	98	97	95	85
chr2	74592532	74592892	DCTN1	0,56	118	120	93	95	132	122	99	76	146	129	133	112	135	131	133	116
chr2	74592962	74593202	DCTN1	0,52	276	281	240	163	230	246	191	132	305	317	273	244	274	295	306	232
chr2	74593318	74593798	DCTN1	0,58	162	141	122	101	150	139	107	87	182	149	160	135	143	146	155	146
chr2	74593804	74594322	DCTN1	0,51	241	218	175	139	215	204	165	132	259	216	239	180	225	224	226	182
chr2	74594392	74594632	DCTN1	0,53	117	104	81	83	117	106	90	57	128	107	118	97	110	118	115	102
chr2	74594726	74595357	DCTN1	0,54	142	139	111	101	132	125	101	78	159	130	142	121	134	137	149	105
chr2	74595825	74596065	DCTN1	0,54	88	77	69	64	91	81	64	49	93	83	93	79	85	80	83	82
chr2	74596117	74596669	DCTN1	0,57	176	168	145	121	170	149	117	101	197	170	168	152	172	162	187	145
chr2	74597023	74598012	DCTN1	0,55	240	236	182	146	222	203	175	134	262	236	241	215	237	236	255	193
chr2	74598024	74598384	DCTN1	0,57	245	238	184	161	229	219	168	144	288	261	249	233	248	264	280	192
chr2	74598579	74598939	DCTN1	0,59	30	31	26	32	43	25	29	23	39	28	34	28	24	35	33	33
chr2	74599944	74600184	DCTN1	0,49	85	88	66	64	96	80	58	47	87	81	87	74	79	81	88	69
chr2	74601344	74601584	DCTN1	0,57	34	32	20	37	33	24	21	25	39	33	38	33	25	40	28	28
chr2	74603758	74603998	DCTN1	0,58	32	27	25	26	32	29	19	24	44	30	28	26	31	28	30	26
chr2	74604455	74604933	DCTN1	0,46	270	266	218	154	228	218	192	133	287	270	252	232	279	265	277	214
chr2	74605069	74605429	DCTN1	0,54	179	179	143	113	154	154	116	96	194	176	187	154	193	178	172	147
chr2	74607027	74607267	DCTN1	0,63	43	46	38	45	54	37	35	29	53	48	49	43	56	38	49	43
chr2	86444077	86444317	REEP1	0,47	126	128	83	91	126	100	85	60	147	113	132	107	109	123	132	101
chr2	86459656	86460016	REEP1	0,63	32	22	25	24	30	28	21	17	34	21	30	24	28	34	31	30
chr2	86479016	86479256	REEP1	0,51	75	73	64	68	84	75	53	57	110	77	69	69	76	101	76	69
chr2	86481756	86481996	REEP1	0,39	151	173	122	116	164	134	113	90	178	141	151	130	157	173	163	123
chr2	86491005	86491245	REEP1	0,47	193	192	155	109	187	154	137	112	222	193	213	179	213	188	192	159
chr2	86509208	86509448	REEP1	0,42	211	230	178	125	213	204	127	120	223	227	237	172	218	235	222	169
chr2	86565052	86565292	REEP1	0,50	225	230	195	142	212	205	164	121	261	224	215	220	247	242	259	203
chr20	30407289	30407529	MYLK2	0,55	68	58	43	45	68	56	38	44	81	56	68	50	63	64	55	56

chr20	30407838	30408438	MYLK2	0,63	37	30	27	27	37	30	24	22	41	28	39	34	34	30	30	31
chr20	30409150	30409630	MYLK2	0,60	29	22	21	21	30	23	16	15	34	21	24	24	22	30	28	24
chr20	30411212	30411452	MYLK2	0,62	19	20	13	17	26	21	14	16	19	18	19	18	16	18	19	14
chr20	30411988	30412228	MYLK2	0,60	37	32	27	30	33	23	25	26	38	29	35	33	24	33	25	24
chr20	30414334	30414814	MYLK2	0,59	73	82	58	54	79	71	63	44	88	70	82	66	68	75	85	69
chr20	30418536	30418999	MYLK2	0,60	45	46	34	34	50	38	31	32	58	39	51	44	48	48	46	36
chr20	30419401	30419992	MYLK2	0,59	142	134	114	91	132	131	103	82	150	146	157	122	147	148	152	124
chr20	30421439	30421679	MYLK2	0,62	54	54	47	43	59	54	46	34	63	39	60	44	54	58	54	47
chr20	56964424	56964664	VAPB	0,67	3	3	1	0	2	4	2	3	4	3	3	3	0	1	2	4
chr20	56993162	56993522	VAPB	0,47	218	233	187	117	188	179	155	113	217	209	220	189	226	216	239	157
chr20	57009589	57009829	VAPB	0,33	260	292	221	144	207	232	186	156	308	290	287	234	261	282	301	193
chr20	57013920	57014160	VAPB	0,31	213	219	167	122	179	179	159	119	219	210	208	185	226	203	210	159
chr20	57015870	57016230	VAPB	0,38	214	245	169	130	172	202	156	119	256	219	212	198	214	207	220	187
chr20	57019031	57019391	VAPB	0,46	199	196	156	118	179	173	147	103	211	201	201	192	197	216	201	168
chr21	33031998	33032238	SOD1	0,66	16	12	17	18	18	19	11	13	18	12	18	13	21	19	13	20
chr21	33036030	33036270	SOD1	0,44	124	130	115	98	131	131	99	77	157	129	131	129	138	137	142	108
chr21	33038676	33038916	SOD1	0,35	171	175	140	109	160	148	119	98	213	175	158	148	158	181	166	140
chr21	33039509	33039749	SOD1	0,41	176	174	126	108	142	169	120	94	183	174	174	141	156	194	166	135
chr21	33040717	33040957	SOD1	0,42	119	119	102	76	109	96	91	70	151	101	128	101	138	114	103	109
chr21	35742723	35743203	KCNE2	0,46	213	210	189	140	194	192	152	109	243	220	218	182	244	228	232	184
chr21	35821437	35822037	KCNE1	0,53	128	126	106	93	130	112	96	82	160	133	133	120	138	127	130	107
chr21	47401692	47401932	COL6A1	0,74	1	1	1	1	0	2	1	0	3	0	0	0	1	0	2	2
chr21	47402492	47402732	COL6A1	0,62	45	40	26	40	48	39	37	28	49	38	45	40	37	42	39	38
chr21	47404102	47404462	COL6A1	0,68	22	13	19	17	23	15	11	12	24	13	16	15	15	13	17	16
chr21	47406339	47406699	COL6A1	0,66	17	13	11	14	18	15	11	9	19	14	14	17	14	13	14	13
chr21	47406793	47407153	COL6A1	0,65	17	22	13	19	25	19	11	17	25	19	24	19	16	23	23	19
chr21	47407334	47407670	COL6A1	0,66	37	44	27	33	44	35	25	28	60	35	50	39	33	39	38	36
chr21	47408904	47409144	COL6A1	0,57	30	30	23	23	32	26	18	26	40	24	28	21	31	33	25	26
chr21	47409426	47409786	COL6A1	0,65	27	23	24	19	34	24	20	20	34	26	32	22	27	31	24	25
chr21	47410119	47410359	COL6A1	0,66	15	17	12	19	19	23	13	13	23	18	25	19	16	20	15	19
chr21	47410593	47411043	COL6A1	0,59	30	24	25	28	36	29	21	21	31	31	31	29	25	31	29	29
chr21	47411847	47412414	COL6A1	0,66	28	24	24	23	32	26	17	21	33	24	24	27	28	23	29	29
chr21	47412572	47412812	COL6A1	0,67	6	3	2	3	8	2	4	4	8	2	3	4	4	2	2	3
chr21	47413991	47414231	COL6A1	0,64	22	14	15	12	19	16	12	12	20	19	19	20	16	16	20	14
chr21	47417245	47417485	COL6A1	0,66	6	5	4	7	9	3	4	3	4	5	5	6	6	5	5	8
chr21	47417524	47417764	COL6A1	0,70	4	6	5	7	6	4	2	4	8	2	6	5	5	6	4	7
chr21	47417939	47418179	COL6A1	0,69	6	5	4	4	10	5	5	7	8	4	5	5	8	5	9	6
chr21	47418209	47418449	COL6A1	0,60	13	10	9	12	15	14	9	10	12	9	19	12	12	13	11	15
chr21	47418721	47418961	COL6A1	0,55	21	24	25	28	30	25	26	23	35	21	37	25	31	30	27	23
chr21	47418979	47419219	COL6A1	0,66	11	9	6	11	10	8	6	6	11	7	11	6	6	11	9	15
chr21	47419468	47419708	COL6A1	0,65	20	21	15	11	27	20	19	16	23	16	25	16	16	21	12	22
chr21	47420142	47420382	COL6A1	0,62	29	30	21	25	36	27	22	21	40	33	32	31	29	32	21	34
chr21	47420616	47420736	COL6A1	0,61	3	3	4	4	6	4	2	1	4	4	4	5	6	6	8	3
chr21	47421113	47421353	COL6A1	0,66	31	27	20	35	33	26	19	25	44	24	42	35	36	38	28	28
chr21	47421809	47422712	COL6A1	0,67	28	22	18	22	26	21	18	18	29	22	27	24	24	25	29	25
chr21	47422928	47423168	COL6A1	0,65	18	19	13	15	23	17	10	11	18	13	23	18	14	15	15	14
chr21	47423195	47424035	COL6A1	0,66	32	29	27	33	38	31	22	24	41	28	34	33	30	35	31	29
chr21	47531327	47531567	COL6A2	0,66	3	3	2	7	6	4	5	5	6	1	7	6	8	3	5	7
chr21	47531831	47532551	COL6A2	0,66	32	25	22	36	39	25	21	19	36	26	35	27	28	33	29	33
chr21	47532607	47532847	COL6A2	0,65	34	27	19	24	35	24	19	20	43	33	34	35	35	31	32	26
chr21	47533834	47534074	COL6A2	0,59	101	102	87	87	106	86	82	59	125	84	117	96	92	116	98	89
chr21	47535696	47536056	COL6A2	0,67	11	11	10	8	16	10	8	10	13	10	14	10	12	12	12	15
chr21	47536183	47536423	COL6A2	0,67	35	32	30	27	47	31	26	21	36	30	37	35	35	32	34	35
chr21	47536466	47536826	COL6A2	0,62	55	57	43	50	62	51	42	47	70	47	74	53	52	56	60	51
chr21	47537220	47537460	COL6A2	0,67	5	5	5	5	8	7	4	4	5	4	8	4	6	6	7	7
chr21	47537698	47537938	COL6A2	0,68	2	4	3	2	3	2	3	3	3	2	4	1	5	4	3	3
chr21	47538438	47538678	COL6A2	0,67	8	9	5	11	12	11	7	8	11	8	14	8	9	7	10	8
chr21	47538868	47539108	COL6A2	0,66	7	5	5	6	8	7	6	7	7	5	8	6	7	8	2	9
chr21	47539612	47539852	COL6A2	0,70	4	4	5	5	6	2	4	3	3	4	6	4	4	3	4	9
chr21	47540339	47540579	COL6A2	0,72	1	1	1	1	3	2	1	2	5	3	2	2	0	3	2	2

chr21	47540885	47541125	COL6A2	0,63	49	42	34	34	52	39	24	36	52	37	53	49	42	44	43	43
chr21	47541380	47541620	COL6A2	0,67	6	3	4	6	4	3	3	2	5	3	5	5	6	2	4	2
chr21	47541926	47542166	COL6A2	0,63	20	14	16	17	24	17	14	12	27	15	23	20	18	17	18	22
chr21	47542307	47542547	COL6A2	0,63	11	10	9	10	13	6	5	4	17	9	8	7	6	10	10	10
chr21	47542699	47542939	COL6A2	0,56	25	22	17	19	29	20	15	14	30	20	29	19	29	25	28	23
chr21	47544475	47544936	COL6A2	0,65	11	8	11	9	11	13	7	10	15	7	11	12	12	9	8	9
chr21	47545082	47546224	COL6A2	0,65	45	42	37	37	52	43	37	31	57	42	51	44	44	45	45	43
chr21	47546315	47546555	COL6A2	0,66	24	23	14	21	22	18	15	16	28	18	19	27	19	12	16	18
chr21	47549017	47549497	COL6A2	0,68	25	21	15	24	24	21	18	17	29	20	25	19	22	18	20	23
chr21	47551781	47552477	COL6A2	0,69	8	6	7	9	11	8	6	7	11	9	8	10	6	12	7	7
chr22	33670331	33670691	LARGE	0,53	129	138	112	86	142	108	86	86	153	119	141	119	137	142	127	112
chr22	33672963	33673323	LARGE	0,57	184	165	144	119	159	153	118	89	200	178	172	146	188	172	177	150
chr22	33679080	33679440	LARGE	0,56	83	84	69	69	98	89	58	64	108	97	88	84	84	91	80	68
chr22	33700166	33700598	LARGE	0,61	46	44	27	28	45	42	31	28	47	35	44	31	45	45	38	39
chr22	33711972	33712332	LARGE	0,61	55	49	46	43	54	49	39	38	60	50	57	48	48	59	58	44
chr22	33733529	33733889	LARGE	0,56	128	155	111	83	133	110	92	83	162	124	137	103	139	148	152	118
chr22	33777847	33778087	LARGE	0,55	89	113	84	69	116	88	78	60	113	99	105	86	86	108	105	93
chr22	33780113	33780353	LARGE	0,49	133	135	99	88	128	112	95	76	134	121	130	111	128	130	124	103
chr22	33828078	33828318	LARGE	0,51	116	124	96	71	115	116	82	69	139	103	122	109	123	127	110	112
chr22	33960739	33961099	LARGE	0,43	181	181	146	119	178	159	131	91	212	179	180	152	182	178	174	142
chr22	34000362	34000602	LARGE	0,56	46	54	37	43	52	58	47	34	75	43	53	51	58	56	59	46
chr22	34022148	34022388	LARGE	0,52	102	109	83	74	98	87	70	59	133	104	115	92	98	103	112	104
chr22	34046263	34046743	LARGE	0,63	43	41	31	33	53	32	27	33	43	39	44	37	45	41	42	45
chr22	34157290	34157530	LARGE	0,52	137	130	111	101	150	132	96	86	181	140	163	137	145	152	139	120
chr22	38369433	38370273	SOX10	0,65	24	20	12	22	30	19	16	14	24	20	24	19	20	24	22	20
chr22	38373767	38374247	SOX10	0,65	20	19	14	21	27	15	16	15	20	17	26	21	21	21	24	27
chr22	38379307	38379787	SOX10	0,70	7	7	7	9	11	4	5	8	9	6	10	7	8	8	7	6
chr22	46067894	46068086	ATXN10	0,73	0	0	0	0	2	0	0	1	0	0	0	2	0	1	0	0
chr22	46085507	46085867	ATXN10	0,39	257	272	200	155	223	225	189	128	275	264	256	224	265	267	273	208
chr22	46088832	46089048	ATXN10	0,34	284	270	207	158	226	241	185	137	279	270	259	237	259	249	271	202
chr22	46096127	46096319	ATXN10	0,34	139	160	116	95	143	117	104	95	158	148	159	134	145	151	137	140
chr22	46098467	46098827	ATXN10	0,34	288	323	231	171	226	242	217	144	311	295	286	250	303	296	330	234
chr22	46114212	46114452	ATXN10	0,42	252	255	212	153	239	230	158	139	281	264	260	212	264	255	280	178
chr22	46125207	46125567	ATXN10	0,39	211	234	156	111	168	172	152	106	214	197	195	160	188	198	190	160
chr22	46134544	46134784	ATXN10	0,36	234	259	188	139	215	202	185	124	263	231	250	202	215	240	245	177
chr22	46136153	46136513	ATXN10	0,35	283	291	210	155	221	236	212	130	292	287	277	241	272	263	295	213
chr22	46202750	46202990	ATXN10	0,42	209	221	170	137	189	188	133	123	242	224	233	192	214	212	221	178
chr22	46238784	46239144	ATXN10	0,45	232	242	188	142	197	213	166	115	267	227	230	217	240	226	260	186
chr22	46239489	46239609	ATXN10	0,31	104	119	108	74	88	111	89	82	146	115	125	121	107	110	110	109
chr3	128514116	128514356	RAB7A	0,47	178	173	139	113	177	153	116	99	182	158	148	161	162	179	160	139
chr3	128516728	128516968	RAB7A	0,43	174	182	118	106	161	157	121	105	187	159	171	155	180	171	157	132
chr3	128525143	128525503	RAB7A	0,49	177	198	161	125	163	168	136	104	194	188	187	157	178	196	173	162
chr3	128526329	128526569	RAB7A	0,56	54	53	40	35	53	37	32	33	63	34	56	43	54	55	44	40
chr3	128532097	128532337	RAB7A	0,51	123	114	122	92	123	104	85	77	151	128	137	111	132	126	122	107
chr3	128889234	128889474	CNBP	0,42	154	171	129	105	163	132	118	93	186	140	171	142	168	165	159	131
chr3	128889843	128890203	CNBP	0,43	252	281	220	157	219	251	189	142	300	271	264	227	274	257	253	204
chr3	128890204	128890684	CNBP	0,43	269	277	239	160	235	230	207	147	302	266	269	232	282	279	270	203
chr3	14166639	14166759	TMEM43	0,73	0	0	0	0	1	1	1	0	0	0	0	0	0	1	1	0
chr3	14170873	14171113	TMEM43	0,49	99	101	86	78	119	93	78	62	122	88	108	92	98	101	75	84
chr3	14172268	14172508	TMEM43	0,60	48	40	41	38	49	30	28	28	49	42	40	36	52	57	37	44
chr3	14173006	14173246	TMEM43	0,48	107	123	84	79	110	92	82	60	142	107	110	94	117	110	110	104
chr3	14173950	14174190	TMEM43	0,60	37	34	30	36	46	33	26	26	43	29	42	40	40	45	33	37
chr3	14174279	14174495	TMEM43	0,57	79	83	57	59	72	70	51	55	94	73	83	70	77	74	76	68
chr3	14175153	14175393	TMEM43	0,60	97	90	71	68	97	83	66	64	115	100	105	84	97	93	100	80
chr3	14176210	14176450	TMEM43	0,54	66	71	50	54	61	50	33	44	84	59	68	59	69	69	64	59
chr3	14176574	14176814	TMEM43	0,61	72	71	62	68	88	79	52	56	102	74	88	66	82	78	80	70
chr3	14177237	14177477	TMEM43	0,59	35	37	36	40	45	39	28	25	52	43	54	37	44	42	41	40
chr3	14180618	14180858	TMEM43	0,61	59	54	52	36	64	44	36	42	58	45	50	46	55	55	48	46
chr3	14183013	14183373	TMEM43	0,60	87	96	75	70	103	80	74	64	116	86	89	80	97	90	79	79
chr3	15493065	15493305	COLQ	0,57	49	58	46	48	70	62	36	34	67	60	67	51	63	57	54	53

chr3	15495309	15495525	COLQ	0,59	24	26	16	17	26	20	17	12	38	25	30	26	25	31	34	21
chr3	15497345	15497585	COLQ	0,62	33	36	16	29	31	28	22	24	31	27	35	30	32	32	35	27
chr3	15497906	15498146	COLQ	0,53	72	70	62	56	76	73	63	42	96	71	80	63	77	77	63	64
chr3	15499642	15499882	COLQ	0,52	104	87	76	80	93	85	73	56	120	92	99	85	94	99	95	79
chr3	15507775	15508015	COLQ	0,58	28	23	18	25	34	29	18	20	40	24	38	30	28	29	26	30
chr3	15511962	15512202	COLQ	0,56	78	92	62	61	92	79	62	50	114	78	89	79	99	86	93	81
chr3	15515404	15515876	COLQ	0,45	201	196	159	119	191	167	141	106	235	194	184	166	186	182	209	157
chr3	15516324	15516564	COLQ	0,48	154	166	123	107	147	132	116	89	187	169	179	134	157	159	154	118
chr3	15516842	15517082	COLQ	0,51	103	109	84	83	119	97	80	66	146	109	109	102	108	121	105	86
chr3	15518548	15518788	COLQ	0,54	87	83	72	65	87	72	65	61	106	75	110	89	78	91	84	77
chr3	15520376	15520616	COLQ	0,54	104	92	72	68	96	88	68	58	107	83	100	81	91	104	81	82
chr3	15520746	15520986	COLQ	0,46	129	125	107	84	113	110	92	73	151	138	130	102	135	137	118	109
chr3	15529643	15529883	COLQ	0,59	27	20	15	23	29	24	17	15	23	22	21	19	23	26	18	26
chr3	15530967	15531207	COLQ	0,57	39	42	34	39	41	35	29	23	42	41	46	39	51	36	32	43
chr3	15540006	15540198	COLQ	0,58	20	19	20	17	26	21	13	12	23	17	24	16	18	21	19	14
chr3	15562959	15563199	COLQ	0,49	147	147	118	116	137	127	100	79	183	125	141	123	138	143	136	135
chr3	193311062	193311302	OPA1	0,66	6	3	4	3	6	5	3	2	8	4	8	6	4	5	6	4
chr3	193332428	193332644	OPA1	0,36	302	289	238	161	238	252	202	145	304	288	308	254	265	315	310	238
chr3	193332656	193332896	OPA1	0,40	304	286	234	167	235	221	180	148	306	282	294	224	279	308	277	206
chr3	193333390	193333630	OPA1	0,32	265	295	217	155	212	230	186	136	286	264	282	225	252	254	281	228
chr3	193334929	193335145	OPA1	0,33	280	311	205	166	238	242	212	147	304	287	278	239	280	294	297	217
chr3	193335479	193335719	OPA1	0,32	258	287	219	150	190	229	185	139	305	270	254	233	272	270	302	196
chr3	193336571	193336811	OPA1	0,34	171	183	137	120	158	169	133	106	190	164	179	165	188	172	176	143
chr3	193343815	193344055	OPA1	0,49	162	142	108	96	141	122	104	80	167	135	154	130	140	140	138	132
chr3	193349307	193349547	OPA1	0,28	264	285	198	156	180	216	174	116	292	240	262	224	266	243	270	201
chr3	193353138	193353378	OPA1	0,30	208	253	169	129	168	173	153	121	237	222	217	187	219	199	224	152
chr3	193354978	193355146	OPA1	0,34	198	236	150	126	170	154	128	104	213	186	188	161	201	183	192	158
chr3	193355677	193355917	OPA1	0,41	119	118	95	75	131	104	83	59	143	112	133	102	123	126	117	106
chr3	193360473	193360920	OPA1	0,33	236	278	196	149	194	219	168	120	253	252	254	213	241	233	243	193
chr3	193361108	193361468	OPA1	0,32	295	334	250	173	222	245	212	148	314	338	303	260	308	296	313	234
chr3	193361708	193361948	OPA1	0,39	111	116	101	67	109	94	77	71	132	93	121	105	115	116	107	87
chr3	193363257	193363672	OPA1	0,29	289	320	245	173	203	274	211	151	339	301	310	270	289	304	345	228
chr3	193364791	193365031	OPA1	0,34	174	185	124	125	170	172	123	113	216	179	177	156	177	188	178	133
chr3	193365770	193366010	OPA1	0,34	219	243	177	128	184	182	135	121	245	218	208	179	231	223	228	180
chr3	193366501	193366741	OPA1	0,30	240	268	198	148	158	229	169	123	280	255	270	212	267	245	254	213
chr3	193372603	193372915	OPA1	0,35	268	290	228	164	215	235	190	147	311	308	277	222	293	278	284	216
chr3	193374764	193375124	OPA1	0,33	259	284	202	156	193	218	189	128	298	253	258	219	255	270	252	196
chr3	193376609	193376849	OPA1	0,39	211	211	166	129	168	183	145	100	231	216	225	199	213	216	240	181
chr3	193377190	193377430	OPA1	0,30	161	154	131	101	140	135	114	85	165	142	163	123	164	143	139	123
chr3	193380500	193380860	OPA1	0,34	219	210	162	118	182	189	137	120	250	211	222	169	216	204	213	159
chr3	193382606	193382846	OPA1	0,32	266	309	213	170	170	232	199	154	306	288	286	266	292	255	296	213
chr3	193384011	193384251	OPA1	0,33	205	244	177	148	186	176	152	113	228	212	217	175	210	206	186	181
chr3	193384893	193385133	OPA1	0,36	270	287	227	158	237	236	193	152	329	273	289	243	285	270	287	231
chr3	193409763	193410003	OPA1	0,27	253	263	206	152	175	217	175	134	294	234	255	218	257	247	269	204
chr3	32148106	32148346	GPD1L	0,76	3	4	0	2	4	2	2	1	4	3	5	2	1	2	1	3
chr3	32169476	32169836	GPD1L	0,39	247	262	214	156	222	226	183	135	287	278	267	224	269	267	273	202
chr3	32179968	32180328	GPD1L	0,48	172	165	141	115	165	149	113	109	194	184	180	154	180	186	169	140
chr3	32181668	32181908	GPD1L	0,52	96	97	76	61	100	85	59	67	107	90	109	88	91	108	95	74
chr3	32188049	32188289	GPD1L	0,41	207	214	173	126	181	192	150	117	226	215	205	196	210	210	194	156
chr3	32200304	32200664	GPD1L	0,60	43	52	30	34	52	37	27	38	50	36	46	44	49	39	50	38
chr3	32200992	32201232	GPD1L	0,40	146	152	111	112	152	139	111	76	179	129	143	130	146	154	147	112
chr3	32207233	32207473	GPD1L	0,43	266	269	209	177	239	237	171	107	301	272	274	220	256	265	271	186
chr3	38591710	38593150	SCN5A	0,58	88	87	69	62	95	76	69	56	105	79	91	77	88	91	88	77
chr3	38595664	38596144	SCN5A	0,53	147	138	112	95	138	141	101	91	162	138	147	137	157	150	147	124
chr3	38597078	38597318	SCN5A	0,58	39	38	30	37	46	47	31	34	55	31	53	42	45	47	46	38
chr3	38597880	38598120	SCN5A	0,50	84	83	69	61	86	72	64	41	104	82	84	72	82	87	86	84
chr3	38598628	38598868	SCN5A	0,51	67	72	58	56	77	64	49	45	90	67	82	70	61	84	61	73
chr3	38601538	38602018	SCN5A	0,56	145	142	119	93	138	137	105	85	171	143	145	131	160	143	154	123
chr3	38603846	38604086	SCN5A	0,63	15	22	10	14	17	10	11	14	20	18	15	14	15	14	17	12
chr3	38607806	38608142	SCN5A	0,58	169	172	140	121	168	152	113	93	189	159	182	154	174	185	193	152

chr3	38616684	38617044	SCN5A	0,59	69	67	62	57	82	64	52	53	88	57	79	54	71	80	69	65
chr3	38618091	38618331	SCN5A	0,62	20	17	15	21	27	26	11	13	27	13	21	16	18	27	22	18
chr3	38620725	38621085	SCN5A	0,65	10	14	7	13	18	14	7	9	15	11	12	16	13	13	12	14
chr3	38622341	38622917	SCN5A	0,63	30	27	23	27	35	27	23	22	32	29	29	27	31	26	24	23
chr3	38627152	38627608	SCN5A	0,56	126	127	85	87	121	114	92	73	141	125	131	104	125	128	135	103
chr3	38628797	38629157	SCN5A	0,57	63	62	44	44	67	58	46	41	78	56	67	54	66	63	69	64
chr3	38639158	38639518	SCN5A	0,54	83	85	60	69	91	70	60	55	98	75	94	70	83	89	71	69
chr3	38640354	38640594	SCN5A	0,64	8	11	5	10	10	10	3	4	8	8	10	7	8	8	9	8
chr3	38645148	38645628	SCN5A	0,61	55	50	37	40	58	46	37	38	66	47	54	48	55	58	55	51
chr3	38646129	38646489	SCN5A	0,55	122	134	103	93	135	120	95	80	171	114	131	126	146	135	144	122
chr3	38647360	38647720	SCN5A	0,55	97	75	71	67	83	89	65	55	108	84	100	87	88	98	89	78
chr3	38648050	38648410	SCN5A	0,61	47	46	37	40	60	47	36	40	61	42	60	46	55	45	48	45
chr3	38649553	38649793	SCN5A	0,53	68	60	46	54	77	65	41	42	76	60	73	51	71	72	63	56
chr3	38651159	38651519	SCN5A	0,61	23	18	13	22	23	23	14	13	27	18	21	17	18	22	16	24
chr3	38655159	38655631	SCN5A	0,49	129	120	119	96	142	122	97	72	154	142	146	120	138	136	133	112
chr3	38662290	38662530	SCN5A	0,54	89	81	68	57	85	86	59	57	102	73	94	72	84	78	101	68
chr3	38663815	38664055	SCN5A	0,62	21	18	12	23	18	16	15	11	24	17	26	25	22	21	19	17
chr3	38671740	38671980	SCN5A	0,54	71	76	47	55	75	62	48	47	86	61	77	64	75	80	67	67
chr3	38674421	38674901	SCN5A	0,60	29	24	21	25	29	21	19	16	26	23	25	23	24	27	27	23
chr3	43732387	43732627	ABHD5	0,75	1	1	1	2	4	3	3	2	4	2	1	1	3	2	1	2
chr3	43740690	43740930	ABHD5	0,40	209	222	162	117	180	177	147	108	218	212	217	173	198	203	198	150
chr3	43743652	43744132	ABHD5	0,42	248	248	201	145	203	214	171	117	262	238	243	205	260	228	252	181
chr3	43753097	43753457	ABHD5	0,39	239	248	188	143	216	200	175	130	266	242	250	216	254	243	250	197
chr3	43756362	43756578	ABHD5	0,32	187	189	157	131	158	167	128	104	201	172	193	158	174	171	171	150
chr3	43759075	43759435	ABHD5	0,42	257	298	239	172	230	235	204	151	308	287	294	242	293	289	296	215
chr3	43759859	43760099	ABHD5	0,38	243	244	173	139	204	196	175	114	263	241	258	225	242	236	251	198
chr3	4558101	4558341	ITPR1	0,37	217	247	198	136	207	212	162	114	257	223	222	208	234	245	215	167
chr3	4562622	4562862	ITPR1	0,41	220	238	178	142	193	193	151	115	242	212	225	171	239	224	228	171
chr3	4669384	4669624	ITPR1	0,51	172	178	127	119	164	150	128	99	205	183	172	147	179	169	172	134
chr3	4680990	4681230	ITPR1	0,46	109	109	94	71	108	107	76	61	121	109	104	89	109	104	107	96
chr3	4683675	4684035	ITPR1	0,43	202	207	156	138	176	173	140	116	207	201	198	180	199	211	205	153
chr3	4685748	4685988	ITPR1	0,52	160	168	116	104	148	145	114	94	181	154	155	140	146	155	149	138
chr3	4686992	4687494	ITPR1	0,47	206	213	175	112	191	188	150	111	225	225	204	183	224	207	209	172
chr3	46899627	46900032	MYL3	0,60	76	66	56	62	84	69	50	52	88	72	82	70	80	82	85	60
chr3	46900871	46901231	MYL3	0,58	88	86	65	70	96	87	70	52	98	85	100	84	90	91	96	83
chr3	46902060	46902583	MYL3	0,60	63	60	54	56	68	62	50	41	85	63	73	67	66	68	66	66
chr3	46904695	46904935	MYL3	0,58	28	25	21	22	23	29	20	20	38	29	33	21	29	30	29	25
chr3	4693734	4693974	ITPR1	0,51	132	136	111	92	150	129	108	95	168	138	158	112	127	160	162	109
chr3	4695427	4695667	ITPR1	0,46	138	159	112	90	135	125	101	81	168	132	158	124	158	141	146	103
chr3	4699704	4700064	ITPR1	0,47	237	248	186	140	198	196	170	124	274	243	247	213	241	229	243	185
chr3	4702601	4702841	ITPR1	0,43	270	312	229	151	228	240	214	147	306	285	268	262	276	286	292	208
chr3	4703665	4704025	ITPR1	0,50	162	169	125	96	154	149	116	87	175	177	167	141	166	156	155	129
chr3	4704639	4704951	ITPR1	0,47	234	232	185	144	220	215	166	126	267	242	233	211	241	235	269	186
chr3	4706720	4707080	ITPR1	0,47	232	231	164	135	212	187	158	121	263	232	251	198	241	238	234	179
chr3	4708966	4709326	ITPR1	0,47	181	157	136	101	153	149	119	94	193	172	172	155	188	165	178	119
chr3	4711277	4711517	ITPR1	0,46	216	229	175	141	195	183	155	132	261	228	223	175	229	249	233	167
chr3	4712331	4712691	ITPR1	0,46	163	142	116	94	123	130	122	79	174	150	140	145	147	154	154	115
chr3	4714765	4715125	ITPR1	0,53	183	162	147	111	177	165	139	98	208	174	204	166	192	181	187	152
chr3	4715840	4716080	ITPR1	0,37	183	208	136	108	181	172	141	97	227	188	195	155	178	201	193	144
chr3	4716661	4717021	ITPR1	0,39	298	302	238	184	238	237	210	145	325	316	289	243	302	296	325	215
chr3	4718211	4718571	ITPR1	0,55	73	73	61	57	74	64	56	45	86	69	69	69	71	79	70	68
chr3	4722185	4722425	ITPR1	0,40	166	185	146	99	181	148	136	93	195	172	178	153	180	176	180	139
chr3	4722954	4723194	ITPR1	0,41	192	216	165	137	192	173	160	107	246	206	220	195	203	214	210	166
chr3	4724975	4725575	ITPR1	0,51	146	156	117	103	145	130	108	93	176	143	158	123	145	151	152	128
chr3	4725877	4726117	ITPR1	0,41	210	227	166	120	185	192	156	117	230	215	214	179	209	217	218	161
chr3	4726701	4726941	ITPR1	0,52	52	50	42	49	63	47	41	29	63	56	66	56	48	50	52	48
chr3	4730121	4730361	ITPR1	0,49	175	178	131	110	152	149	115	100	202	151	173	146	167	160	156	130
chr3	4732747	4733107	ITPR1	0,39	271	296	228	173	239	240	201	142	315	294	293	232	291	279	306	206
chr3	4735119	4735479	ITPR1	0,55	120	115	92	81	116	114	92	79	127	119	133	106	124	116	121	103
chr3	4738769	4739009	ITPR1	0,41	210	193	160	127	190	180	139	110	231	206	192	181	209	188	198	156

chr3	4741440	4741680	ITPR1	0,43	231	216	165	139	188	177	148	124	253	223	215	177	210	219	216	169
chr3	4744453	4744693	ITPR1	0,44	159	167	138	91	146	153	113	94	188	153	180	145	156	166	170	120
chr3	4747762	4748122	ITPR1	0,54	163	151	131	102	151	136	111	92	190	162	164	131	160	140	168	121
chr3	4751902	4752262	ITPR1	0,52	100	98	83	78	100	96	70	59	115	96	102	102	108	111	98	95
chr3	4753376	4753616	ITPR1	0,39	209	235	181	147	191	187	163	110	249	209	227	198	249	224	221	159
chr3	4758946	4759186	ITPR1	0,41	239	242	186	141	215	216	183	132	275	266	255	213	276	260	267	175
chr3	4767127	4767367	ITPR1	0,50	183	179	146	125	172	154	126	91	217	186	198	161	190	196	206	143
chr3	4768713	4768953	ITPR1	0,44	182	177	139	112	174	157	111	90	198	160	164	154	187	158	181	142
chr3	4774717	4774957	ITPR1	0,51	67	63	42	43	60	48	44	33	65	50	61	59	51	54	64	45
chr3	4776792	4777128	ITPR1	0,52	107	115	87	84	115	104	91	68	140	116	122	99	130	112	114	93
chr3	4808123	4808483	ITPR1	0,43	202	202	174	115	180	182	164	107	241	206	200	183	215	214	224	161
chr3	4810141	4810501	ITPR1	0,60	48	45	26	32	46	44	33	26	63	43	54	46	47	45	38	47
chr3	4816845	4817205	ITPR1	0,43	255	263	200	156	220	216	187	122	289	260	274	217	261	258	265	205
chr3	4818882	4819122	ITPR1	0,43	216	237	182	129	188	182	158	118	250	235	223	207	215	233	258	181
chr3	4821161	4821401	ITPR1	0,53	155	159	109	88	147	134	113	83	180	150	158	125	138	153	166	135
chr3	4824243	4824483	ITPR1	0,51	60	62	47	59	70	60	48	43	84	63	75	55	69	72	55	64
chr3	4825431	4825671	ITPR1	0,50	87	80	80	64	90	84	63	60	103	80	94	79	85	84	85	76
chr3	4829546	4829906	ITPR1	0,41	237	265	204	151	216	219	188	139	296	260	247	219	247	251	274	199
chr3	4836689	4836929	ITPR1	0,53	190	177	134	116	169	150	119	100	192	163	196	159	174	182	196	146
chr3	4842016	4842376	ITPR1	0,50	234	245	183	158	221	205	166	145	283	228	253	205	229	252	245	200
chr3	4847688	4848048	ITPR1	0,45	230	223	178	127	203	191	158	131	246	219	218	179	228	200	238	181
chr3	4852866	4853226	ITPR1	0,37	293	321	247	160	221	264	207	151	298	317	302	252	306	288	298	227
chr3	4854751	4854991	ITPR1	0,49	120	143	120	100	129	128	95	84	176	145	151	126	140	141	151	128
chr3	4856056	4856296	ITPR1	0,55	59	55	43	43	48	52	34	34	54	41	45	42	53	51	45	54
chr3	4856639	4856999	ITPR1	0,53	208	227	177	134	192	194	155	107	222	211	221	205	224	201	228	181
chr3	4859665	4860025	ITPR1	0,49	111	120	97	78	119	108	78	77	143	112	114	101	122	109	126	97
chr3	4878436	4878676	ITPR1	0,48	91	97	83	64	100	115	59	63	106	84	105	74	91	89	82	85
chr3	4887745	4887985	ITPR1	0,36	212	243	197	129	192	202	153	120	237	226	223	188	229	229	240	172
chr3	48895053	48895293	SLC25A20	0,48	132	132	92	80	127	117	89	85	143	125	137	114	136	132	130	113
chr3	48895881	48896121	SLC25A20	0,53	93	108	85	71	104	92	61	57	114	93	97	82	100	106	100	81
chr3	48896459	48896699	SLC25A20	0,58	63	68	60	55	79	73	46	53	89	57	69	70	70	75	63	63
chr3	48896892	48897084	SLC25A20	0,45	193	195	144	125	180	178	132	107	214	181	189	162	178	173	178	140
chr3	48899913	48900153	SLC25A20	0,52	124	133	102	86	141	114	97	85	144	121	145	118	127	119	127	115
chr3	48916715	48916955	SLC25A20	0,44	193	190	164	120	192	182	145	102	240	211	215	185	195	192	211	156
chr3	48921373	48921613	SLC25A20	0,51	119	116	87	80	125	100	83	70	141	108	121	114	113	106	107	102
chr3	48929338	48929578	SLC25A20	0,49	109	111	93	65	111	95	85	67	134	103	122	102	119	112	103	91
chr3	48936054	48936294	SLC25A20	0,68	8	7	5	9	13	10	7	6	14	15	8	10	11	8	9	11
chr3	52485236	52485596	TNNC1	0,60	101	90	76	66	97	89	64	60	107	89	106	81	95	97	95	83
chr3	52485696	52485936	TNNC1	0,58	104	103	85	75	96	93	76	71	106	94	102	90	99	94	94	93
chr3	52486064	52486376	TNNC1	0,65	41	36	26	31	43	42	31	29	51	37	49	33	42	43	41	45
chr3	52486393	52486633	TNNC1	0,56	69	68	53	54	75	57	45	50	86	57	82	53	70	73	65	58
chr3	52487899	52488115	TNNC1	0,65	8	5	8	8	16	13	6	5	13	10	11	8	9	14	10	11
chr3	63898206	63898326	ATXN7	0,63	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0
chr3	63898463	63898655	ATXN7	0,69	8	6	7	6	11	8	5	4	10	8	9	7	6	11	9	14
chr3	63898746	63898986	ATXN7	0,56	122	114	95	83	112	100	71	81	126	103	120	99	120	134	99	106
chr3	63937986	63938226	ATXN7	0,37	222	224	183	139	202	199	169	130	255	209	246	197	231	223	218	176
chr3	63953499	63953739	ATXN7	0,54	70	66	64	55	74	73	49	43	81	68	78	73	80	76	63	65
chr3	63965536	63965896	ATXN7	0,46	245	225	181	140	190	216	153	110	245	218	221	211	227	224	241	172
chr3	63967811	63968171	ATXN7	0,45	228	225	175	138	201	206	151	110	242	228	218	203	227	247	231	194
chr3	63968535	63968775	ATXN7	0,45	154	162	127	90	135	142	110	89	167	136	151	132	150	151	158	112
chr3	63973627	63974107	ATXN7	0,47	248	239	204	152	226	202	174	133	265	243	248	208	245	240	243	197
chr3	63975770	63976130	ATXN7	0,57	135	141	110	88	132	136	94	80	158	117	140	122	150	157	146	130
chr3	63976354	63976594	ATXN7	0,52	96	95	83	69	89	81	74	57	109	67	104	91	99	96	92	82
chr3	63981087	63981663	ATXN7	0,51	305	324	259	184	237	269	210	154	323	316	295	259	313	330	343	238
chr3	63981669	63982245	ATXN7	0,51	243	225	196	144	205	201	168	133	264	230	224	205	247	242	238	187
chr3	63983209	63983449	ATXN7	0,42	348	370	317	176	279	301	250	181	385	368	364	307	362	356	380	243
chr3	63985060	63985300	ATXN7	0,49	209	214	177	136	193	177	137	119	241	200	210	188	203	215	212	179
chr3	81539465	81539705	GBE1	0,42	160	158	140	109	142	153	126	89	194	155	177	139	169	172	160	136
chr3	81548198	81548438	GBE1	0,36	246	259	211	148	206	197	163	147	250	245	227	201	260	245	254	181
chr3	81584289	81584529	GBE1	0,38	255	303	222	172	238	253	190	158	288	293	269	235	275	274	300	228

chr3	81585964	81586276	GBE1	0,40	240	271	197	137	213	214	171	133	274	240	246	224	240	248	260	196
chr3	81626981	81627341	GBE1	0,34	289	312	242	158	238	243	206	135	321	305	295	247	279	294	324	216
chr3	81630250	81630490	GBE1	0,36	268	298	191	163	224	239	184	128	297	262	256	216	255	262	281	205
chr3	81635171	81635411	GBE1	0,38	275	274	200	151	231	223	162	148	299	278	271	242	296	265	306	212
chr3	81640131	81640371	GBE1	0,37	240	260	200	139	196	202	169	125	259	246	262	205	267	252	235	189
chr3	81642996	81643236	GBE1	0,36	216	238	200	124	179	175	156	124	238	208	238	183	216	235	248	175
chr3	81691856	81692216	GBE1	0,37	270	297	211	160	210	235	177	130	296	294	273	247	289	292	277	223
chr3	81695467	81695707	GBE1	0,33	189	215	139	127	170	175	140	115	211	204	197	190	211	194	200	152
chr3	81697954	81698194	GBE1	0,33	186	200	157	118	138	175	133	108	220	198	196	182	219	221	212	166
chr3	81698889	81699129	GBE1	0,33	270	313	211	169	217	248	198	137	299	291	289	241	282	288	318	224
chr3	81719926	81720166	GBE1	0,34	203	244	164	121	177	184	152	106	228	200	222	188	222	195	201	155
chr3	81754499	81754859	GBE1	0,32	281	290	222	147	223	231	206	146	299	288	294	241	276	292	317	219
chr3	81810416	81810776	GBE1	0,72	17	13	12	12	19	11	14	10	19	16	21	19	15	18	16	17
chr3	8775499	8775739	CAV3	0,59	133	127	108	102	130	120	92	77	155	132	138	117	138	141	141	111
chr3	8787142	8787622	CAV3	0,60	104	91	88	78	105	90	69	61	116	95	104	88	106	108	110	92
chr3	9691166	9691526	MTMR14	0,67	8	5	6	7	8	10	6	7	7	8	9	7	9	7	6	8
chr3	9695198	9695558	MTMR14	0,52	210	195	163	128	171	178	133	121	222	186	199	168	202	188	201	168
chr3	9703884	9704124	MTMR14	0,51	93	94	62	68	88	95	67	59	95	96	98	79	92	77	83	83
chr3	9710320	9710560	MTMR14	0,51	114	98	87	81	86	93	70	64	124	105	105	86	100	96	105	94
chr3	9711025	9711265	MTMR14	0,59	33	22	18	22	28	24	23	19	34	27	34	19	28	26	30	22
chr3	9712672	9712912	MTMR14	0,47	159	176	139	110	174	155	116	90	201	168	174	164	182	179	194	146
chr3	9714285	9714525	MTMR14	0,51	144	151	112	99	137	121	109	104	176	138	143	125	149	147	142	123
chr3	9718915	9719155	MTMR14	0,46	130	144	97	97	116	113	96	78	160	134	142	113	143	130	136	105
chr3	9719584	9719824	MTMR14	0,57	121	127	89	86	122	112	96	72	148	115	124	118	117	128	136	96
chr3	9724774	9725014	MTMR14	0,42	194	220	168	136	208	187	161	114	248	213	219	196	230	215	232	170
chr3	9726191	9726431	MTMR14	0,58	123	126	109	95	140	107	83	76	131	106	128	113	128	118	124	119
chr3	9726489	9726729	MTMR14	0,54	141	145	121	109	151	124	97	85	172	132	129	118	161	130	156	122
chr3	9726792	9727032	MTMR14	0,57	61	53	37	30	59	46	41	39	66	49	55	48	65	47	47	56
chr3	9729419	9729659	MTMR14	0,56	98	91	87	70	97	87	74	63	106	95	101	92	97	86	103	91
chr3	9730288	9730528	MTMR14	0,52	121	136	105	93	131	107	98	76	156	114	129	115	127	125	125	110
chr3	9730576	9730816	MTMR14	0,61	43	43	29	34	40	30	30	28	52	35	45	38	45	38	32	34
chr3	9731557	9731917	MTMR14	0,59	48	50	37	38	50	44	35	35	60	48	52	47	44	57	48	45
chr3	9739292	9739652	MTMR14	0,59	136	143	123	100	157	137	104	90	175	135	156	127	146	164	153	132
chr3	9743385	9743745	MTMR14	0,58	53	58	49	47	63	54	50	37	68	56	72	58	64	60	58	53
chr4	100495976	100496216	MTTP	0,45	206	221	157	132	194	193	141	114	245	217	197	174	210	219	226	154
chr4	100502975	100503335	MTTP	0,44	192	208	158	107	177	172	151	105	214	210	212	164	205	205	210	156
chr4	100504422	100504782	MTTP	0,35	226	273	198	140	187	218	173	118	278	254	259	217	273	275	264	210
chr4	100510733	100510973	MTTP	0,37	199	228	163	145	178	194	133	109	238	205	216	174	213	202	210	172
chr4	100512329	100512569	MTTP	0,38	221	238	164	146	198	189	154	113	268	227	229	186	235	217	230	168
chr4	100512754	100512994	MTTP	0,31	224	239	180	126	156	189	178	124	258	226	216	194	229	227	235	183
chr4	100515784	100516144	MTTP	0,39	273	277	219	166	243	211	187	131	299	270	285	237	279	262	292	223
chr4	100518122	100518482	MTTP	0,45	160	170	140	104	167	143	123	91	188	152	174	127	176	188	173	144
chr4	100521625	100521985	MTTP	0,37	316	340	248	167	257	267	216	156	364	332	317	265	357	342	341	239
chr4	100522697	100522937	MTTP	0,36	205	221	181	129	180	190	149	121	232	234	216	181	211	222	216	165
chr4	100527830	100528190	MTTP	0,42	172	187	155	125	179	159	133	99	209	184	199	171	198	200	180	147
chr4	100529848	100530208	MTTP	0,39	303	324	255	168	266	271	202	154	326	328	333	269	324	318	335	218
chr4	100532214	100532694	MTTP	0,41	299	331	250	176	242	255	211	153	332	313	307	253	321	306	329	239
chr4	100533979	100534315	MTTP	0,46	187	193	142	112	187	158	131	98	212	189	195	149	178	194	188	143
chr4	100540072	100540312	MTTP	0,32	155	172	134	97	147	149	111	95	191	155	174	132	157	152	165	126
chr4	100542122	100542482	MTTP	0,41	202	183	170	117	183	174	127	110	233	190	202	170	200	196	202	158
chr4	100543739	100544099	MTTP	0,43	208	220	177	126	193	191	139	116	253	219	225	193	228	227	222	165
chr4	113825539	113825779	ANK2	0,25	203	230	161	125	143	177	142	122	214	191	211	185	194	200	221	161
chr4	113970791	113971007	ANK2	0,45	112	118	81	66	114	98	83	68	124	110	114	103	129	113	118	96
chr4	114095502	114095742	ANK2	0,43	119	132	93	78	128	112	104	64	151	116	130	112	121	130	118	107
chr4	114117452	114117692	ANK2	0,43	82	78	55	49	75	68	55	58	89	63	76	59	78	73	75	72
chr4	114120095	114120335	ANK2	0,35	238	271	191	139	189	218	168	116	267	246	241	199	259	256	259	196
chr4	114153245	114153485	ANK2	0,38	299	333	235	195	242	259	205	142	320	304	299	275	314	310	332	220
chr4	114158055	114158415	ANK2	0,47	220	245	163	133	193	183	157	116	241	242	249	187	226	238	222	172
chr4	114158646	114158886	ANK2	0,34	289	332	219	145	212	246	190	141	323	311	276	232	296	296	289	225
chr4	114161569	114161809	ANK2	0,39	213	226	174	123	187	172	159	103	231	222	208	197	220	213	208	165

chr4	114163195	114163435	ANK2	0,44	184	182	142	97	187	157	132	92	202	187	203	162	176	184	189	142
chr4	114170848	114171088	ANK2	0,41	146	154	124	116	136	132	112	90	165	162	155	137	158	165	155	130
chr4	114176809	114177169	ANK2	0,45	218	238	186	144	232	209	166	117	251	230	242	209	231	232	253	182
chr4	114179134	114179374	ANK2	0,40	223	225	163	137	204	195	156	116	259	226	238	176	231	228	214	172
chr4	114179397	114179637	ANK2	0,51	111	96	80	70	101	99	77	56	114	83	100	79	105	104	91	80
chr4	114185981	114186221	ANK2	0,54	77	92	66	65	88	69	63	63	102	78	96	73	95	96	85	72
chr4	114195526	114195886	ANK2	0,48	171	170	143	102	163	153	125	98	208	176	178	155	181	193	196	142
chr4	114198921	114199161	ANK2	0,37	183	200	154	123	169	155	127	116	207	199	215	183	186	196	183	147
chr4	114199544	114199784	ANK2	0,49	146	153	112	99	145	128	98	88	172	140	147	129	140	158	154	123
chr4	114203749	114204109	ANK2	0,40	204	218	177	131	190	184	145	115	251	220	224	181	208	220	216	164
chr4	114208689	114208929	ANK2	0,42	177	189	128	118	169	176	126	97	202	179	185	154	179	187	173	160
chr4	114209472	114209712	ANK2	0,35	200	265	179	132	191	191	152	120	252	257	230	190	217	220	220	164
chr4	114213500	114213740	ANK2	0,59	53	50	56	44	58	56	43	37	72	58	83	49	60	76	58	58
chr4	114214595	114214787	ANK2	0,48	115	114	98	71	126	104	74	68	138	104	114	105	123	105	128	96
chr4	114223838	114224078	ANK2	0,36	245	266	196	135	210	200	165	135	276	255	240	213	247	228	248	176
chr4	114232302	114232638	ANK2	0,39	201	209	157	117	185	169	152	111	228	188	217	182	213	207	205	143
chr4	114238793	114239033	ANK2	0,49	249	239	193	144	212	219	152	116	253	252	239	212	225	242	251	181
chr4	114239604	114239844	ANK2	0,37	193	206	140	109	185	182	125	94	217	192	201	164	192	206	187	147
chr4	114244812	114245052	ANK2	0,42	342	322	283	207	281	253	231	153	367	352	327	291	377	323	364	249
chr4	114251333	114251693	ANK2	0,47	133	139	100	96	117	123	92	80	156	130	141	122	137	140	136	117
chr4	114253056	114253296	ANK2	0,48	131	140	101	81	147	119	89	77	149	138	147	117	148	148	133	121
chr4	114254106	114254442	ANK2	0,45	192	183	150	128	174	174	126	115	223	207	205	174	209	201	201	152
chr4	114256927	114257287	ANK2	0,48	114	109	76	76	115	96	84	73	118	98	110	108	98	112	94	91
chr4	114257654	114258014	ANK2	0,35	260	258	209	145	214	207	173	128	269	275	248	226	250	259	261	194
chr4	114260309	114260549	ANK2	0,38	288	284	249	160	267	249	201	140	329	312	309	252	305	292	313	217
chr4	114262757	114263093	ANK2	0,37	295	300	236	162	246	239	204	141	302	307	295	243	285	287	287	216
chr4	114264115	114264355	ANK2	0,33	292	306	237	174	203	245	210	146	338	312	310	267	288	319	306	214
chr4	114266996	114267236	ANK2	0,39	225	202	155	119	183	171	136	108	219	207	204	172	196	217	208	164
chr4	114269327	114269567	ANK2	0,41	239	220	182	143	179	191	154	121	261	248	221	171	243	226	223	176
chr4	114271274	114271514	ANK2	0,33	249	300	221	173	219	245	176	141	273	275	278	251	269	261	269	199
chr4	114274147	114280507	ANK2	0,46	268	269	216	157	229	222	189	135	290	270	270	226	267	272	279	209
chr4	114281895	114282135	ANK2	0,45	148	135	111	81	147	122	94	81	157	124	138	128	145	137	133	94
chr4	114284439	114284679	ANK2	0,39	161	174	157	124	177	147	124	93	191	192	199	153	183	189	179	140
chr4	114286124	114286364	ANK2	0,41	261	252	191	145	201	197	167	130	260	237	232	202	239	264	246	171
chr4	114288624	114289104	ANK2	0,40	187	202	159	114	174	170	144	100	210	184	210	163	187	191	182	161
chr4	114290575	114291055	ANK2	0,49	224	236	189	142	210	194	172	123	256	239	245	199	235	235	236	186
chr4	114294167	114294702	ANK2	0,40	238	257	206	155	210	219	180	130	283	260	261	223	267	275	265	188
chr4	114302559	114302679	ANK2	0,49	23	24	16	12	26	19	16	11	25	20	18	16	18	12	18	15
chr4	159593505	159593745	ETFDH	0,65	16	23	12	16	16	24	11	16	24	13	25	20	21	23	13	17
chr4	159601508	159601844	ETFDH	0,31	325	343	255	168	232	264	231	166	350	344	333	276	329	328	368	227
chr4	159603281	159603641	ETFDH	0,42	200	207	143	113	176	168	139	104	211	187	199	170	204	197	199	155
chr4	159605697	159605841	ETFDH	0,32	218	201	188	140	219	195	154	125	268	230	247	190	212	218	258	192
chr4	159606191	159606431	ETFDH	0,33	182	186	145	122	168	159	140	89	195	154	176	136	177	162	162	151
chr4	159611418	159611634	ETFDH	0,31	144	137	111	88	133	133	100	81	155	131	148	132	139	140	134	115
chr4	159616541	159616901	ETFDH	0,32	206	251	179	131	192	193	164	125	260	218	233	195	236	230	226	179
chr4	159618600	159618960	ETFDH	0,34	280	295	221	160	221	231	192	136	284	270	263	230	266	264	287	194
chr4	159620030	159620390	ETFDH	0,32	203	214	159	114	171	183	142	107	211	175	192	170	188	210	213	152
chr4	159624478	159624838	ETFDH	0,32	290	314	246	158	210	233	215	135	319	289	282	251	282	269	287	228
chr4	159627251	159627611	ETFDH	0,35	242	246	196	149	204	216	179	121	261	236	241	216	240	241	244	193
chr4	159627711	159628071	ETFDH	0,38	256	261	207	145	220	214	184	125	277	229	256	223	242	246	267	200
chr4	159629471	159629783	ETFDH	0,35	202	231	182	123	174	194	152	116	230	207	192	177	219	189	194	151
chr4	186064441	186064657	SLC25A4	0,70	11	8	5	9	12	9	5	8	15	8	11	9	8	7	6	12
chr4	186065860	186066460	SLC25A4	0,56	150	159	127	106	159	132	112	94	175	138	171	132	148	161	155	134
chr4	186066802	186067162	SLC25A4	0,52	154	158	133	107	144	151	104	92	165	150	156	127	163	157	163	128
chr4	186067866	186068226	SLC25A4	0,42	145	164	112	95	133	122	101	89	174	148	139	113	138	148	134	117
chr4	3475046	3475382	DOK7	0,68	8	6	6	4	7	7	5	3	8	6	5	5	7	5	7	5
chr4	3478060	3478372	DOK7	0,65	24	20	15	22	26	14	15	18	28	20	16	22	19	20	25	18
chr4	3487205	3487445	DOK7	0,69	11	6	2	5	11	3	5	4	11	7	9	8	6	6	6	7
chr4	3491343	3491583	DOK7	0,69	3	8	4	4	10	6	2	6	7	6	6	5	5	4	6	8
chr4	3494376	3495336	DOK7	0,69	14	14	12	17	18	14	9	12	20	12	17	13	15	15	15	17

chr4	52890044	52890404	SGCB	0,44	104	125	95	72	125	107	79	62	133	105	117	99	117	120	116	92
chr4	52894079	52894319	SGCB	0,33	297	330	252	166	239	259	197	148	320	298	301	259	309	309	338	209
chr4	52894811	52895171	SGCB	0,32	331	297	260	186	255	269	222	158	338	327	317	263	344	311	337	232
chr4	52895756	52896116	SGCB	0,39	239	266	196	134	220	204	174	131	257	259	221	206	223	240	247	199
chr4	52899521	52899881	SGCB	0,37	338	363	284	186	272	279	235	169	355	365	346	291	339	331	341	254
chr5	131705560	131706160	SLC22A5	0,70	14	10	11	12	17	14	11	10	16	12	15	12	14	11	10	14
chr5	131714001	131714241	SLC22A5	0,52	147	153	116	92	135	132	99	81	163	159	149	138	161	154	151	124
chr5	131719735	131720095	SLC22A5	0,50	197	207	163	133	183	167	146	110	223	207	217	159	201	207	184	168
chr5	131720925	131721285	SLC22A5	0,47	165	165	131	100	153	137	119	86	178	142	171	138	149	164	154	133
chr5	131722659	131722899	SLC22A5	0,56	96	92	66	70	96	72	53	54	92	80	81	89	104	76	79	69
chr5	131724542	131724782	SLC22A5	0,46	204	209	183	130	187	169	142	107	226	225	211	198	220	208	218	167
chr5	131726308	131726668	SLC22A5	0,50	199	197	155	129	173	183	151	102	222	180	199	174	214	203	209	170
chr5	131728035	131728395	SLC22A5	0,51	163	166	124	102	155	140	116	94	190	164	156	150	161	167	179	136
chr5	131729315	131729555	SLC22A5	0,56	250	241	191	147	203	220	182	118	301	226	251	235	243	253	259	178
chr5	131729800	131730040	SLC22A5	0,41	133	148	126	96	131	122	92	74	163	137	141	107	137	130	139	125
chr5	137206278	137206758	MYOT	0,47	359	359	271	202	277	296	236	165	364	370	351	308	361	375	392	261
chr5	137211424	137211784	MYOT	0,35	294	334	231	153	229	254	208	131	302	302	300	259	292	303	320	230
chr5	137213139	137213379	MYOT	0,37	205	213	155	143	181	191	144	118	241	208	197	194	186	220	223	163
chr5	137216409	137216649	MYOT	0,30	234	270	190	151	166	221	179	127	263	247	234	194	247	246	256	180
chr5	137217607	137217847	MYOT	0,37	202	245	186	117	201	198	181	131	247	237	249	193	235	230	237	174
chr5	137218996	137219356	MYOT	0,39	273	265	202	155	224	218	188	136	286	277	263	235	253	267	274	195
chr5	137221639	137221999	MYOT	0,29	237	245	190	137	177	202	170	122	266	238	240	183	234	228	231	180
chr5	137222499	137222739	MYOT	0,32	215	217	181	132	167	185	148	111	215	178	194	166	191	196	204	159
chr5	137222807	137223167	MYOT	0,34	295	338	263	188	236	268	224	154	345	345	315	268	308	326	344	229
chr5	138282743	138283223	SIL1	0,66	28	24	22	23	32	28	24	16	35	24	35	24	27	26	23	27
chr5	138286761	138287121	SIL1	0,63	21	23	15	17	25	16	9	14	23	16	22	18	18	20	20	17
chr5	138287404	138287644	SIL1	0,62	37	33	30	32	44	34	32	28	48	33	34	30	41	42	40	35
chr5	138356800	138357040	SIL1	0,52	146	158	135	117	143	122	104	80	173	148	163	153	172	156	176	122
chr5	138362405	138362765	SIL1	0,45	218	215	174	135	176	173	146	108	242	210	213	189	223	223	230	151
chr5	138378231	138378447	SIL1	0,45	295	320	253	166	254	217	195	157	301	297	286	228	280	288	311	208
chr5	138386560	138386800	SIL1	0,46	239	255	191	130	208	209	177	116	263	245	233	178	240	224	257	183
chr5	138456672	138456912	SIL1	0,54	116	114	100	92	127	97	87	78	147	95	138	121	115	133	115	91
chr5	138463359	138463599	SIL1	0,54	25	21	15	16	35	23	16	18	22	18	30	23	22	30	22	27
chr5	138643083	138644115	MATR3	0,43	305	319	242	175	252	259	224	154	331	318	298	259	314	311	312	238
chr5	138650274	138650514	MATR3	0,34	264	306	211	157	205	217	201	142	280	270	257	221	253	242	273	190
chr5	138651286	138651526	MATR3	0,29	297	322	249	158	188	259	208	144	306	291	283	233	291	266	296	231
chr5	138651700	138651940	MATR3	0,38	242	239	195	139	205	225	184	143	259	242	263	209	245	243	248	171
chr5	138652647	138652887	MATR3	0,37	168	192	146	118	157	168	130	109	195	196	195	151	190	171	182	161
chr5	138653227	138653467	MATR3	0,32	179	195	144	108	147	151	115	104	206	174	195	161	192	176	190	153
chr5	138654539	138654779	MATR3	0,33	176	186	145	113	172	165	137	101	209	181	195	154	188	171	180	153
chr5	138654926	138655262	MATR3	0,32	246	260	198	143	219	226	182	127	265	226	243	212	240	244	234	189
chr5	138657532	138657772	MATR3	0,33	210	220	155	125	181	168	153	103	235	198	202	187	201	186	193	167
chr5	138658043	138658763	MATR3	0,36	259	289	213	157	226	221	208	131	288	273	268	229	272	266	287	200
chr5	138661059	138661419	MATR3	0,39	212	225	169	128	191	182	162	119	226	207	224	178	212	212	228	176
chr5	138661792	138662032	MATR3	0,30	209	218	170	129	162	170	146	102	218	200	204	173	193	178	184	159
chr5	138664938	138665178	MATR3	0,29	217	244	183	135	168	172	163	131	255	227	225	216	236	233	236	166
chr5	145969409	145969889	PPP2R2B	0,42	269	301	211	153	232	220	172	141	296	266	280	229	283	292	278	220
chr5	145972459	145972699	PPP2R2B	0,41	283	307	266	168	252	262	222	138	333	319	301	257	318	310	338	228
chr5	145979758	145980118	PPP2R2B	0,44	216	241	207	150	222	197	165	121	254	242	234	200	229	236	240	189
chr5	146017715	146018075	PPP2R2B	0,53	121	112	81	81	115	99	81	74	142	115	129	106	109	122	108	101
chr5	146030018	146030378	PPP2R2B	0,42	161	162	144	119	157	141	115	95	189	161	171	166	171	174	168	140
chr5	146070626	146070866	PPP2R2B	0,53	86	90	70	55	92	75	65	60	106	85	85	82	96	97	92	82
chr5	146077492	146077804	PPP2R2B	0,40	245	262	206	147	203	218	154	127	280	249	259	211	252	260	271	188
chr5	146080536	146080776	PPP2R2B	0,42	263	270	209	159	225	218	184	144	310	277	275	215	294	266	310	206
chr5	146235959	146236199	PPP2R2B	0,38	257	253	193	157	206	207	167	131	282	261	250	212	275	252	258	193
chr5	146257479	146257719	PPP2R2B	0,61	35	26	22	26	32	28	25	25	40	25	36	23	33	39	33	36
chr5	146435124	146435316	PPP2R2B	0,51	97	92	82	72	115	83	69	61	109	87	103	77	98	95	105	80
chr5	148384189	148384549	SH3TC2	0,60	29	31	22	23	33	28	20	22	32	25	33	28	37	27	28	29
chr5	148386361	148386697	SH3TC2	0,52	107	102	81	73	108	96	74	65	123	105	120	100	108	114	107	98
chr5	148388356	148388668	SH3TC2	0,51	222	200	165	123	186	183	150	117	233	201	201	173	203	189	222	155

chr5	148389773	148390013	SH3TC2	0,55	92	73	58	50	75	70	53	54	90	70	85	84	82	91	73	67
chr5	148392041	148392401	SH3TC2	0,57	51	43	36	45	62	49	33	33	63	48	53	56	52	59	54	51
chr5	148406044	148408380	SH3TC2	0,55	110	106	85	74	109	100	78	67	126	99	114	97	115	112	104	94
chr5	148411063	148411303	SH3TC2	0,51	74	77	65	60	83	75	56	58	108	74	79	68	79	75	69	76
chr5	148417775	148418135	SH3TC2	0,48	219	239	185	145	186	212	167	121	264	223	234	213	225	247	235	178
chr5	148420083	148420323	SH3TC2	0,41	237	250	211	140	209	233	170	133	268	257	242	196	234	256	255	181
chr5	148420899	148421259	SH3TC2	0,52	128	128	93	85	118	99	81	69	135	111	126	109	128	123	119	105
chr5	148422148	148422508	SH3TC2	0,43	288	305	249	167	274	250	231	143	333	322	297	265	318	299	318	228
chr5	148424028	148424268	SH3TC2	0,47	97	100	77	77	102	91	78	57	110	97	115	84	105	114	98	84
chr5	148427417	148427633	SH3TC2	0,50	58	61	43	47	63	59	42	44	84	60	56	53	73	62	63	52
chr5	148431633	148431873	SH3TC2	0,39	233	263	185	148	199	211	176	114	287	228	258	219	231	243	245	175
chr5	148442439	148442679	SH3TC2	0,63	23	22	15	16	29	20	18	20	26	27	26	27	19	25	23	25
chr5	155756527	155756647	SGCD	0,48	44	34	31	27	44	36	33	36	49	36	47	38	45	38	31	33
chr5	155771412	155771772	SGCD	0,45	217	223	169	131	185	180	144	111	237	200	221	171	227	212	210	176
chr5	155935541	155935781	SGCD	0,40	204	221	182	123	190	191	145	113	239	238	217	182	226	238	237	178
chr5	156016164	156016404	SGCD	0,38	182	186	163	112	142	166	123	102	227	191	195	167	190	195	177	163
chr5	156021929	156022145	SGCD	0,35	172	192	133	109	155	154	129	94	218	176	191	151	167	166	196	145
chr5	156074389	156074629	SGCD	0,42	134	124	110	94	136	121	83	82	147	142	133	122	146	137	131	124
chr5	156184509	156184869	SGCD	0,44	92	99	67	63	101	87	68	60	118	80	104	83	106	97	88	77
chr5	156186134	156186494	SGCD	0,46	167	166	138	104	146	141	107	93	172	147	164	154	165	167	169	136
chr5	36608444	36608804	SLC1A3	0,45	160	176	124	92	136	122	103	82	176	154	164	145	153	153	168	128
chr5	36629545	36629761	SLC1A3	0,42	199	225	184	132	190	190	161	125	235	203	229	176	207	205	205	178
chr5	36671052	36671412	SLC1A3	0,47	163	184	129	109	163	148	122	99	193	181	175	152	184	176	195	141
chr5	36674051	36674291	SLC1A3	0,37	301	331	264	177	262	278	243	168	329	319	309	284	338	318	334	252
chr5	36676961	36677393	SLC1A3	0,44	216	216	172	134	197	186	160	116	233	216	223	190	229	218	220	183
chr5	36679665	36680025	SLC1A3	0,50	150	151	110	99	133	133	101	75	164	146	133	119	150	138	139	127
chr5	36680411	36680747	SLC1A3	0,48	194	182	147	107	195	160	129	103	210	199	184	161	179	207	187	155
chr5	36683912	36684152	SLC1A3	0,54	44	45	31	31	45	40	32	27	59	42	57	46	50	49	44	46
chr5	36686088	36686448	SLC1A3	0,46	196	196	159	124	173	159	144	108	219	193	198	160	198	199	192	168
chr5	38481588	38482428	LIFR	0,42	338	354	269	177	277	285	232	170	369	354	339	281	337	346	369	251
chr5	38482585	38482777	LIFR	0,27	202	205	172	136	180	203	155	120	221	200	214	185	204	181	180	156
chr5	38484803	38485043	LIFR	0,36	277	298	227	150	237	235	197	138	311	284	260	215	291	279	282	202
chr5	38485821	38486181	LIFR	0,43	333	375	275	184	263	275	237	159	354	350	349	300	348	357	354	270
chr5	38489083	38489443	LIFR	0,35	345	363	292	177	247	298	231	173	382	385	321	314	348	358	396	255
chr5	38490222	38490462	LIFR	0,29	336	325	249	181	249	273	222	159	342	285	310	281	300	322	306	250
chr5	38493641	38493977	LIFR	0,38	273	283	227	148	229	225	191	141	284	271	264	226	251	263	278	207
chr5	38496410	38496770	LIFR	0,38	290	300	250	163	242	248	213	149	316	299	299	260	291	306	326	227
chr5	38499529	38499769	LIFR	0,34	213	250	182	147	193	205	172	123	268	238	232	202	248	227	238	162
chr5	38502681	38502921	LIFR	0,32	220	231	191	145	206	188	159	119	265	257	247	198	230	243	242	182
chr5	38503970	38504330	LIFR	0,28	260	282	211	152	188	233	193	147	288	281	283	236	278	284	293	198
chr5	38505911	38506271	LIFR	0,27	257	292	224	156	192	225	195	152	277	261	272	232	268	259	267	203
chr5	38506549	38506789	LIFR	0,39	303	308	227	143	233	252	200	152	317	297	300	241	274	307	315	222
chr5	38510512	38510872	LIFR	0,33	292	334	279	170	238	270	221	156	335	324	313	261	322	325	323	220
chr5	38511798	38512158	LIFR	0,35	214	232	170	126	194	188	149	112	235	228	227	195	228	227	224	166
chr5	38523422	38523782	LIFR	0,32	286	322	253	172	211	254	223	147	302	295	294	247	295	297	334	223
chr5	38527195	38527435	LIFR	0,28	194	201	162	123	168	157	141	115	231	188	186	159	212	213	190	160
chr5	38528806	38528950	LIFR	0,39	193	204	157	132	184	155	128	119	217	184	203	187	206	193	191	161
chr5	38530498	38530858	LIFR	0,38	303	329	249	178	250	278	208	149	329	334	319	266	305	303	323	229
chr5	69345432	69345672	SMN2	0,68	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chr5	69359157	69359397	SMN2	0,33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chr5	69361731	69361971	SMN2	0,34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chr5	69362680	69363376	SMN2	0,38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chr5	69365099	69365219	SMN2	0,40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chr5	69366402	69366642	SMN2	0,34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chr5	69372252	69372492	SMN2	0,27	100	287	0	132	76	169	129	52	210	174	193	174	179	87	91	72
chr5	70220850	70221090	SMN1	0,68	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chr5	70234581	70234821	SMN1	0,33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chr5	70237155	70237395	SMN1	0,34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chr5	70238104	70238800	SMN1	0,38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chr5	70240522	70240642	SMN1	0,40	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

chr5	70241827	70242067	SMN1	0,34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chr5	70247672	70247912	SMN1	0,28	200	190	175	110	126	164	153	170	204	188	194	169	207	190	182	162
chr6	110012551	110012791	FIG4	0,68	4	2	3	1	5	3	4	3	5	3	4	6	4	6	4	5
chr6	110036209	110036449	FIG4	0,32	198	214	174	139	194	181	158	115	247	197	208	186	227	208	217	169
chr6	110037589	110037829	FIG4	0,38	152	174	108	88	144	120	101	64	174	126	167	112	159	150	126	108
chr6	110048255	110048495	FIG4	0,34	239	259	187	143	202	209	172	118	270	233	248	205	235	220	245	197
chr6	110053744	110053984	FIG4	0,28	251	296	219	159	200	229	180	134	283	263	279	218	282	271	282	205
chr6	110056246	110056606	FIG4	0,36	336	387	290	189	272	284	236	161	368	350	345	272	341	332	369	255
chr6	110059471	110059711	FIG4	0,32	269	294	235	154	201	226	172	135	277	254	268	206	263	253	268	187
chr6	110062576	110062816	FIG4	0,39	222	249	178	126	213	214	156	118	235	211	231	202	231	223	243	171
chr6	110064213	110064573	FIG4	0,34	285	295	247	166	225	235	207	137	294	287	284	251	290	273	303	193
chr6	110064806	110065046	FIG4	0,44	206	236	180	128	186	184	136	105	247	220	218	196	221	218	217	169
chr6	110081399	110081639	FIG4	0,37	263	307	221	154	231	215	191	133	297	293	274	241	290	263	284	210
chr6	110083231	110083471	FIG4	0,34	251	276	219	154	214	225	184	152	292	273	265	228	278	263	274	208
chr6	110085066	110085282	FIG4	0,34	122	137	98	93	123	110	95	86	151	129	129	117	138	134	119	108
chr6	110086133	110086469	FIG4	0,36	299	295	241	170	227	240	198	148	316	314	293	239	288	306	312	224
chr6	110087834	110088194	FIG4	0,34	302	308	249	166	247	250	204	142	314	325	294	248	300	301	298	227
chr6	110098073	110098313	FIG4	0,35	204	241	175	121	186	201	149	108	233	226	226	192	208	226	215	175
chr6	110106166	110106286	FIG4	0,34	98	86	78	79	84	81	82	59	112	87	115	83	111	96	92	84
chr6	110107448	110107736	FIG4	0,35	279	275	216	147	235	228	199	146	279	281	279	234	280	275	280	221
chr6	110110718	110110958	FIG4	0,35	181	204	172	107	172	161	137	97	229	206	213	162	196	180	188	137
chr6	110112496	110112856	FIG4	0,52	73	75	54	63	78	62	51	48	88	75	75	70	73	77	72	71
chr6	110113705	110113945	FIG4	0,30	253	262	206	173	204	220	176	152	289	247	259	221	263	245	234	199
chr6	110117890	110118130	FIG4	0,36	193	216	154	107	180	190	132	101	202	190	200	181	208	174	168	153
chr6	110146199	110146559	FIG4	0,47	292	298	227	159	234	261	192	151	296	299	310	246	290	276	299	217
chr6	112430532	112430892	LAMA4	0,46	132	141	108	98	120	107	93	79	131	125	145	100	132	137	127	104
chr6	112435218	112435458	LAMA4	0,41	179	209	155	116	179	168	118	99	197	174	196	155	190	177	195	142
chr6	112435792	112436032	LAMA4	0,41	257	267	204	146	200	210	177	114	274	278	274	206	276	246	274	195
chr6	112437010	112437250	LAMA4	0,40	234	268	196	149	211	216	162	126	283	232	248	218	274	264	262	200
chr6	112438841	112439201	LAMA4	0,43	249	271	201	153	220	192	179	129	280	256	246	220	246	267	245	201
chr6	112440331	112440571	LAMA4	0,46	167	177	146	108	183	153	120	106	217	192	184	159	202	202	179	160
chr6	112441400	112441760	LAMA4	0,39	262	280	193	159	203	200	187	128	279	257	248	228	258	257	263	203
chr6	112443130	112443490	LAMA4	0,42	224	249	187	144	218	194	171	125	246	232	231	202	220	235	234	173
chr6	112450064	112450304	LAMA4	0,37	194	205	168	113	190	202	168	120	235	189	219	174	197	217	191	163
chr6	112451064	112451304	LAMA4	0,38	309	350	259	184	264	250	225	159	323	340	300	265	326	332	357	251
chr6	112452116	112452356	LAMA4	0,44	194	226	170	128	172	190	138	100	226	206	198	180	202	196	198	167
chr6	112453903	112454143	LAMA4	0,35	261	298	235	172	211	231	206	142	306	278	271	262	281	271	311	209
chr6	112454499	112454739	LAMA4	0,44	161	185	131	107	161	151	120	97	177	171	168	155	173	185	176	143
chr6	112455559	112455919	LAMA4	0,37	298	295	245	167	243	235	202	153	319	305	314	245	300	290	306	218
chr6	112457270	112457510	LAMA4	0,37	237	261	185	137	210	208	165	118	292	254	222	223	253	248	272	191
chr6	112460227	112460587	LAMA4	0,44	255	275	209	151	243	225	206	136	298	276	286	237	263	275	293	222
chr6	112460900	112461140	LAMA4	0,43	182	190	161	119	169	168	120	108	215	188	182	160	204	186	201	157
chr6	112461862	112462222	LAMA4	0,36	281	289	222	161	237	230	207	138	285	275	281	231	278	276	297	200
chr6	112462452	112462812	LAMA4	0,38	281	274	216	161	232	246	193	150	303	284	284	230	284	275	288	212
chr6	112463227	112463587	LAMA4	0,47	252	252	204	146	238	224	174	130	264	255	257	222	274	263	265	197
chr6	112465945	112466185	LAMA4	0,43	69	79	55	66	70	63	46	48	84	66	83	75	82	68	69	72
chr6	112469268	112469628	LAMA4	0,45	159	172	142	116	158	160	123	99	193	170	179	143	179	171	188	143
chr6	112471650	112471890	LAMA4	0,48	181	197	148	130	168	165	142	94	222	197	202	178	200	172	189	142
chr6	112475980	112476220	LAMA4	0,39	199	217	151	135	162	159	147	101	209	198	208	183	212	206	215	161
chr6	112476657	112477017	LAMA4	0,39	329	328	249	168	263	279	227	169	353	347	331	269	329	318	369	241
chr6	112479827	112480187	LAMA4	0,33	310	362	273	183	234	272	249	159	377	345	332	272	338	342	376	250
chr6	112486299	112486539	LAMA4	0,45	164	174	121	111	136	131	113	74	167	154	163	128	155	164	168	136
chr6	112493729	112494089	LAMA4	0,51	87	98	77	70	109	89	62	64	113	90	87	82	112	95	94	83
chr6	112496418	112496778	LAMA4	0,49	204	221	172	125	173	177	146	117	256	205	214	177	214	226	234	166
chr6	112499258	112499498	LAMA4	0,36	284	308	234	168	235	233	192	136	300	281	283	254	260	267	306	212
chr6	112506373	112506613	LAMA4	0,38	281	298	238	152	230	224	192	152	298	306	283	231	292	287	314	211
chr6	112508547	112508907	LAMA4	0,48	176	179	127	108	173	138	126	91	184	167	166	154	191	185	176	139
chr6	112510239	112510479	LAMA4	0,44	162	166	128	105	149	140	119	93	190	157	163	136	182	161	156	130
chr6	112512764	112513124	LAMA4	0,44	157	181	155	118	166	162	120	92	193	172	168	150	168	185	170	139
chr6	112522728	112522968	LAMA4	0,38	280	312	220	181	240	236	212	155	304	286	279	250	281	271	294	204

chr6	112528167	112528407	LAMA4	0,47	121	124	98	83	123	101	94	71	136	113	115	100	121	121	114	91
chr6	112537499	112537739	LAMA4	0,42	207	209	160	134	189	184	139	110	216	194	203	195	206	205	217	164
chr6	112574930	112575410	LAMA4	0,60	82	77	63	54	84	75	63	53	94	83	95	72	85	94	88	74
chr6	118879983	118880343	PLN	0,41	358	374	301	190	283	298	264	185	378	377	362	308	370	363	384	273
chr6	129204326	129204566	LAMA2	0,67	19	14	12	15	25	17	11	11	22	15	23	21	20	18	22	21
chr6	129370967	129371327	LAMA2	0,40	291	273	216	150	221	228	183	135	288	292	266	237	278	272	290	210
chr6	129380864	129381104	LAMA2	0,34	222	234	181	130	195	193	136	114	247	208	195	168	224	216	212	166
chr6	129419258	129419618	LAMA2	0,43	255	266	190	154	235	216	186	133	278	274	263	222	255	264	264	196
chr6	129464955	129465315	LAMA2	0,34	264	277	222	147	211	225	174	129	292	261	256	217	263	270	275	207
chr6	129468028	129468268	LAMA2	0,41	212	258	182	134	192	202	154	129	266	231	229	203	234	263	233	198
chr6	129470062	129470302	LAMA2	0,41	196	200	158	106	171	159	136	102	217	183	198	159	190	195	196	154
chr6	129475558	129475918	LAMA2	0,30	206	240	177	131	186	204	165	110	262	251	228	188	223	226	247	181
chr6	129486650	129486890	LAMA2	0,37	206	230	154	113	188	166	134	108	227	211	199	182	213	218	205	182
chr6	129498750	129499110	LAMA2	0,41	267	272	226	148	226	223	188	135	288	293	282	249	271	291	296	216
chr6	129511239	129511599	LAMA2	0,40	263	263	216	142	227	236	178	121	288	281	260	216	262	274	262	208
chr6	129513731	129514091	LAMA2	0,47	135	145	108	88	126	119	99	78	159	121	136	113	151	134	132	108
chr6	129571187	129571427	LAMA2	0,39	255	283	194	138	208	211	171	120	286	263	258	221	254	254	274	188
chr6	129573154	129573514	LAMA2	0,41	237	252	182	136	196	206	167	123	241	241	249	195	243	226	238	176
chr6	129581791	129582031	LAMA2	0,43	269	282	218	161	230	242	205	132	297	281	258	240	272	275	303	223
chr6	129588187	129588427	LAMA2	0,46	164	173	149	95	170	149	109	90	204	172	177	156	189	180	170	149
chr6	129591712	129591952	LAMA2	0,42	293	308	251	163	243	246	201	154	318	286	289	272	293	318	320	222
chr6	129601128	129601368	LAMA2	0,43	227	227	162	146	185	185	153	105	248	234	233	191	237	217	228	192
chr6	129608917	129609277	LAMA2	0,47	202	203	158	115	196	172	133	110	225	202	200	163	211	213	206	171
chr6	129612691	129612931	LAMA2	0,44	161	168	122	111	145	142	116	94	163	171	169	146	177	183	154	130
chr6	129618739	129619099	LAMA2	0,44	179	194	144	116	171	169	122	105	224	180	198	166	178	210	191	150
chr6	129621828	129622068	LAMA2	0,40	217	211	169	132	198	187	159	104	250	219	226	203	222	230	205	165
chr6	129633943	129634303	LAMA2	0,44	200	216	163	133	188	172	141	109	247	217	213	170	213	227	214	168
chr6	129635691	129636051	LAMA2	0,46	106	118	85	79	108	94	79	62	125	108	123	102	115	115	114	103
chr6	129636530	129637411	LAMA2	0,39	283	305	240	166	236	248	188	141	312	306	295	252	306	305	311	231
chr6	129641621	129641861	LAMA2	0,42	186	197	150	136	165	161	128	99	216	195	189	165	210	198	198	133
chr6	129649369	129649609	LAMA2	0,50	86	83	65	69	91	79	66	55	109	88	91	80	108	81	89	75
chr6	129663429	129663669	LAMA2	0,38	272	311	215	151	221	209	208	145	328	291	271	250	284	269	283	222
chr6	129670365	129670605	LAMA2	0,40	192	206	154	132	164	169	127	106	212	181	199	146	203	189	196	154
chr6	129674225	129674585	LAMA2	0,51	96	101	80	68	105	90	67	58	111	93	111	100	103	101	93	90
chr6	129687254	129687614	LAMA2	0,46	164	152	120	92	146	130	112	94	187	161	160	127	159	169	166	132
chr6	129690965	129691205	LAMA2	0,43	131	125	107	77	137	130	87	81	132	112	124	114	138	123	117	109
chr6	129704202	129704442	LAMA2	0,44	96	110	78	67	101	98	78	65	116	88	114	89	95	89	110	79
chr6	129712536	129712896	LAMA2	0,35	224	246	188	132	188	198	169	119	263	236	238	194	231	237	242	184
chr6	129714114	129714474	LAMA2	0,45	149	154	112	98	145	128	112	79	165	136	157	118	153	152	143	129
chr6	129722306	129722546	LAMA2	0,38	146	153	122	91	147	127	104	81	177	142	156	136	154	159	175	120
chr6	129723370	129723730	LAMA2	0,38	266	251	222	166	253	244	189	143	285	260	267	244	263	256	280	209
chr6	129724914	129725154	LAMA2	0,40	259	296	213	152	223	239	198	136	294	293	272	235	310	300	293	198
chr6	129748828	129749068	LAMA2	0,39	211	213	173	127	191	182	158	100	238	218	222	176	208	219	204	172
chr6	129759728	129759968	LAMA2	0,35	170	194	144	121	172	174	133	108	239	196	182	169	204	197	189	171
chr6	129761871	129762231	LAMA2	0,44	239	264	215	161	208	228	190	134	287	262	276	236	279	264	291	184
chr6	129763309	129763429	LAMA2	0,36	61	71	46	51	70	74	56	49	84	61	92	69	66	72	57	49
chr6	129766708	129767068	LAMA2	0,38	245	247	184	140	210	210	161	119	251	235	227	198	241	230	244	170
chr6	129774024	129774384	LAMA2	0,35	280	315	244	157	218	243	201	154	307	293	293	245	294	293	312	219
chr6	129775246	129775486	LAMA2	0,35	98	101	73	56	95	81	64	48	111	81	95	91	100	98	99	67
chr6	129777379	129777739	LAMA2	0,42	216	233	165	126	187	193	161	110	240	235	212	193	234	227	237	182
chr6	129781286	129781526	LAMA2	0,40	194	209	154	120	176	189	147	99	246	192	202	188	214	215	193	167
chr6	129785335	129785695	LAMA2	0,40	239	254	203	142	203	219	173	112	252	248	260	214	247	251	265	189
chr6	129786181	129786541	LAMA2	0,39	253	267	198	144	220	205	180	133	278	242	259	203	258	251	253	182
chr6	129794307	129794547	LAMA2	0,32	250	267	182	140	207	217	179	122	282	234	249	228	255	261	259	189
chr6	129796480	129796600	LAMA2	0,30	75	77	46	58	71	63	46	43	89	69	72	62	71	69	64	67
chr6	129799777	129800017	LAMA2	0,37	293	321	243	177	235	247	204	140	302	283	272	238	293	269	319	217
chr6	129802315	129802675	LAMA2	0,40	230	270	187	155	220	220	187	127	271	253	246	208	244	257	260	183
chr6	129807512	129807872	LAMA2	0,44	239	254	196	148	209	197	165	110	272	234	258	213	242	239	239	191
chr6	129812953	129813313	LAMA2	0,37	306	316	248	167	242	244	216	154	311	312	301	258	291	310	311	222
chr6	129813363	129813723	LAMA2	0,42	284	312	270	169	247	256	230	174	316	306	311	270	317	323	316	229

chr6	129823739	129823979	LAMA2	0,40	179	186	141	104	136	142	137	95	187	157	191	161	173	167	168	144
chr6	129824150	129824510	LAMA2	0,45	184	204	158	114	166	144	135	100	200	181	191	166	200	199	203	156
chr6	129826242	129826602	LAMA2	0,36	209	202	166	135	199	192	152	117	237	222	214	178	217	213	217	179
chr6	129828530	129828890	LAMA2	0,42	285	295	233	161	237	248	210	120	282	274	274	255	290	273	270	218
chr6	129833452	129833692	LAMA2	0,36	190	185	141	110	156	163	136	86	221	200	176	169	199	202	203	151
chr6	129835448	129835808	LAMA2	0,45	212	224	188	146	195	188	161	117	252	219	231	206	233	238	239	178
chr6	129837233	129837593	LAMA2	0,40	269	263	219	162	236	218	177	123	293	272	259	258	265	259	269	186
chr6	133595814	133596054	EYA4	0,34	156	175	127	95	140	146	99	96	170	150	143	133	163	160	167	131
chr6	133703434	133703674	EYA4	0,37	220	243	194	133	195	198	168	123	240	244	252	197	243	232	251	172
chr6	133767709	133767949	EYA4	0,40	202	256	176	116	185	198	154	110	239	215	232	172	220	229	214	177
chr6	133769162	133769402	EYA4	0,44	166	203	133	122	169	160	118	97	211	183	180	166	172	180	162	159
chr6	133777619	133777859	EYA4	0,46	174	187	147	115	168	168	123	102	205	186	173	163	193	195	185	141
chr6	133782164	133782404	EYA4	0,37	242	274	194	150	199	210	158	123	278	243	245	202	240	260	247	171
chr6	133783363	133784010	EYA4	0,43	314	320	259	164	267	277	220	169	351	333	317	270	322	330	328	240
chr6	133785836	133786076	EYA4	0,31	298	310	235	159	189	227	190	139	287	285	289	243	290	293	301	213
chr6	133789606	133789966	EYA4	0,40	293	314	228	162	227	265	196	140	348	325	314	254	325	320	305	222
chr6	133802548	133802788	EYA4	0,43	160	150	124	89	140	138	99	93	170	158	153	124	151	172	153	130
chr6	133804091	133804331	EYA4	0,34	251	287	219	139	207	222	190	143	270	261	266	246	266	269	283	181
chr6	133827194	133827410	EYA4	0,36	256	261	186	148	181	235	178	134	276	247	263	207	231	251	266	172
chr6	133833777	133834257	EYA4	0,38	265	289	213	143	234	235	188	125	301	261	268	216	266	260	272	206
chr6	133836395	133836635	EYA4	0,40	217	214	191	122	191	176	150	108	228	203	225	184	234	208	222	170
chr6	133844134	133844374	EYA4	0,35	248	283	214	152	208	234	188	142	270	283	272	230	277	270	288	192
chr6	133846092	133846452	EYA4	0,40	206	222	161	125	191	173	146	106	237	188	184	181	216	199	201	163
chr6	133849849	133850041	EYA4	0,41	177	194	148	131	144	175	128	104	207	185	211	180	211	195	193	141
chr6	137143798	137143942	PEX7	0,77	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chr6	137146260	137146500	PEX7	0,34	216	220	163	139	176	195	159	111	231	229	216	184	260	210	218	171
chr6	137147351	137147711	PEX7	0,41	168	182	152	109	159	154	134	103	203	192	182	159	176	168	184	151
chr6	137166671	137166911	PEX7	0,35	205	238	158	129	185	188	142	113	222	202	215	189	209	202	207	153
chr6	137167146	137167386	PEX7	0,37	254	259	191	152	237	230	193	128	279	273	231	201	256	253	249	208
chr6	137187697	137187937	PEX7	0,39	158	173	118	101	151	141	101	87	174	160	162	142	161	169	166	121
chr6	137190954	137191170	PEX7	0,38	168	169	125	118	164	137	122	93	192	170	151	135	152	148	152	132
chr6	137193243	137193483	PEX7	0,30	251	305	220	157	192	226	184	143	301	256	286	219	259	255	255	211
chr6	137219209	137219449	PEX7	0,37	251	276	220	157	201	215	167	121	281	260	262	235	246	257	266	204
chr6	137234509	137234749	PEX7	0,35	257	292	217	147	227	226	188	140	273	268	270	226	259	278	259	203
chr6	152443510	152443870	SYNE1	0,55	82	62	52	53	70	64	49	45	72	68	71	62	69	70	64	62
chr6	152451763	152452003	SYNE1	0,45	91	94	67	64	87	85	59	55	108	101	83	69	86	87	90	77
chr6	152453182	152453422	SYNE1	0,51	141	154	121	105	136	125	105	86	177	141	160	142	146	146	146	119
chr6	152454336	152454696	SYNE1	0,39	272	311	229	164	223	241	213	141	315	279	294	243	284	295	305	227
chr6	152456182	152456422	SYNE1	0,36	252	283	217	150	221	228	186	141	285	264	277	227	257	263	272	191
chr6	152457672	152458032	SYNE1	0,51	81	86	66	64	86	70	53	50	88	71	91	71	88	91	69	64
chr6	152461010	152461370	SYNE1	0,53	134	135	98	87	128	117	96	86	147	125	132	124	138	147	143	114
chr6	152462280	152462520	SYNE1	0,48	198	183	151	110	197	158	141	95	220	192	192	175	192	196	171	154
chr6	152464648	152465008	SYNE1	0,47	137	138	113	96	133	137	110	102	164	132	155	126	141	137	147	122
chr6	152466535	152466775	SYNE1	0,48	121	117	95	79	114	102	75	63	130	111	125	103	123	124	123	99
chr6	152469106	152469586	SYNE1	0,57	88	85	75	68	93	81	70	54	127	78	110	96	100	83	84	88
chr6	152470527	152470887	SYNE1	0,41	298	318	255	164	238	243	207	147	321	278	301	267	293	285	312	231
chr6	152472636	152472876	SYNE1	0,38	173	187	143	116	155	155	127	99	195	174	171	156	188	182	169	157
chr6	152473004	152473364	SYNE1	0,51	115	118	94	86	110	99	82	63	146	110	125	98	117	111	101	95
chr6	152475921	152476281	SYNE1	0,43	190	210	154	104	184	163	133	111	238	185	207	167	201	179	206	160
chr6	152476958	152477318	SYNE1	0,47	272	277	201	150	222	226	177	133	279	249	259	232	268	241	263	203
chr6	152485198	152485558	SYNE1	0,50	241	247	214	147	208	210	170	134	259	254	250	211	258	249	258	194
chr6	152497431	152497791	SYNE1	0,43	285	294	226	163	241	234	197	154	307	287	298	258	311	294	299	217
chr6	152501169	152501529	SYNE1	0,38	328	353	273	185	233	282	219	153	331	329	320	293	332	340	338	231
chr6	152510284	152510644	SYNE1	0,42	332	353	259	182	254	273	214	163	314	313	320	284	317	324	351	234
chr6	152522901	152523141	SYNE1	0,43	128	136	95	87	117	116	86	81	141	117	122	119	142	137	117	108
chr6	152527219	152527579	SYNE1	0,43	240	222	188	142	198	203	157	115	240	221	238	208	231	217	241	182
chr6	152529043	152529403	SYNE1	0,48	192	194	148	108	168	166	121	103	215	185	182	162	185	181	192	163
chr6	152532555	152532795	SYNE1	0,34	252	294	210	147	219	218	200	133	300	287	252	241	267	260	285	200
chr6	152534640	152535000	SYNE1	0,37	377	385	293	192	262	285	256	166	394	366	348	300	359	374	383	250
chr6	152536005	152536245	SYNE1	0,40	253	263	195	147	213	201	172	123	292	247	255	202	240	248	236	204

chr6	152539332	152539644	SYNE1	0,35	260	261	195	142	214	226	190	122	273	267	258	219	250	246	259	200
chr6	152540048	152540408	SYNE1	0,44	162	166	140	115	162	152	115	96	197	171	179	140	168	167	170	134
chr6	152541898	152542258	SYNE1	0,45	247	251	188	146	228	219	178	140	272	262	242	221	257	268	254	191
chr6	152542507	152542747	SYNE1	0,35	209	233	187	127	197	197	155	112	240	223	227	189	208	239	225	179
chr6	152545533	152545893	SYNE1	0,35	260	271	210	147	227	224	190	135	289	245	276	213	277	273	252	212
chr6	152546753	152547113	SYNE1	0,39	277	346	255	170	261	269	207	152	326	311	323	261	330	321	323	227
chr6	152551579	152551939	SYNE1	0,33	319	348	274	183	260	265	226	165	354	338	335	278	319	322	322	234
chr6	152552433	152552793	SYNE1	0,39	298	342	267	175	251	262	218	150	335	323	311	261	300	314	342	233
chr6	152553145	152553505	SYNE1	0,33	249	274	199	147	217	205	192	139	267	262	269	210	261	248	261	193
chr6	152554826	152555186	SYNE1	0,42	212	205	170	125	179	175	141	117	215	195	228	186	201	211	212	170
chr6	152555748	152555988	SYNE1	0,39	221	209	199	128	171	183	138	110	246	216	203	179	220	217	205	170
chr6	152557159	152557519	SYNE1	0,35	302	336	251	174	228	257	202	144	341	310	314	260	305	321	308	221
chr6	152557897	152558137	SYNE1	0,49	126	117	115	96	130	133	79	87	142	130	130	124	138	148	139	118
chr6	152560574	152560910	SYNE1	0,42	304	299	221	157	239	240	191	142	300	283	271	226	288	275	315	223
chr6	152563294	152563654	SYNE1	0,42	271	290	219	170	238	257	198	142	297	264	276	237	290	292	300	193
chr6	152565611	152565851	SYNE1	0,44	298	319	226	169	243	255	206	154	302	290	298	240	281	290	311	215
chr6	152570226	152570466	SYNE1	0,35	252	267	213	140	211	255	182	133	279	273	282	229	275	271	276	209
chr6	152575939	152576299	SYNE1	0,35	324	353	281	195	257	273	240	161	373	359	342	281	329	324	362	260
chr6	152576623	152576959	SYNE1	0,40	259	273	216	143	206	231	188	128	284	261	274	220	263	269	265	199
chr6	152577714	152577954	SYNE1	0,45	165	186	135	106	142	158	121	93	193	167	173	147	177	169	183	147
chr6	152583064	152583424	SYNE1	0,34	298	315	268	172	256	267	224	166	355	335	321	285	295	320	345	244
chr6	152589125	152589365	SYNE1	0,43	170	200	133	123	149	159	126	94	204	159	174	155	181	193	168	138
chr6	152590235	152590475	SYNE1	0,54	63	78	54	50	64	64	44	45	81	63	88	70	60	66	69	62
chr6	152599139	152599499	SYNE1	0,53	209	204	176	124	190	175	144	107	219	205	215	176	189	207	189	170
chr6	152602848	152603184	SYNE1	0,45	182	190	154	121	179	168	132	106	220	178	202	160	193	186	173	163
chr6	152605029	152605389	SYNE1	0,49	157	147	125	101	154	132	111	88	173	150	156	134	148	166	158	133
chr6	152614623	152614983	SYNE1	0,48	202	208	161	131	188	181	129	116	214	200	207	169	202	205	214	152
chr6	152614998	152615358	SYNE1	0,44	172	184	148	107	192	150	122	96	212	172	191	148	195	185	189	149
chr6	152621665	152622025	SYNE1	0,46	176	179	141	109	160	155	132	102	199	166	165	143	179	171	164	146
chr6	152622920	152623280	SYNE1	0,58	86	76	59	59	71	70	45	50	88	76	82	70	74	73	77	70
chr6	152629573	152629813	SYNE1	0,41	278	308	243	177	228	247	195	149	323	303	303	255	274	296	285	232
chr6	152630865	152631177	SYNE1	0,53	93	96	61	53	80	67	58	50	102	84	93	73	89	88	73	75
chr6	152631471	152631711	SYNE1	0,41	186	175	142	124	164	155	129	107	199	187	186	144	186	191	179	155
chr6	152631819	152632059	SYNE1	0,44	278	308	239	152	253	248	202	147	302	272	294	262	312	307	309	225
chr6	152637932	152638172	SYNE1	0,33	272	301	225	162	217	245	202	129	315	272	293	212	269	269	290	204
chr6	152639126	152639486	SYNE1	0,40	268	301	215	157	224	233	195	131	294	284	270	235	275	274	274	204
chr6	152639893	152640253	SYNE1	0,34	280	320	243	163	236	238	199	144	313	284	287	244	295	291	286	209
chr6	152642268	152642628	SYNE1	0,37	278	313	231	166	232	250	214	142	293	287	287	262	305	295	316	219
chr6	152642758	152643118	SYNE1	0,36	324	361	286	180	246	283	236	171	361	351	343	301	350	335	352	266
chr6	152644574	152644934	SYNE1	0,46	176	182	147	112	152	150	123	98	197	180	194	164	173	180	183	126
chr6	152646147	152646507	SYNE1	0,47	144	147	113	89	151	119	100	82	166	136	148	123	146	151	144	120
chr6	152646984	152647344	SYNE1	0,35	361	423	320	185	285	295	240	182	388	383	362	313	359	363	429	270
chr6	152647359	152647839	SYNE1	0,45	270	260	224	153	224	220	187	125	278	274	280	229	273	267	268	214
chr6	152650804	152653084	SYNE1	0,46	314	336	261	179	262	270	229	160	354	339	321	277	342	332	347	253
chr6	152655059	152655515	SYNE1	0,36	330	369	291	189	273	270	242	162	361	366	326	277	337	339	378	259
chr6	152657883	152658243	SYNE1	0,44	232	252	195	141	198	201	161	128	237	226	241	206	228	238	244	169
chr6	152660318	152660558	SYNE1	0,38	153	168	120	96	147	116	109	86	178	147	149	149	149	144	149	120
chr6	152665175	152665415	SYNE1	0,46	130	116	94	91	110	106	84	76	153	129	121	117	121	124	126	92
chr6	152668101	152668461	SYNE1	0,41	228	219	185	130	186	191	170	119	225	207	212	183	214	219	228	172
chr6	152671257	152671569	SYNE1	0,50	151	133	118	88	144	122	104	73	164	140	142	120	149	146	136	116
chr6	152671648	152672008	SYNE1	0,42	310	310	256	182	262	265	211	161	329	340	307	266	316	318	342	244
chr6	152673078	152673534	SYNE1	0,43	361	406	335	208	303	306	275	191	385	413	372	326	382	380	429	282
chr6	152674302	152674981	SYNE1	0,44	264	282	239	158	228	235	189	136	306	289	277	243	274	293	309	211
chr6	152675712	152676192	SYNE1	0,47	258	265	218	152	221	222	188	136	276	265	267	221	276	264	277	197
chr6	152679409	152679769	SYNE1	0,37	269	286	227	150	222	240	182	154	282	290	275	245	282	278	284	211
chr6	152680341	152680701	SYNE1	0,43	241	244	181	124	202	199	170	122	274	223	245	190	230	228	239	177
chr6	152683201	152683561	SYNE1	0,46	208	220	177	140	185	189	151	107	247	226	204	186	210	215	252	185
chr6	152685887	152686247	SYNE1	0,42	283	280	240	162	234	233	217	145	289	285	293	231	279	295	289	211
chr6	152688254	152688614	SYNE1	0,44	224	230	170	117	190	194	159	120	252	239	232	174	241	238	216	179
chr6	152690004	152690364	SYNE1	0,52	134	138	95	98	113	112	90	76	156	131	148	109	140	147	151	121

chr6	152690497	152690833	SYNE1	0,49	230	225	188	126	196	198	147	121	253	221	227	209	231	240	230	174
chr6	152694081	152694417	SYNE1	0,36	278	316	237	169	233	242	201	143	321	308	307	244	314	300	289	222
chr6	152697448	152697784	SYNE1	0,36	293	324	245	176	254	255	199	148	326	307	316	262	324	309	342	210
chr6	152697858	152698098	SYNE1	0,39	219	247	168	128	191	190	156	109	234	213	235	203	240	222	235	166
chr6	152702078	152702558	SYNE1	0,51	201	185	159	116	169	181	130	109	207	192	215	162	204	200	203	151
chr6	152706710	152707070	SYNE1	0,37	284	319	252	162	246	251	199	146	311	303	290	250	311	285	304	226
chr6	152708180	152708612	SYNE1	0,41	341	344	275	182	266	292	233	165	351	363	338	282	341	348	340	252
chr6	152711320	152711680	SYNE1	0,42	302	305	236	148	225	238	202	147	311	293	302	256	304	278	325	213
chr6	152712353	152712713	SYNE1	0,53	82	79	62	66	100	82	60	46	99	81	85	80	81	88	87	70
chr6	152716596	152716908	SYNE1	0,36	300	344	268	164	240	273	216	163	326	321	302	261	314	318	349	230
chr6	152717832	152718192	SYNE1	0,38	314	338	246	172	246	261	203	152	342	331	300	269	312	325	345	230
chr6	152719669	152719885	SYNE1	0,42	216	211	158	118	206	172	138	109	236	178	198	184	208	192	182	155
chr6	152720674	152721034	SYNE1	0,43	191	212	157	131	177	180	147	98	218	181	210	160	196	211	200	171
chr6	152722194	152722554	SYNE1	0,35	354	391	290	179	268	289	251	163	394	375	364	305	362	346	421	247
chr6	152725278	152725518	SYNE1	0,32	265	280	211	160	224	230	194	148	279	271	281	261	257	266	280	208
chr6	152728059	152728419	SYNE1	0,40	301	310	257	159	260	265	204	149	315	312	311	272	316	321	324	230
chr6	152730101	152730461	SYNE1	0,33	318	319	251	162	249	268	222	145	312	306	289	264	280	314	317	233
chr6	152730588	152730948	SYNE1	0,35	300	327	247	155	239	258	226	159	328	345	305	253	328	307	336	232
chr6	152734396	152734756	SYNE1	0,36	296	324	259	171	249	264	219	167	313	340	298	260	323	314	328	236
chr6	152737415	152738255	SYNE1	0,47	284	272	228	154	232	226	202	141	285	280	288	245	279	268	293	217
chr6	152740600	152740960	SYNE1	0,33	239	235	173	136	190	183	161	122	247	218	224	190	223	222	224	175
chr6	152746418	152746778	SYNE1	0,36	296	310	248	168	240	268	212	139	315	321	293	257	318	304	356	236
chr6	152748769	152749009	SYNE1	0,38	277	295	235	144	237	231	195	130	288	280	261	219	269	291	303	225
chr6	152749253	152749613	SYNE1	0,52	127	118	89	97	119	103	80	69	143	125	138	119	124	125	121	103
chr6	152751175	152751415	SYNE1	0,29	230	255	198	142	175	193	162	116	259	235	243	189	225	214	241	182
chr6	152751550	152751910	SYNE1	0,39	354	345	293	181	270	308	241	174	374	361	355	310	366	366	388	248
chr6	152754824	152755184	SYNE1	0,35	284	329	263	171	250	263	204	146	316	300	305	242	280	299	318	223
chr6	152756975	152757335	SYNE1	0,37	297	314	239	150	254	256	210	162	329	290	298	260	292	298	322	233
chr6	152762154	152762514	SYNE1	0,30	269	299	232	152	216	226	205	143	290	283	260	236	277	265	281	209
chr6	152763114	152763474	SYNE1	0,55	148	143	114	87	145	122	108	89	166	139	146	130	149	154	152	126
chr6	152765449	152765785	SYNE1	0,34	312	322	250	168	229	246	233	157	330	331	302	262	298	317	323	232
chr6	152768494	152768854	SYNE1	0,42	233	233	195	136	200	215	169	128	275	244	231	215	242	228	246	189
chr6	152770601	152770841	SYNE1	0,38	312	346	259	162	258	252	212	141	328	310	313	269	295	296	323	226
chr6	152771683	152772043	SYNE1	0,45	221	241	175	137	206	185	151	120	250	221	221	197	215	219	221	176
chr6	152772080	152772440	SYNE1	0,38	294	307	247	187	253	259	209	152	323	303	296	246	299	308	305	235
chr6	152774667	152774907	SYNE1	0,52	109	107	77	80	104	82	70	66	129	101	91	93	112	110	95	86
chr6	152776462	152776822	SYNE1	0,44	314	325	261	166	256	290	198	154	345	324	308	273	326	340	351	237
chr6	152776919	152777279	SYNE1	0,39	311	317	244	177	235	268	217	146	340	299	301	264	325	331	325	233
chr6	152779798	152780158	SYNE1	0,39	322	344	250	175	254	274	222	166	336	329	308	273	328	315	343	242
chr6	152782623	152782983	SYNE1	0,42	296	303	233	172	231	239	207	157	325	306	305	271	299	300	324	231
chr6	152783768	152784128	SYNE1	0,42	321	335	265	171	248	275	224	151	350	345	320	273	331	324	349	253
chr6	152784389	152784749	SYNE1	0,35	320	322	235	169	264	264	219	148	333	326	302	253	303	320	340	234
chr6	152786313	152786673	SYNE1	0,40	340	353	277	198	266	275	241	151	372	370	325	298	358	365	384	272
chr6	152787044	152787284	SYNE1	0,37	230	260	201	136	217	217	158	132	278	263	242	221	250	264	243	189
chr6	152792635	152792995	SYNE1	0,38	294	286	234	151	230	241	190	139	319	306	300	246	313	313	304	243
chr6	152793371	152793611	SYNE1	0,39	207	226	152	119	180	174	147	103	221	181	224	189	198	198	215	156
chr6	152804121	152804481	SYNE1	0,43	258	282	186	155	216	227	174	140	259	237	271	221	248	260	246	182
chr6	152805918	152806158	SYNE1	0,34	213	229	176	127	178	173	162	119	231	219	238	199	211	211	224	171
chr6	152809464	152809704	SYNE1	0,28	280	310	240	161	200	265	192	126	304	273	297	223	292	285	318	207
chr6	152819781	152820021	SYNE1	0,29	187	199	161	115	146	182	131	106	209	191	237	155	201	207	197	164
chr6	152823702	152823942	SYNE1	0,35	258	278	212	144	211	217	193	136	303	268	256	223	259	262	270	197
chr6	152826253	152826613	SYNE1	0,41	305	308	234	161	245	249	210	148	313	318	294	251	325	310	311	216
chr6	152831236	152831596	SYNE1	0,40	206	229	161	124	185	192	158	112	243	225	229	193	214	222	244	183
chr6	152832071	152832311	SYNE1	0,34	211	241	167	121	183	175	154	121	238	229	202	172	204	212	209	171
chr6	152832595	152832835	SYNE1	0,33	208	266	187	113	163	194	165	115	219	205	223	193	216	213	231	164
chr6	152841515	152841755	SYNE1	0,40	162	153	132	106	168	142	123	91	178	173	163	154	151	172	157	135
chr6	152847134	152847350	SYNE1	0,41	192	213	165	119	187	191	141	109	211	214	213	181	210	210	212	167
chr6	152861005	152861245	SYNE1	0,35	241	270	191	147	212	211	179	123	258	260	238	226	262	271	266	194
chr6	152949312	152949552	SYNE1	0,48	135	148	134	107	153	129	119	89	176	153	162	141	161	169	163	127
chr6	16306464	16307184	ATXN1	0,50	172	170	143	111	156	157	133	98	195	161	173	152	173	172	180	147

chr6	16326559	16327879	ATXN1	0,64	71	70	56	52	72	62	52	38	76	65	73	62	69	71	70	61
chr6	16327937	16328633	ATXN1	0,63	41	42	31	25	46	33	25	31	40	35	37	32	39	37	40	41
chr6	170866024	170866264	TBP	0,48	101	110	77	73	100	98	78	57	119	94	104	88	92	102	102	83
chr6	170870791	170871007	TBP	0,49	188	187	174	123	193	180	132	126	218	184	204	179	218	185	210	164
chr6	170871116	170871428	TBP	0,55	195	212	163	117	184	168	137	109	234	191	220	182	233	203	210	170
chr6	170873556	170873796	TBP	0,32	230	267	187	128	210	198	164	124	249	234	237	196	213	237	243	181
chr6	170875931	170876171	TBP	0,44	162	193	117	113	161	139	107	102	175	161	173	146	168	164	167	138
chr6	170878603	170878963	TBP	0,35	216	226	175	132	203	185	144	121	256	206	235	184	218	214	212	170
chr6	170880424	170880664	TBP	0,33	271	295	215	156	218	268	181	147	289	281	276	230	264	263	304	216
chr6	170881193	170881433	TBP	0,37	334	341	275	193	264	271	220	154	359	340	310	303	322	344	354	250
chr6	7542053	7542413	DSP	0,70	7	4	4	4	7	6	7	8	4	6	4	6	7	9	6	6
chr6	7555915	7556131	DSP	0,55	125	135	109	108	139	132	96	84	173	139	143	131	141	158	156	119
chr6	7558242	7558602	DSP	0,45	164	176	131	100	178	151	112	100	196	156	166	145	177	167	186	141
chr6	7559365	7559725	DSP	0,51	101	91	86	63	100	90	72	62	111	94	111	84	105	111	106	87
chr6	7562828	7563068	DSP	0,53	103	92	77	67	109	84	71	51	106	97	107	88	99	102	95	97
chr6	7563873	7564113	DSP	0,43	176	180	140	114	170	162	147	99	224	196	180	156	190	182	190	159
chr6	7565492	7565852	DSP	0,48	124	112	95	69	127	117	80	73	153	121	121	104	141	136	136	98
chr6	7566541	7566781	DSP	0,35	182	186	147	121	174	157	145	103	201	167	195	148	194	187	183	138
chr6	7567514	7567754	DSP	0,37	202	237	176	131	192	192	148	124	233	229	219	196	218	210	216	171
chr6	7567956	7568196	DSP	0,50	93	100	72	69	97	99	68	70	126	107	122	97	114	110	100	87
chr6	7568565	7568925	DSP	0,37	307	325	249	171	236	257	200	150	353	319	307	254	320	307	328	236
chr6	7569315	7569675	DSP	0,49	157	170	117	113	151	144	122	91	188	160	158	128	171	150	163	128
chr6	7570612	7570852	DSP	0,54	58	62	38	45	70	59	46	45	67	49	59	45	56	63	59	45
chr6	7571536	7571896	DSP	0,45	183	186	148	119	191	172	139	97	211	195	203	168	209	215	215	151
chr6	7572007	7572367	DSP	0,41	229	221	167	139	197	176	152	114	233	218	211	181	211	233	213	167
chr6	7574269	7574581	DSP	0,35	310	345	237	187	226	256	231	151	351	337	307	275	323	322	349	228
chr6	7574838	7575078	DSP	0,44	128	137	112	94	137	135	104	83	160	135	147	128	153	142	153	122
chr6	7575444	7575804	DSP	0,44	179	205	154	104	169	157	135	102	209	164	212	170	185	197	196	160
chr6	7576481	7576793	DSP	0,38	258	257	205	168	208	192	165	124	263	258	244	212	274	246	255	206
chr6	7577113	7577353	DSP	0,30	221	255	179	134	172	205	159	117	228	232	215	211	228	232	219	172
chr6	7577934	7578150	DSP	0,42	134	146	125	100	156	140	101	80	165	160	177	138	149	146	157	128
chr6	7578625	7578865	DSP	0,34	245	271	226	169	186	226	191	130	289	248	261	212	260	259	291	211
chr6	7579454	7581854	DSP	0,47	164	166	130	104	146	140	113	91	184	156	167	137	170	170	174	134
chr6	7582775	7585967	DSP	0,47	224	226	179	144	201	191	156	117	243	223	229	195	229	224	230	176
chr6	7586019	7586211	DSP	0,42	219	195	174	133	196	173	145	100	220	211	208	170	216	201	218	176
chr7	128470676	128471132	FLNC	0,67	23	17	14	22	20	17	15	14	27	15	17	18	15	22	16	18
chr7	128475323	128475683	FLNC	0,63	54	50	36	47	55	51	40	32	57	49	63	50	44	54	48	48
chr7	128477142	128477382	FLNC	0,67	12	13	15	13	15	14	14	12	19	10	20	17	16	16	12	21
chr7	128477390	128477870	FLNC	0,62	70	63	50	57	73	63	50	44	90	64	74	61	70	80	74	66
chr7	128477959	128478199	FLNC	0,56	150	137	123	111	159	132	114	98	158	132	152	130	148	155	139	129
chr7	128478221	128478936	FLNC	0,64	101	100	79	66	90	80	68	60	106	93	107	91	93	91	100	76
chr7	128480025	128480265	FLNC	0,64	64	59	33	47	58	43	37	34	61	46	56	50	58	50	55	51
chr7	128480544	128480784	FLNC	0,62	33	19	21	21	29	23	21	18	29	22	31	22	24	33	19	27
chr7	128480835	128481075	FLNC	0,65	20	19	16	13	16	17	13	12	21	18	16	17	14	21	14	20
chr7	128481122	128481722	FLNC	0,63	55	52	44	49	57	52	32	40	64	54	59	58	54	55	55	54
chr7	128482176	128482512	FLNC	0,65	43	43	39	49	75	42	39	36	63	50	53	47	51	47	52	47
chr7	128482578	128483058	FLNC	0,64	34	23	28	26	30	24	25	21	29	25	27	25	26	28	25	30
chr7	128483216	128483696	FLNC	0,63	44	44	37	38	45	49	32	32	55	46	51	39	46	48	45	45
chr7	128483784	128484384	FLNC	0,60	45	33	32	32	46	42	30	27	55	34	48	38	38	43	41	45
chr7	128484650	128485370	FLNC	0,63	58	55	45	43	60	48	42	43	69	56	60	51	55	58	59	51
chr7	128485974	128486615	FLNC	0,64	76	69	56	66	79	77	59	51	95	76	84	72	87	90	73	66
chr7	128486698	128487058	FLNC	0,60	49	40	46	49	53	46	37	35	60	47	50	42	50	51	49	50
chr7	128487696	128488176	FLNC	0,67	12	8	6	8	10	7	5	8	11	10	10	7	9	8	10	10
chr7	128488525	128489125	FLNC	0,63	65	57	44	50	60	57	46	49	76	50	66	56	64	67	60	56
chr7	128489126	128489688	FLNC	0,64	36	41	29	31	44	39	31	30	51	36	42	42	38	41	38	42
chr7	128489978	128490098	FLNC	0,70	1	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0
chr7	128490365	128490581	FLNC	0,60	25	25	21	26	28	25	17	17	27	23	26	21	21	26	21	22
chr7	128490746	128491082	FLNC	0,61	37	33	32	30	48	37	26	31	50	41	49	40	44	51	47	41
chr7	128491183	128491783	FLNC	0,60	57	53	35	44	57	45	33	34	65	50	49	47	57	61	49	52
chr7	128492564	128493164	FLNC	0,64	28	19	20	22	25	26	22	20	30	27	29	26	29	26	25	26

chr7	128493466	128493946	FLNC	0,62	57	55	47	42	57	50	44	36	67	56	62	52	55	65	55	52
chr7	128493968	128494328	FLNC	0,68	24	20	17	20	24	22	20	15	30	22	23	23	24	24	21	20
chr7	128494416	128495016	FLNC	0,64	32	24	22	24	31	27	25	22	34	28	29	29	30	28	28	28
chr7	128495190	128495430	FLNC	0,61	75	71	56	78	77	65	58	41	88	74	86	77	80	81	74	67
chr7	128496473	128497073	FLNC	0,63	111	106	85	81	107	100	81	68	124	110	119	97	118	109	126	93
chr7	128497100	128497460	FLNC	0,64	55	51	35	40	53	42	36	30	62	37	49	45	54	54	54	44
chr7	128497986	128498634	FLNC	0,61	78	71	60	58	86	69	53	52	91	73	83	66	78	74	68	58
chr7	143013215	143013575	CLCN1	0,60	21	24	13	15	27	19	11	14	30	20	21	21	25	18	19	21
chr7	143016787	143017027	CLCN1	0,44	98	109	81	77	110	88	70	68	120	90	105	90	103	116	94	86
chr7	143017717	143017957	CLCN1	0,50	107	100	82	64	107	96	83	67	116	90	115	94	118	91	102	85
chr7	143018401	143018641	CLCN1	0,62	136	116	97	103	123	118	86	83	156	122	141	119	131	124	137	118
chr7	143018754	143018994	CLCN1	0,56	158	152	114	108	139	143	106	81	185	153	172	136	147	158	164	121
chr7	143020320	143020560	CLCN1	0,58	90	78	61	66	92	70	65	58	102	66	94	89	69	81	78	80
chr7	143021425	143021665	CLCN1	0,55	181	153	127	113	159	175	124	103	211	168	187	151	180	169	172	130
chr7	143027807	143028047	CLCN1	0,55	113	110	83	67	100	102	85	68	138	105	107	99	103	110	102	88
chr7	143028246	143028486	CLCN1	0,52	255	269	211	148	206	212	191	124	268	260	257	214	254	255	297	193
chr7	143028574	143028814	CLCN1	0,47	258	261	203	155	225	217	186	133	272	251	263	221	237	246	299	192
chr7	143029433	143029673	CLCN1	0,46	127	125	104	94	138	123	98	83	168	123	133	108	140	141	121	105
chr7	143029711	143030071	CLCN1	0,49	175	185	148	114	188	177	139	112	209	181	196	165	199	203	195	155
chr7	143036260	143036500	CLCN1	0,51	171	187	143	120	169	154	137	102	216	168	175	148	168	198	163	149
chr7	143036538	143036778	CLCN1	0,43	203	221	149	141	206	173	154	112	222	214	217	183	207	202	201	155
chr7	143038948	143039308	CLCN1	0,53	174	167	126	113	154	163	113	90	202	167	172	168	175	180	188	142
chr7	143039411	143039651	CLCN1	0,48	126	151	110	94	123	119	100	80	146	115	150	109	142	129	132	121
chr7	143042554	143042914	CLCN1	0,67	9	6	5	6	7	8	8	4	7	5	10	7	8	7	5	6
chr7	143043168	143043408	CLCN1	0,59	209	206	176	134	199	182	148	115	232	189	211	193	225	228	218	170
chr7	143043591	143043831	CLCN1	0,54	206	200	166	140	188	184	144	127	243	189	216	179	225	220	222	171
chr7	143043902	143044142	CLCN1	0,42	129	124	92	91	118	112	99	75	145	129	129	112	139	134	126	102
chr7	143047365	143047845	CLCN1	0,56	116	102	81	79	114	92	84	61	118	103	124	99	107	110	110	92
chr7	143048673	143049129	CLCN1	0,62	40	37	29	36	43	35	29	25	50	31	40	39	46	39	34	36
chr7	150642401	150642641	KCNH2	0,67	5	3	2	3	6	2	3	1	3	4	5	3	3	2	2	1
chr7	150643873	150644209	KCNH2	0,64	21	21	12	19	18	21	14	20	28	19	22	14	21	17	21	15
chr7	150644322	150644898	KCNH2	0,71	9	8	6	7	9	5	6	9	8	7	8	10	8	7	9	7
chr7	150645535	150645727	KCNH2	0,60	24	26	23	25	32	20	14	18	36	21	30	27	25	18	27	30
chr7	150645884	150646220	KCNH2	0,62	49	46	40	40	46	40	35	32	63	38	55	38	44	43	45	44
chr7	150646887	150647607	KCNH2	0,60	49	51	42	37	54	43	39	34	60	48	56	47	47	47	45	39
chr7	150647928	150648288	KCNH2	0,66	32	26	26	22	32	25	22	20	33	27	31	20	27	31	26	24
chr7	150648429	150649029	KCNH2	0,63	35	34	26	31	39	34	25	24	42	35	37	36	33	37	40	35
chr7	150649426	150650026	KCNH2	0,64	68	59	59	54	73	62	52	43	80	51	62	63	69	69	66	57
chr7	150652417	150652657	KCNH2	0,72	0	1	1	2	1	1	0	0	1	1	0	1	2	1	1	0
chr7	150654304	150654664	KCNH2	0,62	40	40	34	32	49	32	28	26	47	32	42	32	38	35	33	36
chr7	150655049	150655481	KCNH2	0,74	3	2	2	3	3	1	0	1	2	2	3	2	3	4	2	3
chr7	150655507	150655699	KCNH2	0,74	2	1	1	0	2	0	0	1	1	1	0	0	1	1	1	0
chr7	150656561	150656921	KCNH2	0,60	59	51	52	55	72	55	44	44	85	60	67	60	67	64	64	63
chr7	150671778	150672114	KCNH2	0,69	18	15	12	15	21	14	8	13	18	15	22	13	16	18	12	17
chr7	150674832	150675048	KCNH2	0,75	3	5	2	4	2	4	0	4	5	2	4	1	1	1	1	2
chr7	151254182	151254422	PRKAG2	0,49	209	211	155	128	162	174	149	108	210	199	202	186	189	188	205	149
chr7	151257536	151257776	PRKAG2	0,44	182	166	141	105	161	136	124	86	200	165	160	144	176	174	176	115
chr7	151261056	151261416	PRKAG2	0,45	231	255	187	145	206	191	170	117	248	228	230	192	255	246	226	190
chr7	151262330	151262570	PRKAG2	0,21	201	214	184	122	191	197	163	110	234	214	231	177	230	199	217	157
chr7	151262708	151263068	PRKAG2	0,42	241	265	201	158	206	207	184	130	279	242	253	205	248	258	271	181
chr7	151265744	151265984	PRKAG2	0,36	197	195	161	112	160	167	145	104	209	197	210	173	195	181	199	152
chr7	151267163	151267403	PRKAG2	0,29	257	292	235	161	229	245	198	154	288	273	279	224	263	265	270	206
chr7	151269689	151269809	PRKAG2	0,30	203	203	163	135	181	204	151	136	213	189	225	198	235	180	237	151
chr7	151271940	151272132	PRKAG2	0,31	217	214	169	120	164	167	147	101	220	175	197	164	195	182	191	155
chr7	151273377	151273593	PRKAG2	0,41	278	309	220	152	230	216	209	155	278	266	263	248	258	270	267	212
chr7	151292348	151292564	PRKAG2	0,35	214	246	188	157	210	214	163	137	238	236	222	201	235	217	212	183
chr7	151329138	151329282	PRKAG2	0,73	1	0	0	1	0	0	1	1	1	0	2	4	3	0	1	2
chr7	151372434	151372794	PRKAG2	0,61	67	70	49	55	56	54	47	41	70	59	76	57	57	66	69	59
chr7	151478137	151478617	PRKAG2	0,61	29	23	21	22	30	28	24	24	31	25	29	21	29	25	28	23
chr7	151483471	151483711	PRKAG2	0,61	18	17	14	14	25	17	12	13	21	13	22	12	18	13	22	13

chr7	30634468	30634828	GARS	0,71	8	4	4	6	9	6	4	5	7	3	8	5	5	8	10	7
chr7	30638342	30638582	GARS	0,39	194	205	167	121	187	175	150	101	227	182	197	184	190	192	208	154
chr7	30639493	30639733	GARS	0,37	224	242	177	129	204	195	161	114	247	214	212	190	228	225	215	174
chr7	30640616	30640856	GARS	0,42	182	193	133	119	166	150	125	100	185	175	186	151	175	182	168	154
chr7	30642573	30642813	GARS	0,34	242	258	206	166	206	207	170	135	270	237	252	226	252	237	252	179
chr7	30643029	30643269	GARS	0,37	164	177	120	109	169	139	117	114	182	184	182	167	177	178	167	142
chr7	30649093	30649453	GARS	0,31	270	313	205	148	200	231	206	134	298	266	275	234	284	282	288	201
chr7	30651606	30651966	GARS	0,36	299	314	240	157	237	241	216	156	322	314	292	263	297	294	339	210
chr7	30655412	30655772	GARS	0,40	227	227	182	131	195	195	158	120	233	217	224	185	206	224	245	167
chr7	30656631	30656991	GARS	0,41	259	260	196	145	228	221	171	133	275	256	253	211	250	249	251	194
chr7	30660942	30661182	GARS	0,38	258	284	192	160	234	223	176	133	283	263	271	229	263	255	265	211
chr7	30661825	30662185	GARS	0,40	241	264	200	148	214	205	183	120	248	239	243	212	248	254	259	183
chr7	30665770	30665986	GARS	0,31	155	185	134	104	168	160	122	109	181	172	180	139	183	164	166	136
chr7	30668110	30668350	GARS	0,35	227	268	173	151	191	215	167	108	243	236	228	219	239	229	233	182
chr7	30670990	30671230	GARS	0,35	249	273	235	143	231	218	194	133	283	252	243	219	252	262	266	191
chr7	30671777	30672137	GARS	0,44	180	186	139	119	174	159	122	105	205	179	189	152	168	181	177	144
chr7	30673293	30673533	GARS	0,41	127	140	107	93	127	119	104	81	135	117	132	111	132	121	119	108
chr7	44102265	44102625	PGAM2	0,60	69	78	61	69	83	67	54	43	98	74	85	69	79	77	77	65
chr7	44104340	44105221	PGAM2	0,63	34	32	25	30	40	32	22	24	44	30	36	31	37	35	33	32
chr7	75931971	75932451	HSPB1	0,72	9	7	4	9	12	9	8	7	8	7	10	10	7	9	8	11
chr7	75933030	75933575	HSPB1	0,64	58	53	46	40	49	50	39	38	63	44	56	53	59	53	46	53
chr7	94214681	94214921	SGCE	0,35	265	286	212	165	210	215	198	136	301	260	263	219	257	280	275	210
chr7	94216986	94217226	SGCE	0,29	276	307	228	162	191	239	190	140	316	263	273	246	271	272	293	212
chr7	94217902	94218142	SGCE	0,32	197	228	182	136	172	180	151	125	229	215	219	200	226	205	230	154
chr7	94228000	94228360	SGCE	0,38	253	241	184	135	202	199	165	115	251	232	238	192	244	230	244	188
chr7	94228901	94229141	SGCE	0,34	225	261	166	135	182	179	147	123	228	216	205	185	221	203	237	165
chr7	94229883	94230243	SGCE	0,39	252	253	192	153	221	200	164	126	288	263	255	215	242	250	253	188
chr7	94232502	94232862	SGCE	0,32	276	296	234	155	224	240	202	143	312	294	295	253	282	302	327	203
chr7	94247988	94248348	SGCE	0,40	284	294	231	165	234	244	203	141	299	283	286	251	303	304	303	206
chr7	94252552	94252792	SGCE	0,26	200	235	188	142	160	207	161	120	242	218	233	186	247	228	227	179
chr7	94257466	94257778	SGCE	0,35	182	215	163	123	161	172	148	107	229	183	211	175	200	196	196	151
chr7	94258971	94259211	SGCE	0,37	184	186	142	114	131	188	128	86	214	186	192	162	193	198	186	156
chr7	94285235	94285475	SGCE	0,68	29	25	33	29	43	36	19	21	40	38	41	42	35	44	41	34
chr8	103220256	103220616	RRM2B	0,32	278	297	215	154	218	244	191	134	296	309	276	243	281	272	286	210
chr8	103224940	103225180	RRM2B	0,32	263	311	224	158	193	248	221	125	286	281	295	219	261	270	278	202
chr8	103226213	103226453	RRM2B	0,38	253	293	214	141	206	221	192	137	298	270	272	231	268	283	286	200
chr8	103230988	103231228	RRM2B	0,37	279	291	222	168	246	241	214	146	299	283	277	233	288	298	287	232
chr8	103236195	103236411	RRM2B	0,34	289	305	247	168	220	245	200	152	316	283	289	255	282	291	327	226
chr8	103237059	103237299	RRM2B	0,39	211	229	186	123	183	195	156	112	243	196	218	182	218	228	220	172
chr8	103238083	103238323	RRM2B	0,33	237	296	201	140	186	224	178	124	257	270	258	228	260	237	274	197
chr8	103244274	103244634	RRM2B	0,38	327	349	270	169	262	274	231	169	337	319	312	257	346	337	351	230
chr8	103250704	103251184	RRM2B	0,66	29	28	28	28	37	30	24	17	38	28	28	33	35	38	29	29
chr8	126036766	126037006	KIAA0196	0,32	179	183	141	114	149	162	105	103	205	174	182	147	185	181	194	131
chr8	126040785	126041025	KIAA0196	0,40	209	209	160	120	171	153	129	109	207	197	201	177	184	201	218	150
chr8	126044430	126044742	KIAA0196	0,50	112	110	89	70	117	87	75	58	117	97	110	94	106	117	107	95
chr8	126049406	126049646	KIAA0196	0,31	201	249	170	118	169	192	147	117	228	220	208	176	205	201	213	148
chr8	126051016	126051256	KIAA0196	0,41	195	216	156	124	189	186	151	110	231	192	213	163	207	197	218	157
chr8	126051968	126052208	KIAA0196	0,43	175	173	144	108	161	162	127	93	175	167	184	163	185	169	180	127
chr8	126055961	126056441	KIAA0196	0,36	308	340	251	161	241	254	209	148	333	310	303	257	294	296	334	237
chr8	126056678	126057038	KIAA0196	0,43	252	276	217	158	229	214	193	135	295	257	262	230	246	267	270	193
chr8	126059390	126059630	KIAA0196	0,42	145	153	115	96	132	128	109	77	170	145	161	130	139	146	143	137
chr8	126061157	126061517	KIAA0196	0,35	302	322	251	170	236	261	216	147	315	326	300	248	306	319	342	222
chr8	126062736	126062976	KIAA0196	0,47	190	199	144	116	168	177	132	102	215	174	205	170	182	192	200	135
chr8	126067752	126067992	KIAA0196	0,38	191	191	164	122	179	168	136	117	194	185	194	185	205	185	206	155
chr8	126068808	126069168	KIAA0196	0,43	180	182	143	117	161	153	122	84	210	182	184	160	171	177	194	143
chr8	126069732	126069972	KIAA0196	0,40	157	150	121	95	149	141	126	97	176	149	163	141	150	153	137	119
chr8	126071383	126071863	KIAA0196	0,39	308	309	238	172	260	246	216	147	316	299	286	250	309	296	319	233
chr8	126073259	126073499	KIAA0196	0,37	252	289	206	162	220	244	174	135	271	286	259	223	263	255	262	195
chr8	126075708	126075948	KIAA0196	0,39	283	323	284	162	241	240	214	155	317	319	318	243	303	293	349	237
chr8	126079778	126080018	KIAA0196	0,36	180	185	131	130	163	165	126	90	208	175	166	155	164	180	170	152

chr8	126085300	126085660	KIAA0196	0,39	346	376	299	172	274	292	242	168	365	370	345	298	345	356	390	245
chr8	126087176	126087416	KIAA0196	0,38	258	303	217	155	219	222	196	137	321	269	251	214	268	265	286	203
chr8	126088485	126088845	KIAA0196	0,43	221	241	177	144	208	187	163	117	268	219	225	200	244	234	260	195
chr8	126090895	126091255	KIAA0196	0,39	234	264	197	144	221	223	167	119	253	235	240	214	254	255	249	189
chr8	126093832	126094072	KIAA0196	0,42	229	228	178	134	195	195	157	121	252	222	213	203	238	251	226	183
chr8	126094546	126094786	KIAA0196	0,25	259	288	218	143	190	216	175	123	249	243	250	206	267	225	251	192
chr8	126095247	126095607	KIAA0196	0,32	279	335	238	179	195	258	211	153	339	328	311	262	301	291	319	243
chr8	126095867	126096227	KIAA0196	0,41	204	219	178	131	167	185	133	112	240	183	204	183	218	203	208	177
chr8	134251061	134251421	NDRG1	0,69	1	4	3	2	2	2	1	3	3	2	3	2	4	3	4	5
chr8	134254171	134254411	NDRG1	0,57	89	81	68	69	96	80	62	58	100	89	98	80	90	93	88	75
chr8	134256495	134256735	NDRG1	0,57	24	34	21	23	37	26	21	17	40	21	31	32	33	33	25	26
chr8	134258762	134259002	NDRG1	0,46	211	253	184	145	191	205	183	104	230	219	226	212	232	233	230	192
chr8	134260023	134260263	NDRG1	0,53	71	73	54	63	89	72	54	45	90	75	78	73	69	77	73	76
chr8	134260865	134261105	NDRG1	0,60	24	20	20	26	37	25	19	16	29	21	25	29	36	31	27	25
chr8	134262608	134262824	NDRG1	0,48	134	153	95	93	117	108	85	84	133	120	140	124	122	135	135	91
chr8	134266689	134266929	NDRG1	0,55	61	57	36	42	69	55	35	35	74	52	50	56	59	46	64	57
chr8	134268942	134269182	NDRG1	0,56	130	113	94	91	116	102	90	91	159	114	133	119	125	133	139	103
chr8	134270518	134270758	NDRG1	0,44	161	170	128	112	163	137	105	90	182	152	160	156	163	157	171	137
chr8	134271321	134271561	NDRG1	0,54	66	78	61	52	79	66	50	41	83	59	68	62	72	76	67	67
chr8	134274229	134274469	NDRG1	0,56	23	18	14	16	25	23	18	15	28	24	18	19	21	20	18	23
chr8	134276722	134276962	NDRG1	0,57	40	53	37	42	53	33	39	33	59	42	62	45	42	53	48	49
chr8	134292372	134292612	NDRG1	0,41	107	125	84	78	121	103	79	70	127	116	128	102	121	111	104	100
chr8	134296461	134296653	NDRG1	0,53	113	117	96	105	127	103	98	75	142	124	146	126	148	131	123	107
chr8	144990266	144996242	PLEC	0,66	41	37	32	35	45	37	29	29	47	36	43	37	42	40	37	39
chr8	144996382	144997885	PLEC	0,67	33	31	24	29	36	27	23	24	36	27	32	33	32	30	31	29
chr8	144997920	145000152	PLEC	0,72	13	11	9	10	15	10	8	11	14	9	14	12	13	13	11	13
chr8	145000860	145002269	PLEC	0,67	28	27	20	24	29	26	17	21	28	25	28	24	25	27	23	27
chr8	145003179	145003515	PLEC	0,68	9	7	4	6	11	7	3	6	11	10	6	10	10	10	6	12
chr8	145003570	145004763	PLEC	0,70	7	6	5	8	7	6	5	6	8	5	7	8	8	6	7	7
chr8	145005644	145005884	PLEC	0,67	10	9	6	11	14	9	7	5	11	8	7	10	10	6	9	8
chr8	145006019	145006235	PLEC	0,67	5	8	3	8	10	5	9	6	8	5	5	6	6	6	4	10
chr8	145006270	145006486	PLEC	0,70	10	13	9	11	15	12	7	8	9	5	12	11	10	10	9	10
chr8	145006503	145007583	PLEC	0,67	22	23	15	23	25	21	16	15	26	18	24	20	26	21	19	19
chr8	145008092	145008332	PLEC	0,71	7	2	2	9	11	5	3	4	8	6	7	4	6	5	6	5
chr8	145008429	145008669	PLEC	0,68	22	15	5	17	19	18	12	16	21	12	19	14	22	15	21	19
chr8	145008721	145009561	PLEC	0,67	36	33	25	32	32	31	23	27	39	26	41	34	33	34	31	35
chr8	145009918	145010278	PLEC	0,65	31	36	20	30	41	29	23	23	38	34	35	32	30	34	29	37
chr8	145011038	145011518	PLEC	0,66	23	15	16	17	32	15	15	14	26	16	25	15	26	22	17	25
chr8	145012243	145012483	PLEC	0,67	9	8	6	10	6	7	5	3	10	8	6	6	10	7	6	7
chr8	145012709	145012949	PLEC	0,65	26	27	25	24	36	24	16	18	35	25	36	31	37	21	28	26
chr8	145013453	145013693	PLEC	0,71	4	5	6	8	8	4	4	5	9	2	5	8	4	5	3	7
chr8	145016501	145016741	PLEC	0,73	1	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1
chr8	145017882	145018122	PLEC	0,72	7	4	4	5	7	2	4	5	8	6	9	4	7	4	4	10
chr8	145018763	145018883	PLEC	0,68	3	1	1	1	2	1	1	2	0	2	1	1	1	2	1	0
chr8	145024252	145024972	PLEC	0,69	9	5	6	7	8	6	6	4	8	4	7	6	8	6	7	8
chr8	145027876	145027996	PLEC	0,70	0	0	0	0	0	0	1	0	1	1	0	0	0	0	1	1
chr8	145047520	145047640	PLEC	0,70	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chr8	145049260	145049620	PLEC	0,66	23	15	10	20	25	22	12	17	25	19	21	22	17	20	18	22
chr8	1791463	1791703	ARHGEF10	0,56	22	20	18	10	21	22	12	18	22	28	19	17	23	28	18	19
chr8	1806089	1806281	ARHGEF10	0,46	39	48	36	37	51	42	32	31	51	43	39	45	41	45	57	46
chr8	1807966	1808446	ARHGEF10	0,63	54	60	47	48	62	57	42	42	79	54	64	59	63	64	58	54
chr8	1812450	1812690	ARHGEF10	0,50	178	179	159	123	187	160	136	88	214	195	180	170	182	172	191	137
chr8	1814609	1814849	ARHGEF10	0,58	126	127	101	91	118	98	88	75	134	113	124	103	123	119	115	100
chr8	1817267	1817507	ARHGEF10	0,44	184	218	163	120	172	169	124	92	220	192	183	154	207	192	198	139
chr8	1824638	1824998	ARHGEF10	0,44	175	177	131	117	156	156	133	104	196	173	198	146	193	170	187	141
chr8	1828151	1828391	ARHGEF10	0,49	105	121	92	73	112	98	88	66	148	104	123	97	107	109	112	106
chr8	1830737	1830977	ARHGEF10	0,64	10	8	9	10	14	5	8	9	10	9	12	9	12	17	8	11
chr8	1833699	1833939	ARHGEF10	0,41	176	176	127	107	154	141	127	100	203	160	185	146	183	170	168	131
chr8	1841653	1841893	ARHGEF10	0,32	244	287	203	161	196	216	209	131	284	267	261	216	242	243	265	210
chr8	1842525	1842837	ARHGEF10	0,52	101	100	93	77	102	92	77	67	121	92	102	92	99	105	92	96

chr8	1844436	1844676	ARHGEF10	0,39	202	222	142	134	182	173	140	111	209	183	176	184	200	196	178	149
chr8	1846524	1846764	ARHGEF10	0,62	78	80	66	56	84	76	58	60	110	70	86	82	78	82	74	81
chr8	1851351	1851711	ARHGEF10	0,47	192	168	142	120	172	147	122	91	201	167	161	137	166	172	166	143
chr8	1853629	1853989	ARHGEF10	0,43	234	264	204	159	191	217	171	124	282	239	251	210	258	235	257	195
chr8	1857368	1857728	ARHGEF10	0,55	105	100	74	79	109	96	78	71	128	92	115	94	117	117	102	86
chr8	1871067	1871307	ARHGEF10	0,32	241	243	181	156	175	218	186	125	247	228	230	199	236	233	234	179
chr8	1871577	1871817	ARHGEF10	0,41	148	161	108	107	131	135	107	79	175	144	139	122	161	142	136	111
chr8	1871869	1872109	ARHGEF10	0,51	134	136	108	82	108	115	83	83	143	129	120	105	143	127	124	107
chr8	1873389	1873629	ARHGEF10	0,54	46	38	29	33	45	35	24	25	49	31	44	41	39	25	33	31
chr8	1874465	1874705	ARHGEF10	0,39	221	234	167	141	185	175	154	110	225	212	210	168	219	217	230	166
chr8	1876524	1876884	ARHGEF10	0,55	131	129	111	86	128	119	110	82	155	142	143	122	132	143	140	115
chr8	1877350	1877710	ARHGEF10	0,53	117	118	93	76	112	103	70	70	122	109	120	110	108	120	113	96
chr8	1881856	1882216	ARHGEF10	0,44	209	206	164	144	163	168	133	115	211	203	208	167	212	206	210	151
chr8	1893553	1893913	ARHGEF10	0,63	50	51	33	38	54	48	34	38	61	45	59	47	47	41	47	55
chr8	1900811	1901051	ARHGEF10	0,63	13	16	11	13	18	16	13	7	25	12	20	16	14	13	13	19
chr8	1904811	1905531	ARHGEF10	0,56	75	72	63	66	86	70	59	52	94	76	84	75	89	79	82	65
chr8	24810260	24810380	NEFL	0,44	63	63	57	48	78	55	46	42	80	54	73	63	66	61	49	56
chr8	24811048	24811384	NEFL	0,55	57	45	45	35	59	42	42	36	59	46	53	44	48	57	52	39
chr8	24811636	24811876	NEFL	0,37	307	329	249	157	269	255	202	146	342	311	294	273	319	302	333	232
chr8	24812907	24814107	NEFL	0,65	50	46	41	46	60	49	39	42	64	50	55	51	56	55	59	55
chr8	63973717	63974077	TTPA	0,36	292	339	259	173	241	253	204	166	353	346	313	263	301	331	346	244
chr8	63976699	63976939	TTPA	0,31	230	245	185	153	181	203	164	123	257	238	236	190	241	226	265	205
chr8	63978379	63978739	TTPA	0,35	292	306	236	167	230	281	212	131	292	317	316	245	279	291	298	215
chr8	63985390	63985750	TTPA	0,38	199	213	167	120	167	175	140	111	223	204	193	160	189	191	207	165
chr8	63998278	63998470	TTPA	0,73	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0
chr8	63998496	63998688	TTPA	0,77	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
chr8	68111070	68111430	ARFGEF1	0,40	275	281	207	140	213	224	180	128	281	276	278	227	257	259	285	210
chr8	68112569	68112809	ARFGEF1	0,42	225	209	179	116	184	191	153	109	238	201	210	156	220	223	242	180
chr8	68113650	68113890	ARFGEF1	0,52	115	114	91	77	110	110	71	67	123	110	117	94	113	109	117	95
chr8	68115283	68115595	ARFGEF1	0,41	278	254	223	171	238	219	201	139	317	284	289	258	262	293	312	215
chr8	68116871	68117111	ARFGEF1	0,41	224	225	184	139	198	202	162	109	245	204	221	204	235	235	221	177
chr8	68123663	68123903	ARFGEF1	0,32	172	169	146	114	154	161	117	97	198	161	171	141	159	159	162	139
chr8	68128740	68128980	ARFGEF1	0,40	162	172	128	108	158	148	114	96	191	159	170	133	170	162	162	133
chr8	68129954	68130434	ARFGEF1	0,35	275	306	231	162	222	238	198	137	313	290	270	242	295	291	285	219
chr8	68131610	68131850	ARFGEF1	0,32	276	332	227	158	241	235	199	156	290	297	281	239	288	287	302	218
chr8	68137082	68137322	ARFGEF1	0,34	234	251	183	130	196	204	163	127	258	235	232	189	222	232	244	177
chr8	68138157	68138517	ARFGEF1	0,34	304	324	239	159	246	248	200	138	334	299	309	262	279	294	294	226
chr8	68139277	68139937	ARFGEF1	0,35	356	395	311	182	259	306	255	174	390	401	356	313	379	372	410	287
chr8	68140108	68140588	ARFGEF1	0,32	295	342	264	178	239	283	228	151	337	319	324	273	303	319	333	259
chr8	68145014	68145254	ARFGEF1	0,33	274	335	255	163	185	251	237	154	320	328	298	286	302	286	344	226
chr8	68150472	68150832	ARFGEF1	0,34	334	365	292	191	254	284	245	152	353	362	344	306	319	355	368	253
chr8	68150868	68151228	ARFGEF1	0,34	344	370	323	203	265	302	244	170	377	363	346	307	372	351	389	255
chr8	68152317	68152533	ARFGEF1	0,37	281	293	209	152	211	230	189	130	268	236	249	213	260	253	275	193
chr8	68163429	68163789	ARFGEF1	0,32	281	294	225	154	225	226	175	134	294	285	283	231	278	276	296	216
chr8	68165615	68165951	ARFGEF1	0,28	271	298	231	160	209	231	194	132	316	278	273	230	276	261	304	215
chr8	68169888	68170128	ARFGEF1	0,32	277	306	213	169	201	230	197	138	300	271	289	230	300	279	293	190
chr8	68170232	68170568	ARFGEF1	0,37	333	350	257	179	262	293	239	165	336	339	344	284	354	347	346	246
chr8	68171985	68172225	ARFGEF1	0,32	251	267	204	147	215	219	185	151	262	239	254	240	243	237	267	201
chr8	68178161	68178521	ARFGEF1	0,37	314	308	257	166	253	256	193	143	333	310	299	259	301	304	316	232
chr8	68179044	68179747	ARFGEF1	0,31	311	352	264	187	249	266	227	161	348	328	334	279	339	329	363	255
chr8	68183873	68184233	ARFGEF1	0,32	243	283	231	157	188	223	176	131	286	252	265	221	273	267	278	203
chr8	68188157	68188397	ARFGEF1	0,36	212	228	170	127	195	206	140	106	230	211	200	191	209	220	218	169
chr8	68189424	68189784	ARFGEF1	0,37	326	347	270	169	244	268	231	164	348	330	329	271	328	341	360	258
chr8	68200124	68200364	ARFGEF1	0,31	318	379	275	171	225	275	229	162	348	351	325	271	310	336	356	247
chr8	68203979	68204459	ARFGEF1	0,38	329	366	272	178	268	275	213	162	356	347	325	260	336	337	357	256
chr8	68208566	68208878	ARFGEF1	0,35	299	320	264	167	248	262	217	162	338	341	318	272	324	313	341	216
chr8	68211445	68211661	ARFGEF1	0,35	178	199	158	113	165	171	118	105	219	178	196	154	197	174	174	164
chr8	68213356	68213692	ARFGEF1	0,36	255	259	208	158	206	214	193	128	291	253	248	212	251	233	262	173
chr8	68214565	68214805	ARFGEF1	0,32	241	297	222	135	206	224	169	136	268	239	276	219	270	241	264	188
chr8	68255340	68255580	ARFGEF1	0,66	37	41	35	38	46	33	19	30	33	40	42	33	32	33	28	37

chr8	75262634	75262874	GDAP1	0,64	11	15	12	9	24	13	13	11	19	14	11	19	15	16	14	18
chr8	75263424	75263760	GDAP1	0,44	268	285	229	142	209	218	173	146	283	268	271	246	261	274	278	214
chr8	75272278	75272638	GDAP1	0,38	280	323	237	166	215	263	201	138	310	305	279	249	284	302	307	206
chr8	75274045	75274285	GDAP1	0,37	213	205	169	124	164	180	157	113	233	195	199	166	216	210	202	165
chr8	75275110	75275350	GDAP1	0,32	173	222	155	116	191	159	140	122	213	213	208	158	225	209	210	161
chr8	75276110	75276710	GDAP1	0,46	206	214	163	119	180	180	147	102	232	207	223	188	218	212	209	169
chr9	108337245	108337485	FKTN	0,35	287	299	241	144	218	245	192	138	327	283	305	238	315	285	321	219
chr9	108358788	108359028	FKTN	0,32	157	187	136	108	145	145	125	93	207	156	186	146	179	174	178	146
chr9	108363347	108363707	FKTN	0,32	305	328	259	181	221	269	224	138	337	317	322	270	314	324	340	241
chr9	108366394	108366874	FKTN	0,39	233	239	188	146	208	183	157	120	250	230	234	193	229	227	230	194
chr9	108370056	108370296	FKTN	0,36	201	219	170	119	195	187	152	100	242	214	224	185	208	209	209	169
chr9	108377503	108377743	FKTN	0,39	194	214	143	125	188	156	124	119	215	193	187	172	198	185	184	146
chr9	108380186	108380426	FKTN	0,30	255	274	203	163	225	212	187	131	291	259	265	212	264	249	265	190
chr9	108382158	108382398	FKTN	0,34	174	196	125	107	144	153	139	109	194	164	178	158	176	164	177	129
chr9	108397258	108397618	FKTN	0,42	253	306	234	157	256	234	183	134	305	299	295	256	294	286	317	216
chr9	111631308	111631548	IKBKAP	0,47	147	145	116	100	127	127	97	91	171	129	154	126	156	144	152	133
chr9	111637092	111637332	IKBKAP	0,37	195	219	185	122	181	185	129	101	233	225	212	189	213	205	219	162
chr9	111640171	111640531	IKBKAP	0,37	308	326	249	182	242	259	210	160	339	325	310	255	317	299	336	227
chr9	111640834	111641074	IKBKAP	0,48	123	137	102	92	125	112	89	65	143	121	140	113	132	117	112	105
chr9	111641661	111641901	IKBKAP	0,46	205	189	158	123	185	159	134	116	217	187	197	191	204	197	193	151
chr9	111642268	111642508	IKBKAP	0,49	135	148	102	90	138	122	88	83	157	119	145	123	137	133	141	108
chr9	111643894	111644134	IKBKAP	0,35	239	280	199	137	185	192	154	126	252	247	232	202	251	234	252	179
chr9	111644315	111644555	IKBKAP	0,47	215	210	165	127	189	165	131	95	231	216	198	168	200	195	223	155
chr9	111651522	111651762	IKBKAP	0,40	236	235	190	133	201	196	156	121	251	200	239	196	262	243	248	174
chr9	111653403	111653763	IKBKAP	0,45	136	147	107	91	136	124	103	78	166	128	143	114	137	147	134	125
chr9	111655194	111655434	IKBKAP	0,39	254	270	220	165	214	241	160	138	281	259	251	231	278	280	282	203
chr9	111656175	111656415	IKBKAP	0,31	242	261	178	127	174	201	156	112	257	257	226	195	229	251	253	178
chr9	111658669	111659029	IKBKAP	0,38	280	309	231	155	238	257	203	142	315	278	283	243	278	281	307	215
chr9	111659155	111659616	IKBKAP	0,45	226	246	188	132	212	179	155	122	275	230	235	195	237	231	251	200
chr9	111660725	111661085	IKBKAP	0,31	260	295	227	151	193	221	194	132	287	249	277	235	272	273	285	213
chr9	111662018	111662258	IKBKAP	0,34	287	328	241	184	243	258	213	153	335	299	291	259	293	318	323	222
chr9	111662477	111662717	IKBKAP	0,45	249	276	211	158	220	202	179	118	289	267	267	231	260	274	263	192
chr9	111663652	111664012	IKBKAP	0,40	261	276	211	150	229	227	177	126	270	260	259	221	270	278	281	185
chr9	111665050	111665290	IKBKAP	0,44	133	151	112	95	142	139	92	83	183	152	163	143	155	149	160	115
chr9	111665775	111666015	IKBKAP	0,39	239	254	205	150	213	207	170	120	276	255	231	212	244	258	247	204
chr9	111668493	111668853	IKBKAP	0,40	204	234	187	129	180	194	145	120	235	205	216	193	200	204	199	157
chr9	111670514	111670754	IKBKAP	0,40	189	215	181	132	207	172	153	117	253	207	233	214	205	221	213	167
chr9	111673194	111673554	IKBKAP	0,41	237	258	190	134	185	197	166	118	244	223	233	186	240	218	246	177
chr9	111674478	111674838	IKBKAP	0,52	181	193	144	114	188	163	132	93	206	182	189	176	195	201	196	153
chr9	111678410	111678650	IKBKAP	0,42	185	196	146	117	189	156	135	108	241	188	188	172	198	181	193	165
chr9	111679768	111680008	IKBKAP	0,40	198	197	144	134	169	173	145	103	232	196	183	166	206	201	198	159
chr9	111681015	111681255	IKBKAP	0,47	222	238	194	138	227	205	163	133	268	267	249	205	255	247	250	194
chr9	111681495	111681711	IKBKAP	0,43	137	115	105	98	125	106	95	83	147	122	129	116	129	111	115	100
chr9	111685044	111685284	IKBKAP	0,41	270	286	247	162	230	233	203	144	304	306	274	271	289	285	294	200
chr9	111688722	111688962	IKBKAP	0,37	207	217	158	121	209	182	164	119	228	213	217	169	211	215	207	163
chr9	111689572	111689812	IKBKAP	0,43	116	132	105	97	119	130	83	81	138	123	123	106	133	116	118	110
chr9	111691944	111692304	IKBKAP	0,42	255	252	209	146	217	210	165	126	273	239	236	210	238	245	257	208
chr9	111693171	111693531	IKBKAP	0,39	207	195	170	127	177	162	143	112	226	203	200	168	203	192	200	142
chr9	113431103	113431343	MUSK	0,42	252	273	194	139	222	217	188	131	300	263	268	228	258	259	261	194
chr9	113444883	113445123	MUSK	0,37	273	296	247	158	219	252	211	143	331	317	300	242	308	278	305	217
chr9	113449292	113449604	MUSK	0,45	163	174	127	115	153	151	114	93	191	175	170	156	175	184	180	150
chr9	113457626	113457866	MUSK	0,32	166	196	127	100	137	147	139	85	185	170	176	143	175	166	175	132
chr9	113459495	113459855	MUSK	0,46	228	228	170	129	180	183	146	104	249	235	234	185	235	234	237	174
chr9	113490862	113491102	MUSK	0,32	263	257	215	141	184	215	201	125	299	280	266	221	239	247	274	208
chr9	113496473	113496713	MUSK	0,52	241	219	207	143	215	201	166	70	257	241	248	217	238	255	242	186
chr9	113509822	113510182	MUSK	0,43	250	253	206	139	206	214	170	110	270	257	250	224	244	250	260	186
chr9	113529990	113530470	MUSK	0,42	179	160	137	116	157	152	120	97	189	180	174	140	185	171	164	135
chr9	113537974	113538334	MUSK	0,42	190	184	138	113	164	166	118	100	201	178	106	160	203	190	178	145
chr9	113538803	113539043	MUSK	0,35	277	307	250	167	199	263	217	156	318	293	144	246	303	304	316	212
chr9	113547015	113547375	MUSK	0,36	355	394	292	175	256	289	234	168	354	371	330	297	361	350	366	257

chr9	113547722	113548082	MUSK	0,42	237	270	200	127	214	207	176	130	284	236	246	191	256	241	267	181
chr9	113549863	113550223	MUSK	0,42	260	298	231	162	268	247	196	135	310	310	290	252	281	271	293	214
chr9	113562506	113563346	MUSK	0,52	200	204	163	127	181	178	135	109	220	202	205	171	201	201	211	163
chr9	119459922	119462082	TRIM32	0,54	137	135	113	94	138	125	97	81	160	140	145	125	145	146	143	122
chr9	124062039	124062231	GSN	0,78	12	9	9	8	18	11	7	5	12	10	15	12	19	14	13	12
chr9	124062309	124062501	GSN	0,51	72	83	56	70	85	82	60	54	93	66	102	89	90	87	83	82
chr9	124064162	124064522	GSN	0,62	49	36	39	41	42	43	33	25	55	41	48	41	44	43	50	48
chr9	124065109	124065445	GSN	0,59	55	54	40	49	55	38	39	30	70	42	56	47	50	52	52	48
chr9	124072862	124073222	GSN	0,55	106	107	98	76	112	108	82	71	126	100	117	95	107	121	114	99
chr9	124074511	124074871	GSN	0,58	23	25	20	17	32	24	16	14	29	23	24	22	22	27	20	22
chr9	124076123	124076339	GSN	0,63	38	40	29	27	39	33	31	25	50	37	37	29	37	37	34	39
chr9	124079309	124079549	GSN	0,58	37	36	26	24	38	35	21	26	46	32	40	34	33	34	40	30
chr9	124080607	124080847	GSN	0,59	35	39	30	42	42	39	27	24	45	33	44	45	41	37	39	44
chr9	124080870	124081230	GSN	0,64	28	26	17	28	31	24	24	21	31	23	29	23	30	31	25	27
chr9	124083492	124083732	GSN	0,58	63	62	42	41	64	51	37	33	62	52	52	48	55	52	57	49
chr9	124086756	124086996	GSN	0,60	9	8	8	6	17	12	9	9	13	8	13	12	15	10	9	12
chr9	124088694	124089054	GSN	0,63	14	15	10	12	17	9	12	10	18	9	17	16	16	18	14	12
chr9	124089492	124089852	GSN	0,61	49	53	34	39	49	40	29	29	56	42	47	43	47	44	42	38
chr9	124091110	124091350	GSN	0,62	39	38	27	30	43	39	25	27	48	34	44	39	44	50	47	43
chr9	124091434	124091674	GSN	0,56	52	58	33	38	54	53	38	42	78	52	54	51	54	50	58	54
chr9	124093575	124093815	GSN	0,56	99	113	78	90	102	94	77	67	131	105	116	94	117	90	98	91
chr9	124094616	124094976	GSN	0,58	41	45	38	42	53	46	32	30	65	48	57	46	53	51	55	46
chr9	131267013	131267253	GLE1	0,65	10	11	13	6	11	8	13	8	13	10	14	11	9	8	10	10
chr9	131271085	131271445	GLE1	0,45	319	354	283	181	263	271	242	169	350	365	344	262	334	325	365	246
chr9	131277782	131277998	GLE1	0,49	89	88	71	69	74	80	61	48	113	77	88	69	86	91	82	70
chr9	131284921	131285161	GLE1	0,46	184	185	132	112	159	155	137	109	195	176	180	163	159	183	171	141
chr9	131285492	131285708	GLE1	0,49	93	92	64	70	90	76	67	48	104	91	90	79	91	87	75	82
chr9	131285817	131286177	GLE1	0,57	55	48	45	45	60	49	36	35	67	45	62	44	53	57	42	52
chr9	131287406	131287766	GLE1	0,54	59	49	42	38	57	49	44	32	63	53	44	50	57	60	57	55
chr9	131289431	131289647	GLE1	0,47	149	132	112	89	142	119	109	77	163	128	149	127	138	136	128	119
chr9	131289649	131289889	GLE1	0,44	227	233	186	140	208	195	152	121	242	206	227	192	222	252	215	191
chr9	131295730	131296314	GLE1	0,44	279	268	216	163	238	219	209	149	293	260	274	222	266	268	271	219
chr9	131298616	131298832	GLE1	0,47	71	71	56	57	74	51	51	39	77	64	60	54	57	67	57	57
chr9	131300196	131300436	GLE1	0,46	130	156	100	88	138	118	88	76	143	126	134	116	142	125	124	113
chr9	131301823	131302063	GLE1	0,46	200	202	157	126	183	158	145	102	226	204	202	172	204	180	199	136
chr9	131302489	131302705	GLE1	0,47	134	159	118	91	131	128	105	81	161	165	151	142	146	144	157	120
chr9	131303294	131303534	GLE1	0,45	286	309	260	166	241	251	208	140	307	300	283	263	297	290	306	224
chr9	132576195	132576555	TOR1A	0,45	250	250	216	134	208	204	172	130	257	263	243	208	247	253	249	164
chr9	132580722	132581274	TOR1A	0,49	230	251	192	150	205	198	182	119	254	234	227	213	237	239	259	203
chr9	132584752	132585232	TOR1A	0,48	210	242	175	132	191	194	169	109	229	198	209	187	217	203	221	163
chr9	132586178	132586418	TOR1A	0,72	0	0	0	1	0	0	1	0	1	0	2	1	0	0	0	0
chr9	134379546	134379786	POMT1	0,55	67	63	46	57	68	53	44	41	66	61	59	57	58	60	63	58
chr9	134381433	134381673	POMT1	0,39	197	202	151	114	167	170	138	102	217	195	185	148	201	185	185	147
chr9	134381752	134381944	POMT1	0,35	211	252	196	148	228	214	162	113	266	253	230	212	209	228	240	176
chr9	134382647	134383007	POMT1	0,56	100	98	82	82	114	88	78	59	134	95	105	93	106	113	98	94
chr9	134384233	134384473	POMT1	0,39	248	266	216	162	225	223	177	128	260	266	258	216	247	241	261	200
chr9	134385049	134385529	POMT1	0,52	205	208	169	139	185	184	153	120	235	211	226	175	197	219	200	169
chr9	134385544	134385904	POMT1	0,49	206	201	161	135	189	190	149	117	232	205	209	173	214	198	215	171
chr9	134386668	134386908	POMT1	0,49	84	95	74	73	97	81	63	58	98	90	98	90	89	92	84	83
chr9	134387389	134387605	POMT1	0,57	36	30	31	38	50	36	23	32	57	33	40	37	47	37	36	40
chr9	134388551	134388791	POMT1	0,63	8	10	6	10	11	11	6	9	8	8	12	7	9	7	8	8
chr9	134390740	134390980	POMT1	0,51	76	90	61	57	100	70	59	57	86	69	84	69	73	80	83	71
chr9	134393757	134393997	POMT1	0,47	106	119	90	77	113	92	86	62	132	106	134	106	123	117	116	92
chr9	134394163	134394403	POMT1	0,63	17	12	15	13	18	15	11	11	19	10	11	14	15	16	12	12
chr9	134394704	134394944	POMT1	0,61	34	24	37	30	34	29	22	24	34	30	29	27	31	29	30	28
chr9	134395403	134395643	POMT1	0,55	53	52	37	46	56	51	32	34	61	52	48	45	66	54	50	42
chr9	134396661	134396877	POMT1	0,56	93	111	70	61	107	86	62	51	113	91	102	88	84	86	83	79
chr9	134397366	134397702	POMT1	0,62	143	155	132	107	153	132	105	87	192	149	161	112	162	157	169	119
chr9	134398225	134398585	POMT1	0,61	33	40	24	29	38	31	19	30	40	30	38	28	33	36	32	35
chr9	135139518	135140478	SETX	0,51	197	203	153	122	186	180	153	111	220	200	211	173	212	205	209	161

chr9	135144925	135145165	SETX	0,42	161	189	144	110	140	157	128	99	209	189	186	161	197	192	172	137
chr9	135147025	135147265	SETX	0,35	282	307	243	172	248	232	202	151	333	301	283	252	295	314	318	214
chr9	135150544	135150904	SETX	0,32	316	354	275	177	233	265	219	154	331	348	315	282	321	326	342	236
chr9	135152372	135152612	SETX	0,35	229	229	182	132	194	184	154	123	243	211	217	177	219	216	211	163
chr9	135153370	135153730	SETX	0,38	238	231	197	139	209	208	159	127	254	234	214	193	227	221	241	194
chr9	135156787	135157027	SETX	0,39	162	177	129	100	155	137	124	83	179	169	174	138	163	159	167	131
chr9	135158545	135158905	SETX	0,40	272	282	223	163	249	255	200	150	290	281	274	251	275	271	273	209
chr9	135161783	135161975	SETX	0,32	304	323	230	173	259	234	199	165	308	287	287	253	286	282	269	210
chr9	135163559	135163751	SETX	0,37	209	202	161	127	193	174	152	121	234	181	217	162	202	206	188	161
chr9	135163867	135164107	SETX	0,37	293	290	241	163	226	241	212	142	327	285	285	247	290	286	303	208
chr9	135171156	135171516	SETX	0,35	293	360	275	167	243	264	235	132	346	330	328	270	312	330	353	255
chr9	135172177	135172537	SETX	0,36	284	309	239	153	220	220	202	135	313	289	271	230	291	278	285	223
chr9	135173402	135173762	SETX	0,37	295	310	242	169	253	268	226	162	319	311	303	251	292	274	325	211
chr9	135175923	135176283	SETX	0,31	293	311	235	182	230	234	231	149	323	307	302	266	288	287	307	239
chr9	135187073	135187313	SETX	0,34	206	226	156	119	174	186	158	121	207	210	204	181	223	218	190	159
chr9	135201638	135205958	SETX	0,38	368	402	304	198	292	312	269	179	396	393	365	311	382	369	402	271
chr9	135206362	135206929	SETX	0,35	263	267	208	150	219	231	178	136	279	244	260	219	273	249	266	206
chr9	135209934	135210174	SETX	0,35	257	274	227	156	227	225	205	127	297	260	275	230	266	267	281	184
chr9	135211636	135211972	SETX	0,35	288	333	256	179	252	257	227	146	304	304	290	275	303	302	329	225
chr9	135218011	135218251	SETX	0,35	255	281	201	142	205	214	188	130	275	260	250	229	259	240	252	187
chr9	135221572	135221932	SETX	0,34	315	342	274	180	233	289	228	151	345	330	322	273	326	322	332	238
chr9	135224546	135224906	SETX	0,43	212	230	172	123	177	189	153	112	218	210	211	179	223	215	217	167
chr9	32973410	32973770	APTX	0,48	163	148	121	108	145	134	107	83	172	142	154	148	173	136	154	122
chr9	32974387	32974627	APTX	0,32	209	233	166	135	187	191	154	123	247	222	212	199	227	214	216	179
chr9	32984549	32984885	APTX	0,52	158	147	126	103	154	148	110	83	186	166	167	141	148	167	161	134
chr9	32985914	32986034	APTX	0,41	52	42	32	32	63	41	39	36	58	44	42	48	53	38	40	38
chr9	32987450	32987930	APTX	0,43	330	343	277	182	294	272	240	177	364	349	346	295	324	344	345	262
chr9	32987981	32988221	APTX	0,39	294	314	255	161	257	260	221	157	334	330	335	253	303	303	331	242
chr9	32989704	32989944	APTX	0,46	187	191	145	108	161	153	136	105	221	185	167	153	194	183	176	137
chr9	33001463	33001703	APTX	0,59	36	27	22	25	33	29	27	27	39	28	31	31	36	35	28	28
chr9	35057046	35057629	VCP	0,53	200	203	174	131	173	170	144	109	220	203	204	184	201	200	202	153
chr9	35058958	35059318	VCP	0,52	192	176	160	130	183	157	138	113	205	185	192	161	207	200	192	153
chr9	35059403	35059859	VCP	0,52	173	169	138	123	152	155	119	104	193	169	189	151	176	168	171	147
chr9	35060235	35060595	VCP	0,47	224	214	183	122	199	191	149	101	240	199	225	186	212	218	207	181
chr9	35060746	35061226	VCP	0,53	176	178	150	112	159	149	124	97	201	166	174	161	179	175	170	148
chr9	35061509	35061749	VCP	0,49	255	290	223	154	211	206	167	131	302	260	262	223	280	258	294	199
chr9	35061933	35062413	VCP	0,49	238	245	191	146	209	193	156	129	246	223	234	209	234	230	233	182
chr9	35062905	35063145	VCP	0,47	173	198	151	123	174	161	124	83	211	196	186	165	190	191	183	155
chr9	35064096	35064336	VCP	0,46	208	195	160	118	196	187	141	112	227	208	210	190	222	191	211	157
chr9	35065192	35065432	VCP	0,47	98	125	84	82	105	91	79	69	129	100	104	93	104	119	102	91
chr9	35066562	35066922	VCP	0,43	201	216	175	139	197	181	161	125	250	202	211	181	211	208	220	147
chr9	35067793	35068153	VCP	0,49	280	300	239	174	239	252	221	150	328	309	281	251	321	297	318	213
chr9	35068183	35068423	VCP	0,47	150	155	121	109	152	145	112	92	174	155	156	132	168	157	148	129
chr9	35072281	35072401	VCP	0,77	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chr9	35681998	35682238	TPM2	0,60	20	21	16	20	30	20	14	14	25	16	21	16	24	23	22	18
chr9	35683066	35683282	TPM2	0,58	30	29	32	34	38	34	23	24	38	33	35	24	24	30	30	29
chr9	35684157	35684635	TPM2	0,50	205	210	162	127	190	187	142	119	239	204	209	178	213	206	210	173
chr9	35684646	35684886	TPM2	0,64	204	210	165	126	173	161	137	113	228	184	198	175	195	206	209	169
chr9	35684978	35685881	TPM2	0,61	119	116	101	81	112	96	81	68	132	123	124	101	120	127	125	102
chr9	35689085	35689325	TPM2	0,61	202	206	154	122	201	173	153	105	224	199	224	182	200	185	215	161
chr9	35689637	35689877	TPM2	0,68	21	20	16	29	31	26	20	22	33	25	29	33	21	27	24	22
chr9	36217299	36217659	GNE	0,50	113	113	85	74	103	95	81	62	122	101	103	91	102	112	106	91
chr9	36218117	36218357	GNE	0,56	145	138	113	89	145	146	100	90	157	123	136	121	164	153	132	115
chr9	36219745	36220105	GNE	0,47	245	247	190	147	221	190	182	133	279	237	246	197	246	231	246	193
chr9	36222734	36223070	GNE	0,49	278	275	220	155	239	235	198	150	287	253	268	230	274	279	288	219
chr9	36223314	36223554	GNE	0,34	232	254	212	148	206	207	170	131	264	248	240	223	255	233	276	220
chr9	36227169	36227529	GNE	0,36	283	287	228	170	246	255	229	148	299	304	289	247	310	281	297	220
chr9	36228941	36229181	GNE	0,39	172	183	139	112	151	149	121	99	208	193	176	144	190	171	184	149
chr9	36233842	36234202	GNE	0,42	200	224	165	120	190	183	156	114	230	197	214	190	215	221	217	164
chr9	36236724	36237084	GNE	0,33	236	256	207	141	201	207	167	123	255	247	233	210	235	245	244	179

chr9	36245953	36246553	GNE	0,46	231	247	189	137	226	203	174	137	266	235	240	214	251	237	247	188
chr9	36249144	36249480	GNE	0,35	266	309	220	172	237	236	199	140	310	271	264	234	291	274	290	203
chr9	36276784	36277144	GNE	0,41	277	316	224	154	234	241	202	129	321	276	268	227	287	295	297	204
chr9	71650600	71650960	FXN	0,77	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0
chr9	71661229	71661469	FXN	0,40	210	241	182	134	200	200	176	131	271	250	249	193	228	235	228	167
chr9	71667997	71668237	FXN	0,39	187	186	151	114	181	165	133	110	220	176	194	160	193	184	180	154
chr9	71679786	71680026	FXN	0,38	171	207	142	137	177	157	141	112	219	198	186	163	181	185	205	151
chr9	71687422	71687758	FXN	0,48	166	183	138	109	156	157	108	103	199	170	183	161	179	171	174	148
chr9	71714713	71714953	FXN	0,50	161	168	132	95	153	143	112	84	183	162	173	131	160	155	164	112
chr9	94794666	94794882	SPTLC1	0,57	36	35	35	29	45	37	26	22	41	29	37	35	35	38	34	28
chr9	94797008	94797248	SPTLC1	0,41	223	233	176	139	190	191	158	116	244	232	213	195	228	218	223	170
chr9	94800468	94800708	SPTLC1	0,51	120	122	103	80	128	104	82	60	141	103	133	113	120	123	122	99
chr9	94808187	94808427	SPTLC1	0,31	286	296	232	146	224	255	196	153	311	282	292	239	279	274	296	192
chr9	94809381	94809621	SPTLC1	0,42	129	135	104	80	143	123	92	76	177	134	138	112	146	132	133	109
chr9	94809822	94810062	SPTLC1	0,39	165	168	140	114	163	142	122	92	193	165	182	157	169	170	192	143
chr9	94812175	94812415	SPTLC1	0,37	222	243	194	136	198	210	164	117	255	238	228	201	229	230	240	178
chr9	94817611	94817851	SPTLC1	0,37	290	284	238	175	224	238	191	136	302	292	276	242	290	273	292	214
chr9	94821405	94821645	SPTLC1	0,38	186	178	140	89	158	162	140	92	215	181	180	162	185	184	188	152
chr9	94830193	94830433	SPTLC1	0,34	266	273	222	149	189	229	192	132	302	281	275	231	260	264	270	193
chr9	94841785	94841905	SPTLC1	0,42	204	187	160	144	187	190	142	112	248	200	205	175	206	201	196	168
chr9	94842237	94842453	SPTLC1	0,34	280	281	209	173	241	248	196	166	289	290	302	235	281	281	294	212
chr9	94843078	94843318	SPTLC1	0,38	225	265	196	147	224	224	163	115	262	251	249	225	256	244	247	170
chr9	94870948	94871188	SPTLC1	0,38	117	113	84	56	99	97	90	52	120	102	106	93	105	109	104	92
chr9	94874670	94874910	SPTLC1	0,34	204	226	166	129	167	185	158	120	245	218	235	183	211	214	237	173
chr9	94877503	94877743	SPTLC1	0,66	20	19	12	23	28	18	13	14	26	18	21	20	22	20	19	19
chrX	103031865	103031985	PLP1	0,44	11	8	5	13	5	11	6	6	6	5	14	4	8	9	11	5
chrX	103040423	103040783	PLP1	0,50	249	127	96	148	101	210	86	123	128	143	249	109	124	131	242	102
chrX	103041284	103041764	PLP1	0,54	133	70	48	91	58	117	49	81	77	71	139	57	70	72	134	53
chrX	103042630	103042990	PLP1	0,49	195	103	69	123	94	171	64	108	108	101	206	87	104	101	199	76
chrX	103043282	103043522	PLP1	0,46	209	126	96	138	103	209	89	131	147	119	248	104	110	123	257	99
chrX	103044174	103044414	PLP1	0,44	244	123	98	158	104	207	91	133	139	139	252	115	141	141	249	98
chrX	103045370	103045610	PLP1	0,48	308	162	144	184	122	258	116	162	171	175	283	140	172	169	381	121
chrX	106871799	106872039	PRPS1	0,55	40	21	15	31	22	39	12	20	25	21	39	21	22	20	31	17
chrX	106882460	106882796	PRPS1	0,40	192	103	80	138	85	178	61	105	115	99	182	89	95	91	198	81
chrX	106884060	106884300	PRPS1	0,41	228	136	94	137	103	182	95	138	112	112	249	99	106	121	233	85
chrX	106885537	106885777	PRPS1	0,41	138	85	65	114	69	154	54	81	97	69	162	65	88	70	162	62
chrX	106888337	106888673	PRPS1	0,48	144	77	54	103	67	128	53	86	79	64	151	76	76	84	146	59
chrX	106890735	106891095	PRPS1	0,42	332	177	127	177	131	271	106	160	171	179	322	140	177	159	353	127
chrX	106893095	106893335	PRPS1	0,41	314	190	154	190	133	277	121	158	179	180	329	136	175	178	371	126
chrX	119562229	119562589	LAMP2	0,36	268	144	113	147	104	222	95	127	137	135	267	120	139	126	265	91
chrX	119565127	119565367	LAMP2	0,40	198	113	82	120	93	169	66	105	98	90	183	82	100	95	179	75
chrX	119572958	119573198	LAMP2	0,36	251	130	103	137	97	203	103	135	130	116	257	103	122	129	255	100
chrX	119575486	119575822	LAMP2	0,37	275	157	122	164	128	250	103	153	156	148	313	120	160	142	310	113
chrX	119576365	119576605	LAMP2	0,37	292	167	147	169	112	235	115	141	160	157	307	135	163	142	317	109
chrX	119580100	119580340	LAMP2	0,40	138	73	60	84	71	131	57	80	80	65	153	65	82	65	158	64
chrX	119581607	119581967	LAMP2	0,40	210	98	86	132	90	184	67	107	98	95	218	79	102	106	200	81
chrX	119582723	119583083	LAMP2	0,34	304	167	127	183	122	282	116	160	161	175	334	140	162	153	335	118
chrX	119589138	119589498	LAMP2	0,37	249	127	103	145	109	212	95	120	136	139	265	110	129	123	257	94
chrX	119590423	119590639	LAMP2	0,30	152	88	58	105	71	136	63	105	86	72	152	67	77	76	136	68
chrX	119602854	119603046	LAMP2	0,65	5	2	1	4	1	3	0	5	3	3	13	3	4	2	8	2
chrX	135278871	135279111	FHL1	0,43	163	77	64	102	82	157	62	86	88	87	173	78	89	87	163	68
chrX	135279162	135279402	FHL1	0,44	174	92	67	112	80	136	54	85	83	84	163	77	78	85	170	64
chrX	135288476	135288836	FHL1	0,58	68	32	32	54	36	60	22	41	47	34	89	34	39	37	63	31
chrX	135289081	135289441	FHL1	0,60	59	28	22	43	35	63	16	41	32	25	69	29	31	30	67	23
chrX	135289851	135290163	FHL1	0,54	137	74	60	96	77	128	53	86	85	76	155	64	81	65	159	56
chrX	135290556	135290892	FHL1	0,50	169	83	61	114	65	164	59	97	89	77	167	72	93	87	161	68
chrX	135291321	135291681	FHL1	0,55	91	40	30	62	48	82	29	56	42	38	97	44	44	41	75	35
chrX	135292008	135292248	FHL1	0,47	138	79	55	96	84	130	48	84	94	80	166	63	74	80	140	64
chrX	149760987	149761227	MTM1	0,33	284	170	114	165	110	247	94	150	148	146	283	120	140	137	319	111
chrX	149764877	149765117	MTM1	0,43	148	77	57	89	63	131	53	64	81	66	152	61	73	68	130	57

chrX	149766982	149767222	MTM1	0,29	250	144	106	159	103	235	102	138	152	131	284	115	113	139	299	105
chrX	149782996	149783236	MTM1	0,34	133	84	55	92	69	131	51	76	84	56	139	59	75	56	138	59
chrX	149787441	149787681	MTM1	0,48	219	123	97	129	96	198	84	121	127	118	239	105	121	107	230	87
chrX	149807337	149807577	MTM1	0,36	179	101	76	130	82	175	59	87	107	100	183	80	103	107	175	70
chrX	149809636	149809996	MTM1	0,42	211	112	85	121	95	197	85	111	118	117	208	96	113	108	220	85
chrX	149814069	149814429	MTM1	0,39	219	129	91	138	112	215	83	120	122	120	246	89	125	128	226	88
chrX	149818101	149818461	MTM1	0,30	280	149	106	152	110	233	103	136	143	127	277	126	135	132	281	96
chrX	149826216	149826576	MTM1	0,37	255	141	104	145	104	221	87	139	136	116	266	111	134	125	267	99
chrX	149828062	149828302	MTM1	0,31	236	133	80	134	100	202	75	110	134	123	239	96	113	117	243	91
chrX	149828780	149829020	MTM1	0,32	253	139	118	156	115	227	100	148	139	137	274	124	136	130	275	101
chrX	149831813	149832173	MTM1	0,37	280	163	118	176	113	240	107	157	149	138	284	129	148	139	294	111
chrX	149839804	149840164	MTM1	0,49	221	114	84	112	92	200	78	116	113	104	229	89	112	111	238	82
chrX	150565686	150565926	VMA21	0,71	3	0	2	2	1	2	0	0	2	0	3	0	0	0	1	0
chrX	150572037	150572277	VMA21	0,36	301	166	127	178	114	263	109	159	144	162	321	137	162	152	340	108
chrX	150573278	150573638	VMA21	0,38	120	58	45	90	60	114	37	79	75	53	130	51	59	56	119	46
chrX	152990631	152991711	ABCD1	0,69	23	9	8	21	14	21	7	14	14	11	25	11	12	8	24	10
chrX	152994596	152994956	ABCD1	0,63	32	16	16	26	20	31	15	22	28	18	40	15	19	16	36	13
chrX	153001466	153002066	ABCD1	0,63	44	20	17	40	28	39	18	27	33	20	49	20	23	26	40	20
chrX	153002537	153002777	ABCD1	0,65	7	1	2	8	2	6	1	7	4	5	8	3	2	3	8	2
chrX	153005438	153005798	ABCD1	0,64	33	13	13	32	13	30	10	23	17	10	33	15	14	14	26	12
chrX	153005920	153006280	ABCD1	0,63	24	15	8	20	14	28	8	22	15	9	31	15	17	14	31	14
chrX	153008362	153008602	ABCD1	0,67	16	11	11	13	12	18	10	14	12	8	25	10	13	12	16	9
chrX	153008617	153008857	ABCD1	0,68	16	12	7	16	13	19	8	14	12	9	17	11	9	11	15	9
chrX	153008885	153009245	ABCD1	0,68	9	5	5	9	6	11	7	11	6	4	11	6	5	4	11	4
chrX	153128053	153128413	L1CAM	0,63	67	39	25	58	32	53	21	46	44	29	70	28	30	33	68	29
chrX	153128733	153129093	L1CAM	0,64	45	18	22	37	26	44	20	34	29	14	48	24	27	28	51	21
chrX	153129284	153129524	L1CAM	0,65	11	5	4	11	6	9	3	5	10	4	12	6	6	7	10	6
chrX	153129674	153131035	L1CAM	0,63	53	24	17	42	28	46	18	36	32	22	58	24	26	27	51	25
chrX	153131096	153131336	L1CAM	0,60	61	33	24	48	35	59	21	50	36	21	65	23	29	37	54	23
chrX	153132034	153132394	L1CAM	0,64	31	10	9	17	10	24	9	16	18	10	25	13	14	14	25	14
chrX	153132423	153132663	L1CAM	0,55	87	41	34	56	49	80	31	60	58	40	92	44	54	48	88	43
chrX	153132729	153133089	L1CAM	0,56	138	70	52	95	70	113	52	76	87	65	147	55	67	71	135	54
chrX	153133175	153134471	L1CAM	0,62	102	49	42	80	53	88	36	63	63	45	107	49	54	51	98	45
chrX	153134866	153135782	L1CAM	0,64	46	21	19	37	25	43	15	35	24	20	54	18	26	22	47	20
chrX	153135819	153136035	L1CAM	0,63	18	10	3	16	12	16	6	7	11	6	15	6	8	6	15	7
chrX	153136139	153136739	L1CAM	0,61	44	23	20	41	27	44	18	32	31	18	52	20	28	29	49	22
chrX	153137527	153137887	L1CAM	0,63	45	24	22	37	27	41	15	33	25	18	45	21	27	23	42	19
chrX	153137979	153138219	L1CAM	0,61	53	26	17	48	29	49	19	36	31	25	49	22	22	16	53	26
chrX	153138629	153138749	L1CAM	0,53	22	10	13	26	16	22	8	21	11	15	26	13	20	16	30	9
chrX	153141133	153141349	L1CAM	0,65	7	2	2	9	3	3	2	6	3	1	7	2	3	2	5	4
chrX	153577130	153577490	FLNA	0,67	30	11	12	25	17	22	10	24	19	11	34	14	14	17	24	14
chrX	153577622	153578666	FLNA	0,62	62	30	25	47	33	55	20	39	34	25	66	25	31	30	64	30
chrX	153579222	153579462	FLNA	0,62	31	16	11	30	18	30	14	23	20	13	40	13	21	11	31	15
chrX	153579886	153580126	FLNA	0,63	26	16	8	22	11	24	8	13	13	12	33	10	12	20	22	14
chrX	153580200	153580440	FLNA	0,61	25	13	14	31	15	28	12	18	17	18	37	17	21	18	32	16
chrX	153580441	153582167	FLNA	0,62	56	26	23	44	32	48	19	39	28	23	54	24	28	27	49	24
chrX	153582226	153583496	FLNA	0,63	63	30	27	50	33	54	20	40	32	28	59	29	31	28	64	30
chrX	153585510	153586076	FLNA	0,65	39	16	16	29	18	31	13	30	20	18	36	20	20	15	35	18
chrX	153586511	153586991	FLNA	0,63	39	16	13	34	23	35	16	25	20	18	37	19	20	17	35	19
chrX	153587253	153589053	FLNA	0,64	42	20	15	34	22	34	14	28	24	15	40	16	23	18	37	20
chrX	153589615	153590215	FLNA	0,58	122	61	46	87	68	118	49	75	70	56	137	59	67	57	127	52
chrX	153590269	153591229	FLNA	0,62	46	29	18	38	29	46	17	34	26	22	52	23	30	22	45	21
chrX	153592324	153592804	FLNA	0,62	23	12	8	27	10	20	7	19	14	14	28	12	9	11	21	13
chrX	153592810	153593376	FLNA	0,63	55	31	26	54	28	53	21	41	34	25	66	25	26	33	56	29
chrX	153593438	153593918	FLNA	0,65	46	24	22	38	25	42	18	26	24	16	50	25	24	20	40	21
chrX	153594324	153595284	FLNA	0,65	50	20	19	41	24	41	11	32	26	19	47	16	24	20	43	21
chrX	153595695	153596513	FLNA	0,65	48	25	19	38	29	48	18	34	25	18	50	23	20	23	47	25
chrX	153599227	153599683	FLNA	0,65	31	13	11	25	22	31	11	21	18	11	37	14	17	15	26	18
chrX	153607765	153608460	EMD	0,66	33	13	13	35	15	34	10	23	20	13	36	14	19	12	31	14
chrX	153608540	153608780	EMD	0,62	46	24	24	36	26	43	11	37	25	21	46	25	26	16	42	21

chrX	153609034	153609634	EMD	0,59	153	69	60	100	74	124	58	95	84	72	147	69	80	78	153	64
chrX	153640161	153640606	TAZ	0,70	20	11	7	19	9	14	7	13	13	8	18	8	10	7	16	11
chrX	153641446	153641686	TAZ	0,61	58	26	22	33	24	48	18	37	31	18	57	24	28	26	43	25
chrX	153641741	153641981	TAZ	0,60	37	18	11	21	25	32	10	23	20	17	33	18	16	15	30	10
chrX	153647803	153648163	TAZ	0,60	59	27	27	48	38	56	25	35	32	24	58	30	30	35	58	24
chrX	153648281	153648696	TAZ	0,63	61	22	26	47	32	49	20	37	33	23	50	30	31	25	52	25
chrX	153648915	153649155	TAZ	0,64	32	18	17	35	19	36	14	30	19	17	39	18	16	20	34	20
chrX	153649172	153649412	TAZ	0,60	38	21	19	35	23	47	14	23	26	20	42	16	18	22	48	24
chrX	31139878	31140118	DMD	0,39	250	142	107	157	108	232	91	137	140	145	270	102	146	137	294	103
chrX	31144654	31144894	DMD	0,39	154	88	63	114	78	138	46	104	97	75	173	67	76	82	159	58
chrX	31152144	31152384	DMD	0,44	111	70	50	91	55	121	42	67	86	60	122	61	74	74	128	59
chrX	31164349	31164589	DMD	0,44	122	53	41	63	63	110	49	68	73	58	141	47	57	52	111	53
chrX	31165313	31165649	DMD	0,49	172	82	63	104	76	140	57	91	92	74	167	76	86	86	172	65
chrX	31187458	31187818	DMD	0,41	223	117	95	131	107	195	84	115	125	110	231	100	123	127	224	91
chrX	31190377	31190617	DMD	0,35	313	163	129	170	124	269	99	134	167	151	317	133	165	151	348	117
chrX	31191568	31191808	DMD	0,39	227	121	90	122	93	193	62	100	124	114	222	91	112	109	211	93
chrX	31195971	31196187	DMD	0,41	93	42	33	57	47	79	29	55	50	35	86	39	52	47	89	32
chrX	31196671	31197031	DMD	0,42	261	138	106	149	120	229	99	138	150	142	281	108	143	161	293	93
chrX	31198422	31198662	DMD	0,39	252	140	98	158	110	234	85	144	143	135	265	112	124	123	261	92
chrX	31200757	31201117	DMD	0,44	148	84	58	95	64	143	51	81	80	74	157	64	70	87	145	72
chrX	31221976	31222336	DMD	0,41	220	116	96	138	97	178	76	127	130	105	226	89	120	108	229	76
chrX	31224621	31224861	DMD	0,38	219	129	98	147	98	201	84	126	137	130	246	108	117	128	244	88
chrX	31227535	31227895	DMD	0,43	261	140	112	147	113	214	88	145	161	137	273	133	140	144	268	100
chrX	31241080	31241320	DMD	0,41	234	117	94	140	99	217	77	112	139	115	242	105	115	116	233	82
chrX	31278982	31279222	DMD	0,37	220	119	94	132	104	207	87	120	137	115	224	93	103	118	248	97
chrX	31284876	31284996	DMD	0,64	1	0	0	0	1	4	1	1	0	0	5	1	0	2	1	0
chrX	31341624	31341864	DMD	0,38	140	82	53	110	72	141	60	89	82	73	163	61	76	75	164	57
chrX	31366591	31366831	DMD	0,38	133	62	55	91	59	130	43	90	73	62	131	62	70	66	113	50
chrX	31462490	31462850	DMD	0,40	248	120	96	144	105	202	82	120	138	121	231	99	121	128	259	98
chrX	31496116	31496596	DMD	0,47	197	86	73	120	88	167	68	106	98	104	202	86	98	107	186	77
chrX	31497039	31497279	DMD	0,40	264	145	104	152	110	216	88	135	141	132	268	115	132	118	279	95
chrX	31514802	31515162	DMD	0,40	201	105	99	122	97	189	72	116	124	106	227	78	111	106	228	87
chrX	31525303	31525663	DMD	0,39	293	163	105	163	119	237	99	144	154	137	259	116	150	144	295	102
chrX	31526219	31526459	DMD	0,39	281	157	115	171	117	221	97	163	153	138	286	122	143	143	296	96
chrX	31645704	31646064	DMD	0,44	275	139	104	146	109	211	94	129	160	138	282	119	130	144	277	88
chrX	31676003	31676363	DMD	0,39	276	163	105	164	121	236	100	149	159	148	280	116	143	143	290	115
chrX	31697417	31697777	DMD	0,35	330	193	132	170	136	268	110	156	190	183	319	136	160	164	343	129
chrX	31747686	31747926	DMD	0,40	250	134	106	135	105	220	94	132	135	119	246	109	135	134	269	92
chrX	31792012	31792372	DMD	0,39	221	130	83	122	100	183	87	125	122	121	232	91	119	118	244	87
chrX	31838025	31838265	DMD	0,41	227	110	90	122	92	196	71	127	136	113	204	97	114	124	224	89
chrX	31854766	31855006	DMD	0,38	293	150	108	159	110	229	85	143	156	133	280	113	147	129	276	106
chrX	31893218	31893578	DMD	0,31	264	136	124	167	110	230	91	140	153	135	285	115	151	155	317	114
chrX	31947607	31947967	DMD	0,41	306	172	128	162	124	270	104	155	172	178	304	143	172	164	362	118
chrX	31950090	31950450	DMD	0,32	319	188	141	170	125	285	126	157	170	187	348	141	163	168	361	119
chrX	31986363	31986723	DMD	0,40	291	154	121	158	127	257	87	145	164	158	292	112	158	149	322	103
chrX	32234926	32235286	DMD	0,33	321	173	0	195	114	265	114	151	168	169	334	137	153	153	344	117
chrX	32305551	32305911	DMD	0,34	266	153	0	141	114	235	100	135	147	141	268	110	138	129	277	103
chrX	32328115	32328475	DMD	0,37	284	145	0	164	118	247	84	141	157	150	272	120	159	146	301	103
chrX	32360127	32360487	DMD	0,45	226	111	0	130	95	214	84	117	137	125	238	106	124	127	253	104
chrX	32361146	32361506	DMD	0,34	320	177	0	183	134	285	106	160	168	187	334	150	178	194	373	134
chrX	32364008	32364248	DMD	0,32	294	165	0	171	87	279	102	163	178	174	327	133	153	179	343	116
chrX	32366463	32366703	DMD	0,33	267	165	0	159	92	236	100	134	161	144	281	118	138	146	297	90
chrX	32380809	32381145	DMD	0,45	244	129	0	133	114	214	84	127	141	117	245	95	128	121	265	96
chrX	32382642	32382882	DMD	0,36	267	119	0	150	102	225	81	139	157	122	238	111	135	132	269	101
chrX	32383046	32383406	DMD	0,40	350	179	0	169	121	284	128	151	179	185	328	139	182	190	366	120
chrX	32398531	32398891	DMD	0,32	364	181	0	179	125	269	128	145	181	186	330	143	175	174	383	119
chrX	32404324	32404684	DMD	0,33	340	193	0	179	130	279	118	140	170	180	342	148	171	167	403	138
chrX	32407524	32407884	DMD	0,36	308	164	0	169	126	268	103	146	157	163	295	132	155	176	327	118
chrX	32408122	32408362	DMD	0,39	303	163	1	171	114	249	100	149	172	167	308	130	156	147	342	131
chrX	32429769	32430422	DMD	0,37	312	163	0	181	127	252	110	153	162	162	310	124	160	167	343	118

chrX	32456252	32456612	DMD	0,34	305	160	0	171	125	283	106	141	172	176	312	148	159	157	325	111
chrX	32459243	32459483	DMD	0,33	278	161	0	186	92	247	98	147	161	163	318	151	155	163	311	106
chrX	32466507	32466843	DMD	0,38	272	149	0	148	112	227	87	130	156	147	273	116	145	145	290	101
chrX	32472767	32473007	DMD	0,36	336	188	0	179	134	265	113	164	187	179	355	148	159	158	351	119
chrX	32481453	32481813	DMD	0,39	270	144	0	147	103	219	90	126	146	129	271	109	148	136	281	107
chrX	32482639	32482879	DMD	0,35	293	147	0	159	117	248	102	144	166	171	295	129	161	179	340	111
chrX	32486540	32486900	DMD	0,35	275	130	0	154	112	223	82	122	152	135	254	115	136	133	286	113
chrX	32490173	32490533	DMD	0,36	261	153	0	147	112	241	86	142	151	145	289	130	146	137	264	104
chrX	32502945	32503305	DMD	0,37	290	155	0	169	126	252	93	143	165	161	299	122	146	160	311	114
chrX	32509334	32509694	DMD	0,39	211	108	0	117	103	182	72	114	116	99	218	89	110	101	205	87
chrX	32519795	32520035	DMD	0,39	294	144	0	167	114	216	95	139	155	140	285	128	149	136	275	106
chrX	32536066	32536306	DMD	0,34	266	134	0	154	106	229	91	138	141	146	265	108	133	131	261	100
chrX	32563183	32563543	DMD	0,42	241	118	108	137	104	211	76	129	124	128	220	95	132	127	235	92
chrX	32583728	32584088	DMD	0,38	315	176	134	186	139	268	106	159	177	158	327	140	168	165	336	131
chrX	32591580	32592032	DMD	0,37	346	192	147	180	135	290	119	168	185	180	344	150	185	177	370	130
chrX	32613813	32614053	DMD	0,34	273	153	110	170	110	242	87	148	164	147	292	134	144	136	280	110
chrX	32632314	32632674	DMD	0,35	290	167	131	160	117	267	107	151	166	157	299	136	155	155	338	100
chrX	32662159	32662519	DMD	0,36	328	188	145	188	129	285	115	167	179	193	349	145	179	179	374	122
chrX	32662994	32663354	DMD	0,36	345	193	139	171	139	286	121	160	175	172	321	140	163	164	369	119
chrX	32715930	32716170	DMD	0,48	113	58	37	72	47	102	36	64	65	49	111	48	60	62	102	42
chrX	32717165	32717501	DMD	0,35	312	168	130	175	115	277	100	149	173	163	324	134	182	155	360	113
chrX	32827548	32827788	DMD	0,39	268	132	108	147	112	213	78	124	148	133	234	104	130	133	265	104
chrX	32834490	32834850	DMD	0,37	320	178	131	170	133	261	112	155	185	167	324	130	164	170	339	128
chrX	32841337	32841577	DMD	0,33	268	156	111	174	100	260	115	136	153	144	284	122	139	152	294	113
chrX	32862818	32863058	DMD	0,44	179	94	71	121	94	151	70	105	101	83	185	90	82	93	192	92
chrX	32867770	32868010	DMD	0,38	248	125	98	126	107	209	89	131	138	139	254	117	153	138	257	95
chrX	33038166	33038406	DMD	0,26	261	143	104	147	94	225	85	122	144	133	262	111	124	120	267	99
chrX	33146212	33146332	DMD	0,41	95	60	34	73	49	106	37	67	68	57	101	43	64	52	99	42
chrX	33229293	33229533	DMD	0,32	272	147	110	152	103	240	89	130	134	155	265	112	136	140	279	103
chrX	33357318	33357438	DMD	0,38	169	73	61	93	75	136	48	84	98	81	165	65	80	73	164	55
chrX	47058139	47059065	UBA1	0,57	174	80	71	117	82	146	61	96	95	83	170	78	87	84	171	71
chrX	47060225	47060465	UBA1	0,62	16	9	7	13	9	21	7	9	9	7	12	4	6	8	17	7
chrX	47060599	47061062	UBA1	0,55	64	30	20	50	28	62	25	40	31	24	64	30	31	26	57	33
chrX	47061483	47062717	UBA1	0,57	140	65	58	101	75	121	51	86	84	65	146	62	76	75	139	61
chrX	47062834	47063146	UBA1	0,55	106	67	44	74	58	95	45	70	59	51	114	48	63	55	99	45
chrX	47065249	47065924	UBA1	0,54	156	78	58	99	77	128	54	91	90	70	157	69	79	76	145	64
chrX	47068933	47069173	UBA1	0,51	154	78	59	123	79	136	59	93	88	79	144	77	79	80	146	62
chrX	47069244	47069604	UBA1	0,58	85	37	35	69	46	82	38	58	48	45	97	39	44	42	81	41
chrX	47070157	47070709	UBA1	0,59	125	64	47	87	60	105	48	70	65	50	117	57	58	62	120	49
chrX	47071746	47071986	UBA1	0,54	90	36	33	49	35	74	28	46	40	32	77	37	39	39	78	34
chrX	47072095	47072664	UBA1	0,52	202	97	85	136	85	167	67	107	95	90	206	88	112	94	199	75
chrX	47073656	47074105	UBA1	0,56	172	83	75	110	73	153	59	95	90	85	168	80	81	81	162	68
chrX	47074140	47074380	UBA1	0,61	49	21	20	35	24	46	17	25	26	19	53	25	20	34	43	20
chrX	66764928	66765168	AR	0,63	25	13	11	24	13	21	10	17	18	10	27	12	9	10	25	16
chrX	66765259	66766339	AR	0,63	51	21	18	40	28	49	18	31	32	22	54	22	26	23	48	23
chrX	66766399	66766711	AR	0,62	56	35	25	52	37	59	18	44	47	37	58	30	37	34	58	29
chrX	66788794	66788914	AR	0,45	45	18	13	30	33	46	13	26	30	23	51	26	23	22	44	26
chrX	66862993	66863353	AR	0,46	262	128	91	161	112	215	93	129	150	140	254	103	139	139	272	90
chrX	66905768	66905984	AR	0,40	226	120	87	137	91	213	67	120	118	119	235	96	124	112	222	91
chrX	66931143	66931575	AR	0,49	156	83	59	101	77	153	63	89	99	79	160	66	93	86	169	68
chrX	66937211	66937571	AR	0,54	156	73	62	99	80	145	55	85	82	83	154	59	73	78	151	57
chrX	66941619	66941859	AR	0,51	214	101	88	121	104	178	68	106	128	94	228	100	111	106	228	84
chrX	66942567	66942927	AR	0,48	241	121	104	128	107	205	84	131	117	117	215	99	121	115	232	93
chrX	66943425	66943785	AR	0,49	298	147	124	161	111	257	95	142	167	163	292	134	149	165	321	114
chrX	70443503	70444463	GJB1	0,58	144	66	54	87	67	122	55	85	78	72	148	67	73	71	153	70
chrX	77359749	77359989	PGK1	0,56	50	26	18	50	30	54	14	32	30	22	59	27	22	32	50	28
chrX	77365268	77365508	PGK1	0,37	184	109	75	120	85	179	55	110	112	98	231	98	99	108	198	89
chrX	77369138	77369764	PGK1	0,47	227	109	90	130	109	191	79	119	125	111	223	103	115	106	228	90
chrX	77372740	77372980	PGK1	0,42	235	121	79	136	93	205	82	120	116	111	226	100	117	99	228	102
chrX	77373487	77373727	PGK1	0,46	83	40	30	57	35	65	26	54	42	46	91	37	43	38	79	23

chrX	77378268	77378508	PGK1	0,38	190	105	77	123	92	165	71	106	109	94	200	75	96	102	207	63
chrX	77378601	77378961	PGK1	0,45	184	92	73	107	73	164	67	96	114	89	175	79	98	92	191	69
chrX	77380279	77380639	PGK1	0,49	123	73	55	90	62	113	44	67	68	67	128	62	64	62	123	61
chrX	77380752	77380992	PGK1	0,49	90	35	36	60	43	75	35	48	58	45	99	43	56	39	75	39
chrX	77381186	77381426	PGK1	0,43	270	139	123	165	125	230	86	146	162	151	293	122	155	139	298	95

Targeted exons not covered by any sequence read are due either to removal of the corresponding oligonucleotide sequences during the design process or removal of a sequence mapping at several genomic positions (eg. SMN1 and SMN2 first 6 exons), or bias in the capture and sequencing (likely for high GC content), or a likely deletion if found in a specific patient

Supplementary Table 3: Variants ranking, conservation scores and amino acid change scores

Patient	Type of mutation	Gene	Mutation nucleotide (protein)	Sequence reads	Prediction with SIFT or polyphen	VaRank ranking for all 267 NMD genes	VaRank ranking for disease class [4]
A	Indel	*MTM1*	c.141-144delAAAG (p.Glu48LeufsX24)	125 / 87	n.a.	2	1
B	Exonic point mutation	*BIN1*	c.1717C>T (p.Gln573X)	0 / 11	n.a.	3	1
C	Large deletion	*DMD*	Deletion ex18-44	n.a.	n.a.	large deletion found	large deletion found
D	Indel + intronic splice site mutation	*SETX*	c.3213-3214insT (p.Gln1072SerfsX3); c.5275-1 G>A	129 / 102; 66 / 67	n.a. - n.a.	2 [2]	1 [2]
E	Indel	*MTM1*	c.156-157ins A (p.Cys53MetfsX8)	6 / 96 [3]	n.a.	2	1
F	Exonic point mutation	*DNM2*	c.1565 G>A (p.Arg522His)	25 / 17	Probably damaging	7	1
G	Intronic, effect on splice	*MTM1*	c.1261-10A>G	0 / 80	n.a.	28	1
H	Indel + exonic point mutation	*SETX*	c.2967-2971delGAAAG (p.Arg989SerfsX5); c.994 C>T (p.Arg332Trp)	57 / 116; 74 / 161	n.a. - Deleterious	2	1 [2]
I	not found	none			n.a.		
J	Exonic point mutation	*TTN*	c.68576C>T (p.Pro22859Leu)	137/131	Deleterious	3	1
K	Exonic point mutation	*COL6A3*	c.6812G>A (p.Arg2271Lys)	37 / 36	Possibly damaging	28	3 [5]
L	not found	none			n.a.		
M	not found	none			n.a.		
N	Exonic point mutation	none (LMNA)	c.1928C>A (p.Thr643Asn); c.1930C>T (p.Arg644Cys 2)	13 / 11; 13 / 11	Deleterious - Deleterious	13	1 [2]
O	Exonic point mutation	*TTN*	c.3100G>A (p.Val1034Met); c.49243G>A (p.Ala16415Thr)	162 / 131; 72 / 75	Deleterious - Deleterious	7	1 [2]
P	Exonic point mutation	*RYR1*	c.8554C>T (p.Arg2852X); c.11557G>A (p.Glu3853Lys)	69 / 45; 23 / 34	n.a. - Deleterious	2 [2]	1 [2]

[1] reads for WT/mutant allele
[2] in a recessive scenario
[3] 94% reads show the variant and the heterozygous limit was set to 80%

Supplementary Table 4: Sequencing, coverage and variant statistics for patients with previously unknown mutations

	Samples	I	J	K	L	M	N	O	P	Average
Sequencing	sequenced nucleotides	1233499104	838038240	1338351264	1006097904	1013384592	1008386208	919090944	1137541248	1061798688
	sequence after filtering (in nt) [2]	430214544	384595344	402688368	330461640	405983664	398940408	409302072	310095288	384035166
	sequence in target regions (in nt)	342340416	312770304	322991856	269129592	315700056	314878752	332151696	246345624	307038537
Coverage	Mean coverage(x)	187	171	176	147	172	172	181	134	168
	Median coverage(x)	179	156	170	138	162	160	169	129	158
	% Base≥ 3x coverage	98	97	98	98	98	98	98	98	98
	% Base≥ 10x coverage	95	93	95	94	95	94	94	94	94
	% fully covered exons	4481	4451	4479	4442	4467	4465	4454	4467	4463
Variants	SNVs	1178	1104	1177	1813	1101	1211	1130	1158	1234
	indels	155	140	143	180	138	152	137	140	148
	total heterozygotes	1011	952	964	1208	922	994	909	902	983
	total homozygotes	322	292	357	785	317	369	358	396	400
	SNVs+ Indels	1333	1244	1320	1993	1239	1363	1267	1298	1382
	SNVs+ Indels without rs number	402	325	337	450	346	383	346	306	362
	novel coding non-synonymous [1]	139 / 0	132 / 2	125 / 5	102 / 0	130 / 3	131 / 2	123 / 2	102 / 3	111 / n.a. [3]
	novel splice site change [1]	5 / 0	5 / 0	7 / 0	3 / 0	9 / 0	7 / 0	7 / 0	5 / 1	6 / n.a. [3]
	novel coding stop (gained/lost) [1]	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	2 / 1	0 / n.a. [3]
	novel coding frameshift [1]	1 / 0	0 / 0	3 / 0	0 / 0	1 / 0	0 / 0	2 / 0	2 / 0	1 / n.a. [3]

[1] before / after fitering and ranking

[2] after filtering duplicate reads and multiple genomic mapping

[3] not applicable

Average enrichment is 1500 fold

4- Discussion and perspectives

Mutations in patients with classical CNM have been identified in three genes: *MTM1* (Laporte et al, 1996), *BIN1* (Nicot et al, 2007) and *DNM2* (Bitoun et al, 2005). Mutations in two other genes (*MTMR14 and RYR1)* in patients with similar CNM phenotypes have been detected as well.

Two heterozygous variations were reported in the Myotubularin-related 14 (*MTMR14)* in patients with sporadic CNM, including one patient with an additional *DNM2* heterozygous change (Tosch et al, 2006). Whether these variations are disease-causing mutations or implicating in phenotype modifications remains to be determined. More recently, Ryanodine receptor 1 (*RYR1*) mutations were mainly detected in patients with partially similar CNM features such as central nuclei and radial arrangement of sarcoplasmic strands in muscle biopsy with autosomal recessive inheritance *1* (Bevilacqua et al, 2011; Jungbluth et al, 2007; Wilmshurst et al, 2010).

About 20% of patients with CNM do not have mutations in the three classical CNM genes (*MTM1*, *DNM2* and *BIN1*) (Biancalana et al, 2012). Thus, it is likely that there are other genes implicated in the pathogenesis of this disorder.

Accordingly, the first biological question and aim of my PhD project was to find novel gene(s) implicated in CNM, using massively parallel sequencing, and the second aim was to test whether massively parallel sequencing can be used as a routine technique in molecular diagnosis of neuromuscular disorders. These points will be discussed separately in this section.

4-1- Aim 1: Gene identification in CNM

In order to potentially identify novel genes implicated in CNM several approaches such as targeted sequencing of 76 or 2500 candidate genes, exome sequencing and whole genome sequencing were performed during this project.

4-1-1- Mutations in known genes

Coding exons and intron-exon boundaries of *MTM1*, *DNM2* and *BIN1* were analyzed by Sanger sequencing in order to select CNM patients without mutations in three classical CNM genes. Some novel and known mutations were detected in *MTM1* as well as *BIN1* in different patients.

In patients with *MTM1* mutations, apart from detection and characterization of mutations, we could show the efficacy of novel anti-myotubularin antibodies. These antibodies can be used for direct Western blotting to detect both exonic and non-exonic mutations and should be able to detect most

of *MTM1* changes using a variety of cell types and muscle tissue without giving the knowledge of exact position of mutations. Furthermore, we showed that RT-PCR followed by sequencing of the PCR products represents a useful approach to detect or/and confirm both exonic mutations and splice alterations due to intronic mutations.

In a CNM patient from a consanguineous family, we detected the first mutation in *BIN1* muscle-specific exon11. This mutation is a homozygous splice acceptor mutation (IVS10-1G>A) affecting the splicing of muscle-specific exon in *BIN1* coding for a phosphoinositide binding domain, resulting in a rapidly progressing myopathy in humans. Amphiphysin 2 is one of the key factors in muscular membrane remodeling. The gene, *BIN1*, has recently been associated with two different muscle disorders: centronuclear myopathy (CNM, MIM #255200) (Nicot et al, 2007) and myotonic dystrophy (DM, MIM #160900 and #602668) (Fugier et al, 2011). However, the muscle-specific role of the ubiquitous protein amphiphysin 2 and the pathological mechanisms underlying the muscle disorders are not well understood. This is mainly due to the lack of faithful animal models. limited progress has been made in the study of the role of Bin1 in mouse model as well, due to the lethality associated with systemic ablation of Bin1 in mice as *Bin1(−/−)* mice die shortly after birth due to hypertrophic dilated cardiomyopathy without apparent defects in vesicle trafficking (Muller et al, 2003).
In our study we identified a new canine model (Great Danes) reproducing the human pathology with similar mutation in the same *BIN1* exon 11 acceptor splice site. Exon 11 of Bin1 consists of $PI(4,5)P_2$ binding domain and is thought to be essential for muscle cell fusion and differentiation as it has been shown to be required for the C2C12 myotube formation (Lee et al, 2002; Wechsler-Reya et al, 1998). As the only reported Bin1 mouse model is lethal, the affected Great Danes are the only characterized mammalian model for CNM linked to BIN1 mutation, and they can be used for preclinical trials of potential therapies.

4-1-2- Targeted sequencing

In order to detect the novel gene(s) implicated in CNM, targeted sequencing of candidate genes was carried out. In NGS of 76 selected genes, a pool of 20 DNAs of patients with CNM without tags was sequenced, using one lane of Illumina GAIIx sequencer, single-read with 72 bp length. One DNA (ACJ7) with a hemizygous c.529-1 G>T mutation in the *MTM1* gene was used as positive control to set the threshold based on the sequencing result for this variation. We wanted to test whether pooling of several samples without tagging which eliminates preparation of costly libraries was possible and what was the detection rate and coverage.

In several studies a similar approach was used for detection of new genes in different disorders (Margraf et al, 2011; Redin et al, 2012; Simpson et al, 2011a). In these studies different numbers of candidate genes were selected, enriched and sequenced in pools of different numbers of tagged samples. In some other studies, either non-tagged samples were sequenced in overlapping pool design, where each individual sample was re-sequenced in several pools (Prabhu & Pe'er, 2009) or several non-tagged samples were pooled and then these pools were tagged and sequenced (Erlich et al, 2009).

The inability to pinpoint the variant carrier among the individuals sequenced in a pool is the most important pitfall in pooling several samples without tagging. Retracing a detected variant back to its carrier requires additional sequencing (or genotyping) of all of these individuals, one at a time. Barcoding (tagging) is an experimental method that involves adding a “signature” nucleotide string to the start of all reads belonging to an individual. These nucleotides serve as the barcode that identifies which individual a given sequenced read came from. This step is a crucial step for later identification of samples with related variations. However, in the time of our study, this approach was costly and there were few numbers of available tags. Thus, pooling of 20 tagged samples was not possible and was not cost-effective. Therefore we pooled 20 samples without tagging.

In our case, the detected variations by NGS should have been verified by Sanger sequencing in all 20 samples. The number of detected variations was high and the NGS data related to the positive control variant did not enable the setup of an efficient threshold for removing false positives. More stringent filtering would remove the change in the positive control, and verifying all variations by Sanger sequencing to distinguish between the false positives and the real variants was not cost-effective. Therefore, pooling of 20 DNAs without tagging followed by NGS of 76 genes in one lane of Illumina GAIIx sequencer, single-read with 72 bp length did not lead to a reliable data. However, this was performed in the early days of pooling approach. Today, the same approach has been used for screening of large cohorts for detection of frequency of very rare variants in large populations for selected genes (Ramsey & Futschik, 2012).

In another targeted sequencing study, DNA of seven patients with CNM was selected and sequenced in the Genoscope sequencing platform (http://www.genoscope.cns.fr/spip/Sequencing.html) in 2009, using the Nimblegen array for capturing 2500 functional and positional candidate genes followed by sequencing in one lane of Roche 454 pyrosequencer. The NGS data analysis was performed only for three patients (C, D & F) with coverage of more than 7x, whereas for four other samples the coverage was not enough, thus further analysis was not carried out (Table8).

In patient C (AHJ97) a homozygous deletion of two nucleotides leading to a frameshift (NM_016653.2 (ZAK):c.490_491del, p.Met164ValfsX24) was detected in exon 7 of the *ZAK* gene. This

variation is segregated in the family as both parents had the same variation in heterozygous status and it is not listed in SNP databases such as dbSNP or 1000 genomes. *ZAK* is located in candidate region based on homozygosity mapping results (in chromosome 2) and it is highly expressed in skeletal muscle. In NHLBI exome variant server, there is not any detected nonsense or splice variations among 13000 chromosomes and most of the detected missenses are very rare suggesting that the deletion of two nucleotides leading to a frameshift is an important change in this gene. Additionally, it is a serine-threonine kinase which can potentially act in the same pathway as MTM1. No previous studies have reported a disease-causing mutation in this gene, so far. All of this data together shows that *ZAK* could be a potential candidate for CNM.

In two CNM sporadic cases, patient D (34263) and patient F (AHH42), there were a lot of variations after NGS of 2500 candidate genes in recessive as well as dominant scenarios. Distinguishing between private, rare polymorphisms and disease-causing mutations was impossible and we could not detect any CNM-causing mutation in these patients. Even by reducing the number of genes from all ~ 30,000 known genes to 2500 candidate genes did not allow us to detect a reliable candidate gene, provided that this gene was among our 2500 selected genes.

To verify whether there is another potential disease-causing variation in patient C, exome sequencing of this affected individual and his parents (trio sequencing) was performed. In this analysis *ZAK* was the best ranked gene as well. Therefore, a panel of 100 patients with CNM was analyzed for all of the 20 exons of *ZAK* by Sanger sequencing, in order to find at least another CNM patient with the mutation in the same gene. Apart from one heterozygous in frame deletion of three nucleotides in exon 19 that was detected in one patient, no other unknown variations were detected.

Re-verification of the clinical and histological data of AHJ97 showed atypical features for CNM such as predominantly affection of shoulder girdle with detachment of two scapulas, diffuse amyotrophy (progressive wasting of muscle tissues) and high CK level (1334 U/l). In three other patients with similar clinical or histopathological features, Sanger sequencing of all 20 exons of *ZAK* did not reveal any mutations.

Whether the homozygous NM_016653.2 (ZAK): c.490_491del, p.Met164ValfsX24 variation in *ZAK* in AHJ97 is a private, rare variation or CNM-causing mutation is not clear, for the moment. In a previous study, Dowling et al., generated zebrafish embryos with reduced myotubularin protein expression, using antisense morpholino technology (Dowling et al, 2009). They showed that in the embryos injected with antisense MTM1 morpholinos (morpholinos to the translation start site, to the splice

donor and acceptor sites of exon 1 and exon 3, respectively) severely impaired motor function, muscle fiber atrophy and the presence of large, abnormally located nuclei were detected. They also identified alterations in the T-tubule and sarcoplasmic reticular networks in morphant zebrafish muscle. They concluded that knockdown of zebrafish myotubularin recapitulates the features of myotubular myopathy, and thus demonstrating that zebrafish is an excellent model for studying the disease. As it was shown that zebrafish is an appropriate animal model for studying CNM and since *ZAK* is an interesting gene based on its function and chromosomal location, we have decided to investigate the effect of knocking down ZAK on zebrafish muscle development and to show the effect of this variation in a suitable animal model. This part of the project is still ongoing by Dr. Ursula Schaffer in our laboratory.

4-1-3- Exome sequencing

In order to identify new genes that potentially cause CNM, 20 CNM cases were exome sequenced during four past years. In 6 of them, we detected disease-causing mutations in *RYR1*. This gene encodes the skeletal muscle ryanodine receptor, which is a calcium channel in sarcoplasmic reticulum as well as a bridging structure connecting the sarcoplasmic reticulum and transverse tubule (Fill et al, 1989). Furthermore, it has role in excitation-contraction coupling in skeletal muscle.

Recently, several variations (dominant, compound heterozygous or homozygous) were reported in *RYR1* gene in patients with CNM phenotype without mutations in three main genes implicated in CNM with neonatal age of onset (Bevilacqua et al, 2011; Jungbluth et al, 2007; Wilmshurst et al, 2010). In these studies, mutations were detected by direct sequencing of cDNA or Sanger sequencing of mutation hot spot regions or all coding exons. Since this gene is a large gene, direct sequencing of all 106 exons and their intron-exon boundaries is not cost-effective and is not performed routinely. This gene is expressed in skeletal muscle, and therefore for cDNA sequencing muscle biopsies are required, however they are not often accessible.

In our case, we successfully detected disease-causing mutations in eight patients from six families with different congenital myopathies by exome sequencing and we showed that NGS is an appropriate technique to detect variations in large genes such as *RYR1*.We provided the evidence that exome sequencing – in combination with clinical and histological analyses - is a fast and reliable technique in congenital myopathies molecular diagnosis.

Apart from CNM sporadic cases with mutations in *RYR1*, seven DNAs from seven different families were used for exome sequencing in different sequencing platforms. The NGS data of these cases were analyzed in different recessive as well as dominant scenarios and numerous variations were detected in each scenario. Therefore, the NGS data of these seven sporadic samples were crossed in order to detect common genes with unknown variations.

Indeed, in several studies the disease-causing mutations were detected by overlapping NGS data of unrelated individuals with similar disorders. In a study, four unrelated individuals affected with Freeman–Sheldon syndrome, also called distal arthrogryposis type 2A, a rare autosomal dominant disorder, were selected and used for exome sequencing. By crossing the NGS data of these four patients and removing variants present in the dbSNP database, eight HapMap exomes and taking the predicted deleteriousness of individual mutations into account, different mutations were detected in a single gene, *MYH3 (Ng et al, 2009).*

In another study, four unrelated individuals affected with Schinzel-Giedion syndrome, characterized by severe mental retardation, distinctive facial features and multiple congenital malformations, were exome sequenced. By overlapping the NGS data of these four patients and removing variants present in dbSNP and in-house SNP databases, different mutations were detected in the *SETBP1* gene (Hoischen et al, 2010).

Compound heterozygous mutations were detected in the *FLVCR2* gene in two unrelated patients with Fowler Syndrome, a rare disorder characterized by hydrocephalus associated with progressive destruction of central nervous system tissue as a result of an unusual and characteristic proliferative vasculopathy. In this study, the NGS data of these two patients were crossed. After removing variations in dbSNP and 1000 genomes, genes with homozygous or compound heterozygous variations in both samples were listed and only one gene, *FLVCR2,* was detected (Lalonde et al, 2010).

Mutations were detected in *MLL2* gene in seven patients with Kabuki syndrome, a rare multiple malformation disorder characterized by a distinctive facial appearance, skeletal abnormalities, cardiac anomalies, and mild to moderate mental retardation, in exome sequencing of ten unrelated patients. In the overlapped data of ten patients, after removing variations present in SNP databases a gene, *MUC16,* was detected which is also known as “black gene”. Several variations are present in this gene in different exome datasets because this gene is a large gene and accumulates numerous variations. Looking to common genes in most of the patients led to find disease-causing mutations in *MLL2* gene in seven patients. Sanger sequencing of the remaining three patients detected mutations in the same gene in two of them. These mutations were missed by exome sequencing due to stringent filtering strategy. Disease-causing mutation in the last patient was not detected (Ng et al, 2010a).

In all of the above mentioned studies, the causative mutations/genes were detected by overlapping NGS data from several unrelated individuals with precisely defined clinical syndromes.
In our case, although CNM is a pathological sign and not a syndrome, i.e, a combination of signs, we decided to overlap the NGS data of our seven CNM sporadic cases to detect common genes in all or most of them. Different loss of function variations were detected in two genes, retinitis pigmentosa 1-like 1 (*RP1L1)* and keratin associated protein 21-1 (*KRTAP21-1)*. These two genes are not expressed in skeletal muscle and have role in visual system and thyroid, respectively. Thus, it is less likely that these genes are implicated in CNM.

The *TTN* gene was detected in almost all of the patients with several variations. Titin, or connectin, is a giant muscle protein expressed in the cardiac and skeletal muscles that spans half of the sarcomere from Z line to M line. Titin plays a key role in muscle assembly, force transmission at the Z line, and maintenance of resting tension in the I band region (Itoh-Satoh et al, 2002). It is a large gene that accumulates numerous variations and several variations are detected in any exome datasets in this gene. This gene is also listed as “black gene” in literature. Thus, to determine whether the detected *TTN* variations in our CNM patients are CNM-causing mutations or polymorphisms further analysis is required.

In other successful studies, all of the samples were sequenced together, using the same enrichment kit as well as the same sequencing technique, leading to homogenous sequenced regions and similar coverage. In our case, for exome sequencing of seven samples, different enrichment kits with different numbers and positions of covering regions and different sequencing machines with different length of reads were used, as the NGS was performed during two years. In order to reduce the bias, the same bioinformatic and filtering analysis was performed for all samples. The sequenced regions as well as the coverage were not similar, leading to omission of some regions in some patients.
The clinical as well as histological findings were not the same in all of seven sporadic CNM patients. Therefore, the possibility of having patients with another disorder rather than CNM, with overlapping clinical or histological features is high. This point could lead to have a bias when the NGS data overlapped; assuming that only the data from patients with precisely defined disorders should be crossed and compared.

Thus, the overlapping the NGS data of seven sporadic CNM patients did not lead to a reliable result due to heterogeneity in clinical and NGS data. In order to use NGS for gene discovery in sporadic

cases, several criteria such as careful choices of samples and controls, similar enrichment, sequencing as well as bioinformatics analysis with good quality NGS data and high coverage should be gathered in order to detect causative mutations.

In previous studies the efficacy of sequencing several family members for detection of causative mutations/genes was shown. In a previous study, exome sequencing was done for two affected members of a family with Miller syndrome, a very rare disorder characterized by the severe micrognathia (undersized jaw), cleft lip and/or palate, and other clinical signs. Mutations were detected in the *DHODH* gene in autosomal recessive inheritance mode (Ng et al, 2010b).
By exome sequencing of four patients in a Chinese four-generation spinocerebellar ataxia family, a disease- causing mutation was found in the *TGM6* gene (Wang et al, 2010a).

In our case, we decided to carry out exome sequencing for two affected individuals from a family (ABJ family) in the Integragen sequencing platform, using Agilent Sureselect liquid capture-whole exome kit version 1 for enrichment followed by NGS, using one lane of Illumina GAIIx sequencer, paired-end with 75bp length.
The NGS data were analyzed for two scenarios: Recessive scenario due to compound heterozygous changes and recessive scenario due to homozygous changes. The linkage analysis in this family had revealed linked regions in chromosome 2 and 9 but the Lod scores were about 2 which were not sufficient for unambiguous mapping of the disease gene. Thus, variations in these two chromosomes as well as variations in other chromosomes were checked in both scenarios.
After the filtration step, all variations were removed and we could not detect any candidate gene by sequencing the two affected members of this family.
One reason could be that a region with a causative mutation was not captured or sequenced.
The percentage of nucleotides with ten reads or more was about 85 in both cases. This means that 15% of targeted nucleotides had coverage less than 10 which especially, is not enough for correct calling of heterozygous variations. In variations with total coverage of 10 reads, distinguishing between real change and background is difficult and most of the variations with total coverage of ten or less are false positives.
As the NGS data of the two affected individuals were crossed and analyzed, the regions with enough coverage in one sample and no or poor coverage in the other sample would eliminate in filtration step.
Since, variations with Rs number in the dbSNP database were removed in the filtration step, causative mutation listed in the dbSNP inaccurately, could be missed. Although the validation status and minor allele frequencies of SNPs in the dbSNP database were taken into account in filtration

step, the variation with Rs number without this information were discarded from the list of variants, as well. Thus it is possible to miss a mutation due to inaccurate listing as polymorphism in the dbSNP database.

Since no promising data was achieved by exome sequencing in ABJ family, whole genome sequencing of one affected patient (ABJ79) and mother (ABJ68) was performed.

4-1-4-Whole genome sequencing

In some studies the causative mutations were detected by whole genome sequencing of affected and non-affected individuals in families. Roach et al., sequenced the entire genome of two affected children with two recessive disorders, Miller syndrome and primary ciliary dyskinesia, and their parents. They could narrow the candidate genes for both of these Mendelian disorders to only four genes (Roach et al, 2010).

In whole genome sequencing of a single patient with a recessive form of Charcot-Marie-Tooth neuropathy the compound heterozygous mutations in the *SH3TC2* gene were detected. (Lupski et al, 2010).

Since we had some non-sequenced regions in exome data in ABJ family, two members of this family, the affected child (ABJ79) and his mother (ABJ68), and three members of AIZ family, the affected child (AIZ36) and his healthy parents (APX27/mother & APX28/father) were selected for whole genome sequencing in the Complete genomics sequencing platform.

4-1-4-1- Whole genome sequencing for the ABJ family

In this family the affected child (ABJ79) and his mother (ABJ68) were selected for whole genome sequencing. The NGS data were analyzed for two possible scenarios: recessive scenario due to compound heterozygous changes, and recessive scenario due to homozygous change.

After filtration and verification of detected variations by Sanger sequencing in two affected members (ABJ75 and ABJ79) and also segregation in family, no candidate was detected. The data for structural and copy number variations were also analyzed and no promising data was achieved.

This could be due to the fact that the region with causative mutation was not sequenced or the causative mutation was missed due to recording as a polymorphism in the dbSNP database inaccurately. Another possibility is that the disease-causing mutation is either a deep intronic change, copy number variation, repeat expansions or a translocation which they were not verified in this study.

4-1-4-2- Whole genome sequencing for the AIZ family (314-1 and parents)

In this family the affected child (AIZ36) and his healthy parents (APX27/mother & APX28/father) were selected for whole genome sequencing. Three scenarios were checked: recessive scenario due to compound heterozygous changes, recessive scenario due to homozygous changes, and *de novo* scenario where the affected child had a variation which was absent in both parents.

In the recessive scenario due to compound heterozygous changes, variations in two genes, *TTN* and *MYO5B*, were detected and Sanger sequencing and segregation confirmed the detected variations. Apart from one variation in *TTN* which was in the exome variant server with low frequency, the other variations were not listed in any SNP databases. Both genes are expressed in skeletal muscle and some variations were detected by exome sequencing of other CNM patients. Some parts of these two genes were not covered and sequenced in different patients, thus all possible variations in these genes were not listed. The listed variations in other patients as well as healthy controls were not verified by Sanger sequencing. Thus, to determine whether these changes are real disease-causing mutations or individual variations or false positives, further verifications are needed.

In the recessive scenario due to homozygous changes, all detected variations were removed after the filtration step.

In the *de novo* scenario, two genes, *CLIP1* and *FLYWCH1*, were detected. Variations in both genes were not present in parents and predicted as deleterious, using polyphen2 and SIFT prediction tools. Both genes are expressed in skeletal muscle.
Looking into NGS data from several CNM patients without detected mutations, we determined that in some of the patients these two genes were not covered and sequenced completely, thus all of the variations in these two genes were not listed. However, some variations were detected in each patient. The listed variations in other patients as well as healthy controls were not verified by Sanger sequencing. Thus, to determine whether these changes are real disease-causing mutations or individual variations or false positives, further verifications are needed.

In conclusion, by whole genome sequencing of this family, four potential candidate genes, *TTN*, *MYO5B, CLIP1* and *FLYWCH1* were detected. Analysis of other detected variations in other samples by Sanger sequencing could remove false positives or could confirm any of these genes.
Functional studies, such as performing mutagenesis and verifying the effect of change in muscle is another possible approach, as well.

4-2- Aim 2: Improving diagnostic ofneuromuscular disorders using next generation sequencing

In this project, we performed targeted enrichment of 267 known NMD genes followed by NGS in patients affected by different neuromuscular diseases with or without known mutations.

DNA multiplexing (4 tagged samples were sequenced together in one lane of Illumina GAIIx sequencer) and blind variant ranking retrieved successfully different mutation types such as intronic, exonic, non-synonymous, insertions and small and big deletions for diseases with different segregations. We could retrieve mutations in all eight patients with known disease-causing changes. Analysis of eight other patients without molecular characterization identified causative mutations in several of them.

A faster molecular diagnosis of NMD will have major impacts on patients as it will improve disease management and genetic counseling, and will allow access to therapy or inclusion into therapeutic trials. As an example, the identification of *RYR1* mutations in patient P is of major medical importance as the treatment of RYR1 patients with salbutamol has shown significant amelioration of muscle weakness.

We could not detect disease-causing mutations in four patients. One of these patients was first clinically diagnosed with demyelinating polyneuropathy, but clinical and biochemical re-analyses in parallel to NGS suggested he had a mitochondrial disease which implicated genes were not covered by our design.

In the other patient, two heterozygous changes including one laminopathy causing mutation were detected. Since both changes were always in the same reads, we believed that both changes were on the same allele, and thus cannot be the sole cause of the axonal neuropathy. This hypothesis was just based on NGS data, as we did not have access to parents DNAs to confirm or refute it.

For the other two patients, who were previously excluded for several candidate genes by Sanger sequencing, mutations were also missed by our approach. One reason could be that the disease-causing mutation may be a deep intronic change, copy number variation, repeat expansions or a translocation for which detection was not tested in our study. Alternatively, these patients may also be mutated in a gene not linked to NMD at the time of our targeting library design.

As no previous large scale sequencing study targeting several NMD genes was reported at that time, our aim was to pilot an efficient screening strategy in an attempt to improve the clinical and molecular investigations of neuromuscular diseases from a very heterogeneous panel of patients.

Such targeted parallel sequencing of all candidate genes is especially suitable for diseases with high genetic heterogeneity, as it is the case for NMD, and should ease the identification of allelic diseases.

In addition, targeted sequencing has a higher coverage and leads to a smaller list of variants as it focuses on a subset of genes, whereas the sensitivity and heterozygosity assessment decrease following exome or genome sequencing due to lower coverage. Other advantages such as less incidental findings, high throughput and cost-effective molecular analysis are reachable, using targeted sequencing.

Other points were discussed in review paper in introduction section.

4-3- General discussion

Nowadays, with the use of NGS, the disease gene/variation identification challenge shifts from identification to interpretation.

In each study any of the targeted, exome or whole genome sequencing can represent a suitable strategy. For diagnosis approaches, targeted sequencing can be more useful than exome or genome sequencing due to higher coverage, a smaller list of variants as well as less incidental findings, high throughput and cost-effectiveness.

For gene discovery, although the whole genome is sequenced, only the protein coding regions of the genome are analyzed. As whole genome sequencing with sufficient depth of read is still costly to be used as the main tool for disease gene discovery, sequencing the exome is the most applied NGS technique for gene discovery. In figure 34, whole genome sequencing is compared to exome sequencing.

	Whole-genome sequencing (WGS)	Exome sequencing
Cost	Still costly, but decreasing rapidly	Reduced cost is a tenth to a third of WGS
Technical	No capture step, automatable	Capture step, technical bias
Variation	Uncovers all genetic and and genomic variation (SNVs and CNVs) Discovery of functional coding and noncoding variation ~3.5 million variants	Focuses on ~1% of the genome Limited to coding and splice-site variants in annotated genes ~20,000 variants
Disease	Suitable for mendelian and complex trait gene identification, as well as sporadic phenotypes caused by *de novo* SNVs or CNVs	Good for highly penetrant mendelian disease gene identification

Figure 34: comparison between whole genome and exome sequencing.

Reference: (Gonzaga-Jauregui et al, 2012)

Normally, between 20,000 and 50,000 variations are detected per exome (Gilissen et al, 2012). Efficient filtration strategy could help to reduce the number of candidates and leads to discover the disease-causing gene/variation. Schematic filtration workflow for genome as well as exome is shown in figure 35.

It is important to emphasize that the pathogenic variation may be discarded during the filtration step. A variant that is present at low frequency in a heterozygous state in the normal population can be removed even though it causes disease if present in homozygous state. For instance, in a study, one of the detected pathogenic mutations was recorded in the dbSNP (Walsh et al, 2010).

Another step which may remove pathogenic variation, is filtration based on computational predictions of the functional impact of new variations. It was shown that many known disease-causing mutations are predicted to be benign or polymorphic, using such prediction algorithms (Hicks et al, 2011). Thus, these programs can help the prioritization of variants and are not useful for elimination step.

Therefore, as several variations identify in each exome or genome by NGS, it is important to carefully consider filtration strategies for efficiently prioritizing pathogenic variants.

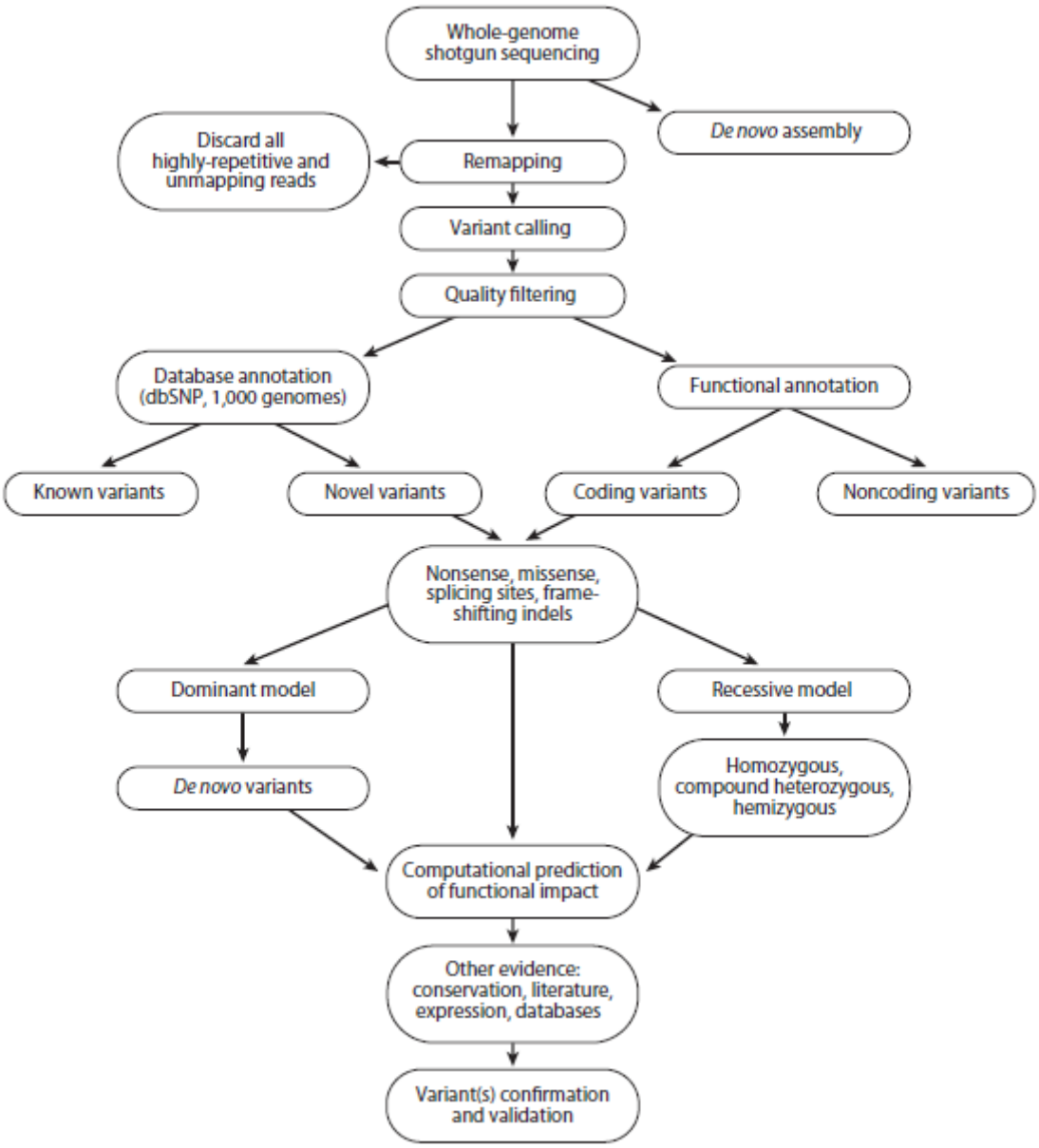


Figure 35: Schematic filtration workflow for genome as well as exome datasets. After sequencing, mapping is carried out against the human reference genome in order to obtain the list of variants. After removing low-quality variants, several filtration steps such as removing variants present in SNP databases or removing non-coding variants are performed. List of extracted variants are analyzed in different scenarios related to mode of inheritance. For few remaining candidates, more criteria such as predicted effect of change, conservation or expression profiles are taken into account in order to prioritize the variations.

Reference: (Gonzaga-Jauregui et al, 2012)

There are several reasons for missing the disease-causing variation, using NGS technique. The mutations can be missed due to: lack of sequence coverage or being in non-captured region,

bioinformatics issues such as false calling of homozygous/heterozygous variations, misinterpretation of variations, being a large indel or structural variation or repeat expansion as well as a problem in copy number or being intronic mutations. There are some problems in the reference genome as well, such as presenting the minor-allele nucleotide instead of major allele in some genes (Fuentes Fajardo et al, 2012) or digenicity may underlie the disorder. Therefore, the disease gene/mutation identification is not successful all the time, using NGS technique.

In order to use NGS in gene discovery as well as diagnosis approaches efficiently, several improvements should be considered. Errors due to biases in library construction, PCR steps and sequencing should be reduced by improving technical issues. Bioinformatic programs and tools should be improved to lessen the mapping and variant calling errors. The reference genome should be assessed and corrected. And finally more control samples from different populations should be sequenced and accessible publically in order to detect rare polymorphisms.

4-4-Perspectives

Several approaches can be performed in order to confirm or refute of any of detected candidate genes. As it is not clear that whether the homozygous NM_016653.2 (ZAK): c.490_491del, p.Met164ValfsX24 variation in *ZAK* in AHJ97 is a private, rare variation or CNM-causing mutation, investigation of the effect of knocking down ZAK on zebrafish muscle development is ongoing to see whether this gene has any role in muscle.

Several variations were detected in the *TTN*, *MYO5B, CLIP1* and *FLYWCH1* in different patients. These variations could be verified by Sanger sequencing in order to detect false positives. If the variations in any of these genes are confirmed, Sanger sequencing of a panel of CNM patients without molecular characterization will be carried out, in order to detect more samples with mutations in the same gene.

Functional studies, such as performing mutagenesis and verifying the effect of change in muscle is another possible approach, as well.

5- Conclusion

Although massively parallel sequencing is an appropriate technique to use for gene discovery as well as in diagnostic laboratories, some issues such as bioinformatics and technical problems, volume of data, incidental finding and correct data interpretation need to be addressed.

The overall rate of success for NGS is uncertain, as only successful studies have been reported to date. In our case, the two aims of my PhD project, detection of novel gene in CNM and NGS using in neuromuscular disorder diagnosis laboratories routinely, were addressed and several promising data were achieved. Looking to literature shows that only the detection of the disease-causing variation and gene is not enough and further robust functional analysis and suitable animal model will be needed in order to understand the disease mechanisms and biological pathways.

In conclusion, during my PhD project, mutations in *RYR1* were detected in some of CNM patients by exome sequencing. Some potential candidate genes such as *ZAK, TTN*, *MYO5B, CLIP1* and *FLYWCH1* were detected in other patients without mutations in classical CNM genes. In addition, in the project using NGS for diagnostic purposes, several unknown mutations were detected in different genes, in patients with different neuromuscular disorders and the usefulness of the technique for diagnostic application was shown.

References

Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P, Kondrashov AS, Sunyaev SR (2010) A method and server for predicting damaging missense mutations. *Nat Methods* **7**(4)**:** 248-249

Albers CA, Cvejic A, Favier R, Bouwmans EE, Alessi MC, Bertone P, Jordan G, Kettleborough RN, Kiddle G, Kostadima M, Read RJ, Sipos B, Sivapalaratnam S, Smethurst PA, Stephens J, Voss K, Nurden A, Rendon A, Nurden P, Ouwehand WH (2011) Exome sequencing identifies NBEAL2 as the causative gene for gray platelet syndrome. *Nat Genet* **43**(8)**:** 735-737

Alonso JL, Cavaliere MJ, Gagioti SM, Atalla AA, Nascimento I, Dias JC (1981) [Myotubular myopathy: clinical, electrophysiological and histological study of a case]. *Arq Neuropsiquiatr* **39**(4)**:** 450-472

Amburgey K, McNamara N, Bennett LR, McCormick ME, Acsadi G, Dowling JJ (2011) Prevalence of congenital myopathies in a representative pediatric united states population. *Ann Neurol* **70**(4)**:** 662-665

Andersen PS, Havndrup O, Hougs L, Sorensen KM, Jensen M, Larsen LA, Hedley P, Thomsen AR, Moolman-Smook J, Christiansen M, Bundgaard H (2009) Diagnostic yield, interpretation, and clinical utility of mutation screening of sarcomere encoding genes in Danish hypertrophic cardiomyopathy patients and relatives. *Hum Mutat* **30**(3)**:** 363-370

Antonarakis SE, Beckmann JS (2006) Mendelian disorders deserve more attention. *Nat Rev Genet* **7**(4)**:** 277-282

Bang ML, Centner T, Fornoff F, Geach AJ, Gotthardt M, McNabb M, Witt CC, Labeit D, Gregorio CC, Granzier H, Labeit S (2001) The complete gene sequence of titin, expression of an unusual approximately 700-kDa titin isoform, and its interaction with obscurin identify a novel Z-line to I-band linking system. *Circ Res* **89**(11)**:** 1065-1072

Barak T, Kwan KY, Louvi A, Demirbilek V, Saygi S, Tuysuz B, Choi M, Boyaci H, Doerschner K, Zhu Y, Kaymakcalan H, Yilmaz S, Bakircioglu M, Caglayan AO, Ozturk AK, Yasuno K, Brunken WJ, Atalar E, Yalcinkaya C, Dincer A, Bronen RA, Mane S, Ozcelik T, Lifton RP, Sestan N, Bilguvar K, Gunel M (2011) Recessive LAMC3 mutations cause malformations of occipital cortical development. *Nat Genet* **43**(6)**:** 590-594

Becker J, Semler O, Gilissen C, Li Y, Bolz HJ, Giunta C, Bergmann C, Rohrbach M, Koerber F, Zimmermann K, de Vries P, Wirth B, Schoenau E, Wollnik B, Veltman JA, Hoischen A, Netzer C (2011) Exome sequencing identifies truncating mutations in human SERPINF1 in autosomal-recessive osteogenesis imperfecta. *Am J Hum Genet* **88**(3)**:** 362-371

Bentley DR, Balasubramanian S, Swerdlow HP, Smith GP, Milton J, Brown CG, Hall KP, Evers DJ, Barnes CL, Bignell HR, Boutell JM, Bryant J, Carter RJ, Keira Cheetham R, Cox AJ, Ellis DJ, Flatbush MR, Gormley NA, Humphray SJ, Irving LJ, Karbelashvili MS, Kirk SM, Li H, Liu X, Maisinger KS, Murray LJ, Obradovic B, Ost T, Parkinson ML, Pratt MR, Rasolonjatovo IM, Reed MT, Rigatti R, Rodighiero C, Ross MT, Sabot A, Sankar SV, Scally A, Schroth GP, Smith ME, Smith VP, Spiridou A, Torrance PE, Tzonev SS, Vermaas EH, Walter K, Wu X, Zhang L, Alam MD, Anastasi C, Aniebo IC, Bailey DM, Bancarz IR, Banerjee S, Barbour SG, Baybayan PA, Benoit VA, Benson KF, Bevis C, Black PJ, Boodhun A, Brennan JS, Bridgham JA, Brown RC, Brown AA, Buermann DH, Bundu AA, Burrows JC, Carter NP, Castillo N, Chiara ECM, Chang S, Neil Cooley R, Crake NR, Dada OO, Diakoumakos KD, Dominguez-Fernandez B, Earnshaw DJ, Egbujor UC, Elmore DW, Etchin SS, Ewan MR, Fedurco M, Fraser LJ, Fuentes Fajardo KV,

Scott Furey W, George D, Gietzen KJ, Goddard CP, Golda GS, Granieri PA, Green DE, Gustafson DL, Hansen NF, Harnish K, Haudenschild CD, Heyer NI, Hims MM, Ho JT, Horgan AM, Hoschler K, Hurwitz S, Ivanov DV, Johnson MQ, James T, Huw Jones TA, Kang GD, Kerelska TH, Kersey AD, Khrebtukova I, Kindwall AP, Kingsbury Z, Kokko-Gonzales PI, Kumar A, Laurent MA, Lawley CT, Lee SE, Lee X, Liao AK, Loch JA, Lok M, Luo S, Mammen RM, Martin JW, McCauley PG, McNitt P, Mehta P, Moon KW, Mullens JW, Newington T, Ning Z, Ling Ng B, Novo SM, O'Neill MJ, Osborne MA, Osnowski A, Ostadan O, Paraschos LL, Pickering L, Pike AC, Chris Pinkard D, Pliskin DP, Podhasky J, Quijano VJ, Raczy C, Rae VH, Rawlings SR, Chiva Rodriguez A, Roe PM, Rogers J, Rogert Bacigalupo MC, Romanov N, Romieu A, Roth RK, Rourke NJ, Ruediger ST, Rusman E, Sanches-Kuiper RM, Schenker MR, Seoane JM, Shaw RJ, Shiver MK, Short SW, Sizto NL, Sluis JP, Smith MA, Ernest Sohna Sohna J, Spence EJ, Stevens K, Sutton N, Szajkowski L, Tregidgo CL, Turcatti G, Vandevondele S, Verhovsky Y, Virk SM, Wakelin S, Walcott GC, Wang J, Worsley GJ, Yan J, Yau L, Zuerlein M, Mullikin JC, Hurles ME, McCooke NJ, West JS, Oaks FL, Lundberg PL, Klenerman D, Durbin R, Smith AJ (2008) Accurate whole human genome sequencing using reversible terminator chemistry. *Nature* **456**(7218)**:** 53-59

Bevilacqua JA, Monnier N, Bitoun M, Eymard B, Ferreiro A, Monges S, Lubieniecki F, Taratuto AL, Laquerriere A, Claeys KG, Marty I, Fardeau M, Guicheney P, Lunardi J, Romero NB (2011) Recessive RYR1 mutations cause unusual congenital myopathy with prominent nuclear internalization and large areas of myofibrillar disorganization. *Neuropathol Appl Neurobiol* **37**(3)**:** 271-284

Biancalana V, Beggs AH, Das S, Jungbluth H, Kress W, Nishino I, North K, Romero NB, Laporte J (2012) Clinical utility gene card for: Centronuclear and myotubular myopathies. *Eur J Hum Genet*

Biancalana V, Caron O, Gallati S, Baas F, Kress W, Novelli G, D'Apice MR, Lagier-Tourenne C, Buj-Bello A, Romero NB, Mandel JL (2003) Characterisation of mutations in 77 patients with X-linked myotubular myopathy, including a family with a very mild phenotype. *Hum Genet* **112**(2)**:** 135-142

Bilguvar K, Ozturk AK, Louvi A, Kwan KY, Choi M, Tatli B, Yalnizoglu D, Tuysuz B, Caglayan AO, Gokben S, Kaymakcalan H, Barak T, Bakircioglu M, Yasuno K, Ho W, Sanders S, Zhu Y, Yilmaz S, Dincer A, Johnson MH, Bronen RA, Kocer N, Per H, Mane S, Pamir MN, Yalcinkaya C, Kumandas S, Topcu M, Ozmen M, Sestan N, Lifton RP, State MW, Gunel M (2010) Whole-exome sequencing identifies recessive WDR62 mutations in severe brain malformations. *Nature* **467**(7312)**:** 207-210

Bitoun M, Bevilacqua JA, Eymard B, Prudhon B, Fardeau M, Guicheney P, Romero NB (2009) A new centronuclear myopathy phenotype due to a novel dynamin 2 mutation. *Neurology* **72**(1)**:** 93-95

Bitoun M, Bevilacqua JA, Prudhon B, Maugenre S, Taratuto AL, Monges S, Lubieniecki F, Cances C, Uro-Coste E, Mayer M, Fardeau M, Romero NB, Guicheney P (2007) Dynamin 2 mutations cause sporadic centronuclear myopathy with neonatal onset. *Ann Neurol* **62**(6)**:** 666-670

Bitoun M, Maugenre S, Jeannet PY, Lacene E, Ferrer X, Laforet P, Martin JJ, Laporte J, Lochmuller H, Beggs AH, Fardeau M, Eymard B, Romero NB, Guicheney P (2005) Mutations in dynamin 2 cause dominant centronuclear myopathy. *Nat Genet* **37**(11)**:** 1207-1209

Bitoun M, Stojkovic T, Prudhon B, Maurage CA, Latour P, Vermersch P, Guicheney P (2008) A novel mutation in the dynamin 2 gene in a Charcot-Marie-Tooth type 2 patient: clinical and pathological findings. *Neuromuscul Disord* **18**(4)**:** 334-338

Bolze A, Byun M, McDonald D, Morgan NV, Abhyankar A, Premkumar L, Puel A, Bacon CM, Rieux-Laucat F, Pang K, Britland A, Abel L, Cant A, Maher ER, Riedl SJ, Hambleton S, Casanova JL (2010) Whole-exome-sequencing-based discovery of human FADD deficiency. *Am J Hum Genet* **87**(6)**:** 873-881

Botstein D, Risch N (2003) Discovering genotypes underlying human phenotypes: past successes for mendelian disease, future approaches for complex disease. *Nat Genet* **33 Suppl:** 228-237

Braslavsky I, Hebert B, Kartalov E, Quake SR (2003) Sequence information can be obtained from single DNA molecules. *Proc Natl Acad Sci U S A* **100**(7)**:** 3960-3964

Buj-Bello A, Laugel V, Messaddeq N, Zahreddine H, Laporte J, Pellissier JF, Mandel JL (2002) The lipid phosphatase myotubularin is essential for skeletal muscle maintenance but not for myogenesis in mice. *Proc Natl Acad Sci U S A* **99**(23)**:** 15060-15065

Byun M, Abhyankar A, Lelarge V, Plancoulaine S, Palanduz A, Telhan L, Boisson B, Picard C, Dewell S, Zhao C, Jouanguy E, Feske S, Abel L, Casanova JL (2010) Whole-exome sequencing-based discovery of STIM1 deficiency in a child with fatal classic Kaposi sarcoma. *J Exp Med* **207**(11)**:** 2307-2312

Caliskan M, Chong JX, Uricchio L, Anderson R, Chen P, Sougnez C, Garimella K, Gabriel SB, dePristo MA, Shakir K, Matern D, Das S, Waggoner D, Nicolae DL, Ober C (2011) Exome sequencing reveals a novel mutation for autosomal recessive non-syndromic mental retardation in the TECR gene on chromosome 19p13. *Hum Mol Genet* **20**(7)**:** 1285-1289

Chanzy S, Routon MC, Moretti S, de Gennes C, Mselati JC (2003) [Unusual good prognosis for X-linked myotubular myopathy]. *Arch Pediatr* **10**(8)**:** 707-709

Chen YZ, Bennett CL, Huynh HM, Blair IP, Puls I, Irobi J, Dierick I, Abel A, Kennerson ML, Rabin BA, Nicholson GA, Auer-Grumbach M, Wagner K, De Jonghe P, Griffin JW, Fischbeck KH, Timmerman V, Cornblath DR, Chance PF (2004) DNA/RNA helicase gene mutations in a form of juvenile amyotrophic lateral sclerosis (ALS4). *Am J Hum Genet* **74**(6)**:** 1128-1135

Claeys KG, Maisonobe T, Bohm J, Laporte J, Hezode M, Romero NB, Brochier G, Bitoun M, Carlier RY, Stojkovic T (2010) Phenotype of a patient with recessive centronuclear myopathy and a novel BIN1 mutation. *Neurology* **74**(6)**:** 519-521

Clark MJ, Chen R, Lam HY, Karczewski KJ, Euskirchen G, Butte AJ, Snyder M (2011) Performance comparison of exome DNA sequencing technologies. *Nat Biotechnol* **29**(10)**:** 908-914

Clarke NF, Waddell LB, Cooper ST, Perry M, Smith RL, Kornberg AJ, Muntoni F, Lillis S, Straub V, Bushby K, Guglieri M, King MD, Farrell MA, Marty I, Lunardi J, Monnier N, North KN (2010) Recessive mutations in RYR1 are a common cause of congenital fiber type disproportion. *Hum Mutat* **31**(7)**:** E1544-1550

Consortium IHGS (2004) Finishing the euchromatic sequence of the human genome. *Nature* **431**(7011)**:** 931-945

Craig DW, Pearson JV, Szelinger S, Sekar A, Redman M, Corneveaux JJ, Pawlowski TL, Laub T, Nunn G, Stephan DA, Homer N, Huentelman MJ (2008) Identification of genetic variants using bar-coded multiplexed sequencing. *Nat Methods* **5**(10)**:** 887-893

de Gouyon BM, Zhao W, Laporte J, Mandel JL, Metzenberg A, Herman GE (1997) Characterization of mutations in the myotubularin gene in twenty six patients with X-linked myotubular myopathy. *Hum Mol Genet* **6**(9)**:** 1499-1504

DeJesus-Hernandez M, Mackenzie IR, Boeve BF, Boxer AL, Baker M, Rutherford NJ, Nicholson AM, Finch NA, Flynn H, Adamson J, Kouri N, Wojtas A, Sengdy P, Hsiung GY, Karydas A, Seeley WW, Josephs KA, Coppola G, Geschwind DH, Wszolek ZK, Feldman H, Knopman DS, Petersen RC, Miller BL, Dickson DW, Boylan KB, Graff-Radford NR, Rademakers R (2011) Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron* **72**(2)**:** 245-256

Donner K, Sandbacka M, Lehtokari VL, Wallgren-Pettersson C, Pelin K (2004) Complete genomic structure of the human nebulin gene and identification of alternatively spliced transcripts. *Eur J Hum Genet* **12**(9)**:** 744-751

Dowling JJ, Vreede AP, Low SE, Gibbs EM, Kuwada JY, Bonnemann CG, Feldman EL (2009) Loss of myotubularin function results in T-tubule disorganization in zebrafish and human myotubular myopathy. *PLoS Genet* **5**(2)**:** e1000372

Dressman D, Yan H, Traverso G, Kinzler KW, Vogelstein B (2003) Transforming single DNA molecules into fluorescent magnetic particles for detection and enumeration of genetic variations. *Proc Natl Acad Sci U S A* **100**(15)**:** 8817-8822

Drmanac R, Sparks AB, Callow MJ, Halpern AL, Burns NL, Kermani BG, Carnevali P, Nazarenko I, Nilsen GB, Yeung G, Dahl F, Fernandez A, Staker B, Pant KP, Baccash J, Borcherding AP, Brownley A, Cedeno R, Chen L, Chernikoff D, Cheung A, Chirita R, Curson B, Ebert JC, Hacker CR, Hartlage R, Hauser B, Huang S, Jiang Y, Karpinchyk V, Koenig M, Kong C, Landers T, Le C, Liu J, McBride CE, Morenzoni M, Morey RE, Mutch K, Perazich H, Perry K, Peters BA, Peterson J, Pethiyagoda CL, Pothuraju K, Richter C, Rosenbaum AM, Roy S, Shafto J, Sharanhovich U, Shannon KW, Sheppy CG, Sun M, Thakuria JV, Tran A, Vu D, Zaranek AW, Wu X, Drmanac S, Oliphant AR, Banyai WC, Martin B, Ballinger DG, Church GM, Reid CA (2010) Human genome sequencing using unchained base reads on self-assembling DNA nanoarrays. *Science* **327**(5961)**:** 78-81

Duerr RH, Taylor KD, Brant SR, Rioux JD, Silverberg MS, Daly MJ, Steinhart AH, Abraham C, Regueiro M, Griffiths A, Dassopoulos T, Bitton A, Yang H, Targan S, Datta LW, Kistner EO, Schumm LP, Lee AT, Gregersen PK, Barmada MM, Rotter JI, Nicolae DL, Cho JH (2006) A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. *Science* **314**(5804)**:** 1461-1463

Emery AE (1991) Population frequencies of inherited neuromuscular diseases--a world survey. *Neuromuscul Disord* **1**(1)**:** 19-29

Erlich Y, Chang K, Gordon A, Ronen R, Navon O, Rooks M, Hannon GJ (2009) DNA Sudoku--harnessing high-throughput sequencing for multiplexed specimen analysis. *Genome Res* **19**(7)**:** 1243-1253

Erlich Y, Edvardson S, Hodges E, Zenvirt S, Thekkat P, Shaag A, Dor T, Hannon GJ, Elpeleg O (2011) Exome sequencing and disease-network analysis of a single family implicate a mutation in KIF1A in hereditary spastic paraparesis. *Genome Res* **21**(5)**:** 658-664

Fabrizi GM, Ferrarini M, Cavallaro T, Cabrini I, Cerini R, Bertolasi L, Rizzuto N (2007) Two novel mutations in dynamin-2 cause axonal Charcot-Marie-Tooth disease. *Neurology* **69**(3)**:** 291-295

Fedurco M, Romieu A, Williams S, Lawrence I, Turcatti G (2006) BTA, a novel reagent for DNA attachment on glass and efficient generation of solid-phase amplified DNA colonies. *Nucleic Acids Res* **34**(3)**:** e22

Fill M, Ma JJ, Knudson CM, Imagawa T, Campbell KP, Coronado R (1989) Role of the ryanodine receptor of skeletal muscle in excitation-contraction coupling. *Ann N Y Acad Sci* **560:** 155-162

Fischer D, Herasse M, Bitoun M, Barragan-Campos HM, Chiras J, Laforet P, Fardeau M, Eymard B, Guicheney P, Romero NB (2006) Characterization of the muscle involvement in dynamin 2-related centronuclear myopathy. *Brain* **129**(Pt 6)**:** 1463-1469

Fischer M, Snajder R, Pabinger S, Dander A, Schossig A, Zschocke J, Trajanoski Z, Stocker G (2012) SIMPLEX: Cloud-Enabled Pipeline for the Comprehensive Analysis of Exome Sequencing Data. *PLoS One* **7**(8)**:** e41948

Flusberg BA, Webster DR, Lee JH, Travers KJ, Olivares EC, Clark TA, Korlach J, Turner SW (2010) Direct detection of DNA methylation during single-molecule, real-time sequencing. *Nat Methods* **7**(6)**:** 461-465

Fuentes Fajardo KV, Adams D, Mason CE, Sincan M, Tifft C, Toro C, Boerkoel CF, Gahl W, Markello T (2012) Detecting false-positive signals in exome sequencing. *Hum Mutat* **33**(4)**:** 609-613

Fugier C, Klein AF, Hammer C, Vassilopoulos S, Ivarsson Y, Toussaint A, Tosch V, Vignaud A, Ferry A, Messaddeq N, Kokunai Y, Tsuburaya R, de la Grange P, Dembele D, Francois V, Precigout G, Boulade-Ladame C, Hummel MC, Lopez de Munain A, Sergeant N, Laquerriere A, Thibault C, Deryckere F, Auboeuf D, Garcia L, Zimmermann P, Udd B, Schoser B, Takahashi MP, Nishino I, Bassez G, Laporte J, Furling D, Charlet-Berguerand N (2011) Misregulated alternative splicing of BIN1 is associated with T tubule alterations and muscle weakness in myotonic dystrophy. *Nat Med* **17**(6)**:** 720-725

Fujimura-Kiyono C, Racz GZ, Nishino I (2008) Myotubular/centronuclear myopathy and central core disease. *Neurol India* **56**(3)**:** 325-332

Gazzerro E, Bonetto A, Minetti C (2011) Caveolinopathies: translational implications of caveolin-3 in skeletal and cardiac muscle disorders. *Handb Clin Neurol* **101:** 135-142

Gilissen C, Arts HH, Hoischen A, Spruijt L, Mans DA, Arts P, van Lier B, Steehouwer M, van Reeuwijk J, Kant SG, Roepman R, Knoers NV, Veltman JA, Brunner HG (2010) Exome sequencing identifies WDR35 variants involved in Sensenbrenner syndrome. *Am J Hum Genet* **87**(3)**:** 418-423

Gilissen C, Hoischen A, Brunner HG, Veltman JA (2012) Disease gene identification strategies for exome sequencing. *Eur J Hum Genet* **20**(5)**:** 490-497

Gitcho MA, Bigio EH, Mishra M, Johnson N, Weintraub S, Mesulam M, Rademakers R, Chakraverty S, Cruchaga C, Morris JC, Goate AM, Cairns NJ (2009) TARDBP 3'-UTR variant in autopsy-confirmed frontotemporal lobar degeneration with TDP-43 proteinopathy. *Acta Neuropathol* **118**(5)**:** 633-645

Gonzaga-Jauregui C, Lupski JR, Gibbs RA (2012) Human genome sequencing in health and disease. *Annu Rev Med* **63:** 35-61

Gotz A, Tyynismaa H, Euro L, Ellonen P, Hyotylainen T, Ojala T, Hamalainen RH, Tommiska J, Raivio T, Oresic M, Karikoski R, Tammela O, Simola KO, Paetau A, Tyni T, Suomalainen A (2011) Exome sequencing identifies mitochondrial alanyl-tRNA synthetase mutations in infantile mitochondrial cardiomyopathy. *Am J Hum Genet* **88**(5)**:** 635-642

Greenway MJ, Alexander MD, Ennis S, Traynor BJ, Corr B, Frost E, Green A, Hardiman O (2004) A novel candidate region for ALS on chromosome 14q11.2. *Neurology* **63**(10)**:** 1936-1938

Gross EA, Callow MG, Waldbaum L, Thomas S, Ruggieri R (2002) MRK, a mixed lineage kinase-related molecule that plays a role in gamma-radiation-induced cell cycle arrest. *J Biol Chem* **277**(16)**:** 13873-13882

Haack TB, Danhauser K, Haberberger B, Hoser J, Strecker V, Boehm D, Uziel G, Lamantea E, Invernizzi F, Poulton J, Rolinski B, Iuso A, Biskup S, Schmidt T, Mewes HW, Wittig I, Meitinger T, Zeviani M, Prokisch H (2010) Exome sequencing identifies ACAD9 mutations as a cause of complex I deficiency. *Nat Genet* **42**(12)**:** 1131-1134

Hadano S, Hand CK, Osuga H, Yanagisawa Y, Otomo A, Devon RS, Miyamoto N, Showguchi-Miyata J, Okada Y, Singaraja R, Figlewicz DA, Kwiatkowski T, Hosler BA, Sagie T, Skaug J, Nasir J, Brown RH, Jr., Scherer SW, Rouleau GA, Hayden MR, Ikeda JE (2001) A gene encoding a putative GTPase regulator is mutated in familial amyotrophic lateral sclerosis 2. *Nat Genet* **29**(2)**:** 166-173

Hammans SR, Robinson DO, Moutou C, Kennedy CR, Dennis NR, Hughes PJ, Ellison DW (2000) A clinical and genetic study of a manifesting heterozygote with X-linked myotubular myopathy. *Neuromuscul Disord* **10**(2)**:** 133-137

Harms MB, Sommerville RB, Allred P, Bell S, Ma D, Cooper P, Lopate G, Pestronk A, Weihl CC, Baloh RH (2012) Exome sequencing reveals DNAJB6 mutations in dominantly-inherited myopathy. *Ann Neurol* **71**(3)**:** 407-416

Hayward C, Colville S, Swingler RJ, Brock DJ (1999) Molecular genetic analysis of the APEX nuclease gene in amyotrophic lateral sclerosis. *Neurology* **52**(9)**:** 1899-1901

Hedberg C, Melberg A, Kuhl A, Jenne D, Oldfors A (2012) Autosomal dominant myofibrillar myopathy with arrhythmogenic right ventricular cardiomyopathy 7 is caused by a DES mutation. *Eur J Hum Genet* **20**(9)**:** 984-985

Herman GE, Kopacz K, Zhao W, Mills PL, Metzenberg A, Das S (2002) Characterization of mutations in fifty North American patients with X-linked myotubular myopathy. *Hum Mutat* **19**(2)**:** 114-121

Hicks S, Wheeler DA, Plon SE, Kimmel M (2011) Prediction of missense mutation functionality depends on both the algorithm and sequence alignment employed. *Hum Mutat* **32**(6)**:** 661-668

Hoischen A, van Bon BW, Gilissen C, Arts P, van Lier B, Steehouwer M, de Vries P, de Reuver R, Wieskamp N, Mortier G, Devriendt K, Amorim MZ, Revencu N, Kidd A, Barbosa M, Turner A, Smith J, Oley C, Henderson A, Hayes IM, Thompson EM, Brunner HG, de Vries BB, Veltman JA (2010) De novo mutations of SETBP1 cause Schinzel-Giedion syndrome. *Nat Genet* **42**(6)**:** 483-485

Hu LJ, Laporte J, Kress W, Kioschis P, Siebenhaar R, Poustka A, Fardeau M, Metzenberg A, Janssen EA, Thomas N, Mandel JL, Dahl N (1996) Deletions in Xq28 in two boys with myotubular myopathy and abnormal genital development define a new contiguous gene syndrome in a 430 kb region. *Hum Mol Genet* **5**(1)**:** 139-143

Isidor B, Lindenbaum P, Pichon O, Bezieau S, Dina C, Jacquemont S, Martin-Coignard D, Thauvin-Robinet C, Le Merrer M, Mandel JL, David A, Faivre L, Cormier-Daire V, Redon R, Le Caignec C (2011) Truncating mutations in the last exon of NOTCH2 cause a rare skeletal disorder with osteoporosis. *Nat Genet* **43**(4)**:** 306-308

Itoh-Satoh M, Hayashi T, Nishi H, Koga Y, Arimura T, Koyanagi T, Takahashi M, Hohda S, Ueda K, Nouchi T, Hiroe M, Marumo F, Imaizumi T, Yasunami M, Kimura A (2002) Titin mutations as the molecular basis for dilated cardiomyopathy. *Biochem Biophys Res Commun* **291**(2)**:** 385-393

Jeannet PY, Bassez G, Eymard B, Laforet P, Urtizberea JA, Rouche A, Guicheney P, Fardeau M, Romero NB (2004) Clinical and histologic findings in autosomal centronuclear myopathy. *Neurology* **62**(9)**:** 1484-1490

Johnson JO, Mandrioli J, Benatar M, Abramzon Y, Van Deerlin VM, Trojanowski JQ, Gibbs JR, Brunetti M, Gronka S, Wuu J, Ding J, McCluskey L, Martinez-Lage M, Falcone D, Hernandez DG, Arepalli S, Chong S, Schymick JC, Rothstein J, Landi F, Wang YD, Calvo A, Mora G, Sabatelli M, Monsurro MR, Battistini S, Salvi F, Spataro R, Sola P, Borghero G, Galassi G, Scholz SW, Taylor JP, Restagno G, Chio A, Traynor BJ (2010) Exome sequencing reveals VCP mutations as a cause of familial ALS. *Neuron* **68**(5)**:** 857-864

Jones SM, Howell KE, Henley JR, Cao H, McNiven MA (1998) Role of dynamin in the formation of transport vesicles from the trans-Golgi network. *Science* **279**(5350)**:** 573-577

Jungbluth H, Wallgren-Pettersson C, Laporte J (2008) Centronuclear (myotubular) myopathy. *Orphanet J Rare Dis* **3:** 26

Jungbluth H, Zhou H, Sewry CA, Robb S, Treves S, Bitoun M, Guicheney P, Buj-Bello A, Bonnemann C, Muntoni F (2007) Centronuclear myopathy due to a de novo dominant mutation in the skeletal muscle ryanodine receptor (RYR1) gene. *Neuromuscul Disord* **17**(4)**:** 338-345

Kaplan JC (2011) The 2012 version of the gene table of monogenic neuromuscular disorders. *Neuromuscul Disord* **21**(12)**:** 833-861

Kerem B, Rommens JM, Buchanan JA, Markiewicz D, Cox TK, Chakravarti A, Buchwald M, Tsui LC (1989) Identification of the cystic fibrosis gene: genetic analysis. *Science* **245**(4922)**:** 1073-1080

Kirchhoff M, Rose H, Gerdes T, Lundsteen C (2001) [Detection of submicroscopic chromosome abnormalities by comparative genomic hybridization]. *Ugeskr Laeger* **163**(41)**:** 5652-5657

Klein A, Lillis S, Munteanu I, Scoto M, Zhou H, Quinlivan R, Straub V, Manzur AY, Roper H, Jeannet PY, Rakowicz W, Jones DH, Jensen UB, Wraige E, Trump N, Schara U, Lochmuller H, Sarkozy A, Kingston H, Norwood F, Damian M, Kirschner J, Longman C, Roberts M, Auer-Grumbach M, Hughes I, Bushby K, Sewry C, Robb S, Abbs S, Jungbluth H, Muntoni F (2012) Clinical and genetic findings in a large cohort of patients with ryanodine receptor 1 gene-associated myopathies. *Hum Mutat* **33**(6)**:** 981-988

Kondo E, Nishimura T, Kosho T, Inaba Y, Mitsuhashi S, Ishida T, Baba A, Koike K, Nishino I, Nonaka I, Furukawa T, Saito K (2012) Recessive RYR1 mutations in a patient with severe congenital nemaline myopathy with ophthalomoplegia identified through massively parallel sequencing. *Am J Med Genet A* **158A**(4)**:** 772-778

Krawitz PM, Schweiger MR, Rodelsperger C, Marcelis C, Kolsch U, Meisel C, Stephani F, Kinoshita T, Murakami Y, Bauer S, Isau M, Fischer A, Dahl A, Kerick M, Hecht J, Kohler S, Jager M, Grunhagen J, de Condor BJ, Doelken S, Brunner HG, Meinecke P, Passarge E, Thompson MD, Cole DE, Horn D, Roscioli T, Mundlos S, Robinson PN (2010) Identity-by-descent filtering of exome sequence data identifies PIGV mutations in hyperphosphatasia mental retardation syndrome. *Nat Genet* **42**(10)**:** 827-829

Kurotaki N, Imaizumi K, Harada N, Masuno M, Kondoh T, Nagai T, Ohashi H, Naritomi K, Tsukahara M, Makita Y, Sugimoto T, Sonoda T, Hasegawa T, Chinen Y, Tomita Ha HA, Kinoshita A, Mizuguchi T, Yoshiura Ki K, Ohta T, Kishino T, Fukushima Y, Niikawa N, Matsumoto N (2002) Haploinsufficiency of NSD1 causes Sotos syndrome. *Nat Genet* **30**(4)**:** 365-366

Kwiatkowski TJ, Jr., Bosco DA, Leclerc AL, Tamrazian E, Vanderburg CR, Russ C, Davis A, Gilchrist J, Kasarskis EJ, Munsat T, Valdmanis P, Rouleau GA, Hosler BA, Cortelli P, de Jong PJ, Yoshinaga Y, Haines JL, Pericak-Vance MA, Yan J, Ticozzi N, Siddique T, McKenna-Yasek D, Sapp PC, Horvitz HR, Landers JE, Brown RH, Jr. (2009) Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. *Science* **323**(5918)**:** 1205-1208

Laing NG (2012) Genetics of neuromuscular disorders. *Crit Rev Clin Lab Sci* **49**(2)**:** 33-48

Lalonde E, Albrecht S, Ha KC, Jacob K, Bolduc N, Polychronakos C, Dechelotte P, Majewski J, Jabado N (2010) Unexpected allelic heterogeneity and spectrum of mutations in Fowler syndrome revealed by next-generation exome sequencing. *Hum Mutat* **31**(8)**:** 918-923

Lander ES, Botstein D (1987) Homozygosity mapping: a way to map human recessive traits with the DNA of inbred children. *Science* **236**(4808)**:** 1567-1570

Langmead B, Trapnell C, Pop M, Salzberg SL (2009) Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol* **10**(3)**:** R25

Laporte J, Biancalana V, Tanner SM, Kress W, Schneider V, Wallgren-Pettersson C, Herger F, Buj-Bello A, Blondeau F, Liechti-Gallati S, Mandel JL (2000) MTM1 mutations in X-linked myotubular myopathy. *Hum Mutat* **15**(5)**:** 393-409

Laporte J, Guiraud-Chaumeil C, Vincent MC, Mandel JL, Tanner SM, Liechti-Gallati S, Wallgren-Pettersson C, Dahl N, Kress W, Bolhuis PA, Fardeau M, Samson F, Bertini E (1997) Mutations in the MTM1 gene implicated in X-linked myotubular myopathy. ENMC International Consortium on Myotubular Myopathy. European Neuro-Muscular Center. *Hum Mol Genet* **6**(9)**:** 1505-1511

Laporte J, Hu LJ, Kretz C, Mandel JL, Kioschis P, Coy JF, Klauck SM, Poustka A, Dahl N (1996) A gene mutated in X-linked myotubular myopathy defines a new putative tyrosine phosphatase family conserved in yeast. *Nat Genet* **13**(2)**:** 175-182

Lee E, Marcucci M, Daniell L, Pypaert M, Weisz OA, Ochoa GC, Farsad K, Wenk MR, De Camilli P (2002) Amphiphysin 2 (Bin1) and T-tubule biogenesis in muscle. *Science* **297**(5584)**:** 1193-1196

Lee WH, Bookstein R, Hong F, Young LJ, Shew JY, Lee EY (1987) Human retinoblastoma susceptibility gene: cloning, identification, and sequence. *Science* **235**(4794)**:** 1394-1399

Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* **25**(14)**:** 1754-1760

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R (2009) The Sequence Alignment/Map format and SAMtools. *Bioinformatics* **25**(16)**:** 2078-2079

Li H, Ruan J, Durbin R (2008a) Mapping short DNA sequencing reads and calling variants using mapping quality scores. *Genome Res* **18**(11)**:** 1851-1858

Li R, Li Y, Kristiansen K, Wang J (2008b) SOAP: short oligonucleotide alignment program. *Bioinformatics* **24**(5)**:** 713-714

Liu CM, Wong T, Wu E, Luo R, Yiu SM, Li Y, Wang B, Yu C, Chu X, Zhao K, Li R, Lam TW (2012) SOAP3: ultra-fast GPU-based parallel alignment tool for short reads. *Bioinformatics* **28**(6)**:** 878-879

Logan CV, Lucke B, Pottinger C, Abdelhamed ZA, Parry DA, Szymanska K, Diggle CP, van Riesen A, Morgan JE, Markham G, Ellis I, Manzur AY, Markham AF, Shires M, Helliwell T, Scoto M, Hubner C, Bonthron DT, Taylor GR, Sheridan E, Muntoni F, Carr IM, Schuelke M, Johnson CA (2011) Mutations in MEGF10, a regulator of satellite cell myogenesis, cause early onset myopathy, areflexia, respiratory distress and dysphagia (EMARDD). *Nat Genet* **43**(12)**:** 1189-1192

Lupski JR, Reid JG, Gonzaga-Jauregui C, Rio Deiros D, Chen DC, Nazareth L, Bainbridge M, Dinh H, Jing C, Wheeler DA, McGuire AL, Zhang F, Stankiewicz P, Halperin JJ, Yang C, Gehman C, Guo D, Irikat RK, Tom W, Fantin NJ, Muzny DM, Gibbs RA (2010) Whole-genome sequencing in a patient with Charcot-Marie-Tooth neuropathy. *N Engl J Med* **362**(13)**:** 1181-1191

Majczenko K, Davidson AE, Camelo-Piragua S, Agrawal PB, Manfready RA, Li X, Joshi S, Xu J, Peng W, Beggs AH, Li JZ, Burmeister M, Dowling JJ (2012) Dominant Mutation of CCDC78 in a Unique Congenital Myopathy with Prominent Internal Nuclei and Atypical Cores. *Am J Hum Genet* **91**(2)**:** 365-371

Mamanova L, Coffey AJ, Scott CE, Kozarewa I, Turner EH, Kumar A, Howard E, Shendure J, Turner DJ (2010) Target-enrichment strategies for next-generation sequencing. *Nat Methods* **7**(2)**:** 111-118

Margraf RL, Durtschi JD, Dames S, Pattison DC, Stephens JE, Voelkerding KV (2011) Variant identification in multi-sample pools by illumina genome analyzer sequencing. *J Biomol Tech* **22**(2)**:** 74-84

McKusick VA (2007) Mendelian Inheritance in Man and its online version, OMIM. *Am J Hum Genet* **80**(4)**:** 588-604

Mejaddam AY, Nennesmo I, Sejersen T (2009) Severe phenotype of a patient with autosomal recessive centronuclear myopathy due to a BIN1 mutation. *Acta Myol* **28**(3)**:** 91-93

Metzker ML (2010) Sequencing technologies - the next generation. *Nat Rev Genet* **11**(1)**:** 31-46

Monnier N, Romero NB, Lerale J, Landrieu P, Nivoche Y, Fardeau M, Lunardi J (2001) Familial and sporadic forms of central core disease are associated with mutations in the C-terminal domain of the skeletal muscle ryanodine receptor. *Hum Mol Genet* **10**(22)**:** 2581-2592

Muller AJ, Baker JF, DuHadaway JB, Ge K, Farmer G, Donover PS, Meade R, Reid C, Grzanna R, Roach AH, Shah N, Soler AP, Prendergast GC (2003) Targeted disruption of the murine Bin1/Amphiphysin II gene does not disable endocytosis but results in embryonic cardiomyopathy with aberrant myofibril formation. *Mol Cell Biol* **23**(12)**:** 4295-4306

Murdock DR, Clark GD, Bainbridge MN, Newsham I, Wu YQ, Muzny DM, Cheung SW, Gibbs RA, Ramocki MB (2011) Whole-exome sequencing identifies compound heterozygous mutations in WDR62 in siblings with recurrent polymicrogyria. *Am J Med Genet A* **155A**(9)**:** 2071-2077

Musunuru K, Pirruccello JP, Do R, Peloso GM, Guiducci C, Sougnez C, Garimella KV, Fisher S, Abreu J, Barry AJ, Fennell T, Banks E, Ambrogio L, Cibulskis K, Kernytsky A, Gonzalez E, Rudzicz N, Engert JC,

DePristo MA, Daly MJ, Cohen JC, Hobbs HH, Altshuler D, Schonfeld G, Gabriel SB, Yue P, Kathiresan S (2010) Exome sequencing, ANGPTL3 mutations, and familial combined hypolipidemia. *N Engl J Med* **363**(23)**:** 2220-2227

Nance JR, Dowling JJ, Gibbs EM, Bonnemann CG (2012) Congenital myopathies: an update. *Curr Neurol Neurosci Rep* **12**(2)**:** 165-174

Ng PC, Henikoff S (2003) SIFT: Predicting amino acid changes that affect protein function. *Nucleic Acids Res* **31**(13)**:** 3812-3814

Ng SB, Bigham AW, Buckingham KJ, Hannibal MC, McMillin MJ, Gildersleeve HI, Beck AE, Tabor HK, Cooper GM, Mefford HC, Lee C, Turner EH, Smith JD, Rieder MJ, Yoshiura K, Matsumoto N, Ohta T, Niikawa N, Nickerson DA, Bamshad MJ, Shendure J (2010a) Exome sequencing identifies MLL2 mutations as a cause of Kabuki syndrome. *Nat Genet* **42**(9)**:** 790-793

Ng SB, Buckingham KJ, Lee C, Bigham AW, Tabor HK, Dent KM, Huff CD, Shannon PT, Jabs EW, Nickerson DA, Shendure J, Bamshad MJ (2010b) Exome sequencing identifies the cause of a mendelian disorder. *Nat Genet* **42**(1)**:** 30-35

Ng SB, Nickerson DA, Bamshad MJ, Shendure J (2010c) Massively parallel sequencing and rare disease. *Hum Mol Genet* **19**(R2)**:** R119-124

Ng SB, Turner EH, Robertson PD, Flygare SD, Bigham AW, Lee C, Shaffer T, Wong M, Bhattacharjee A, Eichler EE, Bamshad M, Nickerson DA, Shendure J (2009) Targeted capture and massively parallel sequencing of 12 human exomes. *Nature* **461**(7261)**:** 272-276

Nicot AS, Toussaint A, Tosch V, Kretz C, Wallgren-Pettersson C, Iwarsson E, Kingston H, Garnier JM, Biancalana V, Oldfors A, Mandel JL, Laporte J (2007) Mutations in amphiphysin 2 (BIN1) disrupt interaction with dynamin 2 and cause autosomal recessive centronuclear myopathy. *Nat Genet* **39**(9)**:** 1134-1139

North K (2008) What's new in congenital myopathies? *Neuromuscul Disord* **18**(6)**:** 433-442

Norton N, Li D, Rieder MJ, Siegfried JD, Rampersaud E, Zuchner S, Mangos S, Gonzalez-Quintana J, Wang L, McGee S, Reiser J, Martin E, Nickerson DA, Hershberger RE (2011) Genome-wide studies of copy number variation and exome sequencing identify rare variants in BAG3 as a cause of dilated cardiomyopathy. *Am J Hum Genet* **88**(3)**:** 273-282

O'Roak BJ, Deriziotis P, Lee C, Vives L, Schwartz JJ, Girirajan S, Karakoc E, Mackenzie AP, Ng SB, Baker C, Rieder MJ, Nickerson DA, Bernier R, Fisher SE, Shendure J, Eichler EE (2011) Exome sequencing in sporadic autism spectrum disorders identifies severe de novo mutations. *Nat Genet* **43**(6)**:** 585-589

O'Sullivan J, Bitu CC, Daly SB, Urquhart JE, Barron MJ, Bhaskar SS, Martelli-Junior H, dos Santos Neto PE, Mansilla MA, Murray JC, Coletta RD, Black GC, Dixon MJ (2011) Whole-Exome sequencing identifies FAM20A mutations as a cause of amelogenesis imperfecta and gingival hyperplasia syndrome. *Am J Hum Genet* **88**(5)**:** 616-620

Ozgul RK, Siemiatkowska AM, Yucel D, Myers CA, Collin RW, Zonneveld MN, Beryozkin A, Banin E, Hoyng CB, van den Born LI, Bose R, Shen W, Sharon D, Cremers FP, Klevering BJ, den Hollander AI, Corbo JC (2011) Exome sequencing and cis-regulatory mapping identify mutations in MAK, a gene encoding a regulator of ciliary length, as a cause of retinitis pigmentosa. *Am J Hum Genet* **89**(2)**:** 253-264

Phillips MS, Fujii J, Khanna VK, DeLeon S, Yokobata K, de Jong PJ, MacLennan DH (1996) The structural organization of the human skeletal muscle ryanodine receptor (RYR1) gene. *Genomics* **34**(1)**:** 24-41

Pierce SB, Walsh T, Chisholm KM, Lee MK, Thornton AM, Fiumara A, Opitz JM, Levy-Lahad E, Klevit RE, King MC (2010) Mutations in the DBP-deficiency protein HSD17B4 cause ovarian dysgenesis, hearing loss, and ataxia of Perrault Syndrome. *Am J Hum Genet* **87**(2)**:** 282-288

Prabhu S, Pe'er I (2009) Overlapping pools for high-throughput targeted resequencing. *Genome Res* **19**(7)**:** 1254-1261

Prior TW (2010) Perspectives and diagnostic considerations in spinal muscular atrophy. *Genet Med* **12**(3)**:** 145-152

Ramensky V, Bork P, Sunyaev S (2002) Human non-synonymous SNPs: server and survey. *Nucleic Acids Res* **30**(17)**:** 3894-3900

Ramsey DM, Futschik A (2012) DNA pooling and statistical tests for the detection of single nucleotide polymorphisms. *Stat Appl Genet Mol Biol* **11**(5)

Redin C, Le Gras S, Mhamdi O, Geoffroy V, Stoetzel C, Vincent MC, Chiurazzi P, Lacombe D, Ouertani I, Petit F, Till M, Verloes A, Jost B, Chaabouni HB, Dollfus H, Mandel JL, Muller J (2012) Targeted high-throughput sequencing for diagnosis of genetically heterogeneous diseases: efficient mutation detection in Bardet-Biedl and Alstrom syndromes. *J Med Genet* **49**(8)**:** 502-512

Renton AE, Majounie E, Waite A, Simon-Sanchez J, Rollinson S, Gibbs JR, Schymick JC, Laaksovirta H, van Swieten JC, Myllykangas L, Kalimo H, Paetau A, Abramzon Y, Remes AM, Kaganovich A, Scholz SW, Duckworth J, Ding J, Harmer DW, Hernandez DG, Johnson JO, Mok K, Ryten M, Trabzuni D, Guerreiro RJ, Orrell RW, Neal J, Murray A, Pearson J, Jansen IE, Sondervan D, Seelaar H, Blake D, Young K, Halliwell N, Callister JB, Toulson G, Richardson A, Gerhard A, Snowden J, Mann D, Neary D, Nalls MA, Peuralinna T, Jansson L, Isoviita VM, Kaivorinne AL, Holtta-Vuori M, Ikonen E, Sulkava R, Benatar M, Wuu J, Chio A, Restagno G, Borghero G, Sabatelli M, Heckerman D, Rogaeva E, Zinman L, Rothstein JD, Sendtner M, Drepper C, Eichler EE, Alkan C, Abdullaev Z, Pack SD, Dutra A, Pak E, Hardy J, Singleton A, Williams NM, Heutink P, Pickering-Brown S, Morris HR, Tienari PJ, Traynor BJ (2011) A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron* **72**(2)**:** 257-268

Rhead B, Karolchik D, Kuhn RM, Hinrichs AS, Zweig AS, Fujita PA, Diekhans M, Smith KE, Rosenbloom KR, Raney BJ, Pohl A, Pheasant M, Meyer LR, Learned K, Hsu F, Hillman-Jackson J, Harte RA, Giardine B, Dreszer TR, Clawson H, Barber GP, Haussler D, Kent WJ (2010) The UCSC Genome Browser database: update 2010. *Nucleic Acids Res* **38**(Database issue)**:** D613-619

Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, Zielenski J, Lok S, Plavsic N, Chou JL, et al. (1989) Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* **245**(4922)**:** 1066-1073

Roach JC, Glusman G, Smit AF, Huff CD, Hubley R, Shannon PT, Rowen L, Pant KP, Goodman N, Bamshad M, Shendure J, Drmanac R, Jorde LB, Hood L, Galas DJ (2010) Analysis of genetic inheritance in a family quartet by whole-genome sequencing. *Science* **328**(5978)**:** 636-639

Romero NB (2010) Centronuclear myopathies: a widening concept. *Neuromuscul Disord* **20**(4)**:** 223-228

Ronaghi M, Karamohamed S, Pettersson B, Uhlen M, Nyren P (1996) Real-time DNA sequencing using detection of pyrophosphate release. *Anal Biochem* **242**(1)**:** 84-89

Ronaghi M, Uhlen M, Nyren P (1998) A sequencing method based on real-time pyrophosphate. *Science* **281**(5375)**:** 363, 365

Rosen DR, Siddique T, Patterson D, Figlewicz DA, Sapp P, Hentati A, Donaldson D, Goto J, O'Regan JP, Deng HX, et al. (1993) Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* **362**(6415)**:** 59-62

Saporta AS, Sottile SL, Miller LJ, Feely SM, Siskind CE, Shy ME (2011) Charcot-Marie-Tooth disease subtypes and genetic testing strategies. *Ann Neurol* **69**(1)**:** 22-33

Schessl J, Medne L, Hu Y, Zou Y, Brown MJ, Huse JT, Torigian DA, Jungbluth H, Goebel HH, Bonnemann CG (2007) MRI in DNM2-related centronuclear myopathy: evidence for highly selective muscle involvement. *Neuromuscul Disord* **17**(1)**:** 28-32

Siepel A, Bejerano G, Pedersen JS, Hinrichs AS, Hou M, Rosenbloom K, Clawson H, Spieth J, Hillier LW, Richards S, Weinstock GM, Wilson RK, Gibbs RA, Kent WJ, Miller W, Haussler D (2005) Evolutionarily conserved elements in vertebrate, insect, worm, and yeast genomes. *Genome Res* **15**(8)**:** 1034-1050

Simpson DA, Clark GR, Alexander S, Silvestri G, Willoughby CE (2011a) Molecular diagnosis for heterogeneous genetic diseases with targeted high-throughput DNA sequencing applied to retinitis pigmentosa. *J Med Genet* **48**(3)**:** 145-151

Simpson MA, Irving MD, Asilmaz E, Gray MJ, Dafou D, Elmslie FV, Mansour S, Holder SE, Brain CE, Burton BK, Kim KH, Pauli RM, Aftimos S, Stewart H, Kim CA, Holder-Espinasse M, Robertson SP, Drake WM, Trembath RC (2011b) Mutations in NOTCH2 cause Hajdu-Cheney syndrome, a disorder of severe and progressive bone loss. *Nat Genet* **43**(4)**:** 303-305

Spiro AJ, Shy GM, Gonatas NK (1966) Myotubular myopathy. Persistence of fetal muscle in an adolescent boy. *Arch Neurol* **14**(1)**:** 1-14

Stenson PD, Mort M, Ball EV, Howells K, Phillips AD, Thomas NS, Cooper DN (2009) The Human Gene Mutation Database: 2008 update. *Genome Med* **1**(1)**:** 13

Susman RD, Quijano-Roy S, Yang N, Webster R, Clarke NF, Dowling J, Kennerson M, Nicholson G, Biancalana V, Ilkovski B, Flanigan KM, Arbuckle S, Malladi C, Robinson P, Vucic S, Mayer M, Romero NB, Urtizberea JA, Garcia-Bragado F, Guicheney P, Bitoun M, Carlier RY, North KN (2010) Expanding the clinical, pathological and MRI phenotype of DNM2-related centronuclear myopathy. *Neuromuscul Disord* **20**(4)**:** 229-237

Tanner SM, Orstavik KH, Kristiansen M, Lev D, Lerman-Sagie T, Sadeh M, Liechti-Gallati S (1999a) Skewed X-inactivation in a manifesting carrier of X-linked myotubular myopathy and in her non-manifesting carrier mother. *Hum Genet* **104**(3)**:** 249-253

Tanner SM, Schneider V, Thomas NS, Clarke A, Lazarou L, Liechti-Gallati S (1999b) Characterization of 34 novel and six known MTM1 gene mutations in 47 unrelated X-linked myotubular myopathy patients. *Neuromuscul Disord* **9**(1)**:** 41-49

Tennyson CN, Klamut HJ, Worton RG (1995) The human dystrophin gene requires 16 hours to be transcribed and is cotranscriptionally spliced. *Nat Genet* **9**(2)**:** 184-190

Tewhey R, Warner JB, Nakano M, Libby B, Medkova M, David PH, Kotsopoulos SK, Samuels ML, Hutchison JB, Larson JW, Topol EJ, Weiner MP, Harismendy O, Olson J, Link DR, Frazer KA (2009) Microdroplet-based PCR enrichment for large-scale targeted sequencing. *Nat Biotechnol* **27**(11)**:** 1025-1031

Thompson HM, Cao H, Chen J, Euteneuer U, McNiven MA (2004) Dynamin 2 binds gamma-tubulin and participates in centrosome cohesion. *Nat Cell Biol* **6**(4)**:** 335-342

Tosch V, Rohde HM, Tronchere H, Zanoteli E, Monroy N, Kretz C, Dondaine N, Payrastre B, Mandel JL, Laporte J (2006) A novel PtdIns3P and PtdIns(3,5)P2 phosphatase with an inactivating variant in centronuclear myopathy. *Hum Mol Genet* **15**(21)**:** 3098-3106

Tosch V, Vasli N, Kretz C, Nicot AS, Gasnier C, Dondaine N, Oriot D, Barth M, Puissant H, Romero NB, Bonnemann CG, Heller B, Duval G, Biancalana V, Laporte J (2010) Novel molecular diagnostic approaches for X-linked centronuclear (myotubular) myopathy reveal intronic mutations. *Neuromuscul Disord* **20**(6)**:** 375-381

Toussaint A, Cowling BS, Hnia K, Mohr M, Oldfors A, Schwab Y, Yis U, Maisonobe T, Stojkovic T, Wallgren-Pettersson C, Laugel V, Echaniz-Laguna A, Mandel JL, Nishino I, Laporte J (2011) Defects in amphiphysin 2 (BIN1) and triads in several forms of centronuclear myopathies. *Acta Neuropathol* **121**(2)**:** 253-266

Trapnell C, Salzberg SL (2009) How to map billions of short reads onto genomes. *Nat Biotechnol* **27**(5)**:** 455-457

Trump N, Cullup T, Verheij JB, Manzur A, Muntoni F, Abbs S, Jungbluth H (2011) X-linked myotubular myopathy due to a complex rearrangement involving a duplication of MTM1 exon 10. *Neuromuscul Disord* **22**(5)**:** 384-388

Tsai TC, Horinouchi H, Noguchi S, Minami N, Murayama K, Hayashi YK, Nonaka I, Nishino I (2005) Characterization of MTM1 mutations in 31 Japanese families with myotubular myopathy, including a patient carrying 240 kb deletion in Xq28 without male hypogenitalism. *Neuromuscul Disord* **15**(3)**:** 245-252

Unsworth KE, Mazurkiewicz P, Senf F, Zettl M, McNiven M, Way M, Holden DW (2007) Dynamin is required for F-actin assembly and pedestal formation by enteropathogenic Escherichia coli (EPEC). *Cell Microbiol* **9**(2)**:** 438-449

Valouev A, Ichikawa J, Tonthat T, Stuart J, Ranade S, Peckham H, Zeng K, Malek JA, Costa G, McKernan K, Sidow A, Fire A, Johnson SM (2008) A high-resolution, nucleosome position map of C. elegans reveals a lack of universal sequence-dictated positioning. *Genome Res* **18**(7)**:** 1051-1063

Vasli N, Laugel V, Bohm J, Lannes B, Biancalana V, Laporte J (2012) Myotubular myopathy caused by multiple abnormal splicing variants in the MTM1 RNA in a patient with a mild phenotype. *Eur J Hum Genet* **20**(6)**:** 701-704

Vissers LE, de Ligt J, Gilissen C, Janssen I, Steehouwer M, de Vries P, van Lier B, Arts P, Wieskamp N, del Rosario M, van Bon BW, Hoischen A, de Vries BB, Brunner HG, Veltman JA (2010) A de novo paradigm for mental retardation. *Nat Genet* **42**(12)**:** 1109-1112

Vissers LE, Lausch E, Unger S, Campos-Xavier AB, Gilissen C, Rossi A, Del Rosario M, Venselaar H, Knoll U, Nampoothiri S, Nair M, Spranger J, Brunner HG, Bonafe L, Veltman JA, Zabel B, Superti-Furga A (2011) Chondrodysplasia and abnormal joint development associated with mutations in IMPAD1, encoding the Golgi-resident nucleotide phosphatase, gPAPP. *Am J Hum Genet* **88**(5)**:** 608-615

Vissers LE, Veltman JA, van Kessel AG, Brunner HG (2005) Identification of disease genes by whole genome CGH arrays. *Hum Mol Genet* **14 Spec No. 2:** R215-223

Voelkerding KV, Dames SA, Durtschi JD (2009) Next-generation sequencing: from basic research to diagnostics. *Clin Chem* **55**(4)**:** 641-658

Wallgren-Pettersson C, Clarke A, Samson F, Fardeau M, Dubowitz V, Moser H, Grimm T, Barohn RJ, Barth PG (1995) The myotubular myopathies: differential diagnosis of the X linked recessive, autosomal dominant, and autosomal recessive forms and present state of DNA studies. *J Med Genet* **32**(9)**:** 673-679

Walsh T, Shahin H, Elkan-Miller T, Lee MK, Thornton AM, Roeb W, Abu Rayyan A, Loulus S, Avraham KB, King MC, Kanaan M (2010) Whole exome sequencing and homozygosity mapping identify mutation in the cell polarity protein GPSM2 as the cause of nonsyndromic hearing loss DFNB82. *Am J Hum Genet* **87**(1)**:** 90-94

Wang JL, Yang X, Xia K, Hu ZM, Weng L, Jin X, Jiang H, Zhang P, Shen L, Guo JF, Li N, Li YR, Lei LF, Zhou J, Du J, Zhou YF, Pan Q, Wang J, Li RQ, Tang BS (2010a) TGM6 identified as a novel causative gene of spinocerebellar ataxias using exome sequencing. *Brain* **133**(Pt 12)**:** 3510-3518

Wang K, Li M, Hakonarson H (2010b) ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res* **38**(16)**:** e164

Wechsler-Reya RJ, Elliott KJ, Prendergast GC (1998) A role for the putative tumor suppressor Bin1 in muscle cell differentiation. *Mol Cell Biol* **18**(1)**:** 566-575

Wheeler DA, Srinivasan M, Egholm M, Shen Y, Chen L, McGuire A, He W, Chen YJ, Makhijani V, Roth GT, Gomes X, Tartaro K, Niazi F, Turcotte CL, Irzyk GP, Lupski JR, Chinault C, Song XZ, Liu Y, Yuan Y, Nazareth L, Qin X, Muzny DM, Margulies M, Weinstock GM, Gibbs RA, Rothberg JM (2008) The complete genome of an individual by massively parallel DNA sequencing. *Nature* **452**(7189)**:** 872-876

Wilmshurst JM, Lillis S, Zhou H, Pillay K, Henderson H, Kress W, Muller CR, Ndondo A, Cloke V, Cullup T, Bertini E, Boennemann C, Straub V, Quinlivan R, Dowling JJ, Al-Sarraj S, Treves S, Abbs S, Manzur AY, Sewry CA, Muntoni F, Jungbluth H (2010) RYR1 mutations are a common cause of congenital myopathies with central nuclei. *Ann Neurol* **68**(5)**:** 717-726

Worthey EA, Mayer AN, Syverson GD, Helbling D, Bonacci BB, Decker B, Serpe JM, Dasu T, Tschannen MR, Veith RL, Basehore MJ, Broeckel U, Tomita-Mitchell A, Arca MJ, Casper JT, Margolis DA, Bick DP, Hessner MJ, Routes JM, Verbsky JW, Jacob HJ, Dimmock DP (2011) Making a definitive diagnosis: successful clinical application of whole exome sequencing in a child with intractable inflammatory bowel disease. *Genet Med* **13**(3)**:** 255-262

Xu B, Roos JL, Dexheimer P, Boone B, Plummer B, Levy S, Gogos JA, Karayiorgou M (2011) Exome sequencing supports a de novo mutational paradigm for schizophrenia. *Nat Genet* **43**(9)**:** 864-868

Yang Y, Hentati A, Deng HX, Dabbagh O, Sasaki T, Hirano M, Hung WY, Ouahchi K, Yan J, Azim AC, Cole N, Gascon G, Yagmour A, Ben-Hamida M, Pericak-Vance M, Hentati F, Siddique T (2001) The gene encoding alsin, a protein with three guanine-nucleotide exchange factor domains, is mutated in a form of recessive amyotrophic lateral sclerosis. *Nat Genet* **29**(2)**:** 160-165

Yu X, Guda K, Willis J, Veigl M, Wang Z, Markowitz S, Adams MD, Sun S (2012) How do alignment programs perform on sequencing data with varying qualities and from repetitive regions? *BioData Min* **5**(1)**:** 6

Zanoteli E, Oliveira AS, Schmidt B, Gabbai AA (1998) Centronuclear myopathy: clinical aspects of ten Brazilian patients with childhood onset. *J Neurol Sci* **158**(1)**:** 76-82

Zuchner S, Dallman J, Wen R, Beecham G, Naj A, Farooq A, Kohli MA, Whitehead PL, Hulme W, Konidari I, Edwards YJ, Cai G, Peter I, Seo D, Buxbaum JD, Haines JL, Blanton S, Young J, Alfonso E, Vance JM, Lam BL, Pericak-Vance MA (2011) Whole-exome sequencing links a variant in DHDDS to retinitis pigmentosa. *Am J Hum Genet* **88**(2)**:** 201-206

Zuchner S, Noureddine M, Kennerson M, Verhoeven K, Claeys K, De Jonghe P, Merory J, Oliveira SA, Speer MC, Stenger JE, Walizada G, Zhu D, Pericak-Vance MA, Nicholson G, Timmerman V, Vance JM (2005) Mutations in the pleckstrin homology domain of dynamin 2 cause dominant intermediate Charcot-Marie-Tooth disease. *Nat Genet* **37**(3)**:** 289-294

Diagnostic génétique et identification de nouveaux gènes impliqués dans les maladies neuromusculaires par séquençage haut débit.

Introduction et but du projet :

Objectif 1 :

Les maladies neuromusculaires (NMD) sont des maladies génétiques qui affectent les muscles, les nerfs et les jonctions neuromusculaires (JNM = le lien entre les fibres nerveuses et musculaires). Elles sont rares, souvent graves avec différents âges d'apparition allant de l'enfance à l'âge adulte et représentent une lourde charge pour les patients, leurs familles et le système de santé publique (Emery, 1991). Les principaux symptômes de la plupart des maladies neuromusculaires sont la fatigue et la faiblesse musculaires ainsi que l'atrophie musculaire. Les maladies neuromusculaires sont l'un des troubles les plus hétérogènes génétiquement avec plus de 200 gènes impliqués. Ce haut degré d'hétérogénéité génétique est problématique pour les laboratoires de diagnostic car la vérification de tous les gènes impliqués dans une maladie prend du temps et coûte cher.

Plusieurs grands gènes sont mutés dans différents troubles neuromusculaires tels que DMD (MIM # 300377) qui s'étend sur plus de 2,3 Mb avec 79 exons (Tennyson et al, 1995). Ceci représente un difficulté pour les laboratoires de diagnostic et la plupart du temps, même les gènes de grande taille tel que TTN qui sont connus pour être impliqués dans une maladie, ne sont pas entièrement testés et seules les régions connues pour accumuler des mutations sont d'abord analysées (Andersen et al, 2009).

L'hétérogénéité clinique est l'autre problème dans le diagnostic des maladies neuromusculaires. Par exemple des mutations dans le gène cavéoline 3 (MIM # 601253) ont été détectées dans quatre différents phénotypes: « Rippling muscular disease », dystrophie musculaire des ceintures, myopathie distale et hyperCKemia (Gazzerro et al, 2011). Les patients peuvent avoir un chevauchement de ces symptômes, ou la même mutation peut provoquer divers phénotypes cliniques avec différentes sévérités. Ainsi, il est important de détecter les mutations pathogènes pour le conseil génétique.

Il ya un chevauchement des phénotypes cliniques et histologiques de la plupart des maladies neuromusculaires qui rend nécessaire le diagnostic définitif. Par exemple, les découvertes récentes dans les myopathies congénitales telles que les myopathies némaline, à cores et centronucléaires ne montrent aucun signe spécifique dans près de la moitié des cas, tandis que plusieurs patients ont des manifestations cliniques et histopathologiques diverses mais qui se chevauchent (Nance et al, 2012). Dans les laboratoires de diagnostic quand une substitution est trouvée dans l'un des gènes impliqués, toute recherche complémentaire est arrêtée, ce qui peut conduire à manquer la véritable mutation. Ainsi, il est parfois difficile et long d'identifier une mutation dans une analyse gène par gène et si aucun changement n'est constaté, les patients subissent plusieurs tests fastidieux, coûteux et parfois douloureux (Prior, 2010). Le dernier obstacle est la méconnaissance des gènes non identifiés dans différentes maladies neuromusculaires.

Pour résoudre ces problèmes et tester si le séquençage haut-débit peut être utilisé comme une technique de routine dans le diagnostic moléculaire des maladies neuromusculaires, le premier objectif de mon projet

de thèse était d'utiliser le séquençage haut-débit pour trouver des mutations chez les patients avec différentes maladies neuromusculaires.

Objectif 2 :

Les myopathies centronucléaires (CNM) sont des troubles neuromusculaires héréditaires et un type de myopathies congénitales avec différentes sévérités et âges d'apparition, principalement caractérisées par la présence au centre des fibres musculaires d'un ou plusieurs noyaux intériorisés (Fujimura-Kiyono et al, 2008; Jungbluth et al , 2008). Aujourd'hui, pour l'évaluation globale des CNM, les données cliniques, histopathologiques et s'il est possible de l'IRM musculaire doivent être évaluées pour orienter le test génétique (Biancalana et al, 2012).

Des mutations chez les patients atteints de CNM classique ont été identifiées dans trois gènes: MTM1 (MIM # 300415) (Laporte et al, 1996), BIN1 (MIM # 601248) (Nicot et al, 2007) et DNM2 (MIM # 602378) (Bitoun et al, 2005).

Des mutations dans deux autres gènes ont été détectées chez des patients atteints d'autres phénotypes CNM partiellement similaires. Deux variants hétérozygotes ont été rapportés dans MTMR14 (MIM # 611089) chez les patients atteints de CNM sporadique, dont un patient portant également une substitution hétérozygote dans DNM2 (Tosch et al, 2006). Il reste à déterminer si ces variations sont des mutations pathogènes ou impliquant des modifications du phénotype.Des mutations dans RYR1 (MIM # 180901) ont été détectée chez des patients présentant une transmission autosomique récessive et des caractéristiques similaires aux CNM, tels que les noyaux internalisés (Bevilacqua et al, 2011; Jungbluth et al, 2007; Wilmshurst et al, 2010).

La prévalence des CNM (âge <18 ans) a été estimée à moins de 1 sur 100,000 (Amburgey et al, 2011) et est répartie entre MTM1 (45%), DNM2 (15%), RYR1 (10-15%) et BIN1 (<5%) (Biancalana et al, 2012). Environ 20% des patients CNM n'ont pas de mutation dans ces cinq gènes impliqués. Les mutations de ces patients ont besoin d'être identifiées. Le deuxième objectif de mon projet de thèse a été de trouver ces gène (s) en utilisant le séquençage haut-débit.

Résultats:

Résultats pour le premier objectif:

Pour identifier les mutations en cause chez les patients atteints de maladies neuromusculaires et pour valider une approche de capture de séquence et de séquençage haut débit (NGS=Next generation sequencing), nous avons capturé et séquencé 267 gènes impliqués dans les maladies neuromusculaires chez les 16 patients atteints de différentes maladies neuromusculaires avec ou sans mutations connues (8 patients avec mutations connues et 8 sans mutations connues). Nous avons pu retrouver toutes les mutations chez les 8 patients ayant des mutations connues, y compris des mutations ponctuelles introniques / exoniques, une insertion / délétion, une grande délétion et des variations hétéro / homo / hémizygotes. Chez les patients avec des variations inconnues, nous avons pu identifier des mutations dans 4 des 8 patients.

L'un de ces quatre patients avait été préalablement diagnostiqué avec une polyneuropathie démyélinisante, mais de nouvelles analyses cliniques et biochimiques menées en parallèle de l'étude NGS a suggéré qu'il avait une maladie mitochondriale dontles gènes ne sont pas couverts par notre capture.

Le deuxième patient a montré 2 changements faux-sens dans LMNA, y compris le changement p.Arg644Cys, précédemment liée à diverses laminopathies; basée sur les données de NGS, nous montrons que les deux changements sont sur le même allèle et ne peuvent donc pas être la seule cause de la neuropathie axonale. Cependant, nous n'avons pas eu accès aux ADN des parents pour vérifier si les deux changements sont sur le même allèle.

Pour les 2 autres patients, qui étaient auparavant exclus pour plusieurs gènes candidats par séquençage Sanger, les mutations causant la maladie peuvent être un changement intronique, des expansions répétées ou une translocation, mutations qui n'ont pas été testés dans cette étude. Par ailleurs, ces patients peuvent également être mutés dans un gène non lié aux NMD au moment de la construction de notre bibliothèque cible.

Un diagnostic moléculaire plus rapide des NMD aura des impacts majeurs sur les patients, car il améliore la gestion des maladies et le conseil génétique, et permettra l'accès à des thérapies ou l'inclusion dans les essais thérapeutiques. A titre d'exemple, l'identification des mutations RYR1 dans l'un des patients est d'une importance médicale majeure car le traitement des patients atteints de RYR1 avec le salbutamol a montré une amélioration significative de la faiblesse musculaire. Nous avons publié ces données récemment (Vasli et al, 2012).

Les résultats pour le deuxième objectif:

Pour répondre à la deuxième question biologique, nous avons ciblé et séquencé des gènes candidats (76 gènes candidats ou 2500 gènes candidats), des exomes et génomes entiers, dans des plusieurs familles et des cas sporadiques avec CNM. Dans certains cas, nous avons trouvé des mutations dans RYR1. Ce gène code pour le récepteur de la ryanodine du muscle squelettique ; c'est un canal calcium du réticulum sarcoplasmique, ainsi qu'une structure de pontage connectant le réticulum sarcoplasmique et des tubules transverses (Fill et al, 1989). Nous avons soumis ces données récemment pour publication.

Nous avons trouvé également une délétion homozygote exonique de deux nucléotides dans un gène non caractérisé dans l'une des familles après séquençage ciblé de 2500 gènes candidats par Roche 454 pyrosequenceur et également séquençage de l'exome du patient par Illumina HiSeq2000. Ce changement n'est pas présent dans les bases de données de polymorphismes comme dbSNP, 1000 génomes ou »exome variant server ». Les données d'expression ont montré une baisse du niveau d'expression de ce gène. Une RT-PCR de ce gène a également montré une réduction du niveau de l'ARN dans le cas concerné comparant à des contrôles. La mère et le père sont porteurs hétérozygotes de la variation. Nous avons séquencé les exons codants et les frontières intron-exon de ce gène dans un panel de 100 patients atteints de CNM afin de trouver un autre patient ayant des mutations dans ce même gène. Nous avons trouvé une délétion hétérozygote de 3 nucléotides, chez un autre patient qui n'est pas un changement fort. Nous n'avons pas trouvé d'autres changements chez d'autres patients. Maintenant, l'analyse fonctionnelle chez le poisson zèbre est en cours pour tester si la délétion de ce gène a un effet sur le muscle.

En séquençant l'exome et le génome complet de personnes affectées et non affectées dans une autre famille, nous avons identifié de probables mutations dans le gène TTN codant pour la titine,

Conclusions et perspectives:

Pour le premier objectif, nous avons démontré que cette stratégie peut détecter différents types de mutations. Toutes les mutations ont été détectées à partir du séquençage haut-débit et l'analyse d'un cas index unique, contrairement aux études précédentes où le séquençage comparatif de plusieurs exomes individuels avait été utilisé pour identifier les mutations causales.

Un défi majeur dans l'analyse des données de NGS est l'identification de la variation pathogène parmi une grande liste des variants. Notre analyse en aveugle basée sur le classement des variants et le type de la maladie a permis d'identifier toutes les mutations connues. Cependant, les données cliniques, histologiques et moléculaires détaillées étaient nécessaires pour les étapes de confirmation, c'est à dire faire correspondre les données génétiques avec le phénotype.

Un tel séquençage haut-débit ciblé sur tous les gènes candidats est particulièrement adapté pour des maladies avec une hétérogénéité génétique élevée, comme c'est le cas pour les NMD, et devrait faciliter l'identification des maladies alléliques, c'est à dire différentes maladies causées par des mutations du même gène. En outre, cette stratégie permet l'analyse des gènes de grande taille, tels que TTN, qui ne sont pas systématiquement vérifiés entièrement par séquençage Sanger, même s'ils sont connus pour être impliqués dans des maladies.

Les principales conditions pour une utilisation ultérieure de la stratégie NMD-seq comme une approche de routine dans les laboratoires de diagnostic génétiques sont la reproductibilité, la sensibilité de détection et l'étude de cohortes hétérogènes de patients dont la caractérisation clinique est parfois incomplète comme ce fut le cas dans notre étude. Nous proposons que cette stratégie puisse être une approche de dépistage préliminaire, potentiellement avant des investigations plus invasives et de longue haleine, comme les biopsies.

Pour le deuxième objectif, nous avons trouvé des mutations pathologiques chez certains des patients CNM dans un gène (RYR1) connus pour être impliqué dans CNM, et dans le gène TTN qui était précédemment impliqué dans d'autres myopathies. Dans certains autres patients nous avons trouvé des gènes candidats potentiels et une analyse fonctionnelle pour prouver l'implication de ces gènes dans CNM est en cours.

Nous concluons que le séquençage haut débit est une technique solide pour l'identification des gènes si une cohorte homogène de patients est disponible et est également utile pour l'utiliser comme une technique de routine dans le diagnostic moléculaire.

References:

Emery AE (1991) Population frequencies of inherited neuromuscular diseases--a world survey. Neuromuscul Disord 1(1): 19-29

Tennyson CN, Klamut HJ, Worton RG (1995) The human dystrophin gene requires 16 hours to be transcribed and is cotranscriptionally spliced. Nat Genet 9(2): 184-190

Andersen PS, Havndrup O, Hougs L, Sorensen KM, Jensen M, Larsen LA, Hedley P, Thomsen AR, Moolman-Smook J, Christiansen M, Bundgaard H (2009) Diagnostic yield, interpretation, and clinical utility of mutation screening of sarcomere encoding genes in Danish hypertrophic cardiomyopathy patients and relatives. Hum Mutat 30(3): 363-370

Gazzerro E, Bonetto A, Minetti C (2011) Caveolinopathies: translational implications of caveolin-3 in skeletal and cardiac muscle disorders. Handb Clin Neurol 101: 135-142

Nance JR, Dowling JJ, Gibbs EM, Bonnemann CG (2012) Congenital myopathies: an update. Curr Neurol Neurosci Rep 12(2): 165-174

Prior TW (2010) Perspectives and diagnostic considerations in spinal muscular atrophy. Genet Med 12(3): 145-152

Fujimura-Kiyono C, Racz GZ, Nishino I (2008) Myotubular/centronuclear myopathy and central core disease. Neurol India 56(3): 325-332

Jungbluth H, Wallgren-Pettersson C, Laporte J (2008) Centronuclear (myotubular) myopathy. Orphanet J Rare Dis 3: 26

Laporte J, Hu LJ, Kretz C, Mandel JL, Kioschis P, Coy JF, Klauck SM, Poustka A, Dahl N (1996) A gene mutated in X-linked myotubular myopathy defines a new putative tyrosine phosphatase family conserved in yeast. Nat Genet 13(2): 175-182

Nicot AS, Toussaint A, Tosch V, Kretz C, Wallgren-Pettersson C, Iwarsson E, Kingston H, Garnier JM, Biancalana V, Oldfors A, Mandel JL, Laporte J (2007) Mutations in amphiphysin 2 (BIN1) disrupt

interaction with dynamin 2 and cause autosomal recessive centronuclear myopathy. Nat Genet 39(9): 1134-1139

Bitoun M, Maugenre S, Jeannet PY, Lacene E, Ferrer X, Laforet P, Martin JJ, Laporte J, Lochmuller H, Beggs AH, Fardeau M, Eymard B, Romero NB, Guicheney P (2005) Mutations in dynamin 2 cause dominant centronuclear myopathy. Nat Genet 37(11): 1207-1209

Tosch V, Rohde HM, Tronchere H, Zanoteli E, Monroy N, Kretz C, Dondaine N, Payrastre B, Mandel JL, Laporte J (2006) A novel PtdIns3P and PtdIns(3,5)P2 phosphatase with an inactivating variant in centronuclear myopathy. Hum Mol Genet 15(21): 3098-3106

Bevilacqua JA, Monnier N, Bitoun M, Eymard B, Ferreiro A, Monges S, Lubieniecki F, Taratuto AL, Laquerriere A, Claeys KG, Marty I, Fardeau M, Guicheney P, Lunardi J, Romero NB (2011) Recessive

RYR1 mutations cause unusual congenital myopathy with prominent nuclear internalization and large areas of myofibrillar disorganization. Neuropathol Appl Neurobiol 37(3): 271-284

Jungbluth H, Zhou H, Sewry CA, Robb S, Treves S, Bitoun M, Guicheney P, Buj-Bello A, Bonnemann C, Muntoni F (2007) Centronuclear myopathy due to a de novo dominant mutation in the skeletal muscle ryanodine receptor (RYR1) gene. Neuromuscul Disord 17(4): 338-345

Wilmshurst JM, Lillis S, Zhou H, Pillay K, Henderson H, Kress W, Muller CR, Ndondo A, Cloke V, Cullup T, Bertini E, Boennemann C, Straub V, Quinlivan R, Dowling JJ, Al-Sarraj S, Treves S, Abbs S, Manzur AY, Sewry CA, Muntoni F, Jungbluth H (2010) RYR1 mutations are a common cause of congenital myopathies with central nuclei. Ann Neurol 68(5): 717-726

Amburgey K, McNamara N, Bennett LR, McCormick ME, Acsadi G, Dowling JJ (2011) Prevalence of congenital myopathies in a representative pediatric united states population. Ann Neurol 70(4): 662-665

Biancalana V, Beggs AH, Das S, Jungbluth H, Kress W, Nishino I, North K, Romero NB, Laporte J (2012) Clinical utility gene card for: Centronuclear and myotubular myopathies. Eur J Hum Genet

Fill M, Ma JJ, Knudson CM, Imagawa T, Campbell KP, Coronado R (1989) Role of the ryanodine receptor of skeletal muscle in excitation-contraction coupling. Ann N Y Acad Sci 560: 155-162

Nasim VASLI

Genetic diagnosis and identification of novel genes in neuromuscular disorders using next generation sequencing

Résumé :

Les maladies neuromusculaires sont des maladies souvent très sévères et très handicapantes, et un fardeau pour les patients, leurs familles, ainsi que pour le système de santé. Le but de ce projet était de mettre au point et de valider une approche de capture de séquence et de séquençage haut débit pour identifier les mutations en cause chez les patients atteints de maladies neuromusculaires et également trouver les nouveaux gènes qui sont impliqués dans une sous-classe de myopathies, les myopathies centronucléaires. Nous avons montré que l'approche de capture de séquence et de séquençage haut débit peux être utile dans le domaine des maladies neuromusculaires car elle est moins coûteuse que les approches conventionnelles « gène par gène » mise en œuvre dans les laboratoires de diagnostics génétiques.
Cette stratégie devrait élargir les spectres cliniques connus et identifier de nouvelles maladies alléliques (des mutations dans un gène causant différentes maladies). De plus, cela sera utile pour l'élargissement des connaissances sur les corrélations génotypes-phénotypes qui sont nécessaires à une prise en charge plus adaptée et au développement de stratégies thérapeutiques.

Mot clé : les maladies neuromusculaires- séquençage haut débit, les myopathies centronucléaires

Summary:

Neuromuscular disorders (NMD) are genetic diseases affecting muscles, nerves and neuromuscular junctions. They are rare and often severe with different age of onset from childhood to adulthood with significant burden to the patients, their families and public health system. For testing the possibility of using massively parallel sequencing as a routine technique in molecular diagnosis of neuromuscular disorders, the first aim of my PhD project was to use massively parallel sequencing technique in patients with different NMDs for disease-causing mutation detection.
The second aim of my PhD project was to find novel gene(s) implicated in centronuclear myopathies (CNM). CNM are inherited neuromuscular disorders and a type of congenital myopathies, characterized mainly by presence of central and one or more internalized nuclei in muscle fibers with different severities and age of onset, using massively parallel sequencing. About 20% of CNM patients don't have any mutations in four implicated genes. Disease- causing mutation(s)/ gene(s) in these patients need to be identified.
We could show that next generation sequencing is a robust technique for gene identification if a homogenous cohort of patients is available and also is useful to use as a routine technique in molecular diagnosis as it is time and cost effective technique.

Keywords: Neuromuscular disorders, massively parallel sequencing, centronuclear myopathies