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Genetic analysis of generalized epilepsies in a sudanese cohort and functional validation in the worm *C. elegans*

Maha Dahawi

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**Université de Khartoum
Sorbonne Université
Ecole doctorale Cerveau, Comportement, Cognition (ED3C)**

Genetic analysis of genetic generalized epilepsies in a Sudanese cohort and functional validation in the model *C. elegans*

Présentée par
Maha Hassan Osman Ali DAHAWI

Pour obtenir le grade de Docteur
Spécialité Neurosciences
Soutenue publiquement le 25 octobre 2021

Devant un jury composé de:

Pr. Martin CATALA
Dr. Thomas BOULIN
Pr. Eric PASMANT
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Co-directrice (Soudan)

س

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الم

*Take the **first** step in faith. You don't have to see the whole staircase, just take the first step.*

Martin Luther King

Seek the company of the wise, who know. Agree with what they say, for one understands only that with which one agrees. Be sincere in what you say - a single tongue should not speak two different words. No deceit or fraud should enter into your thoughts. Do not belittle anyone or anything, for everyone and everything in its inner being wishes for the same thing.

Ibn Arabi

Our deepest fear is not that we are inadequate. Our deepest fear is that we are powerful beyond measure. It is our light, not our darkness that most frightens us. We ask ourselves, 'Who am I to be brilliant, gorgeous, talented, fabulous?' Actually, who are you not to be? You are a child of God. Your playing small does not serve the world. There is nothing enlightened about shrinking so that other people won't feel insecure around you. We are all meant to shine, as children do. We were born to make manifest the glory of God that is within us. It's not just in some of us; it's in everyone. And as we let our own light shine, we unconsciously give other people permission to do the same. As we are liberated from our own fear, our presence automatically liberates others.

Marianne Williamson

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Abstract in English

Genetic generalized epilepsy (GGE) is a common form of epilepsy, including childhood absence epilepsy, juvenile absence epilepsy, juvenile myoclonic epilepsy, and GGE with tonic-clonic seizures alone. Since large families with GGE are rare in western countries, most studies were performed in American and European cohorts of sporadic cases. They suggested that susceptibility genes for GGE are numerous and their variants rare, challenging their identification. They also underlined that the inheritance of GGE is likely oligogenic. Up to now, only few genes including *GABRA1* and *SCN1A* have been reported as susceptibility genes for GGE.

In this project, our aim was to study familial forms of GGEs from Sudan as a different approach to answer the question of their genetic determination. We identified for the first time *ADGRV1* ultra rare/rare variants in 5 of 20 (25%) Sudanese families with GGE. In these families, there was a statistically significant association between GGE and *ADGRV1* variants proposing its oligogenic contribution to GGE. We identified a novel gene, *FAT1*, encoding an atypical cadherin involved in neurite outgrowth, in a family with an autosomal recessive form of Generalized tonico-clonic epilepsy. Novel candidate genes, *DCHS1*, *ASTN2* and *FBXO42*, were also identified in large families with GGE.

In parallel, we explored *C. elegans* as an animal model for functional validation of candidate genes in epilepsy. We performed knockdown and knock-in techniques on known and novel genes responsible for epilepsy. Behavioral tests showed statistical difference between mutant lines compared to controls. We also explored the oligogenic hypothesis in the worm by testing double mutants.

Our project paved the way in answering the oligogenicity puzzle in GGEs and the use of *C. elegans* as the first functional screening of candidate genes for epilepsy.

Key words: Genetic generalized epilepsy, *ADGRV1*, *FAT1*, *DCHS1*, *ASTN2*, oligogenic, *C. elegans*

Résumé en français

L'épilepsie généralisée génétique (EGG) est une forme courante d'épilepsie, qui comprend l'épilepsie-absence infantile, l'épilepsie-absence juvénile, l'épilepsie myoclonique juvénile et l'EGG avec crises tonico-cloniques seules. Les grandes familles avec EGG étant rares dans les pays occidentaux, la plupart des études ont été réalisées sur des cas sporadiques de cohortes américaines et européennes. Ces études ont suggéré qu'il existait de nombreux gènes de susceptibilité à l'EGG et que leurs variants étaient rares, ce qui rend difficile leur identification. Il a également été souligné que l'hérédité de l'EGG est probablement oligogénique. Jusqu'à présent, seuls quelques gènes, dont *GABRA1* et *SCN1A*, ont été identifiés comme gènes de susceptibilité à l'EGG.

Dans ce projet, notre objectif était d'étudier les formes familiales d'EGG au Soudan, comme approche complémentaire pour identifier leur déterminisme génétique. Nous avons identifié pour la première fois des variants ultra rares/rares d'*ADGRV1* dans 5 des 20 familles soudanaises (25%) atteintes d'EGG. Dans ces familles, il y avait une association statistiquement significative entre l'EGG et les variants d'*ADGRV1*, montrant qu'*ADGRV1* était un gène de susceptibilité à l'EGG. Nous avons également identifié un nouveau gène, *FAT1*, codant pour une cadhérine atypique impliquée dans la croissance des neurites, dans une famille présentant une forme autosomique récessive d'EGG avec crises tonico-cloniques. De plus, de nouveaux gènes candidats, *DCHS1*, *ASTN2* et *FBXO42*, ont été identifiés dans de grandes familles atteintes d'EGG.

En parallèle, nous avons exploré le nématode *C. elegans* comme modèle animal pour la validation fonctionnelle de gènes candidats à l'épilepsie. Nous avons effectué des techniques de knock-down et knock-in sur des gènes connus et nouveaux, responsables de l'épilepsie. Les tests comportementaux ont montré une différence statistique entre les lignées mutantes et témoins. Nous avons également exploré l'hypothèse oligogénique chez le nématode, en testant des doubles mutants.

Notre projet a ouvert la voie pour répondre à l'énigme de l'oligogénicité dans les EGG ainsi qu'à l'utilisation de *C. elegans* comme premier criblage fonctionnel de gènes candidats de l'épilepsie.

Mots-clés: Epilepsie généralisée génétique, *ADGRV1*, *FAT1*, *DCHS1*, *ASTN2*, oligogénique, *C. elegans*

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List of acronyms

ABPE: atypical benign partial epilepsy

ACMG: American College of Medical Genetics and Genomics

ADNFLE: autosomal dominant nocturnal frontal lobe epilepsy

ADEAF: autosomal dominant epilepsy with auditory features

AD: Alzheimer's disease

ADGRV1: adhesion G protein-coupled receptor V1

ADHD: attention deficit hyperactivity disorder

AE: absence epilepsy

AED: antiepileptic drug

AMP: Association for Molecular Pathology

ALS: amyotrophic lateral sclerosis

AR: autosomal recessive

ASD/ID: autism spectrum disorder/intellectual disability

A β : β -amyloid peptide

BECTS: benign epilepsy with centrotemporal spikes

BFNE: benign familial neonatal epilepsy

BFNIS: benign familial neonatal-infantile seizures

BAM: binary alignment map

BMEI: benign myoclonic epilepsy of infancy

Bp: base pairs

C. elegans: *Caenorhabditis elegans*

CACNA1H: Calcium Voltage-Gated Channel Subunit Alpha1 H

CADD: combined annotation dependent depletion

CAE: childhood absence epilepsy

CBZ: carbamazepine

CGC: Caenorhabditis Genetics Center

Chr: chromosome

CHRNA2: cholinergic Receptor Nicotinic Alpha 2 subunit

CHRNA4: cholinergic receptor nicotinic alpha 4 subunit

CHRN2: cholinergic receptor nicotinic beta 2 subunit

CNS: central nervous system

crRNA: CRISPR RNA

CSTB: cystatin B

CSWS: continuous spike and waves during sleep

CT: computed tomography

DCX: doublecortin

DEE: developmental and epileptic encephalopathies

DOORS: deafness, onychodystrophy, osteodystrophy, mental retardation and seizures syndrome

DS: Dravet syndrome

DSD: disorders of sexual development

dsDNA: double stranded DNA

DEE: developmental and epileptic encephalopathies

DEPDC5: dishevelled, Egl-10 and pleckstrin domain containing protein 5

DNA: deoxyribonucleic acid

dNTP: deoxyribonucleotide triphosphate

DSB: double stranded break

DSD: disorder of sexual development

EBT: elution buffer tween

EE: epileptic encephalopathy

EEG: electroencephalogram

EME: early myoclonic encephalopathy

EOAE: early onset absence epilepsy

EOEE: early onset epileptic encephalopathy

EZ: epileptogenic zone

FALS: familial amyotrophic lateral sclerosis

FCD: focal cortical dysplasia

FE: focal epilepsy

FFEVF: familial focal epilepsy with variable foci

FIG4: Phosphoinositide 5-Phosphatase

FIME: familial infantile myoclonic epilepsy

fMRI: functional magnetic resonance imaging

FOXG1: forkhead box G1

FOXO: forkhead family of transcription factors

FS: febrile seizure

GABA(A): gamma-aminobutyric acid receptor subtype A

GABRG2: $\gamma 2$ GABA(A) receptor subunit

GAERS: genetic absence epilepsy rat of Strasbourg

GATOR1: Gap Activity TOWard Rags 1

GEF+: generalized epilepsies with febrile seizures plus

GEFS+: genetic epilepsy with febrile seizures plus

GEPR: genetic epilepsy-prone rat

GGE: genetic generalized epilepsy

GGE-TCS: GGE with tonic-clonic seizures

GOF: gain of function

GSWD: generalized spike-wave discharge

GTCS: generalized tonic-clonic

GTCS: generalized tonic-clonic seizure

G6PD: glucose-6-phosphate dehydrogenase

HD: Huntington's diseases

HDR: homology dependent repair

HIC: high-income countries

HME: hemimegalencephaly

HTT: Huntingtine protein

ICK: intestinal cell kinase

ID: intellectual disability

IDT: integrated DNA technologies

IED: interictal epileptic discharges

IGE: idiopathic generalized epilepsy

IGF1R: insulin like growth factor 1 receptor

ILAE: International League Against Epilepsy

INSR: insulin receptor

INSRR: insulin receptor related receptor

IPTG: isopropyl β -D-1-thiogalactopyranoside

IQ: intellectual quotient

IS: infantile spasms

IT15: Interesting Transcript 15

IZ: irritative zone

JAE: juvenile absence epilepsy

JME: juvenile myoclonic epilepsy

KChIP: Kv channel interacting protein

KCNIP1: potassium voltage-gated channel interacting protein 1

KCNQ2: potassium voltage-gated channel subfamily q member 2

KD: knock-down

KI: knock-in

KIF13B: kinesin family member 13B

KLP-4: Kinesin-Like Protein

Kv: voltage-dependent K^+

LB: Luria Broth

LGS: Landau-Kleffner syndrome

LGS: Lennox-Gastaut syndrome

LIS1: lissencephaly-1

LMIC: low-and-middle-income country

LMN: lower motor neuron

LM-PCR: ligation-mediated PCR

LOF: loss of function

LTG: lamotrigine

MAF: minor allele frequency

MAST4: microtubule associated serine/threonine kinase family member 4

MPSI: migrating partial seizures of infancy

MRI: magnetic resonance imaging

mTOR: mechanistic target of rapamycin

mTORC1: mechanistic target of rapamycin complex 1

M-CAP: mendelian clinically applicable pathogenicity

nAChR: nicotinic acetylcholine receptor

NEESB: neonatal epileptic encephalopathy with suppression burst

NHEJ: nonhomologous end joining

NGM: nematode growth medium

NGS: next generation sequencing

NMJ: neuromuscular junction

NPRL2: nitrogene permease regulator-like 2

NPRL3: nitrogene permease regulator-like 3

OS: Ohtahara syndrome

Pcnxl2: pecanex-like 2

PCR: polymerase chain reaction

PD: Parkinson's disease

PFA: paraformaldehyde

PKG: protein kinase G

PME: progressive myoclonic epilepsy

PTZ: pentylenetetrazole

PWE: people living with epilepsy

RME: ring motor neurons

RIS: single ring interneuron

RNAi: RNA interference

Shh: sonic hedgehog

SIFT sorting intolerant from tolerant

SNCA: synuclein alpha

SNR: substantia nigra pars reticulata

SOD1: superoxide dismutase

SPRI: solid phase reversible immobilization

ssODN: single stranded oligodeoxyribonucleotides

SUDEP: sudden unexpected death in epilepsy

SWD: spike-wave discharge

SWLB: single worm lysis buffer

TLE: temporal lobe epilepsy

Tg: tottering model

Tm: melting temperature

ULD: Unverricht-Lundborg disease

UMN: upper motor neuron

Unc: uncoordinated

VCF: variant calling format

VPA: sodium valproate

VUS: variants of uncertain significance

WES: whole-exome sequencing

WGS: whole-genome sequencing

WWE: women with epilepsy

YVS: Yunis-Varón syndrome

3D: three-dimensional



Introduction

Chapter 1: Epilepsy, a global view

I. Definition and classification

1. Definition

Epilepsy is one of the most common brain conditions affecting over 70 million people worldwide. Epilepsy exists when someone has an epileptic seizure, and their brain "demonstrates a pathologic and enduring tendency to have recurrent seizures" (Fisher et al., 2014). An epileptic seizure is defined as: "a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain". Epilepsy manifests as spontaneous epileptic seizures and has many neurobiological, cognitive, and psychological consequences with serious risks of injury, impairment of brain function, or even death of the patient.

The global burden of epilepsy reports 13 million disability-adjusted lives due to epilepsy per year. There is an unequal geographical distribution of epileptic patients since more than 70% of them live in low-to-middle-income countries. Overall, a global drop in the number of epilepsy-related deaths has been observed between 1990 and 2016. However, the most negligible improvements have been in low sociodemographic countries, and there is an urgent need for improvement (G. Singh & Sander, 2020a; Thijs, Surges, O'Brien, & Sander, 2019).

2. Classification

Seizure and epilepsy classifications were previously created by the International League Against Epilepsy (ILAE) in 1981, updated several times. These classifications are remarkably crucial for the clinicians/care teams, patients/families, and researchers. For the patients, it offers and clarifies a nameable diagnosis/etiology and improves understanding of the disease. For doctors and the patient's care team, these classifications are fundamental in facilitating communication and discussion. From a therapeutic perspective, having these classifications enables the investigation of drugs or surgical treatments.

Classically, two major seizure types have been identified depending on the locus of onset: partial or focal seizures, arising from focal cortical disturbances, and generalized seizures, characterized by synchronous discharge of both hemispheres (**Figure 1**). Clinical and

electrophysiological (using electroencephalogram, EEG) findings are both required to differentiate between them.

Previous ILAE classifications divided seizures into partial and generalized, with partial seizures subsequently divided further into simple and complex, based on the retainment of consciousness. Partial seizures are encountered as the most common seizure type, comprising around 60%, while genetic generalized seizures appear in around 30 to 40% of cases. (Fisher et al., 2017).

Based on decades of clinical experience, the ILAE appointed a novel operational classification of seizure types and epilepsies (**Figure 1**). The new 2017 classification, compared to the old classifications, uses alternative terms with several significant additions. These changes made it possible for the inclusion/classification of previously unclassifiable seizure and epilepsy types. It also reflected the greater understanding of epilepsies and their underlying mechanisms following the significant scientific advances. It presents three levels: (i) seizure type, where it assumes that the patient has epileptic seizures, as well-defined by the new 2017 ILAE seizure classification; (ii) a diagnosis of epilepsy type, including focal epilepsy, generalized epilepsy, combined generalized and focal epilepsy, and an unknown epilepsy group; (iii) epileptic syndrome, where a specific syndromic diagnosis is made (Pack, 2019; Scheffer et al., 2018).

ILAE 2017 Classification of Seizure Types Expanded Version

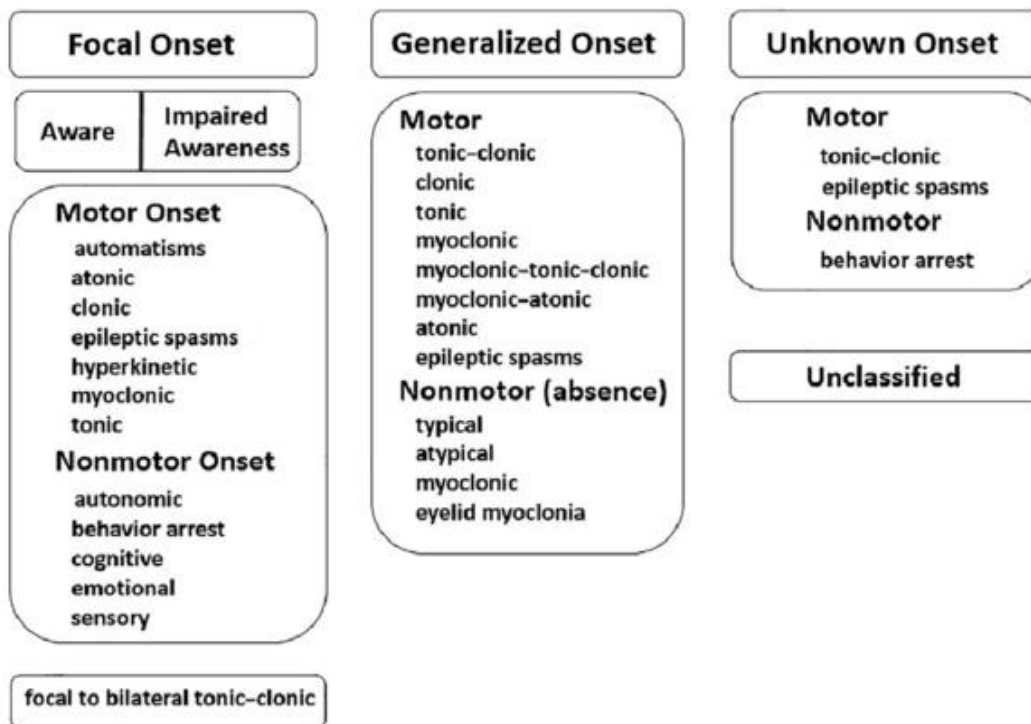


Figure 1. International League Against Epilepsy (ILAE) classification of seizure types.

Adapted from operational classification of seizure types by the ILAE.

II. Syndromic diagnosis of epilepsy

The identification of an epileptic syndrome requires specific clinical information, such as the age of onset, seizure description, predisposing factors, associated central nervous system (CNS) symptoms and signs, severity, EEG findings, and brain imaging. To some extent, and particularly in children, epileptic syndromes have different outcomes and responses to treatment. For epidemiological purposes, epileptic syndromes can be classified into four different prognostic groups:

- Excellent prognosis (20-30%) characterized by a high probability of spontaneous remission like neonatal seizures, benign partial epilepsies, benign myoclonic epilepsy in infancy, epilepsies provoked by specific modes of activation, i.e., reflex epilepsies.
- Good prognosis (30-40%) characterized by easy pharmacological control and the possibility of spontaneous remission, including infantile absence epilepsy and some focal epilepsies.

- Drug-dependent prognosis (about 10-20%), in which seizures may respond to drugs, with a tendency to relapse after treatment withdrawal, including juvenile myoclonic epilepsy and most focal epilepsies (symptomatic or cryptogenic).
- Poor prognosis (about 20%) in which seizures tend to recur despite intensive treatment, including epilepsies associated with congenital neurological defects, progressive neurological disorders, along with symptomatic or cryptogenic focal ([Annapurna Poduri, MD, MPH1 and Daniel Lowenstein, 2014](#); [J. W. Sander, 1993](#)).

III. Etiology

There is a range of etiological groups - without a hierarchical sequence - which includes: structural, genetic, infectious, metabolic, and immune, as well as an unknown group. A patient's epilepsy may be classified into more than one etiological category. The structural etiology is crucial for epilepsy surgery, while the genetic etiology is very important for genetic counselling/consideration of novel therapies such as mTOR therapies ([Goldstein & Hauptman, 2021](#); [Scheffer et al., 2018](#)). The etiology can also vary with geographical location. In South Sudan, for example, onchocerciasis (also known as river blindness) is one of the commonest causes of epilepsy ([Jada et al., 2020](#)).

Excitability and synchrony of neuronal circuits theory proposed an explanation of why normal individuals can develop unprovoked seizures without identifying a locus of onset. In addition, first-degree relatives of patients with idiopathic generalized epilepsy (IGE) have a roughly two to threefold elevated risk of being affected ([Ottman, Annegers, Risch, Hauser, & Susser, 1996](#)).

IV. Epilepsy in low-and-middle-income countries

Epilepsy is universally reported; however, the distribution of patients differs between low-to middle-income countries (LMIC) and high-income countries (HIC). The most noticeable difference relates to the age of onset. HIC follows a bimodal curve with a noticeably higher incidence in early childhood and the elderlies ([Hesdorffer et al., 2011](#)). In LMIC, the incidence is more significant in older children and young adults than in other age groups. In LMIC, the higher incidence of common infections associated with seizure disorders can be an explanation in these age groups (including neurocysticercosis, falciparum malaria,

meningitis, and encephalitis) (Carter et al., 2004; Hesdorffer et al., 2011). One hundred twenty-five thousand patients die each year of epilepsy, and over 80% of these deaths take place in LMIC, including 13 African countries (G. Singh & Sander, 2020b).

The distribution of cases in developing countries is also different: the median lifetime epilepsy prevalence is 5.8 per 1000, whereas in rural areas of developing countries is 15.4 per 1000 (Bashir & Cumber, 2019; Ngugi et al., 2011). Most of these studies were only on convulsive epilepsies but not on non-convulsive seizures (e.g., absences). Given also the difficulty in recalling the seizure, these studies are likely underestimating the prevalence of lifetime epilepsy by two to four times (Edwards et al., 2008).

Chapter 2: Epilepsy from a Sudanese perspective

I. Epidemiology

In Sudan, the prevalence of epilepsy could be underestimated in areas where the condition is stigmatized, leading to the concealment or denial of the diagnosis. Therefore, considering the cultural, social, and economic factors is vital in prevalence estimation in these countries. Keeping that in mind, epilepsy accounts for 1.6 annual mortality rates with 238.7 disability-adjusted life years per 100 000 (Bashir & Cumber, 2019). The latest prevalence of epilepsy estimated in Khartoum (the capital of Sudan) state in school children (4/1000) is higher than those reported previously from Khartoum province in 1983 (0.9/1000) (Mohamed et al., 2017).

II. Stigma and quality of life

In Sudan, literature has well-described epilepsy stigma and its related elements of labelling, isolation, and discrimination experienced by patients and their families. It significantly affects the living standards and social life of patients and their families. In a cross-sectional descriptive study conducted on 270 people with epilepsy (between the ages of 16-70 years) focusing on understanding the different types of stigma among people living with epilepsy (PWE), it was demonstrated that 16.6% of the patients had a positive self-stigma score, half of them having coaching stigma (the attitude of parents ashamed of their children's disease, and as a result, teaching their children that epilepsy is undesired or a burden that they all must tolerate). Twenty-five percent have a courtesy stigma (describing the overriding impact of the stigma that extends to affect their families, relatives, and close social circles) (Hamid, Taha, Balla, & Hussien, 2013). One-third of people with epilepsy had a poor coping score that was strongly determined by self-stigma and courtesy stigma score (Hamid et al., 2013). This study, among others, highlights the problem of stigma and directs towards finding the proper tools for raising awareness towards addressing it.

In another cross-sectional study evaluating the quality of life in PWE living in Khartoum state, interviews were taken from people living in cities, suburbs, and adjacent villages. The questionnaire explored seizure control, adverse effects of antiepileptic medications,

physical harm, social/psychological impact, and stigmatization. A significant number of respondents declared that epilepsy has a negative influence on many aspects of their daily life activities, education, and social life. Seventeen percent were unoccupied or had their job stopped because of their illness. Relationships with partners were affected in 20% of cases. Fifteen percent seemed stigmatized by their illness. None of the participants had obtained any formal health education or counselling about epilepsy (Sokrab, Sokrab, & Elzubeir, 2012).

Traditional and spiritual healers negatively affect the diagnosis and treatment of patients with epilepsy. In a cross-sectional study to evaluate the impact of spiritual and traditional beliefs of caregivers on the management of children with epilepsy in Sudan: one-third of the studied population attributed epilepsy to supernatural causes, more than two-thirds reported the use of both traditional and spiritual medicine, more than half used different religious methods to treat epilepsy. Around half of the participants believed that religious and or traditional treatment was truly effective in the management of epilepsy, one-third used herbs in the treatment of epilepsy (Mohammed & Babikir, 2013).

III. Medical staff in Sudan

There is scarcity and unequal distribution of neurologists in Sudan, being mainly located in the capital Khartoum. Consequently, a large number of patients with epilepsy are treated by doctors from other specialties and medical degrees, including medical and house officers. A study done in Sudan in 2017, evaluating the knowledge of doctors (house officers (n=34), medical officers (n=60), registrars (n=52), and specialists (n=8) on women issues related to epilepsy, found that the majority of participants knew the role of folic acid in reducing teratogenicity of antiepileptic drugs (AEDs) (89%) and that women with epilepsy (WWE) should not stop taking their AEDs during pregnancy (76.6%). Two-thirds of participants knew that most WWE have healthy children (68.2%) and that enzyme-inducing AEDs can execute a role in reducing the effectiveness of some contraceptives (72.1%). Half of the participants knew the association between AEDs and osteomalacia (50%), and more than one-third (38.3%) knew that women taking AEDs could safely breastfeed (38.3%). On the other hand, few of them (20.1%) knew that WWE have a higher incidence of sexual dysfunction compared with healthy women, and only some (15.6%) knew that estrogen has a proconvulsant effect while progesterone has anticonvulsant qualities (Caksen, Aktar, Yildirim, & Ceylaner, 2019).

This study shows that the knowledge of Sudanese doctors was not satisfactory, and that further education is crucial to minimize the knowledge gap.

Pharmacists play a vital role in educating epileptic patients about their disease/medications and the possibility of drug-drug interactions. Another descriptive cross-sectional study conducted on young pharmacists (less than 30 years and with less than 5 years of experience), to evaluate the level of knowledge about antiepileptic drugs in Khartoum, found that only 14.7% of them had good knowledge about the AEDs, side effects, and drug-drug interactions, another element to be addressed in epilepsy in Sudan (Ali, Mahgoub, Nimir, & Ali, 2019).

IV. Consanguinity

Consanguinity (derived from the Latin word *consanguinitas*, 'blood relation') is defined as a reproductive relationship between two biologically related individuals sharing a common ancestor. For thousands of years and in different populations, consanguinity has been practiced. Currently, more than 1.2 billion of the global population practice consanguineous marriages (Fareed & Afzal, 2017). The prevalence of consanguineous marriage varies in different countries, is associated with many factors such as religion, ethnicity, demography, residence (living in rural or urban areas), isolation of the population, educational level, socioeconomic status, consanguinity among parent's marriages, and responder's attitude towards consanguinity (Ahmad & Bradby, 2007).

In European history, Roman civil law prohibited marriage if the couple was within four degrees of consanguinity. The church adopted the same rules; however, the authorities did not reach all of Europe, and consanguinity has been common, especially in cultural and geographic isolates (Bouchard, 1981).

A significant cluster of countries with high levels of consanguinity is listed in most communities of North Africa, Middle East, and West Asia, a transverse belt that runs from Pakistan and Afghanistan in the East, to Morocco in the West, and in South India, with intra-familial unions collectively accounting for 20-50% of all marriages (Bittles & Black, 2010). In some regions of Pakistan, most marriages have been consanguineous (Anwar, Khyatti, & Hemminki, 2014).

In Sudan and specifically in the West, consanguinity was reported to be as high as 67.5% in sickle cell anemia (Daak et al., 2016). In another study on disorders of sexual development (DSDs), parental consanguinity was seen in up to 70% of cases showing the strong association between consanguinity and the manifestation of these different disorders in families. Importantly, the detrimental effects of inbreeding are the consequence of homozygosity of harmful genes (Ellaithi, Kamel, & Saber, 2012). The consanguinity rate was found to be around 54% in a study on 202 school children with varying types of epilepsy in Khartoum (Mohamed et al., 2019).

Consanguinity is known for its harmful effect, though there are documented benefits of it, especially for Malaria. Theoretically, beneficial effects of inbreeding could be expected in those who are homozygous for protective recessive and codominant genes. It is argued that the most common monogenetic conditions in humans, namely, alpha-thalassemia, glucose-6-phosphate dehydrogenase (G6PD) deficiency, hemoglobin C, and Duffy antigen-negative red blood cells, which have evolved under pressure from Malaria, had their survival and selection enhanced by consanguineous marriages in malaria-infested regions of the world (Denic & Nicholls, 2007).

Chapter 3: Clinical description of epilepsies

I. Focal epilepsy

Focal seizures originate and involve circuits in one hemisphere of the brain, commonly limbic or neocortical (Fisher et al., 2017). Previously, focal epilepsies were thought to be underlined mainly by cerebral structural abnormalities, especially in drug-resistant epilepsy (Berg et al., 2009; L. M. Li et al., 1995). However, an increasing number of inherited and *de novo* genetic mutations have been found in non-lesional focal epilepsy (Perucca et al., 2017).

Focal epilepsy syndromes include (i) temporal lobe epilepsy (TLE), (ii) autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE), and (iii) autosomal dominant epilepsy with auditory features (ADEAF).

TLE is the most common form of adult localization-related epilepsy, in which hippocampal onset accounts for at least 80% of all temporal lobe seizures (Tatum, 2012).

ADNFLE is defined by clusters of nocturnal motor seizures, which are often stereotyped and brief (5 seconds to 5 minutes). ADNFLE is inherited in an autosomal dominant manner with a penetrance rate of 70%, as most individuals diagnosed with ADNFLE have an affected parent (Kurahashi & Hirose, 1993).

ADEAF is a focal epilepsy syndrome with auditory symptoms and/or receptive aphasia as prominent ictal manifestations. The most common auditory symptoms are simple imprecise sounds, including humming, buzzing, or ringing. ADEAF is inherited in an autosomal dominant manner. Most individuals with ADEAF have an affected parent with a very low proportion of *de novo* pathogenic variants (Michelucci & Nobile, 1993).

II. Epileptic encephalopathy

They are severe, early-onset conditions characterized by refractory seizures, developmental delay or regression associated with ongoing epileptic activity, and mostly poor prognosis. Dravet, Ohtahara, and West syndromes are some of the highly well-studied EEs (C. T. Myers & Mefford, 2015). Treatment of an infantile-onset epileptic encephalopathy even in later adult life can enhance the outcome as documented in Dravet syndrome, where modification of anti-seizure therapy resulted in improved seizure control, cognition, and quality of life (Catarino et al., 2011).

The term 'epileptic encephalopathy' (EE) also refers to disorders in which frequent, often abundant, epileptiform activity continues even when no clinical seizures are apparent (Scheffer & Liao, 2020).

Most EEs begin in infancy and childhood, though they can occur at any age, including adult life, as seen in the progressive myoclonic epilepsies (Catarino et al., 2011).

III. Genetic generalized epilepsy

Genetic generalized epilepsy (GGE) is a common type of epilepsy representing up to one-fourth of all epilepsies. The ILAE Commission on the classification defined GGE as "forms of generalized epilepsies in which all seizures are initially generalized (absences, myoclonic jerks, and generalized tonic-clonic seizures), with an EEG expression that is generalized bilateral, synchronous, symmetrical discharge" (Epilepsy, 1985).

Many studies lead to the advancement of the discovery of monogenic causes of epilepsy. However, most IGEs are complex and, few IGE susceptibility genes are shared across the different subtypes (Prasad, Satyanarayana, & Munshi, 2013).

GGEs can be divided into several subtypes: juvenile absence epilepsy (JAE), juvenile myoclonic epilepsy (JME), childhood absence epilepsy (CAE), and epilepsy with generalized tonic-clonic seizures (**Figure 2**). In addition to the above mentioned, several rare GGE syndromes have been identified, some of which were associated with intellectual disability, including benign myoclonic epilepsy of infancy (BMEI), early-onset epilepsy, myoclonic-astatic epilepsy, epilepsy with myoclonic absences, eyelid myoclonic with absences, and absence status epilepsy (Prasad et al., 2013).

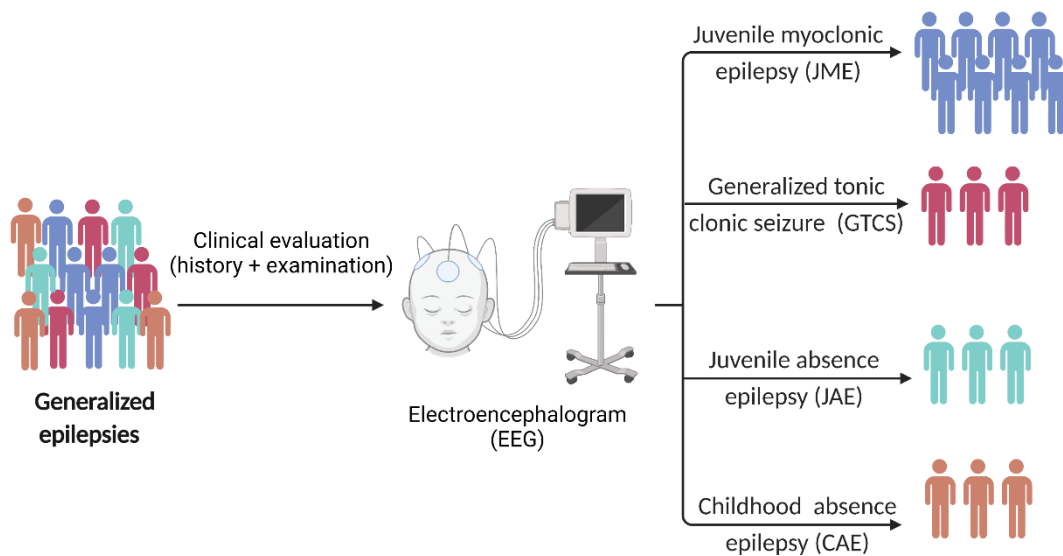


Figure 2. Genetic generalized epilepsies.

Steps to reach a diagnosis: history taking, examining the patients, EEG test. Four types are shown with JME as the commonest type.

1. Childhood or Juvenile Absence Epilepsy (CAE or JAE)

In 1924, Adie described childhood absence epilepsy (pyknolepsy) as an "abrupt onset seizure, occurring between 4 and 12 years of age, with epileptic seizures of very frequent short duration recurring daily for weeks, months, or years" ([ADIE, 1924](#)).

With a prevalence of 10-12% in children with epilepsy younger than 16 years, absence epilepsy is considered the most common pediatric epilepsy syndrome. It is characterized by the classical triad of frequent absence seizures, normal cognition, and generalized 3 Hz spike-wave discharges on EEG ([Epilepsy, 1985](#)). CAE occurs between 3 and 8 years, while JAE arises between 9 and 12 years of age ([Delgado-Escueta et al., 1994](#)).

Diagnostic criteria

The clinical characteristics of CAE are the following ([Guilhoto, 2017](#)):

- Children of school age (peak manifestation 6-7 years).
- Very frequent (several to many/day) absences.
- EEG with bilateral, synchronous, symmetrical spike waves, usually at 3 Hz.
- Development of GTCS often occurs during adolescence.

The clinical characteristics of JAE are ([Guilhoto, 2017](#)):

- Absences are the same as in CAE though retropulsive movements are less common.
- Clinical manifestation occurs around puberty.
- Seizure frequency is lower than in pyknolepsy.
- Frequent association with GTCS, which precede absence seizures more often than in pyknolepsy.
- Not infrequently, the presence of myoclonic seizures.
- Spike waves (SW) often > 3 Hz.
- Excellent response to therapy.

Treatment and prognosis

The drug of choice for CAE with absence seizures is ethosuximide. Valproic acid and lamotrigine can also be used. Compared to ethosuximide, valproic acid has additional adverse effects (like high-frequency tremor, altered mental status, and weight gain). Lamotrigine is less effective ([Kessler & McGinnis, 2019](#)).

A retrospective study found that the prognosis for seizure-free off medications in CAE has improved markedly over time, with a tremendous increase during the 1970s and 1980s. The reported remission rate ranges from 56% to 84% ([Grosso et al., 2005](#)). This improvement is related to the earlier diagnosis and the increasing use of effective medications ([Matricardi, Verrotti, Chiarelli, Cerminara, & Curatolo, 2014](#)).

2. Juvenile myoclonic epilepsy

JME is the most frequent generalized epilepsy syndrome. Described by Janz and Christian ([Baykan & Wolf, 2017](#)) as a single or arrhythmic bilateral, predominantly brachial myoclonus most of the time in an awakening state, this phenotype could be combined with generalized tonic-clonic seizures (GTCS) and absences in one-third of the cases ([Epilepsy, 1985](#)). Patients seek medical attention for the first time after their first generalized tonic-clonic seizure; nevertheless, and in most cases, this seizure was preceded by many myoclonic jerks not recognized to be a seizure by the patient. A detailed history with an accurate description of the seizures will diagnose a JME (as many families reported it as clumsiness of their child).

Patients with JME are highly susceptible to certain trigger factors such as sleep deprivation, stress, alcohol consumption, or photostimulation.

Classically, JME is considered a GGE subphenotype; however, this concept is now evolving, being replaced by a description of a system disorder of the brain ictogenesis in bilateral networks involving primarily the thalamus and selective areas of the neocortex (Wolf et al., 2015).

Current diagnostic criteria:

In 2011, an international workshop of clinical experts in JME summarized diagnostic criteria for JME as follows: two diagnostic groups could then be set, one narrower and one wider.

Class I diagnostic criteria are:

- Myoclonic jerks without loss of consciousness repeatedly occurring on awakening, i.e., within two hours after awakening.
- Routine sleep or sleep deprivation EEG shows normal background activity and ictal generalized high amplitude polyspikes (or polyspike and wave) with concomitant myoclonic jerks.
- Normal intelligence.
- Age at onset of between 10 and 25 years (Kasteleijn-Nolst Trenité et al., 2013).

Class II set of criteria include:

- Myoclonic jerks predominantly occurring after awakening.
- Myoclonic jerks are facilitated by sleep deprivation/stress and provoked by visual stimuli or praxis or GTCSs preceded by myoclonic jerks.
- Normal background on EEG and at least one occurrence of interictal generalized spike or polyspike waves, with some asymmetry (Wolf et al., 2015).

The diagnostic EEG findings of JME include normal background activity with generalized 3-6 Hz SWs of frontal predominance, mostly fragmented irregular polyspike waves, detected mainly at sleep onset or on awakening. However, there is high variability in EEG findings in patients (Serafini, Rubboli, Gigli, Koutroumanidis, & Gelisse, 2013).

Personality traits and sleep pattern in JME

Janz and Christian's observations on JME patient behavior were mainly in adults. Indeed, "their mental behavior is very often characterized by unsteadiness, lack of discipline, hedonism, and indifference towards their disease. They promise more than they can deliver. Their behavior often has effects on their therapy. Claiming they adhere to all prescriptions, but in reality, they skip attending control visits and taking their medication regularly. Their mood changes rapidly and frequently; they are easy to encourage and to discourage; they are gullible and unreliable" (Janz & Christian, 1957).

Their sleep pattern was another thing to be observed; according to the characteristics of circadian rhythm, patients with JME are categorized into "morning types" and "evening types". Morning types usually wake up early, go to bed early, whereas evening types wake up late in the morning and fall asleep late in the evening; they prefer doing the main work in the afternoon and evening. The therapeutic efficacy of the evening types is affected due to the circadian misalignment risk. Patients with JME are more likely to become evening types compared with patients with focal epilepsy or healthy controls (Kendis, Baron, Schuele, Patel, & Attarian, 2015). Previously, in Janz and Christian's work on 47 patients with JME, a typical sleep-wake cycle and typical personality traits were recognized: they wake up late and go to bed late, and their main work is prolonged until the afternoon or evening (Janz & Christian, 1957). Another study by Pung and Schmitz on a group of 20 patients with JME in comparison with patients having TLE confirmed that patients with JME exhibit a characteristic circadian rhythm and personality traits.

Treatment and prognosis

The selection of treatment in JME is based on clinical experience, retrospective studies, and prospective open-label trials. JME has a particular pharmacological sensitivity with a good response rate in general. The antiepileptic drugs (AED) of choice include sodium valproate (first-line management), lamotrigine, levetiracetam, topiramate, and zonisamide (Crespel et al., 2013).

JME is considered to be a lifelong disease, and the medications need to be continued. There is a high risk of seizure recurrence if the medications are stopped. Indeed, only 10% of

patients remained seizure-free after drug discontinuation ([Martínez-Juárez et al., 2006](#); [Panayiotopoulos, Obeid, & Tahan, 1994](#)).

IV. GGE with GTC alone

A generalized tonic-clonic seizure (GTC), earlier known as "Grand Mal" seizure, is a motor seizure with a tonic phase followed by clonic muscle contractions. GTC seizure is usually associated with impaired awareness or complete loss of consciousness. It arises within and rapidly involves bilateral cortical, subcortical, and brainstem networks of the brain ([Fisher et al., 2017](#)).

GTCs can occur secondary to structural, infectious, metabolic, or immune-related pathologies; however, the etiology of most of the generalized tonic-clonic seizures is related to a genetic cause.

Diagnosis

The age range of generalized seizures is between 5 and 40 years old; the diagnosis of a GTC seizure is based on the patient's history and physical examination. Asking about auras should be inquired, a detailed history is needed regarding any environmental or physiologic triggers (fever, menstrual period, lack of sleep, stress, intense emotions, strenuous exercise, loud music, flashing lights, etc.), immediately preceding the seizure. It is therefore essential to ask the patients, after the first episode of GTC seizure, about previous staring spells (absence seizures) or early morning myoclonic jerks. GTC seizures begin with an abrupt loss of consciousness without any aura. The tonic phase starts with a generalized stiffening of the body with or without cyanosis. Sequentially, clinical features evolve into clonic jerking followed by postictal sleepiness, confusion, or agitation ([Gavvala & Schuele, 2016](#)).

At a second step, a detailed physical examination is equally indispensable. A postictal state of headache, confusion, somnolence, personality, and mood changes are common and characteristic of GTC seizures. Examination of the mouth can show lateral tongue bites, which can be seen in approximately 22% of patients with epileptic seizures. Back pain after a fall can result from a vertebral compression fracture. Nuchal rigidity from meningeal inflammation or asterix from the metabolic disorder can be an indication of the etiology of the seizure.

Focal weakness or asymmetry on examination can direct towards focal seizures evolving into bilateral tonic-clonic seizures. Skin examination can reveal signs of neurocutaneous syndromes associated with epilepsy. Signs of urinary incontinence due to the relaxation of the urinary sphincter can be seen in an immediate postictal phase (Gavvala & Schuele, 2016).

Treatment and prognosis

Antiepileptic drugs (AED) are the mainstay of treatment. First-line medications are valproic acid, topiramate, and lamotrigine. Side effects of Valproic acid include hepatotoxicity, teratogenicity, and pancreatitis. It is contraindicated in patients with suspected mitochondrial disorders due to the high risk of hepatic failure. Valproate can also cause neural tube defects, decreased intellectual quotient (IQ) scores, and autism following *in utero* exposure. Consequently, valproate should not be administered to women of childbearing potential unless seizures are refractory to other AEDs. If used in women of childbearing potential, folic acid supplementation and effective contraception should be applied. Lamotrigine causes severe skin rashes. The incidence of these rashes, including Stevens-Johnson syndrome, is around 0.08% to 0.3% in the adult population; however, it is higher in pediatric patients occurring 2 to 8 weeks after starting the medication (Perucca, Scheffer, & Kiley, 2018).

The prognosis for generalized tonic-clonic seizures depends on the cause of the seizure and risk factors for the patient. The three factors consistently shown to have increased risk of seizure recurrence are the number of seizures, the occurrence of a neurological disorder, and an abnormal inter-ictal EEG. The risk of recurrent episodes is about 40% based on observational studies. It is highest during the period immediately after the initial attack and decreases with time (Rizvi, Ladino, Hernandez-Ronquillo, & Téllez-Zenteno, 2017).

V. Diagnostic tools

1. Structural abnormalities detection

Structural imaging in epilepsy is based on a balance between diagnostic accuracy and clinical feasibility (Wellmer et al., 2013). In epileptic patients with cerebral structural abnormalities, modern neuroimaging techniques are used, such as computed tomography (CT) and magnetic resonance imaging (MRI). CT imaging detects major malformations like tumors and infarcts,

while MRI allows the detection of more subtle abnormalities such as hippocampal sclerosis (Wiesmann, 2003). Combined MRI and fluorodeoxyglucose-positron emission tomography (FDG-PET) are used in focal cortical dysplasia patients (FCD) (Baldassari et al., 2019). Other screening includes the three-dimensional (3D) 1 mL isotropic voxel, volumetric T1-weighted imaging used in malformations of cortical development (Sidhu, Duncan, & Sander, 2018).

2. Electroencephalogram

An EEG detects abnormalities in the electrical activity of the brain. During the procedure, electrodes consisting of small metal discs with thin wires are pasted onto the scalp. The electrodes detect tiny electrical charges that result from the activity of the brain cells.

The EEG is a crucial tool in epilepsy. The EEG evaluation in a patient with potential epilepsy serves in answering several questions based on the clinical question asked. In patients with recent onset of paroxysmal episodes/attacks, it may help diagnose if these are of epileptic origin. In patients with newly diagnosed or suspected epilepsy, it may increase diagnostic certainty and show the risk of seizure recurrence, and can also directly define the epilepsy syndrome. In focal drug-refractory epilepsy, EEG is central in the presurgical diagnosis of epilepsy to localize the irritative zone (IZ) and the seizure onset zone as markers of the extension of the epileptogenic zone (EZ) (Rosenow, Klein, & Hamer, 2015). The EEG has a high temporal resolution providing a good overview as it samples from a large portion of the brain.

However, it carries a relatively low spatial resolution unless recorded with special invasive electrodes. Scalp electrodes only detect brain activity synchronized in at least six or more square centimeters of the cortex, (Fernández Torre et al., 1999) as cerebral activity is reduced by the impedance of the cerebrospinal fluid, meninges, skull, and scalp. Moreover, surface EEG can only record from parts of the cerebral cortex not buried in the sulci. In addition, the insular and mesiobasal aspects of the brain are not covered by surface EEG. Despite these limitations, surface EEG remains the most relevant test in diagnosing epilepsy for the foreseeable future. However, caution is needed when interpreting EEG studies because even EEG interpretations of experienced epileptologists may have only low-to-moderate reliability (Adam Moser, Kevin Range, 2008). The generalized spike-wave discharge (GSWD) >2.5 Hz (variations across sub-syndromes) is considered as the EEG hallmark in GGE. Importantly, the term generalized does not necessarily mean that the discharge starts at the same time across

all the brain areas, It describes the EEG phenomenology, in which the GSWD involves all areas of the cerebrum, either apparently symmetrically with bilateral synchrony or with variable side or regional emphasis (Elmali, Auvin, Bast, Rubboli, & Koutroumanidis, 2020; Koutroumanidis et al., 2017).

Chapter 4: Epilepsy from a genetic perspective

Recently, there has been a speedy expansion in the knowledge of genetics in epilepsy. It is estimated that genetic epilepsies constitute 30% to 40% of all epilepsies (McTague, Howell, Cross, Kurian, & Scheffer, 2016; Orsini, Zara, & Striano, 2018; Striano, Vari, Mazzocchetti, Verrotti, & Zara, 2016).

Most of the mutations detected in the past were in the subunits of neuronal ion channels affecting the excitation-inhibition balance leading to the recurrence of seizures (Villa & Combi, 2016). More recently, many genes encoding proteins with various functions have been associated with different types of epilepsy or epileptic encephalopathies (McTague et al., 2016).

I. Genes in epilepsy

Monogenic forms of epilepsies

More than 200 genes are responsible for epileptic encephalopathies and familial forms of epilepsy. The main gene families are summarized in table 2. We will focus on several illustrative examples.

1. Ion voltage-gated channels: one gene, many types of epilepsy

The involvement of ion channels in epilepsies has been well shown in different epileptogenic syndromes. There is an enrichment of ion channel genes in the brain, wherein their dysfunction has a direct effect on action potential (Seitter & Koschak, 2018). Most of the epileptogenic causing variants are in ion-voltage channel genes, reinforcing the role of channelopathies in different types of epilepsies (T. Jiang, Tan, Tan, & Yu, 2014). As an example, variants in *SCN1A*, *SCN2A*, or *SCN8A*, encoding three α subunits of the sodium (Na⁺) voltage-gated channel, all highly expressed in the CNS (

Figure 3), have been shown to cause different epilepsies in humans (Goldin & Escayg, 2010).

SCN1A gene, encoding the $\alpha 1$ subunit, is a good example of channelopathies. It is involved in a wide spectrum of phenotypes, associating febrile and afebrile seizures that range from genetic epilepsy with febrile seizures plus (GEFS+), afebrile seizures of different types (generalized or focal), to the Dravet syndrome, an epileptic encephalopathy.

Patients with GEFS+ have most often a good response to antiepileptic treatment and do not present cognitive decline. In contrast, Dravet syndrome (DS) is an epileptic encephalopathy with an infantile-onset that also associates febrile and afebrile seizures (Claes et al., 2001; Depienne et al., 2009a). Initial seizures are usually prolonged and triggered by fever, and from the second year of life, a pattern of multiple afebrile seizure types including focal, myoclonic, absence, and atonic seizures start to appear, while development progressively slows or declines, evolving toward intellectual disability in most cases (Dravet, 2011). Both syndromes are due to loss of function (LOF) variants, but the mutational pattern is different in the different phenotypes; in the GEFS+ syndrome, patients have only transmitted missense *SCN1A* variants, while Dravet patients carry missense or frameshift variants as well as gene rearrangements (Depienne et al., 2010) (90% among them occur *de novo*) (Depienne et al., 2009b).

Pathogenic variants in *SCN2A* are also reported in a spectrum of neurodevelopmental disorders, from benign familial neonatal-infantile seizures to epileptic encephalopathies. Up to this date, more than 300 patients with *SCN2A* variants have been published, the majority presenting with epilepsy.

Large cohort studies and variant-specific electrophysiology have allowed the description of different *SCN2A*-epilepsy phenotypes delineating the different phenotypes of *SCN2A*. Variants in *SCN2A* exert opposing effects on voltage-gated Na⁺ channel (Nav1.2) function in benign familial neonatal-infantile seizures (BFNIS), developmental and epileptic encephalopathies (DEE), and autism spectrum disorder (ASD/ID). Gain of function (GOF) variants lead to DEE (less than three months) and BFNIS, while variants associated with reduced channel activity (LOF) result in ASD/ID and later onset DEE. Moreover, the degree to which the GOF variants potentiate Nav1.2 activity differentiates DEE from BFNIS, with “severe” variants leading to DEE and “milder” variants leading to BFNIS (Ben-Shalom et al., 2017; Sanders et al., 2018).

SCN8A encodes the voltage-gated sodium channel Nav1.6, primarily expressed in excitatory neurons with high concentrations at the initial axon segment and the node of Ranvier. Pathogenic variants in *SCN8A* are associated with a large spectrum of epilepsy phenotypes ranging from mild phenotype childhood seizures with normal cognition, intermediate phenotype treatable seizures with mild ID to severe early-onset developmental and epileptic encephalopathies (K. M. Johannesen et al., 2019).

Potassium (K^+) channels play another crucial role in epilepsies, considered as the largest family of the voltage-gated cation superfamily. Compared to sodium (Na^+) and calcium (Ca^{2+}) channels, whose roles are to initiate and conduct action potentials, K^+ channels display a myriad of different types and roles such as keeping neuronal excitation within limits, control action potentials and excitability properties of neurons ([Anderson & Greenberg, 2001](#)).

The voltage-dependent K^+ (Kv) family is the largest K^+ channel family. Most monogenic K^+ channel epilepsies are related to this group. It includes 12 subfamilies (Kv1-12) composed of 40 individual α subunits (genes), 12 of which are strongly implicated in human monogenic epilepsy.

One of the long-studied genes in this category is *KCNQ2*. Its related disorders are considered the longest studied disorders, most variants have been identified in this gene to date. *KCNQ2*-related disorders showed a continuum of overlapping neonatal epileptic phenotypes caused by pathogenic variants at the heterozygous state in *KCNQ2*. The clinical features range from *KCNQ2*-related benign familial neonatal epilepsy (*KCNQ2*-BFNE) at the mild end of the clinical spectrum to *KCNQ2*-related neonatal epileptic encephalopathy (*KCNQ2*-NEE) at the severe end ([Miceli et al., 1993](#)).

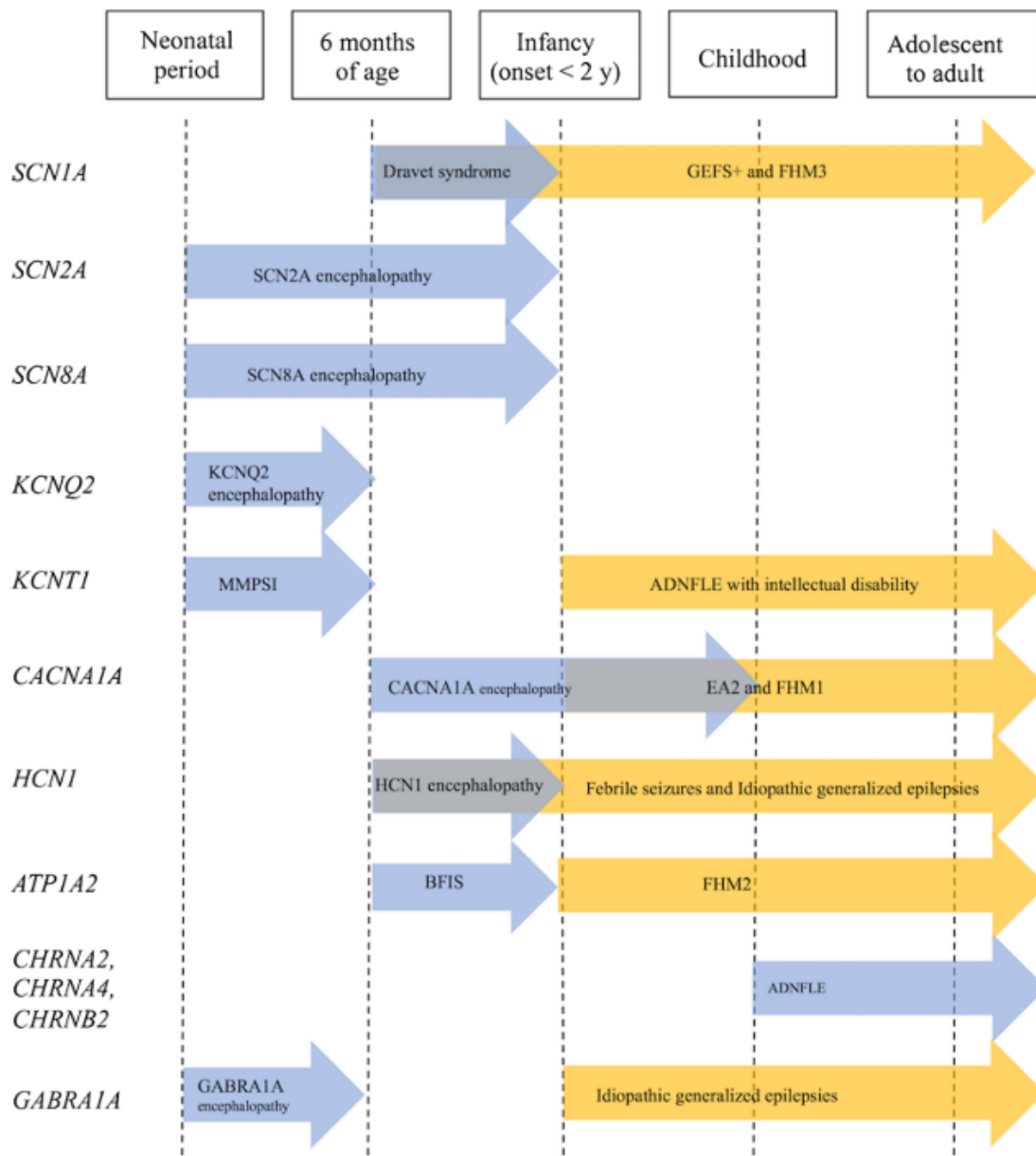


Figure 3. Age of onset & related phenotypes of brain channelopathies.

Pediatric onset in cyan, adult onset in yellow (Bartolini et al., 2020).

2. Ligand-gated channels

The first monogenic pathogenic variant to be identified in the history of epilepsy genetics was related to the Nicotinic acetylcholine receptor (nAChR). It is composed of 3 domains: the extracellular, transmembranous, and intracellular domains. The receptor functions in response to ligands that act as agonists or antagonists, causing activation or inactivation of the receptor (Rossman, 2011). Pathogenic variants in the alpha-4 subunit of the nAChR encoded by *CHRNA4* were reported in patients with autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) (N. M. Allen, Weckhuysen, Gorman, King, & Lerche, 2020; Bartolini et

al., 2020). ADNFLE also results from pathogenic GOF variants in both the alpha 2 (CHRNA2) or the beta 2 (CHRN2) subunits of the receptor. The penetrance of ADNFLE is about 70-80%. Most pathogenic variants are located in the pore-forming domain (Steinlein, Kaneko, & Hirose, 2012) (Table 1).

Pathogenic variants in *GABRG2*, the gene encoding the $\gamma 2$ gamma-aminobutyric acid receptor subtype A (GABA(A) receptor subunit, were first described in two families with epilepsy associated with febrile seizures close to GEFS+. In the first family, a K289M variant affects a highly conserved residue located in the extracellular loop between transmembrane segments M2 and M3. Furthermore, analysis of the mutated and wild-type alleles in *Xenopus Laevis* oocytes confirmed the predicted effect of the variant, resulting in a decrease in the amplitude of GABA-activated currents (Baulac et al., 2001). Another variant was described (c.245G→A nucleotide substitution in exon 2 [R43Q]) in a family where the two main phenotypes were childhood absence epilepsy (CAE) and febrile seizures (FS). Here the variant in *GABRG2* abolished the in vitro sensitivity to Diazepam, highlighting the possibility that endozepines have a physiological role in preventing seizures (Robyn H. Wallace et al., 2001).

To study the connectivity within and between the somatosensory cortex, putamen, and ventral thalamus by functional MRI (fMRI), five patients of a family with early-onset absence epilepsy and/or febrile seizures due to the *GABRG2* R43Q variant were studied vs. five age-matched controls. It was shown that there was an increased fMRI connectivity within the somatosensory cortex and between the putamen and ventral thalamus in all individuals with the *GABRG2* pathogenic variant compared with controls (Pedersen et al., 2019). Bigger cohorts are needed to emphasize this conclusion.

GABRG2 is also involved in Dravet syndrome; next-generation sequencing (NGS) was performed on 870 patients with Dravet syndrome and identified nine variants in three different GABAA receptor subunit genes (GABRs). Three of them were in *GABRG2* (c.269C>G, p. T90R; c.1025C>T, p. P342L) presented as *de novo* cases (Hernandez et al., 2021).

3. Genes with other functions

A novel class of genes belonging to the mTORC1 pathway has been involved in inherited focal epilepsies: examples are *DEPDC5* (dishevelled, Egl-10 and pleckstrin domain-containing protein 5), *NPRL2* (nitrogen permease regulator-Like 2), and *NPRL3* (nitrogen permease regulator like 3). Collectively, the three proteins form the GATOR1 complex working as an inhibitor of the mTORC1 pathway in mammalian cells (Dibbens et al., 2013).

With more than 180 unrelated families described up to now, genes of the GATOR1-mTORC1 pathway (*DEPDC5*, *NPRL2*, and *NPRL3*) are collectively the most frequently mutated genes in focal epilepsies, among which *DEPDC5* is predominantly found (85% of all cases) (Baldassari et al., 2019; Baulac, 2016) (Table 1).

Classification of genes by function	Phenotypes involved	References
Chromatin remodelling		
CHD2	EOEE, LGS, EE	(Carvill et al., 2013)(Chénier et al., 2014; Lund, Brodtkorb, Øye, Røsby, & Selmer, 2014; Suls et al., 2013)
Ion channels and neurotransmitter receptors		
GABRA1	DS, IS, JME, CAE	(Carvill et al., 2014; Cossette et al., 2002; Lachance-Touchette et al., 2011; Maljevic et al., 2006)
GABRG2	GEF+, DS	(Baulac et al., 2001),(Robyn H. Wallace et al., 2001)(Hernandez et al., 2021)
GABRB3	IS, LGS	(A. S. Allen et al., 2013)
GRIN2A	CSWS, BECTS, ABPE, EE	(Conroy et al., 2014; DeVries & Patel, 2013; Dymont et al., 2015; Lemke et al., 2013; Lesca et al., 2013; Venkateswaran et al., 2014)
GRIN2B	IS, LGS, FE/ID, ID, ASD	(A. S. Allen et al., 2013)(O’Roak et al., 2014)(Lemke et al., 2014)
HCN1	EOEE	(Nava et al., 2014)
KCNA2	EE	(Syrbe et al., 2015)
KCNQ2	BFNS, EOEE, EE	(N. M. Allen et al., 2014; Richards et al., 2004; N. A. Singh et al., 2003; Weckhuysen et al., 2013, 2012)
KCNT1	MPSI, ADFLE	(Barcia et al., 2012; Heron et al., 2012; Ishii et al., 2013; McTague et al., 2013)
CHRNA4	ADNFLE	(N. M. Allen et al., 2020)(Bartolini et al., 2020)
CHRNA2	ADNFLE	(Bartolini et al., 2020)
CHRN2	ADNFLE	(Bartolini et al., 2020)
SCN1A	GEF+, FS, DS, EE, FE	(Goldin & Escayg, 2010)(Depienne et al., 2010)
SCN2A	EE, BFNIS	(Sanders et al., 2018)(Ben-Shalom et al., 2017)

SCN8A	EE, EOEE	(de Kovel et al., 2014; Estacion et al., 2014; Ohba et al., 2014; Vaher et al., 2014; Veeramah et al., 2012)
Intracellular signalling		
GNAO1	OS, IS, EE	(Nakamura et al., 2013)(“De Novo Mutations in Synaptic Transmission Genes Including DNMT1 Cause Epileptic Encephalopathies.” 2017)
SYNGAP1	EE, ID, ASD	(Berryer et al., 2013; Carvill et al., 2013; de Ligt et al., 2012; Hamdan, Daoud, et al., 2011)
TBC1D24	MPSI, DOORS, EOEE, FE + ID, FIME, PME	(Afawi et al., 2013; Corbett et al., 2010; Falace et al., 2010; Guven & Tolun, 2013; Milh et al., 2013; Poulat et al., 2015; Stražišar, Neubauer, Paro Panjan, & Writzl, 2015)
Metabolism		
SLC13A5	EOEE	(Thevenon et al., 2014)
SLC25A22	NEESBs, MPSI, EME	(Cohen et al., 2014; Kodera et al., 2013; Molinari et al., 2009; Poduri et al., 2013)
Synaptic vesicle cycle		
STXBP1	EOEE, OS, IS, DS, EE	(Deprez et al., 2010; Hamdan, Gauthier, et al., 2011; Hamdan et al., 2009; Mastrangelo et al., 2013; Michaud et al., 2014; Otsuka et al., 2010)
SNAP25	EE	(Rohena et al., 2013)
mTOR signalling		
DEPDC5	FFEVF, ADNFLE, BECTS, FCD, HME	(Dibbens et al., 2013; Ishida et al., 2013; Lal et al., 2014; Picard et al., 2014)
MTOR	FCD	(Lim et al., 2015; Nakashima et al., 2015)

Table 1. Examples of epilepsy genes in the last decade categorized based on their function.

ABPE atypical Benign partial epilepsy, ADNFLE autosomal dominant nocturnal frontal lobe epilepsy, ASD autism spectrum disorder, BECTS benign epilepsy with centrotemporal spikes, BFNS benign familial neonatal seizures, CAE childhood absence epilepsy, CSWS continuous spike and waves during sleep, DOORS deafness, onychodystrophy, osteodystrophy, mental retardation and seizures syndrome, DS Dravet syndrome, EE epileptic encephalopathy, EME early myoclonic encephalopathy, EOEE early-onset epileptic encephalopathy, FCD Focal cortical dysplasia, FE focal epilepsy, FFEVFP familial focal epilepsy with variable foci, FIME familial infantile myoclonic epilepsy, GGE genetic generalized epilepsy, GEF+ generalized epilepsies with febrile seizures plus, HME hemimegalencephaly, ID intellectual disability, IS infantile spasms, JME juvenile myoclonic epilepsy, LGS Landau–Kleffner syndrome, MPSI migrating partial seizures of infancy, NEESBs neonatal epileptic encephalopathy with suppression bursts, OS Ohtahara syndrome, PME progressive myoclonus epilepsy.

II. Genes in GGE

GGE is classified as (i) complex forms causing sporadic cases due to common variants, (ii) polygenic forms causing sparse familial clustering determined by rare variants, or (iii) monogenic epilepsy leading to multigenerational pedigrees ([Koeleman, 2018](#)).

In all epilepsies and among relatives of all probands, the cumulative incidence of epilepsy to age 40 was 4.7%, and risk was increased 3.3-fold (95% confidence interval 2.75-5.99) compared with population incidence. However, the risk was increased to the greatest extent in relatives of probands with idiopathic generalized epilepsies (standardized incidence ratio 6.0) ([Peljto et al., 2014](#)). Very few genes are reported in monogenic forms, *GABRA1* gene, encoding the $\alpha 1$ subunit of the GABAA receptor or in the *EFHC1* gene, encoding the Myoclonin-1, though its causative role is debated (see below).

***GABRA1* and autosomal dominant JME**

Variants in genes encoding GABAA receptor subunits are a rare cause of GGE.

Variants in *GABRA1*, encoding the $\alpha 1$ subunit of the GABA(A) receptor, are involved in a phenotypic spectrum of epilepsies, including JME and photosensitive IGE. Autosomal dominant JME was the first epilepsy phenotype linked to a *GABRA1* mutation more than a decade ago. It was reported that the c.965C>A (p. Ala322Asp) variant in *GABRA1* segregated in affected individuals of a large French-Canadian family with JME. This variant was absent in controls. Compared with wild-type receptors, the mutated GABA(A) receptors show a lesser amplitude of GABA-activated currents *in vitro*, emphasizing that the seizures may result from LOF of this inhibitory ligand-gated channel ([Cossette et al., 2002](#)).

The de novo variant c.541C>T, (p. Pro181Ser) in *GABRA1* was documented in a more recent report on monozygotic twin sisters with genetic generalized epilepsy: one had juvenile absence seizures promptly responding to first-line medications, while the second developed severe treatment-refractory epilepsy with febrile, absence, atonic, and tonic-clonic seizures. This shows marked intrafamilial variability in *GABRA1*-related epilepsy ([Krenn et al., 2019](#)).

In a larger study including 16 novel probands and three at-risk relatives with disease-causing variants in the *GABRA1* gene, the phenotypic spectrum varied from unclassified epilepsy, JME,

photosensitive GGE, GEF+, to severe epileptic encephalopathies. Predominant seizure types in all patients were tonic-clonic in 56% and myoclonic seizures in 31%. EEG showed a generalized photo paroxysmal response in 37%. Four selected mutations studied functionally showed a LOF without a clear genotype-phenotype correlation ([K. Johannesen et al., 2016](#)).

In a cohort of French-Canadian families with GGE, the effects of three mutations of the *GABRA1* gene (D219N, A322D, and K353delins18X) were functionally studied. Firstly, cortical organotypic cultures from *GABRA1* (flox/flox) mice were prepared, endogenous *GABRA1* alleles were inactivated, then mutant $\alpha 1$ subunits were transfected in single glutamatergic pyramidal cells or basket GABAergic interneurons by biolistic transfection: it was found that *GABRA1*(-/-) GABAergic cells showed reduced innervation field, which was rescued by co-expressing $\alpha 1$ -A322D and $\alpha 1$ -WT but not $\alpha 1$ -D219N. Furthermore, the expression of the most severe *GABRA1* missense variant ($\alpha 1$ -A322D) induced an obvious increase of spine density in pyramidal cells along with an increase in the number of mushroom-like spines. In addition, $\alpha 1$ -A322D expression in GABAergic cells mildly increased perisomatic bouton density, whereas other mutations did not alter bouton formation. Collectively, these results suggest that the effects of different *GABRA1* variants on GABAergic bouton and dendritic spine formation are specific to the mutation and cannot always be explained by a simple LOF gene model ([Lachance-Touchette, Choudhury, Stoica, Di Cristo, & Cossette, 2014](#)).

More studies showed that the observed LOF due to reduced protein stability and surface expression of GABAA receptors could lead to a subsequent reduction of inhibitory inputs in neurons ([Cossette et al., 2002](#); [Maljevic et al., 2006](#); [Yalçın, 2012](#)) (**Table 1**).

III. EFHC1 and ICK, two debated causing genes for JME

EFHC1, encoding the Myoclonin 1, was the second gene related to AD JME. However, its responsibility was largely debated. Indeed, different studies on *EFHC1* gene in JME led to different conclusions.

Five missense *EFHC1* variants were shown segregating in 6 unrelated Mexican families with JME; these variants were absent in 382 control individuals. Overexpression of *EFHC1* in mouse hippocampal primary culture neurons induced apoptosis that was noticeably lowered by the variants ([Suzuki et al., 2004](#)).

Another study in Mexico showed that the frequency of *EFHC1* variants in 41 sporadic cases with JME was 7.3%, concluding that variants in the Myoclonin1/*EFHC1* gene are an important cause of JME in Mexican patients ([Jara-Prado et al., 2012](#)).

A study screened highly penetrant variants in *EFHC1* across multiple populations with JME: the *EFHC1* coding sequence was sequenced in 23 Hispanic JME patients and 23 patients with non-JME genetic generalized epilepsy versus 60 matched controls. Sequencing was also done for specific *EFHC1* variants in GGE cases/controls from other ethnic backgrounds: 17 African American IGE patients along with their 24 matched controls and 92 Caucasian JME patients with 103 matched controls. The identified variants were reported to be pathogenic but were also found in un-phenotyped individuals in public databases. It was found that the *EFHC1* P77T-R221H (rs149055334-rs79761183) JME haplotype was found in one Hispanic control and in two African American controls. Public databases also showed that the *EFHC1* P77T-R221H JME haplotype was present in a phenotyped West African ancestry population. It was shown that it could be found at appreciable frequency in healthy individuals with no family history of epilepsy, questioning the causality of the above variants in different populations ([Subaran, Conte, Stewart, & Greenberg, 2015](#)).

Another study on 125 individuals (n=100 with JME and n=25 with other GGEs) questioned whether testing for *EFHC1* variants should be included in the diagnostic epilepsy gene panels: *EFHC1* coding exons were amplified and sequenced, pathogenicity was predicted using the American College of Medical Genetics and Genomics (ACMG)/Association for Molecular Pathology (AMP). Variant's screening revealed 11 missense variants in 44 probands with JME (44%) and one of seven individuals with generalized tonic-clonic seizures on awakening (14%). Six of the 11 variants (54%) were classified as 'benign', and the remaining variants were considered as variants of uncertain significance (VUS). It is a suggestive evidence that *EFHC1* testing lacks a scientific foundation based on the disputed nature of the gene-disease relationship ([Gonsales et al., 2020](#)).

EFHC1 was not the only gene to be questioned. The c.914A>C (p.K305T) variant in intestinal cell kinase (*ICK*) was identified at first in a large family with JME and screened in 310 additional patients. Seven percent of the patients carried a likely pathogenic variant at the heterozygote state ([Bailey et al., 2018](#)); thus, this gene was considered responsible for autosomal dominant JME. However, the investigators of the EuroEPINOMICS-CoGIE, EpiPGX, Epi4K, and EPGP

Consortia attempted to replicate these initial findings in a cohort of 1149 familial cases of GGE versus 5911 ethnically matched controls by whole-exome sequencing (WES). No evidence of enrichment in *ICK* variant was found in GGE or JME patients compared to controls. Non-synonymous variants at a minor allele frequency (MAF) $\leq 0.1\%$ were never detected in 357 patients with JME ([Lerche, Berkovic, & Lowenstein, 2019](#)).

IV. Genes in differential diagnoses of GGE

Due to clinical overlapping, GGE can be confused with some epileptic syndromes. However, these later can be suspected when generalized seizures are refractory to treatment. For example, in subcortical band heterotopia with X-linked inheritance, women with a variant in the *DCX* gene (encoding Doublecortin) can present with normal intellect and GTCS with generalized spike and wave discharges over 2.5 Hz at EEG. In this case, MRI is vital for the diagnosis ([Gleeson et al., 2000](#); [Palmini et al., 1991](#)).

The progressive myoclonic epilepsies (PMEs) are a group of neurodegenerative disorders described by myoclonic and convulsive seizures, action myoclonus, tremor, ataxia, and often cognitive decline, with an EEG showing generalized spike and waves. The most known cause of PME is Unverricht-Lundborg disease (ULD), an autosomal recessive disease caused by variants in the *CSTB* gene encoding cystatin B. In the North-European population, the phenotype is always severe with cognitive decline, in the Mediterranean basin its different, the clinical presentation of ULD can imitate JME with GTCS, ataxia and action myoclonus appear later, leading to the diagnosis of ULD. In rare and mild cases of ULD, the clinical picture can continue to mimic JME until at least the middle age of life ([Kälviäinen, 2015](#); [Pennacchio et al., 1996](#)).

V. GGE as a complex epilepsy

Most if not all human traits are influenced by genetic variation. In general, determining the impact of genetic variation versus the environment covers a wide range of effects. Inspired by the term "fog of war" to describe ambiguity in military operations, "the fog of genetics" came as an expression of the uncertainty in genetics as we try to understand the genetic basis of human traits and diseases. It is evident that we are missing a lot of important information.

Most human traits and diseases have a complex non-mendelian genetic design; the path is clearer for scientists to detect genetic variants with high penetrance and strong, early-onset phenotypes.

For most phenotypes, a lot of significant elements of heritability are missed, namely contributions from unknown genetic variants that can explain the variation of a phenotype (Magalhães & Wang, 2019; McClellan & King, 2010).

The presence of familial aggregation, the mono- versus dizygotic studies (Kjeldsen, Corey, Christensen, & Friis, 2003), the absence of obvious cause sustains an underlying genetic cause for epilepsy. It is particularly challenging in common epilepsies like GGE, as little is known about its genetic architecture.

Extensive efforts have been made to identify susceptibility variants involved in GGE. We can delineate three eras defined by experimental approach and corresponding molecular tools.

In the first era (1997-2012), the gene candidate approach was applied to familial forms or sporadic cases. Very few variants in *GRIK1* (Glutamate receptor, ionotropic, kainate 1), *GABRB3* (gamma-aminobutyric acid type A receptor subunit beta 3), *KCNQ2*, and *KCNQ3* (potassium voltage-gated channel subfamily Q member 2 or 3) reached statistical significance (T. Sander, Hildmann, et al., 1997; Urak, Feucht, Fathi, Hornik, & Fuchs, 2006), though these results were not reproducible.

In the second era of Genome-Wide Association Study (GWAS), including thousands of patients and controls (from 2012 to 2014), GGE was associated with some SNPs in the vicinity of genes: in 1q43 (*CHRM3*) (253), 2p16.1 (*VRK2*; *FANCL*), 2q23 (*ZEB2*), 2q24.3 (*SCN1A*), 4p15 (*PCDH7*) and 17q21.32 (*PNPO*). Some genes, including *SCN1A* were good candidates by function (Epilepsy-austin@unimelb.edu.au, 2014), again, these results were not reproducible.

Finally, the advent of WES allowed performing association studies on rare variants: the European Epi4K consortium in 2017 (641 patients/3877 controls) (A. S. Allen et al., 2017), the EPI25 international consortium in 2019 (3108 sporadic cases/8300 controls) (Berkovic & Neale, 2019) and in 2021 (5048 sporadic cases/14100 controls) (Motelow et al., 2021). At a single gene level, these studies failed to reach any statistical significance; however, they supported the hypotheses that GGE (i) are genetically highly heterogeneous, (ii) involve rare or ultra-rare variants, and (iii) are determined by an oligogenic mode of inheritance. They showed that GGE patients have an excess of ultra-rare, deleterious variants in

constrained genes and in genes previously associated with epilepsy. Moreover, inhibitory GABA(A) receptor genes, as well as cation channels, were enriched for missense variants in GGE compared to controls, whereas no enrichment was seen in excitatory receptors (Berkovic & Neale, 2019).

In a complementary approach, our team, in collaboration with Pr Riadh Gouider's group (Service of neurology, Razi hospital, Tunis), studied 30 Tunisian GGE families using a targeted panel of 30 candidate genes for GGE. We suggested the oligogenicity of GGE in this population and the involvement of *CACNA1H* and *MAST4* in GGE (Landoulsi et al., 2018a) (Figure 4) is a demonstrative example of monogenic versus oligogenic approaches.

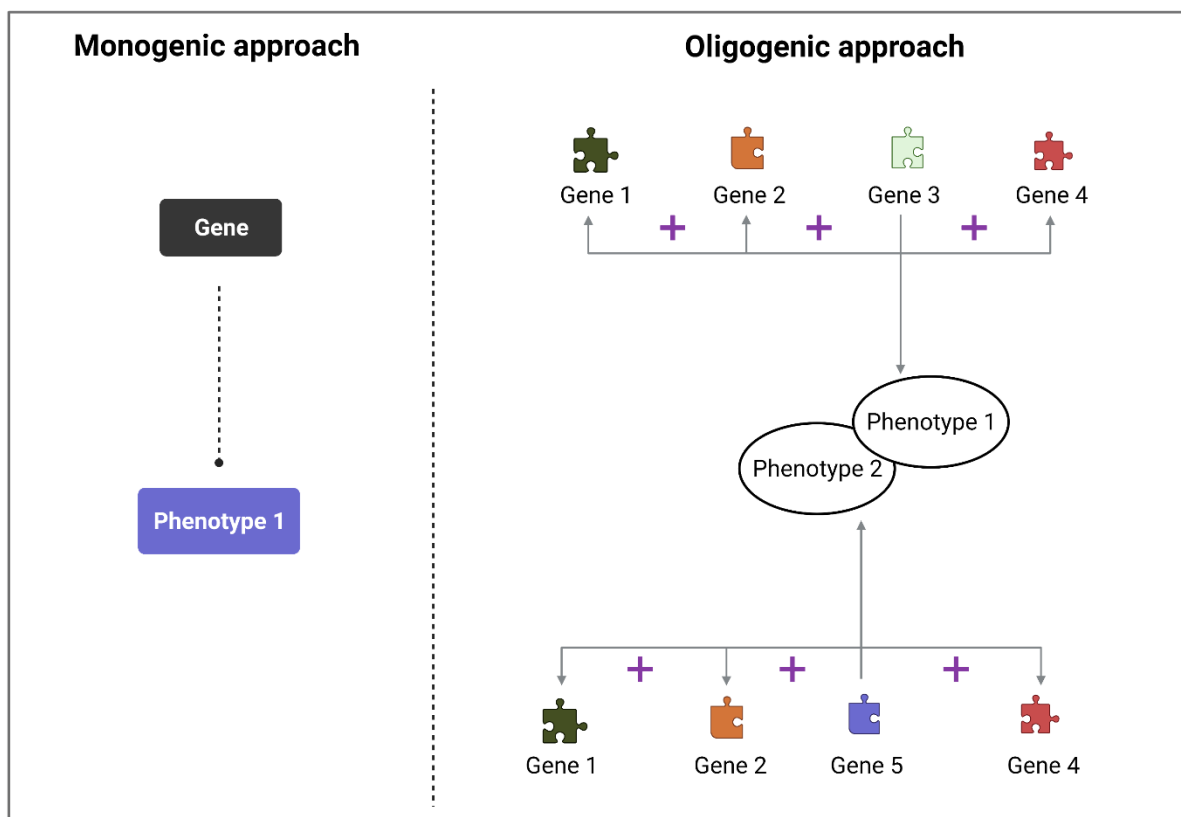


Figure 4. Monogenic versus oligogenic epilepsies

Monogenic epilepsies are caused by one gene; in oligogenic epilepsies, different genes interact to solve the puzzle of the different phenotypes.

Chapter 5: Functional models in genetic generalized epilepsies

Epilepsy has been studied and modelled in a wide variety of *in vitro* and *in vivo* models. No single animal model of epilepsy fully represents this disease, given the wide range of conditions and phenotypes. In general, an animal model of epilepsy refers to an induced or inherited propensity for developing seizures.

Seizures can be observed as a result of genetic manipulation in nearly every animal with a nervous system, and as a consequence, the potential range is huge (**Figure 5**). Ideally, these animals should be able to demonstrate the causal mechanism(s) underlying the disease in humans, the phenotypic features of the human condition, and the treatment responses. Theoretically, the perfect model would satisfy all three criteria, though every single model demonstrates some limitations (Oliva et al., 2014). In 1869, John Hughlings Jackson reported the spontaneously arising seizures in dogs, followed by the studies of David Ferrier on rabbits, Guinea pigs, cats, and dogs showing that the stimulation of the cortex in these mammals generates clonic seizures resembling humans (Ferrier, 1873).

In the late seventies, a non-human primate animal (the baboon) model for generalized photosensitive epilepsy emerged. This animal shows generalized interictal epileptic discharges (IEDs) activated by sleep and rare generalized myoclonic or tonic-clonic seizures. A second observation was that intermittent flashing lights activated interictal discharges (photo paroxysmal response) or seizures (photo convulsive response) in the baboon. Photosensitivity in noncaptive baboons varies according to the region of origin, being detected in the Casamance region in Senegal, in about 60% of the animals (Szabó et al., 2005).

In the early eighties, the well-known/well-studied mouse model, the tottering model (tg), was described. The tg mutant strain, due to a spontaneous pathogenic variant in *Cacna1a* encoding the $\alpha 1$ subunit, is one of a few recognized models for absence epilepsy. The recessive ataxic tottering-6j mice had a base substitution (C-to-A) in the consensus splice acceptor sequence linked to exon 5 of the $\alpha 1$ subunit of the Cav2.1 channel gene, resulting in the skipping of exon five and deletion of part of the S4-S5 linker, S5, and part of the S5-S6 linker in domain I of the $\alpha 1$ subunit of the Cav2.1 channel (T. Y. Kim et al., 2015). Tottering seizures are notably similar to human absence epilepsy: the mouse reveals the characteristic behavioral arrest, assuming a fixed staring posture. Normal electroencephalographic

recordings were reported except during the frequent, spontaneous seizures when generalized bilateral spike and wave discharges were reported. Tg mice are responsive to anti-absence drugs (Frankel, Mahaffey, McGarr, Beyer, & Letts, 2014; Levitt & Noebels, 1981). Three additional genes, encoding $\beta 4$, $\alpha 2\delta 2$, and $\gamma 2$ subunits of voltage-gated calcium channels cause absence seizures in other spontaneous AR mouse models: *Cacnb4* in lethargic (lh); *Cacna2d2* in ducky (du) and *Cacng2* in stargazer (stg) lines (Everett et al., 2007).

In the nineties: other molecular mechanisms were reported in rats and mice. Examples are the altered biophysical properties of T-type calcium channels in the genetic absence epilepsy rat of Strasbourg (GAERS) model; the changes in the subunit composition of GABA_A receptors in the GAERS model, and the increased numbers of GABA_B receptors in the lethargic mouse (lh/lh mouse) model. In generalized convulsive seizures, neuronal populations within the inferior and superior colliculi have a role in regulating seizures in the genetic epilepsy-prone rat (GEPR) model, and subpopulations within the substantia nigra pars reticulata (SNR) appear to regulate seizures in the fluorothyl model (Hosford, 1995).

In 2005, another mouse model for absence epilepsy (AE) was developed to study mutations in the GABA receptor gamma 2. To better understand the molecular basis of AE in patients, a mouse model was generated, harboring a gamma two subunit point variant (R43Q) (found in a large Australian family). Mice heterozygous for the mutation showed behavioral arrest associated with 6-to-7 Hz spike-and-wave discharges, blocked by Ethosuximide (T. H. Kim, Reid, & Petrou, 2015; Tan et al., 2007).

In 2009, a novel mechanism of AE was identified, with the discovery of absence seizures given by several mouse mutant alleles of the *SCN8a* gene, encoding the voltage-gated sodium channel NaV1.6 (Papale et al., 2009). NaV1.6 is implicated in many mouse models of AE, including a missense mutation identified in a chemical mutagenesis screen (*SCN8a* V929F) (Oliva et al., 2014).

To tap on the complexity of generalized epilepsies and the effect of modifier genes, another model emerged in 2014. *Gria4* deficient mice emerged as a model of AE, one for which the common laboratory inbred strain C3H/HeJ (HeJ) harbors a natural intracisternal A-particle retrotransposon (IAP) insertion in *Gria4* that reduces its expression 8-fold. Between C3H and non-seizing strains such as C57BL/6, genetic modifiers change disease severity. By creating

new *Pcnx12* frameshift alleles using TALEN mutagenesis, it was shown that *Pcnx12* deficiency is accountable for mitigating the seizure phenotype – making *Pcnx12* the first known modifier gene for AE. This finding emphasized the genetic complexity between strains, directing for the use of another C3H substrain to map additional modifiers, including validation of a Chr 15 locus that dramatically affects the severity of SWD episodes (Frankel et al., 2014).

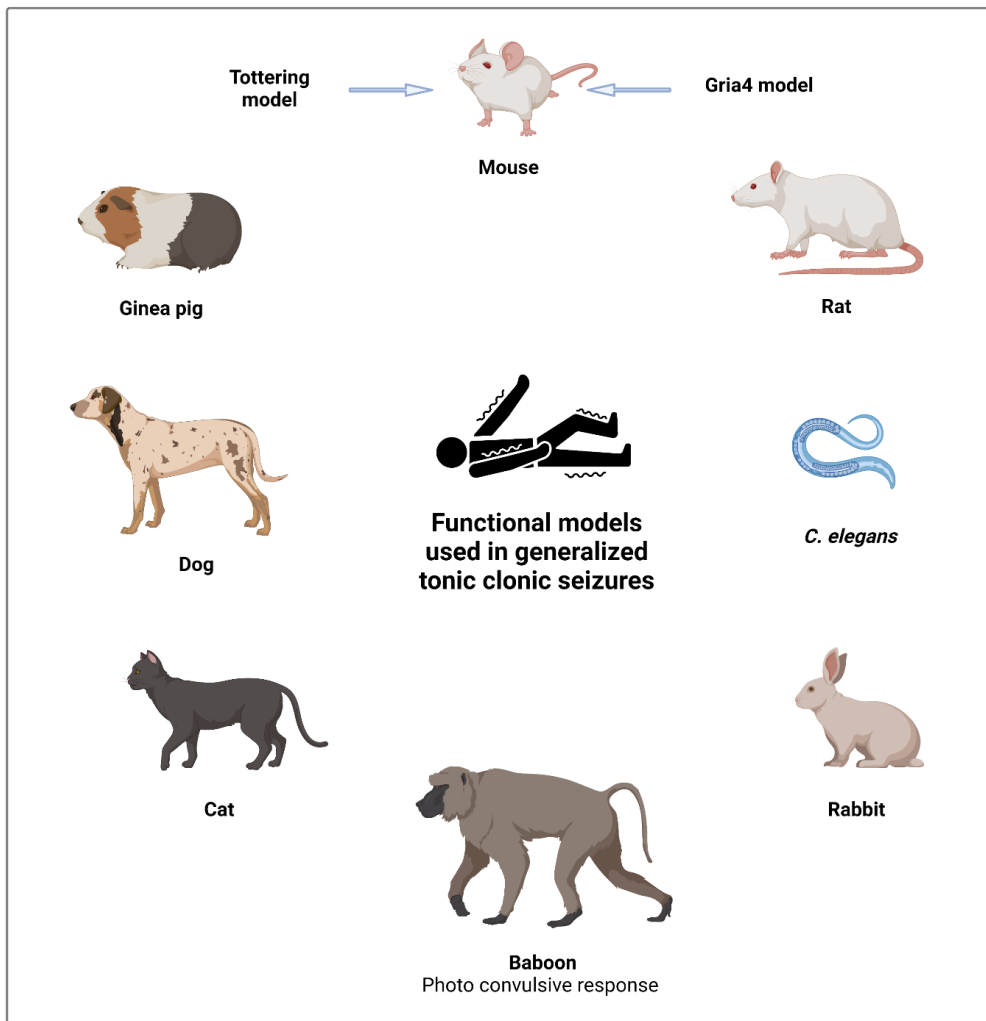


Figure 5. Different animal models used in genetic generalized epilepsy studies.

I. General remarks on *C. elegans* as a model organism

In 1963, Sydney Brenner wrote:

"Classical problems of molecular biology have either been solved or will be solved in the next decade" and proposed that there will be a dramatic improvement in the field "notably the development and the nervous system" by exploring the tiny roundworm *C. elegans* (Corsi, Wightman, & Chalfie, 2015a).

The non-parasitic nematode *C. elegans* is one of the most attractive animal models contributing significantly to understanding many human diseases. It offers the value of a whole-animal model yet the simplicity of single cells. It has many advantages: small size and transparent body (adults are approximately 1.5 mm in length and 80 μm in diameter), short life cycle (approximately three days at 20°C), ability to self-fertilize, and high reproductive rate (>300 offspring per hermaphrodite).

C. elegans has different biological systems like the muscles, intestine, hypodermis, gonads, and a nervous system of 302 neurons in hermaphrodites. *C. elegans* lab assays are fast and of low cost, amenable to high-throughput analysis, and genetically tractable with a fully sequenced genome (Jeffrey R. Wozniak, Ph.D., Edward P. Riley, Ph.D., Michael E. Charness, 2019; Jeong, Lee, & Lee, 2018; Johnson, Jenn, Barclay, Burgoyne, & Morgan, 2010; Markaki & Tavernarakis, 2020).

All the connections in the nervous system are well mapped/described (White, Southgate, Thomson, & Brenner, 1986). It has a transparent body allowing for different tools like calcium imaging (Kerr et al., 2000), optogenetics, and fluorescent-based techniques. Together, these tools allowed examining single neurons as well as the whole nervous system (Richards et al., 2018) (Figure 6).

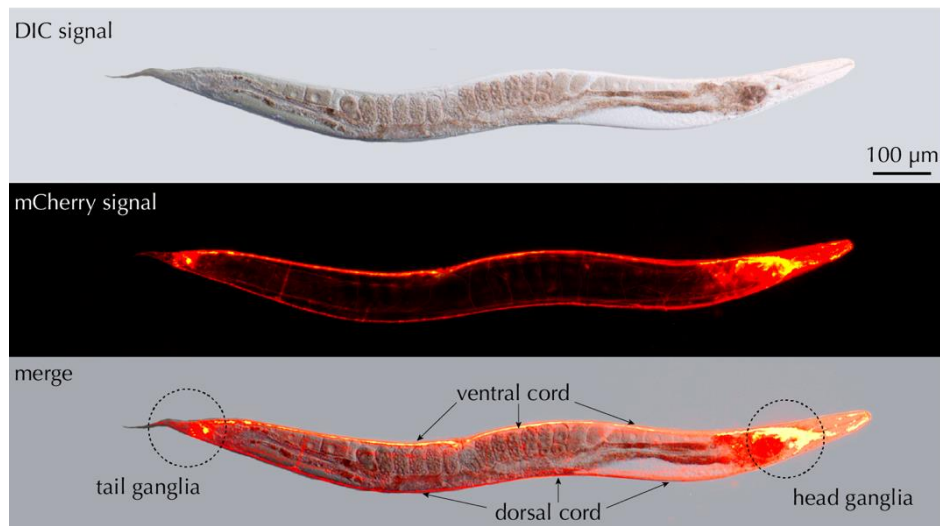


Figure 6. The nervous system of the *C. elegans*.

The picture represents a young adult animal expressing, with the *unc-119* regulatory sequence, the mCherry protein in the entire nervous system. Images were taken using a fluorescent macroscope (*Nikon*) with a x5 objective.

The entire network encloses less than 10,000 chemical synapses, containing 1500 neuromuscular junctions (NMJs), and about 800 gap junctions (Mulcahy et al., 2018). This anatomical simplicity contrasts with the complexity of the molecular repertoire expressed in the nervous system. Though *C. elegans* contains 108 times lesser neurons than humans, its genome encloses about 22 000 genes, highly comparable with the human gene content (Hobert, 2013).

C. elegans was the first multicellular organism to have its genome entirely sequenced. Its protein-coding genome is well conserved, presenting approximately 40% of all orthologs and paralogs between *C. elegans* and humans (“Genome sequence of the nematode *C. elegans*: a platform for investigating biology,” 1998; W. Kim, Underwood, Greenwald, & Shaye, 2018; Shaye & Greenwald, 2011). Conserved gene systems include the neurotransmitter biosynthetic enzymes, synaptic release mechanisms, and neurotransmitter receptors. Most ion channels and second messenger pathways are also well conserved. Voltage-gated potassium and calcium channels are abundant, though the worm lacks the gene for the voltage-activated sodium channel (Bargmann, 1998).

Mapping of the GABAergic system by anti-GABA immunostaining recognized 26 neurons in the *C. elegans* nervous system (**Figure 7**): 19 motoneurons (D-class) establish NMJs on body-wall muscles, four motoneurons (RMEs) control head muscles, two neurons (AVL and DVB) innervate intestinal muscles and the interneuron RIS ([McIntire, Reimer, Schuske, Edwards, & Jorgensen, 1997](#)).

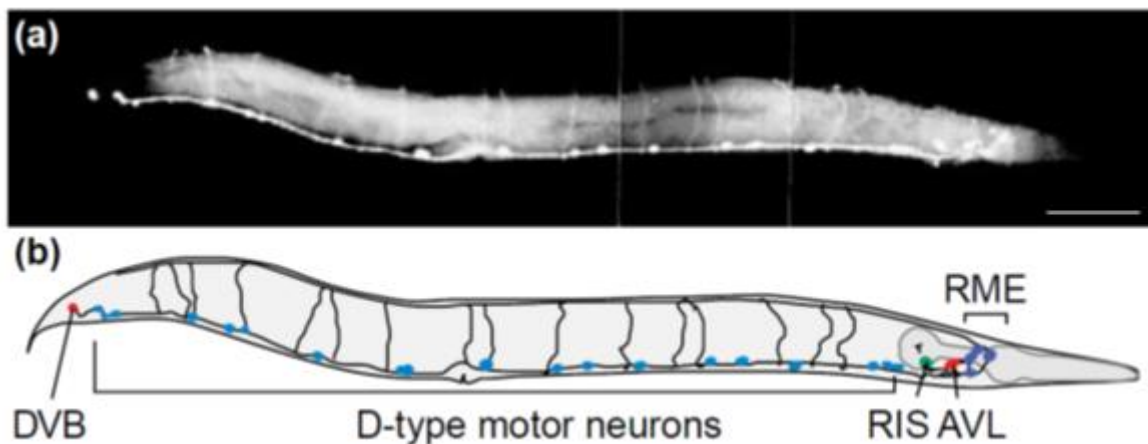


Figure 7. GABAergic neurons in the *C. elegans*.

GABA nervous system. **(a)** Fluorescent micrograph of an adult wild-type *Caenorhabditis elegans* hermaphrodite stained with antiserum raised against GABA. Twenty-six neurons stain for GABA. Anterior is to the right, and the right side of the body is shown. Scale bar, 0.1 mm. **(b)** Schematic drawing of the positions of the 26 GABA-containing neurons in the animal above. The neurons are color-coded based on their synaptic outputs: the D-type DD and VD motor neurons innervate the body muscles; RME neurons innervate the head muscles; AVL and DVB innervate the enteric muscles, and RIS is an interneuron. ([Schuske, Beg, & Jorgensen, 2004](#)).

II. *C. elegans* as a model in different neurological diseases

C. elegans is used as a model in different neurodegenerative diseases with progressive loss of structure/function of neurons, such as amyotrophic lateral sclerosis, Parkinson's, and Huntington's diseases (ALS, PD, and HD, respectively) in which proteinopathies are involved ([Alexander, Marfil, & Li, 2014](#); [Corsi, Wightman, & Chalfie, 2015b](#); [Peng, Trojanowski, & Lee, 2020](#)).

In PD, it provides several advantages as an *in vivo* model. The most meaningful benefits include the vast array of genetic tools available and the ability to screen for disease modifiers speedily and cost-effectively ([Ma et al., 2018](#)). In parallel, the main shortcomings are (i) the absence of a homolog of *SNCA*, the gene coding to the α -synuclein with its important role in PD ([Ma et](#)

al., 2018), (ii) the challenge of performing molecular biology precisely on dopamine neurons (which make up only 8 of 959 cells), and the fact that neuronal connectivity diverges from humans. With all these shortcomings in mind, the model system has facilitated numerous major contributions to the understanding of PD (Cooper & Van Raamsdonk, 2018).

ALS is another degenerative disease affecting motor neurons resulting in progressive muscle weakness. Among its familial inheritance (10% of the cases) is the *SOD1* gene (coding for the superoxide dismutase) (Al-Chalabi, van den Berg, & Veldink, 2017). *SOD1* gene mutation is present in around 2% of families presenting with familial amyotrophic lateral sclerosis (FALS) (Van Damme, Robberecht, & Van Den Bosch, 2017). *C. elegans* is also used as a model of ALS in co-expressing mutant human *SOD1* and green fluorescent proteins (GFPs) in muscle tissues (Oeda et al., 2001). The model facilitated studying oxidative stress in these genes and the role of autophagy in ALS (J. Li, Huang, & Le, 2013).

Moreover, *C. elegans* has been utilized to model AD, characterized by the presence of extra-neuronal deposits of insoluble β -amyloid peptide ($A\beta$) and of tau-associated neurofibrillary tangles in the brain (Goedert, 2015; Hardy & Selkoe, 2002; Link, 1995; Lu et al., 2014). The contribution of the causative neurotoxic peptides, amyloid- β , and tau, to disease-like neurodegeneration, was clearly demonstrated in *C. elegans*. Through its technological advances, the model expanded the possibilities for intervention by offering researchers the possibility of defining functional modifiers of neurodegeneration, a vital step in the discovery of improved therapies for AD (Griffin, Caldwell, & Caldwell, 2017).

HD is another example, characterized by a progressive decline in motor control and cognition associated with aging. It is an autosomal-dominant disorder affecting the *IT15* gene coding for the Huntingtin (HTT) protein with an increase of polyglutamine repeats in the N-terminus. Many models expressing various mutated HTT were developed in *C. elegans* (Nollen et al., 2004; Satyal et al., 2000). These different transgenic lines were used to study the cellular mechanisms that play a role in the toxicity and aggregation processes. Neuroprotective roles against polyQ neurotoxicity were described in signalling pathways involving the DAF-16, a homolog for FOXO (transcription factor family) (Cordeiro et al., 2020; Tauffenberger, Chitramuthu, Bateman, Bennett, & Parker, 2013).

The above-mentioned examples showed the diversity in using *C. elegans* in neurodegenerative diseases; fewer studies were done on *C. elegans* in epilepsy.

III. *C. elegans* in epilepsy

In 2004, the emergence of epileptic-like convulsions was described in association with cortical malformations. In humans, variants in the *LIS1* gene lead to a disorganized and smooth cerebral cortex caused by a failure in neuronal migration leading to lissencephaly. Among the clinical consequences of lissencephaly are mental retardation and intractable epilepsy. The nematode *C. elegans* provides a chance to study such convulsions in an animal with a defined nervous system. A *lis-1* allele (*pnm-1*) mutation-induced convulsions mimicking epilepsy were studied in combination with a chemical antagonist - pentylentetrazole PTZ - of GABA neurotransmitter signalling. Identical convulsions were attained using *C. elegans* mutants defective in GABA transmission, putting in mind that none of these mutants or the antagonist alone caused convulsions, indicating a threshold was exceeded in response to this combination. The phenotype associated with convulsions was described as anterior head bobbing and posterior immobilization, detected in various GABA mutants, including alleles of a GABA synthesis gene *unc-25*(e156), GABA vesicular transporters *unc-46*(e177), *unc-47*(e307), and the GABAA receptor *unc-49*(e407). A comparable phenotype was detected in *pnm-1*(*LIS-1* allele) (Williams, Locke, Braden, Caldwell, & Caldwell, 2004b).

Head bobbing phenotype was also described in the *EFHC-1* gene, a gene potentially linked to JME, (chapter 4). It was shown that *EFHC-1* functions within specialized non-motile mechanosensory cilia, where it regulates neuronal activation and dopamine signalling. Furthermore, *EFHC-1* is also localized at the synapse, where it modulates dopamine signalling in cooperation with the orthologue of an R-type voltage-gated calcium channel. It was concluded that there was a dual-regulation of neuronal excitability at sites of neuronal sensory input (cilium) and neuronal output (synapse). Such a distributed regulatory mechanism may be vital for establishing neuronal activation thresholds under physiological conditions, and when disrupted, may embody a novel pathomechanism for epilepsy (Loucks et al., 2019).

Another group focused on the cGMP/protein kinase G (PKG) pathway, showing that increased PKG activity decreased seizure duration in *C. elegans* utilizing a recently developed

electroconvulsive seizure assay. In this study, it was shown that treating worms with either a potassium channel opener (Retigabine) or published pharmaceuticals that increase PKG activity significantly reduces seizure recovery time. The study suggested that PKG signalling modulates downstream K⁺ channel conductance to control seizure recovery time in *C. elegans* (Breiding, 2014).

In another study on the *STXBP1* gene, a gene involved in a variety of infantile epilepsy syndromes, an in vivo model was developed to test the pathogenicity of different variants in a cost-effective manner. The CRISPR-Cas9 method was used to introduce a null mutation into the *unc-18* gene (the *C. elegans* orthologue of *STXBP1*), creating a paralyzed worm strain. A rescue was then introduced with transgenes encoding the human *STXBP1*/Munc18-1 protein. Behavioral tests, electrophysiological, and biochemical approaches were then used to reach a conclusion about the different variants (Zhu et al., 2020).

C. elegans was also used as high-throughput AEDs screening, and in parallel, to have a better understanding of the mechanism of action of the AEDs. Head-bobbing convulsions induced by PTZ were observed in liquid-based incubation. It was shown that convulsions induced within minutes of PTZ exposure to *unc-49* mutants were strongly inhibited by the AED Ethosuximide. This protective activity was independent of ethosuximide's implied target, the T-type calcium channel, as a null mutation in the worm *cca-1* ortholog did not affect Ethosuximide's anticonvulsant action (S. Q. Wong et al., 2018).

In our research, we combined different techniques like RNA interference (RNAi) and CRISPR-Cas9 to test different variants in familial generalized epilepsies.

Aims of the thesis

My doctoral project presented in this dissertation is focused on the genetic characterization of generalized epilepsies in a Sudanese cohort that consisted of 20 Sudanese families with a consanguinity rate of 60%. They were from inbred communities from Al Jazira, the White Nile region, the central and northern region of Sudan. In some non-consanguineous families, the parents were not related, but consanguinity existed in maternal/paternal branches (**Figure 8, Table 2**).

Aim 1

So far, studies on large international cohorts have pointed to the fact that GGEs have a complex mode of inheritance. Few variants have been reported in likely susceptibility genes for GGE, mostly in non-African populations, though, to our knowledge, no studies were performed in the Sudanese population.

We investigated 20 Sudanese families, including 54 individuals, by WES in order to identify susceptibility variants responsible for GGE. We will analyze the characteristics and the segregation in families of the candidate variants and discuss the mode of inheritance in these familial forms.

Aim 2

The second objective is to find a suitable functional model for developing countries as genetically engineered rodents are extensively used as models for epilepsy. *C. elegans* could be considered as the first step of a multi-level functional approach, consisting of screening numerous candidate variants generated by our genetics studies. We tested this model for two rare missense variants identified both at the homozygous state in a large Tunisian sibling with CAE. Indeed, it is conceivable to study the consequences of combinations of variants in *C. elegans* since double or triple KD or KI can be done, especially by CRISPR-Cas9 technology.

Table 2. Names of the regions in Sudan from which the families originated.

Family	Region in Sudan
F20	North state Dongula
F2	Khartoum
F6	Gadarif
F7	Elobeid Kurdufan North
F3	Khartoum
F8	Aljazera
F9	Aljazera
F10	Aljazera
F11	White Nile
F12	Obeid Kurdufan North
F13	Alshimalya
F4	Atbara
F1	Aljazera
F14	River Nile state Almahmya
F15	Aldiaain west
F16	Alshimalya
F17	Alkhujalab Khartoum
F5	Aljazeera
F19	Khartoum
F18	Aljazeera

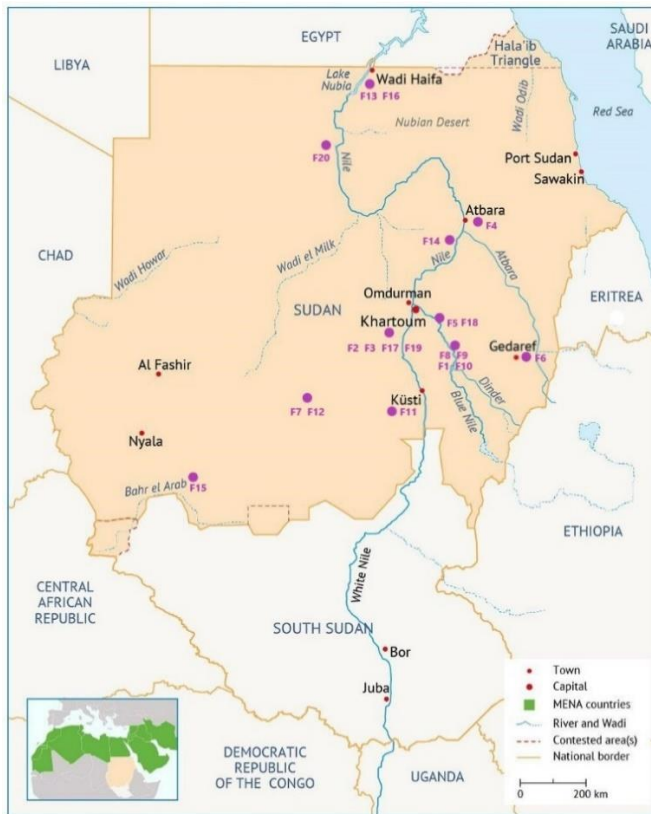


Figure 8. Distribution of Sudanese families in different regions of Sudan.



Materials and methods

Chapter 6: Materials and methods of the genetic data

The materials and methods section will be divided into two parts, referring to the genetic part and the *C. elegans* part of my thesis work. For a whole overview of the genetic part, please refer to **Figure 9**.

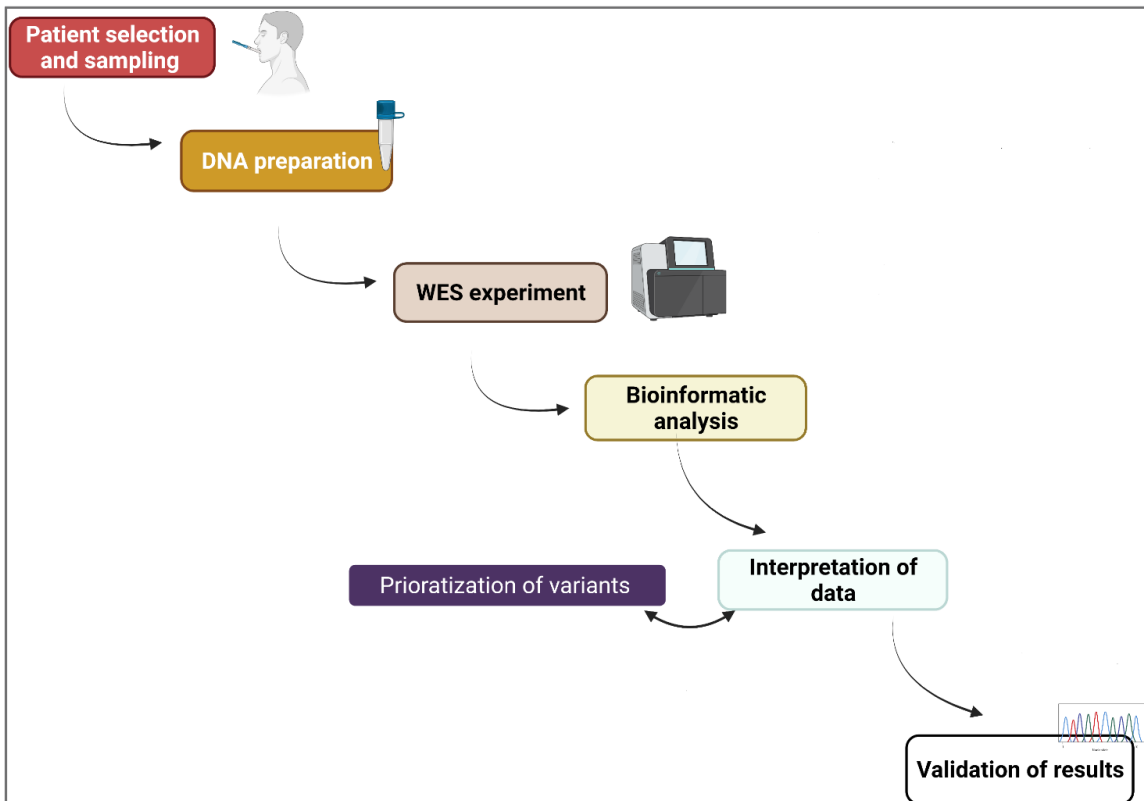


Figure 9. Schematic representation of the method of collection/analysis of the samples.

I. Dealing with patients

1. Ethics

This study was prospectively reviewed and approved by the national health research ethics committee, Federal ministry of health, Sudan (1-4-18). Written informed consent was given by all participants. For patients less than 18 years, informed consent was obtained from a parent/a legal guardian.

2. Subject's recruitment

We investigated 20 Sudanese families, including 54 individuals. In each family, we recruited the proband (n=20) along with all approachable affected family members (n=34) and available asymptomatic relatives (n=84). Inclusion criteria for patients were a clinical presentation of generalized epilepsies with a positive family history of epilepsy. Patients with focal seizures or epileptic encephalopathies were excluded.

Patients were examined and diagnosed by the referring consultant neurologists/neuro-pediatricians, followed by a second standardized phenotyping by clinicians of the research team (EM, AM). Healthy relatives were examined to exclude subtle phenotypes.

An electroencephalogram (EEG) was performed for at least one patient per family.

3. Recruitment method

Recruitment was done either through a direct referral of the patients from the neurologists or (in most cases) by screening the library of EEGs in Professor Mohamed Salah Almajzoub's clinic and contacting the patients and their clinicians for further information.

4. Clinical phenotyping

Patients were clinically examined and diagnosed by the referring consultant neurologist/neuro-pediatrician. Then, we performed a second examination and filled a questionnaire. One of the significant challenges we faced was the stigma against epilepsy in some families living in rural areas. Therefore, counseling the families by phone was a vital initial step to explain the nature of our research.

A. History

Points covered in the history include:

- Personal data, including contact details and origin/tribe.
- Family history of medical disorders, epilepsy, or other neurological or non-neurological disorders.

- A detailed description of the family (pedigree obtained).
- A detailed history (mainly from the patient's relatives as witnesses) of the disease from the age at onset till the date of examination.

B. Examination

General and neurological assessment included higher functions, UMN (upper motor neuron), LMN (lower motor neuron) and gait examination, extrapyramidal signs, and any type of deformity.

C. Investigation

EEG was performed in at least one patient per family in all families. MRI was done in few patients.

D. Sampling

Saliva (2 mL) samples were collected using Oragene Discover DNA collection kits (DNA Genotek Inc., Ottawa, ON, Canada), and genomic DNA was purified using the prepIT.L2P kit (DNA Genotek Inc., Ottawa, ON, Canada) following the protocol provided by the manufacturer. DNA quality and quantity control were done using standard agarose gel electrophoresis, NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA), and Qubit® fluorometer (Thermo Scientific, Wilmington, DE, USA).

II. Laboratory protocols

1. Standard agarose gel electrophoresis

Agarose gel is used for quality checking of genomic DNA to assess its integrity and exclude its degradation. It was also used to separate and size PCR products according to their precise molecular weight.

Preparation of an agarose gel concentration (0.8 to 2%) depends on the targeted DNA. The gel was prepared using a standard method: heating 0.8-2 grams of agarose powder dissolved in 100 mL of (0.5-1x) TBE (Tris/Boris/EDTA) buffer solution in a microwave oven for 2-3 minutes.

Then, 3 μL (concentration= 10 mg/mL in H_2O) of an intercalating agent as ethidium bromide was added under the safety hood after cooling the solution to less than 60°C using adequate laboratory practice guidelines for safety use of biohazardous substances. The DNA samples are loaded together with a graded band size ladder into the sample wells, the gel is run at a voltage and for a time period that will achieve optimal separation (20 to 30 minutes at 120 mV voltage). Visualization of gel under UV illuminator and capture of necessary images was done.

2. Primer design, PCR amplification, and Sanger sequencing

Primer design

Specific primers were designed using Primer-3Plus software. The sequence as an input was obtained from Ensembl genome browser; the sequence is around 18 to 25 base pairs (bp). Primer specificity was tested using the BLAST tool of the genome browsers (UCSC and NCBI). *In silico* PCR tool was also used (UCSC genome browser). Oligo calc was used to assess the GC concentration, potential hairpin formation, and self-annealing. Primers are designed in a region without frequent variants.

PCR

Amplification of regions in specific genes was performed using a classical polymerase chain reaction (PCR) procedure that included preparation of a master mix composed of a buffer, dNTPs, Taq polymerase, specific primers (forward/F and reverse/R), H_2O , and the DNA template (patient DNA sample). The touchdown protocol is composed of three steps:

1. Denaturation ($94\text{-}95^\circ\text{C}$).
2. Hybridization (annealing) (range including the melting temperature (T_m) of the primer, typically 54°C to 65°C).
3. Extension (synthesis) (72°C).

Touchdown is a flexible way of getting the DNA product; it is used in the following way:

1. The first cycles are performed at high annealing temperature to tolerate the specificity of the polymerization.
2. It then declines gradually to cover a broader range of annealing temperatures.

ExoSAP purification

ExoSap purification is an enzymatic purification method used to clean the PCR product from primers and dNTPs to exclude background noise in the result obtained. After mixing of specific buffers and the two enzymes: the mix was added to the PCR product to attain a final concentration of the exonuclease I (EI) (20 U/ μ L) and the alkaline phosphatase (AP) (1 U/ μ L), the product was then incubated for 15 min at 37°C (to activate the enzyme) then at 80°C for 5 min (to inactivate the enzyme).

EI role is to remove single-stranded primers, AP role is to remove the phosphates of the remaining dNTPs.

Sanger sequencing

Sanger sequencing is a method for determining the nucleotide sequence of DNA. It is the gold standard experiment to validate and confirm the segregation of variants within families.

It is performed at the GATC® platform. One sense primer solution (forward or reverse) at 5 pM concentration combined with a diluted PCR product/sample are prepared to be sent to the external facility. Sequencher 4.9 is then used to interpret the results.

3. Whole Exome Sequencing, next generation sequencing

Whole-exome sequencing (WES) is a targeted approach focusing on the protein-coding sequence of the genome, and is a highly successful strategy to identify genetic changes in epilepsy.

1. Genomic DNA preparation and fragmentation:

40 DNA samples from patients were prepared, a starting quantity of DNA of 1 μ g (50 μ L at a concentration of 20 ng/ μ L) is used as recommended by Roche NimbleGen. DNA quality is tested using Nanodrop® and gel electrophoresis. Samples were subjected to a PCR test before library preparation to exclude the presence of any PCR inhibitors. The genomic DNA fragmentation was performed using a Covaris shearing instrument.

2. End repair:

Both 5' and 3' ends of the fragmented double-stranded DNA (dsDNA) were turned into blunt ends with the use of Kapa® end repair master mix.

3. A-tailing:

Addition of a stretch of dAMP to the 3' end of the amplified and cleaned dsDNA fragments is performed using a Kapa® A-tailing buffer and enzyme in a thermocycler-based step followed by a Solid Phase Reversible Immobilization (SPRI) cleaning step. This kit employs a highly efficient PCR cleaning system that utilizes the SPRI magnetic-based technology with the help of paramagnetic beads. The cleaning system allows easy removal of contaminants as primers, primer-dimers, salts, and dNTPs to leave only the proper amplified dsDNA fragments.

4. Adapter ligation:

SeqCap® adapter Kit A and B (with 12 adapters each) allow indexing of the 40 samples with 40 different adapters.

5. Dual-SPRI size selection:

Fragments with sizes between 250-450 bp are selected.

6. Capture steps:

i. Pre-captured ligation-mediated LM-PCR is followed by SPRI cleaning step.

ii. Measurement of the product using NanoDrop® spectrophotometer/Qubit® fluorometer. It is run in Caliper® Bioanalyzer DNA 1000 chip to assure that the size of the fragments ranged between 150-500 bp (average 300 bp).

iii. Multiplexing in one tube: fragments are mixed at equal amounts (by mass) of each amplified DNA sample library to obtain a single pool with a combined mass of at least 1.2 µg. 1 µg of the Multiplex DNA Sample Library Pool will be used in the sequence capture.

iv. Hybridization and capture step:

Hybridization of the biotinylated specific target probes (oligos that are complementary to the sequencing primer site inside the paired-end adapter sequence). After adding the Multiplex Hybridization Enhancing Oligo pool, DNA samples are dried using a DNA vacuum concentrator, then resuspended utilizing a mix of the hybridization component and hybridization buffer. Next, the sample is incubated in a heat block of 95°C for 10 minutes to allow denaturation of the double-stranded DNA multiplex, then transferred to an aliquot of COT Human (contained in the SeqCap EZ® Accessory Kit v2).

Incubation in a thermocycler at 47°C (with a heated lid turned on at 57°C) allows the hybridization to take place over 64-72 hours. The capture of the sample hybridized with biotinylated probes by Streptavidin Dynabeads® was incubated with the sample at 47°C for 45 min, followed by a wash process.

v. Post-captured LM-PCR followed by SPRI cleaning step.

7. The product is measured using NanoDrop® spectrophotometer and run in Caliper® Bioanalyzer DNA 1000 chip to assure that the size of the fragments ranged between 150-500 bp (average 300 bp).

8. Library preparation for NextSeq-500 sequencing.

Based on the concentrations obtained with Qubit® fluorometer, the most concentrated multiplexed amplified captured sample is diluted to the concentration of the less concentrated one ($C1 \cdot V1 = C2 \cdot V2$). Then, using the elution buffer tween (EBT), the library is diluted to 4 nM to obtain the appropriate concentration of the NextSeq sampling using a formula provided by the manufacturer. Next, the sample is chemically denatured using NaOH followed by the addition of a special hybridization buffer (HT1) supplied with the NextSeq sequencing kit in order to dilute the library to a final concentration of 1.8 pM. The library is then loaded into the designated reservoir in the prefilled cartridge reagent provided by Illumina.

NextSeq-500 sequencing

The Illumina reagent cartridge (NextSeq 500 HighOutput kit 150 cycles) then thoroughly washed and dried flow cell and the buffer cartridge are loaded into the NextSeq500 machine, and the sequencing process is launched after assuring that the waste cartridge is empty.

The DNA fragments bind to the surface of the flow cell through annealing of the P5 and P7 sequences of the adapters to complementary sequences on the flow cell. DNA polymerase then produces clusters of 1 million copies of clonal DNA fragments originating from each single DNA molecule. As sequencing is carried out from both ends, it is called paired-end sequencing, and the reads obtained from each end are called R1 and R2. The index sequence of each adapter allows the machine to affiliate each sequence to the correct sample.

Read mapping and variant detection

The NextSeq-500 output data is in the form of eight FastQ files per sample (four for Read 1 and four for Read 2: Nextseq-500 flow cell contains four physical lanes). Base-calling read mapping and quality control are performed using a locally developed bioinformatic pipeline. The reference sequence for read mapping and alignment is based on GRCh37 assembly. The output is a variant calling format (VCF) and a BAM (for alignment, reads, and coverage).

Variant selection and prioritization

Analysis was carried out through analyzing excel sheets of thousands of unfiltered variants (synonymous variants and MAF>5% were excluded).

For gene/variant prioritization, a minimal threshold depth of 20x was selected. To ensure the inclusion of all valid variants, we set a relaxed minor allele frequency (MAF) filter of <1% at the offset of our analysis for homozygous variants. The filter was tightened at a downstream step to <0.1% for heterozygous variants. Functional consequences of variants selected were stop codons, frameshift, splice site variants, and missense changes.

Pathogenicity prediction algorithms were used: CADD of a score of 20 or more, M-CAP, SIFT, Polyphen-2, Mutation Taster (**Figure 10**).

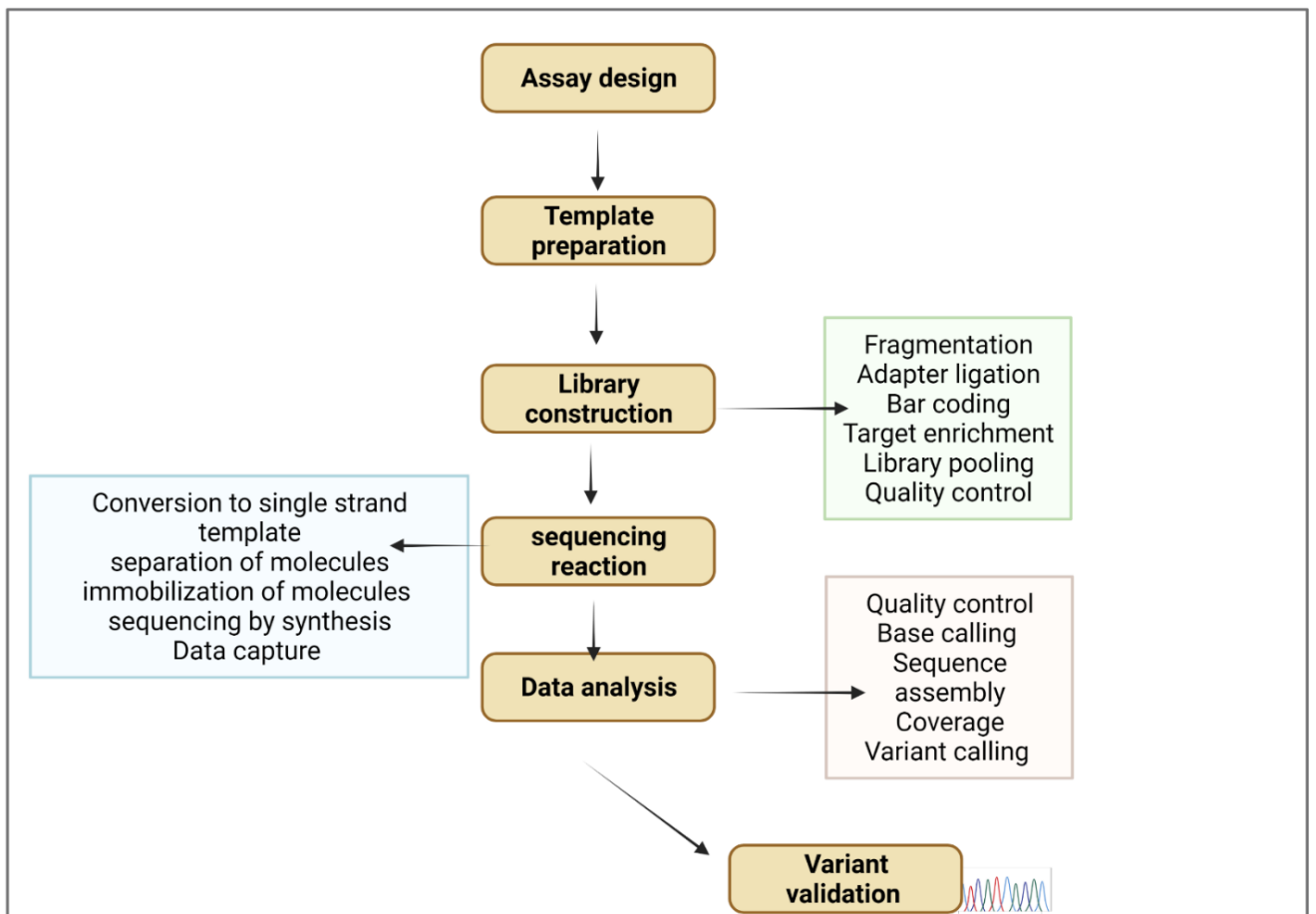


Figure 10. Next generation sequencing simplified steps.

Adapted from (Ilyas, 2017)

Chapter 7: Materials and methods of *C. elegans*

I. Solutions and media

- M9 buffer: 22mM KH₂PO₄, 42mM Na₂HPO₄, 86mM NaCl, 1mM MgSO₄ in 1 L dH₂O, mixed and autoclaved.
- Nematode growth medium (NGM) agar: 50mM NaCl, 0.25% of bacto-peptone, 1.7% agar, in dH₂O. Autoclaved and, after the medium cooled, the following solutions were added using a sterile technique: 1mM CaCl₂, 1mM MgSO₄ and 25mM potassium phosphate, 5µg/mL cholesterol (stock of 5 mg/mL in EtOH) prior to pouring petri plates.
- Single worm lysis buffer (SWLB): 50 mM Tris-HCl pH 8.0, 100 mM NaCl, 50 mM EDTA, 1% SDS, 30 mM, 100 µg/mL proteinase K. Buffer was freshly made just before use.
- *E. coli* OP50 bacteria culturing.

Luria Broth (LB) agar was used for streaking of isolated colonies of *E. coli* OP50 bacteria. LB preparation: LB broth was prepared with 25 g in 1 L of dH₂O and autoclaved for sterilization. Bacteria incubating and storage: a single OP50 colony each time was used to inoculate 200 mL of LB. The inoculated medium was left overnight at 37°C incubator room with 225 rpm shaking to grow and was then subsequently stored at 4°C for up to two weeks.

- Bleach solution: hypochlorite sodium 2.5%, 0,5mM KOH in 5 mL d H₂O.
- Freezing solution 2X: 50mM Potassium phosphate buffer, 100mM NaCl, Glycerol 30% in dH₂O. After autoclave, we added 0.3 mM of MgSO₄.
- IPTG: 1 M stock in sterile H₂O. Stored at -20°C.

II. Nematode handling

Individual animals were manipulated with a worm pick, consisting of a piece of thin platinum wire with a flattened end. The platinum wire cools quickly when flamed, allowing sterilization of the pick. For handling large amounts of animals, agar chunks were cut out from old NGM cultures and transferred into fresh, seeded plates with the aid of a sterilized scalpel. N2 Bristol and most other strains were maintained in a 15°C incubator. Temperature-sensitive mutants were treated according to their characteristics and the aims of the study ([Stiernagle, 2006a](#)).

Harvesting nematode eggs

Nematodes were washed off NGM plates using M9 buffer (into a 15 mL tube). The animals were centrifuged at 300 rpm for one minute. Excess buffer was removed. A bleach solution (7 mL) was added to the volume of the worm pellet. Samples were shaken during a 5-minute incubation. They were checked under the stereomicroscope for release of eggs and fragmentation of adult bodies. Samples were centrifuged at 300 g for 1 minute to pellet eggs. Bleach solution was removed, and eggs were washed three times with centrifugation using M9 buffer.

Freezing and thawing nematode stocks

The wild-type and mutant strains are stored at -80°C. Two to three large (12 cm) Petri dishes of the desired strain for storing were left to grow, then synchronized for obtaining L1 larvae. Each plate was then washed with 8 mL of M9 buffer to make three or four 0.5 mL aliquots, to which an equal volume of freezing solution was added.

Tubes were mixed and immediately placed in a polystyrene box at -80°C. After 48h, one tube was thawed and plated as a tester for the other tubes ([Stiernagle, 2006a](#)).

III. *C. elegans* crosses

To generate the crosses, the following steps were followed:

Ten males (generated by heat shock) were crossed with 1 hermaphrodite (10 plates were generated). A successful cross was assessed by the ratio of male/hermaphrodite worms in the progeny (if the ratio was close to 50% the cross was considered successful). F1 virgin hermaphrodites were selected and were transferred to fresh NGM petri dishes and were left to produce progeny for 24 hours before being removed from the plates. F2 worms were separated in fresh NGM plates (60 plates) and let grow until there were few F3 generation worms. The F2 hermaphrodite are then picked and digested for genotyping and sequenced through Sanger sequencing. (**Figure 11**). All procedures were done near the flame to maintain sterile conditions.

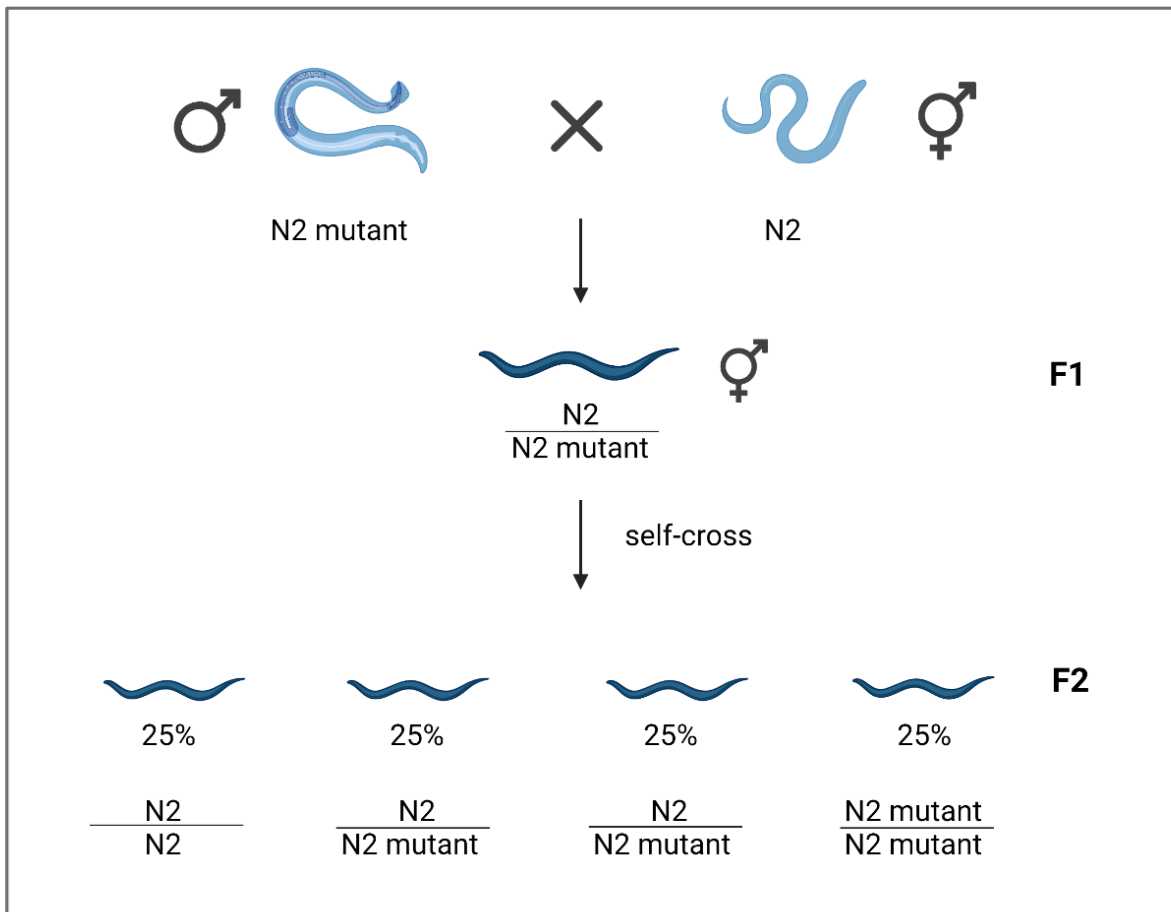


Figure 11. Crossing steps of *C. elegans* animals to obtain mutants of interest.
Adapted from (Zuryn & Jarriault, 2013).

***C. elegans* lysis and genomic DNA extraction**

A single worm was picked from each plate and was placed in a sterile 0.5 mL tube with 12.5 μ L of SWLB. Samples were incubated at -80°C for 10 minutes to freeze-fracture. They were then removed and put for 1 hour at 60°C for lysis, followed by 15 minutes at 95°C for proteinase K inactivation. Samples were stored at -20°C as DNA for PCR.

Single worm PCR on *C. elegans* and primers

To perform PCR, we used 2 μ L of the digested DNA and the Promega GoTaq DNA polymerase kit.

IV. RNAi protocol

For the knock down of the identified epilepsy genes (*KIF13B*, *FOXG1*, *STXBP1*), the following protocol is used (Figure 12).

- dsRNA plasmids: *KIF13-B* and *FOXG1* are ordered from Arhinger Library (Source Bioscience). *STXBP1* was designed in the lab and ordered from GenScript.
- The stock of bacteria was thawed.
- Day 1: Under the hood, 5 mL of LB was added, and then 1 mL of the bacteria was added and incubated overnight (O/N).
- Day 2: another set of tubes (LB plus bacteria) were prepared. We added 1 mL of prepared bacteria, Ampicillin (50 µg/mL), and 1mM of IPTG in each tube with a final volume of 5 mL. The preparation was incubated at 37°C for 6 hours with 225 rpm shaking.
- Before spreading the bacteria into the NGM Plate, fresh plates were left in the incubator overnight at 37°C.
- Before adding the bacteria, 200 µL of IPTG (1M) was spread and let dry for 10 min under sterile conditions.
- 200 µL of incubated bacteria was added and spread the same way (O/N incubation).
- The following morning N2 worms in L1 stage were added.
- Worms were left to grow at the incubator (20°C).

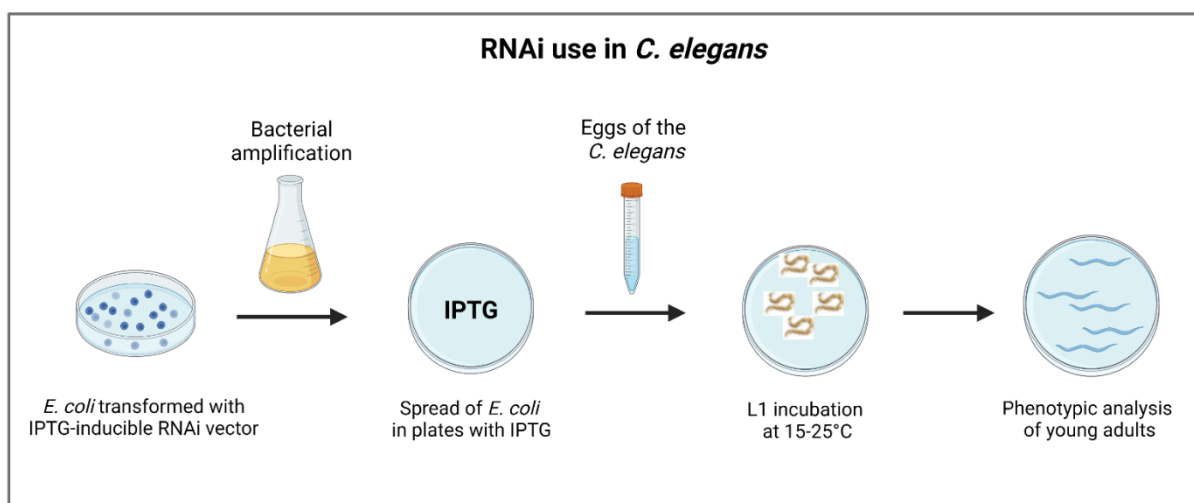


Figure 12. Scheme of the RNAi protocol on *C. elegans* animals.

V. CRISPR-Cas9 genome-editing technique

CRISPR-Cas9 is a tool that enables to edit genomic sequences. In our work, it is used as a knock-in technique to introduce the 2 missense mutations in 2 genes of interest (*KIF13B* and *IGF1-R*).

Steps of CRISPR-Cas9 in *C. elegans*

1. The CRISPR-Cas9, an endonuclease, can be directed by a small guide RNA (CRISPR RNA, crRNA) to create a double-stranded break (DSB) in the genome near the site of the desired DNA sequence change.
2. DSBs are lethal events that need to be repaired by DNA repair cell machinery via either the nonhomologous end-joining (NHEJ) or homology-dependent repair (HDR) pathways.
3. The HDR pathway uses a homologous DNA template to the sequences flanking the DSB and adds the desired edited sequence (which includes the mutations) to the targeted locus
4. In this case, knock-in of specific mutations (precision genome editing) requires HDR of the DSB by synthetic donor DNAs containing the desired edits. Single-stranded oligodeoxyribonucleotides (ssODNs) engage in a high-efficiency HDR mechanism that requires only ~35 nucleotides of homology with the targeted locus to introduce edits ranging from 1 to 1,000 nucleotides (**Figure 13**).

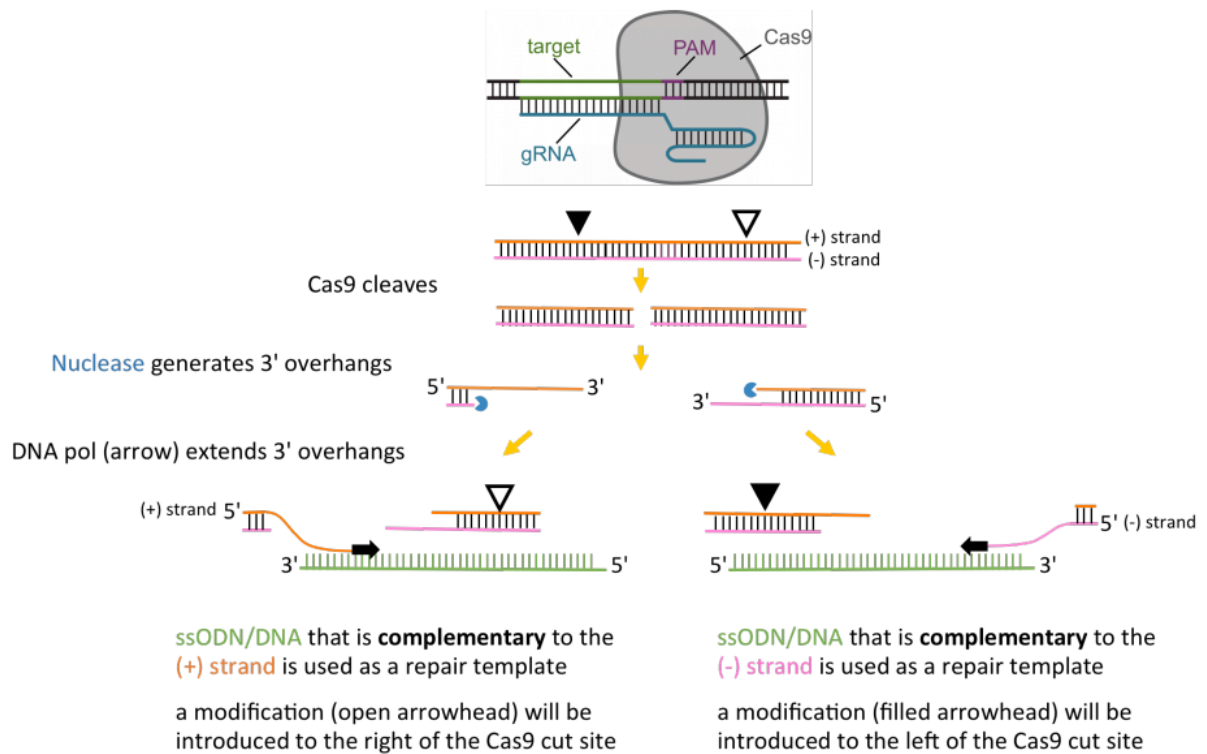


Figure 13. CRISPR-Cas9 genome-editing technique.

Using Cas9 induced DNA double strand cleavage to insert a gene via homology directed repair. (From <https://flycrispr.org>).

1. KLP-4 missense mutation

KLP-4 is described as a *C. elegans* ortholog of human *KIF13B* (kinesin family member 13B). The gene editing CRISPR-Cas9 technique is used to reproduce the missense mutation c.2372A>G (p.Tyr791Cys) seen in patients in *KIF13B* gene.

KLP-4 alignment using *Clustal Omega* tool shows that Tyr791 mutation corresponds to Phe829 in *KLP-4*. Overall homology around 45% (Figure 14, Table 3).

KIF13B	-----NPVIRSYFKRADPFYDEQENHSLIGVANVFLESLEFYDVKLQYAVPIINQ	825
KLP-4	TPHASP MKIAGIPMNECSSLVIDPFFESQEHHNLVGVANVFLEVLFDLRLDYQVPIISQ	863
	*: .. **::: .***:.*:***** *:***:::* *****	

Figure 14. Location of the missense mutation in *KIF13B*, *KLP-4*.

Injection and selection

- Cas9 protein from IDT: 0.25 µg/µL
- Duplex crRNA-MCP061/tracrRNA: 3 pmol/µL
- ssODN oMCP878: 110ng/µL
- pRF4: 40ng/µL
- 16 N2 worms (wild-type strain) were injected.
- 67 F1 were isolated(offspring) from 13 different P0 (injected animals), PCR was done to detect the mutation, homozygote worms were selected (**Table 3**)

2. IGF1R missense mutation

The *daf-2 C. elegans* gene is described as an ortholog of human *IGF1R* (insulin like growth factor 1 receptor), *INSR* (insulin receptor) and *INSRR* (insulin receptor related receptor).

The aim is to reproduce the mutation c.1532G>A (p.Arg511Gln) seen in patients in *IGF1R* gene in *C. elegans* ortholog gene *daf-2*.

IGF1R-DAF-2c alignment with *Clustal Omega* tool shows that Arg511 corresponds to Ser669 in DAF-2c (**Figure 15, Table 4**).

```

IGF1R      FTSTTTSKNRRIITWHRYPDP--YRDLISFTVYYKEAPFKNVTE-YDGQDACGSNSWNM      551
DAF-2c     VSITAVNADSVFFSWPSFNITDIDQRKFLGYELFFKEVPRIDENMTIEEDRSACVDSWQS      712
          .: *:. . : : : : * . . * * . . . : : : * . * : . : : . : * :

```

Figure 15. Location of the missense mutation in *IGF1R*, *DAF-2c*.

Injection and selection

Mix injection:


- Cas9 protein from IDT: 0.25 µg/µL
- Duplex crRNA-MCP062/tracrRNA: 3 pmol/µL
- ssODN oMCP888: 110 ng/µL
- pRF4 : 40 ng/µL
- Inject 12 N2 worms were injected (P0).
- 62 F1 were isolated. PCR analysis was done, homozygous lines were isolated (**Table 3**).

Guide: KLP-4	crRNA-MCP061 : 5' TGCTCTGGCTTTCGAAGAA –TGG
Mutation F829C	CCATTCTTCGAAAGCCAAGAGCAC-> CCATTCTgCGAgtctCAgGAaCAC
Lines generated	MCP236 <i>klp-4(bab236[klp-4 F829C])</i> MCP237 <i>klp-4(bab236[klp-4 F829C])</i> MCP238 <i>klp-4(bab236[klp-4 F829C])</i> MCP239 <i>klp-4(bab236[klp-4 F829C])</i>

Table 3. Summary of the CRISPR-Cas9 for *KLP-4* mutation.

Guide: IGF1-R	crRNA-MCP062 : 5' TATATCGGTAATGTTGAATG – AGG
Mutation S669Q	TTCTTTAGTTGGCC <u>IC</u> ATTC -> TTCTTctccTGGCC <u>acag</u> TTC
Lines generated	MCP268 <i>daf-2(bab268[daf-2 S669Q])</i> MCP269 <i>daf-2(bab269[daf-2 S669Q])</i>

Table 4. Summary of the CRISPR-Cas9 for *daf-2* mutation.



Results

In the two next chapters (8 and 9), we investigated a cohort of 20 multigenerational families with genetic generalized epilepsy (GGE) in order to identify susceptibility genes. Initially, our Sudanese team identified the largest families (with at least 2 patients) and carefully studied their phenotype. EEG was recorded in at least the index case and if needed, an MRI or CT scan was performed. A questionnaire was systematically filled up for patients and their available relatives. A saliva sample was taken, transferred to Paris and DNA was extracted and kept at -20°C at the Paris Brain Institute (ICM). In each family, since it was possible to identify members who kept the family history, their interviews allowed reconstructing extended pedigrees (at least on three or four generations), defining the status for the disease of family members and the consanguinity loops. In parallel, 119 Sudanese control DNAs were collected. The second step of the project began by the classical work of geneticist estimating the mode of inheritance of GGE in each pedigree. However, for the molecular approach, we hypothesized that at least one variant was shared by all patients within a family. We performed WES in all index cases (n=20). Moreover, DNAs from affected members in families with at least 3 sampled patients were also included in the WES study. The interpretation of the results focused on these largest pedigrees. Since the studies reported by international consortia revealed the huge genetic heterogeneity of susceptibility genes in GGE, we expected to mainly identify private variants in each family. This is why we decided to work on a first family with 3 available sibs with the same phenotype (Generalized tonico-clonic seizures). In parallel, we completed or enlarged other families. Fortunately, we identified good candidate genes, *ADGRV1* and *KCNIP1*, and variants in *ADGRV1* were frequent (25% of families) in the Sudanese population.

Chapter 8: Involvement of *ADGRV1* gene in familial forms of Genetic Generalized Epilepsy (accepted in *Frontiers in Neurology*)

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ADGRV1, Genetic generalized epilepsy, absence epilepsy, susceptibility gene, oligogenism.

Summary**Background:**

Genetic generalized epilepsies (GGE) including childhood absence epilepsy (CAE), juvenile absence epilepsy (JAE), juvenile myoclonic epilepsy (JME), and GGE with tonic-clonic seizures alone (GGE-TCS), are common types of epilepsy mostly determined by a polygenic mode of inheritance. Recent studies showed that susceptibility genes for GGE are numerous and their variants rare, challenging their identification. In this study, we aimed to assess GGE genetic etiology in a Sudanese population.

Methods:

We performed whole-exome sequencing (WES) on 40 patients' DNA from 20 Sudanese families with GGE searching for candidate susceptibility variants, which were prioritized by CADD software and functional features of the corresponding gene. We assessed their segregation in 138 individuals and performed genotype-phenotype correlations.

Results:

In a family including three sibs with GGE-TCS, we identified a rare missense variant in *ADGRV1* encoding an adhesion G protein-coupled receptor V1, which was already involved in the autosomal recessive Usher type C syndrome. In addition, five other *ADGRV1* rare missense variants were identified in four additional families and absent from 119 Sudanese controls. In

one of these families, an *ADGRVI* variant was found at a homozygous state, in a female more severely affected than her heterozygous brother, suggesting a gene dosage effect. In the five families, GGE phenotype was statistically associated with *ADGRVI* variants ($OR=0.9 \cdot 10^3$).

Conclusion:

This study highly supports, for the first time, the involvement of *ADGRVI* missense variants in familial GGE and that *ADGRVI* is a susceptibility gene for CAE/JAE and GGE-TCS phenotypes.

INTRODUCTION

Genetic generalized epilepsies (GGEs) are common types of epilepsy, accounting for around one-fourth of epilepsies. Four clinical entities have been defined based on the main seizure type and age at onset: childhood absence epilepsy (CAE), juvenile absence epilepsy (JAE), juvenile myoclonic epilepsy (JME), and GGE with tonic-clonic seizures (TCS) (GGE-TCS) (1)(2). The cumulative incidence up to 40 years was 8% for first-degree relatives of GGE patients (3).

In rare families with JME, segregating as an autosomal dominant disease, variants have been reported in the *GABRA1* gene, encoding the $\alpha 1$ subunit of the GABAA receptor (4) or in the *EFHC1* gene, encoding the Myoclonin-1(5)(6). However, the causative role of the *EFHC1* variants has been debated (7). By combining genetic and electrophysiological approaches, rare coding variants in genes encoding subunits of the GABAA receptor, especially *GABRB2* and *GABRA5*, have been implicated in a cohort including American, European, and Turkish sporadic patients with GGE (8). In addition, whole-exome sequencing (WES) studies have been performed on large international cohorts of sporadic cases (9)(10)(11). They led to the hypotheses that GGEs (i) are genetically highly heterogeneous, (ii) involve rare or ultra-rare variants, and (iii) are determined by a polygenic mode of inheritance (12)(13). In these conditions, few genes have been reported as likely susceptibility genes for GGE, including *SCN1A* and *SLC6A1* encoding the $\alpha 1$ subunit of the sodium voltage-gated channel and a gamma-aminobutyric acid (GABA) transporter, respectively (14)(15). Since most studies have been performed on Western populations, rare susceptibility genes as *SCN1A* have also been associated with GGE in other geographical areas (16)(17).

Loss of function variants in *ADGRV1* gene encoding the adhesion G protein-coupled receptor V1 caused the rare autosomal recessive form IIC of Usher syndrome (18). While the *Frings* mouse, carrying the homozygous c.6835delG (p.Val2250*) variant in *Mass1* (the mouse

orthologous gene of *ADGRVI*), displayed generalized auditory-induced seizures (19). More recently, few studies suggested that *ADGRVI* gene could be a susceptibility gene in different types of epilepsy like focal epilepsy (20), epileptic encephalopathy, or myoclonic epilepsies (21).

In this study, we collected 20 GGE families from Sudan, including 54 affected individuals, and performed WES in one or more affected individuals per family. In five families (25%), all patients were heterozygous for ultra-rare/rare missense variants in *ADGRVI* gene.

PATIENTS and METHODS

Ethical approval

This study was approved after review from the National Health Research Ethics Committee, Federal Ministry of Health, Sudan (1-4-18). All participants gave their written informed consent. For patients under the age of 18, informed consent was provided by a parent or legal guardian.

Patient's cohort and DNA sampling

We investigated 20 Sudanese families, including 54 individuals. In each family, we recruited the proband (n=20) along with all reachable affected family members (n=34) and available asymptomatic relatives (n=84). Patients' inclusion criteria were clinical presentation of generalized epilepsies with a positive family history of epilepsy (i.e., any family history of epilepsy was recorded). Patients with focal seizures or epileptic encephalopathies were excluded (Table 1).

Patients were examined and diagnosed by the referring consultant neurologists/neuropediatricians, followed by second standardized phenotyping by clinicians of the research team (MSE, MA). Healthy relatives were examined to exclude subtle neurological signs. An electroencephalogram (EEG) was performed for at least one patient per family.

In each family, we approached elderly members with solid knowledge about the family history. Their recurrent and intensive interviews allowed reconstructing extended pedigrees (at least on three or four generations), defining consanguinity loops and the status of the disease of family members.

Saliva samples (2 mL) were collected using Oragene Discover DNA collection kits (DNA Genotek Inc., Ottawa, ON, Canada), and genomic DNA was purified using the prepIT.L2P kit

(DNA Genotek Inc., Ottawa, ON, Canada) following the protocol provided by the manufacturer. DNA quality and quantity assessment was done using standard agarose gel electrophoresis, NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA), and Qubit® fluorometer (Thermo Scientific, Wilmington, DE, USA).

Whole-exome sequencing

WES was performed on 40 samples of affected individuals, including three affected siblings from F1 family (Figure 1) and the proband of the 19 remaining families. Sequencing libraries were prepared using the NimbleGen SeqCap EZ Exome v3 array (Nimblegen Inc., Madison, WI) and sequenced as 150-bp paired-end reads on the Nextseq 500 platform (Illumina, San Diego, CA, USA), at the iGenSeq sequencing platform at the ICM (Paris). Reads were processed following a standard analysis pipeline at the Department of medical genetics, Pitié-Salpêtrière hospital, Paris. Overall sequence quality was assessed with FastQC v0.11.8, the reads were then matched to the reference human genome sequence (hg19) using the Burrows-Wheeler Aligner BWA-mem v0.7.17, the alignment files were sorted and indexed using Samtools v1.9, and Sambamba v0.7.0 was used to flag duplicates. Variants were called using GATK Software v4.1.4 on Gencode v30 basic CDS (coding DNA sequence) targets. Multi-allelic variants were split, and indels were normalized using vt 0.57721.

Variant selection, prioritization, and validation

All non-silent variants shared by the three affected individuals of the F1 family were selected, with an allele frequency <1% in the closest control populations with available WES or WGS data. African controls were extracted from GnomAD database (<https://gnomad.broadinstitute.org/>), and Northeast Africa/Arabian Peninsula (NEA/AP) controls were extracted from GME variome database (<http://igm.ucsd.edu/gme/>). Synonymous variants

(without splicing effect), 5' and 3' UTR variants, and <-10 bp or >+10 bp intronic variants were not taken into account. Candidate variants were checked on bam using IGV, then prioritized according to (i) ClinVar dataset (<http://www.ncbi.nlm.nih.gov/clinvar>) to identify variants already considered as pathogenic or likely pathogenic in a disease, (ii) the pathogenicity prediction according to CADD (v1.4) with a score >20 predicting the 1% most deleterious variants in the gene (22), and (iii) the functional features of the corresponding gene (expression in Brain and number of publications related to this gene with keywords, "epilepsy", "seizure", "brain" and "neuron").

Segregation of the two candidate variants within the families and their frequency in 119 Sudanese non-epileptic controls were determined by Sanger sequencing. One sense or reverse primer solution at 5 pM concentration combined with a diluted PCR product was sent to the GATC® platform where BigDye chemistry on PCR amplicons and an AB13730 sequencer (Applied Biosystems) were used. Sequence analysis was then performed using Sequencher 4.9 software.

Statistical tests

To test for an association between potentially pathogenic variants and GGE, we computed an odds ratio (likelihood ratio) between the H0 and H1 models. The H0 model assumed that the presence of a variant in sibs is independent of the pathology and thus segregated according to Mendelian proportions: 0.5 chances of having one variant for each sib (a single parent has a variant), except for family F4, where the chance is 0.75, as both parents carry one variant). Therefore, the overall H0 likelihood is $L_{H0} = 0.5^3 \times 0.5^2 \times 0.5^2 \times 0.75^2 \times 0.5^2 \sim 1.1 \cdot 10^{-3}$. Alternatively, we used an H1 model where the presence of a variant in a sib was a necessary (but not sufficient) condition to develop the pathology. As all patients carried a variant, we had

$L_{H1} = 1$. The odds ratio was then $L_{H1}/L_{H0} \sim 910.2$, indicating that it was much more likely that carrying a variant was a necessary (but not sufficient) condition to be affected.

Fisher's exact test was used to compare qualitative variables. A p-value <0.05 was considered statistically significant. Two-tailed hypotheses were considered throughout the study.

RESULTS

Description of the cohort

The cohort comprised 20 unrelated families (two to five affected members each) from rural areas in Sudan. Fifteen families had a homogeneous type of GGE, including twelve with GGE-TCS, two with CAEs/JAEs, and one with JME. Five families included patients with different epileptic phenotypes (CAE, JME, or GGE-TCS). In total, 37 among 54 patients had TCS (68.5%), 10/54 CAE/JAE (18.5%) and 7/54 JME (12.9%) (Table 1).

To enrich the cohort in autosomal recessive (AR) GGE, we preferentially select families with consanguineous index cases. Thus, the rate of consanguinity (RC) in index cases was 0.7 (14/20). However, if we extend to all siblings with at least one affected member, the RC was 0.43 (34/60), which is very close to RC in the Sudanese population ranging between 40% and 49% (23).

Clinical findings in family F1

The study included a family F1 with nine siblings in the fifth generation, including three affected and one healthy male, and five healthy females (Figure 1). Their parents were not related. A maternal aunt was also affected by GGE but was not available for sampling. The clinical features of the affected members are reported in Table 2. The three affected brothers showed symptoms of GGE-TCS. The average age at onset of seizures was 19.6 years. Seizures

occurred mostly during their working hours as farmers in the field and were witnessed by their coworkers. No preceding aura was reported. The frequency and intensity of the seizures varied among siblings. No history of developmental delay or febrile seizures was noticed. No additional neurological signs were found at clinical examination, and cognition was normal. The three patients were treated with sodium valproate. The patient V-4 showed a more severe phenotype in terms of seizure frequency (twice per week) and inefficacy of sodium valproate, while his brothers were free of seizures with this treatment. EEG of V-4 showed generalized spike-wave or polyspike-wave discharges.

Genetic results in family F1

WES was performed on the DNA samples of the three affected brothers (V-1, V-2 and V-4). The selection process did not retain any variants at the homozygous, composite heterozygous, or hemizygous state. Among the 22 shared heterozygous prioritized filtered variants (Supplementary Table 1), we identified variants in *ADGRV1* and *KCNIP1* gene, already flagged as related to epilepsy, making them the best candidates by function. *ADGRV1* encodes the adhesion G protein-coupled receptor V1, highly expressed in different brain structures during development (Human Brain Transcriptome: <https://hbatlas.org/hbtd/images/wholeBrain/GPR98.pdf>), and *KCNIP1* a neuronal K⁺ channel-interacting protein considered as a modulator of the GABAergic system (24). The *ADGRV1* c.13919G>A (p.Gly4640Glu) variant was predicted deleterious by different pathogenicity prediction tools, including CADD (with a high score of 29.4) and M-CAP. It has never been reported in GnomAD African population (and with an overall frequency of 2.10^{-5} in GnomAD), and its frequency is 0.0037 in NEA/AP controls from GME variome and 0.008 in 119 Sudanese controls (2/238 control chromosomes) (Table 3). This variant came from the branch of the affected aunt (Figure 1).

In *KCNIP1*, the missense variant c.403G>A (p.Asp135Asn) was predicted possibly pathogenic by CADD (with a high score of 34) and M-CAP, was present at the heterozygous state in the two affected brothers V-1 and V-2, and at the homozygous state in the more severely affected one V-4 (Table 3). Since this variant was located at the first base of exon 5, SpliceAI software predicted no effect on splicing. This variant was absent in the GnomAD (including Africans), GME database, and Sudanese controls.

Search for additional variants in *ADGRVI* and *KCNIP1* genes

We then searched for candidate variants in *ADGRVI* or *KCNIP1* genes in index cases of the 19 remaining families using WES data. Our filtering criteria were the same as for WES: non-silent variants with a MAF <1% and for missense, a CADD score >20 predicting the 1% most deleterious variants in the gene (22). No candidate variants were identified in *KCNIP1*. In contrast, we identified five variants in *ADGRVI* in four additional pedigrees with at least two affected members (Table 3). In each family, the variant was identified in all patients (Figure 1) and was not present in Sudanese controls (Table 3). Two missense variants segregating *in cis* were identified in family F2: c.1718G>T (p.Gly573Val) and c.9440G>A (p.Arg3147Gln). They had the same MAF in both the European and African controls in GnomAD, strongly suggesting that they were in linkage disequilibrium (Table 3); CADD score was higher (=24.9) for the c.1718G>T (p. Gly573Val) compared to c.9440G>A (p. Arg3147Gln) variant (=22.6). A cumulative deleterious effect on the protein function cannot be excluded for these *in-cis* variants.

Ten affected sibs carried missense *ADGRVI* variants at the heterozygous state and one, (IV-6) in family F4, at a homozygous state in the five families. The likelihood ratio between the (H1) model where the presence of a variant in sibs is a necessary (but not sufficient) condition to develop GGE versus the hypothesis of independence (H0) was statistically significant

(OR=0.91 10^3) (see Patients and Methods). Though these variants were identified in all genotyped patients, they were rarely (1/5) found in their healthy sibs. The healthy carrier of an *ADGRVI* variant in family F1 (V-5) was a 20 years-old female, the youngest among her three affected brothers. She had no history of any neurological disorder, and her complete neurological examination was normal. However, we could notice that one brother had his first seizure at 23 years (Table 2). Moreover, she did not carry the second variant in *KCNIP1* present in her three affected sibs (Figure 1).

The frequency of identified *ADGRVI* variants is higher in GGE patients (5/20; 5 alleles/40 alleles) compared to the 119 Sudanese controls (2/119; 2 alleles/238 alleles) (Fischer's exact test- $p=0.8 \cdot 10^{-3}$).

Four identified *ADGRVI* variants were localized in the calx-beta domains of the protein, a highly repetitive ectodomain; however, variants (p. Gly573Val) in F2 and (p. Pro5732Leu) in F4 were not located in annotated protein domains (Table 3 and Figure 2).

Genotype-phenotype correlation

In families with *ADGRVI* variants, there was inter- and intra-familial variability of the phenotype in terms of age at onset, type and frequency of seizures or response to treatment. Both patients in family F2 had CAE/JAE, all patients in family F4 and F5 had GGE-TCS, while in family F3, one patient developed JAE (IV-9) and the other GGE-TCS (IV-5) (Figure 1, and Table 2).

In family F4, patient IV-6 with an *ADGRVI* variant at the homozygous state (likely because of an endogamous marriage) showed a more severe phenotype and less response to treatment than her heterozygous affected brother IV-10. IV-6 had 3.5 seizures/week compared with 1.5 for IV-10. She was treated first by Carbamazepine, and then Sodium Valproate (VPA) was added.

Under this bi-therapy, her seizures were less frequent but persisted. Conversely, her brother was free of seizures under Carbamazepine monotherapy.

DISCUSSION

Among the different approaches to unravel susceptibility variants contributing to complex traits, genetic studies focused on limited geographic areas have so far been viewed as a useful complementary strategy to large-scale case-control studies (25)(26).

In this study, we investigated familial forms of GGE in rural areas of Sudan. Examining the pedigrees (Figure 1), it was difficult to define a monogenic mode of inheritance in families with *ADGRVI* variants. Family F1 had patients from two generations, indicating a possible autosomal dominant (AD) inheritance with low penetrance. In family F2, patients were found in independent branches of the pedigree. In family F3, an autosomal recessive (AR) transmission could be suspected since the parents of the two sibs were closely related; however, they presented two different types of GGE (JAE versus GGE-TCS). In families F4 and F5, the two affected sibs were consanguineous, but relatives from previous generations, a great uncle in F4 and an uncle in F5, were also affected.

Another possibility is that GGE in *ADGRVI*-linked families derived from a polygenic inheritance: patients are most often sibs or isolated cases (Figure 1), who could receive a deleterious combination of susceptibility variants in different genes, separately transmitted by their healthy parents (14)(17)(27)(28). This model is compatible with the co-occurrence of the phenotype with the variants in the *ADGRVI*-related families.

Little has been published on the effects of consanguinity on complex disorders. In a population with both a high RC and a founder effect, as in Sudan, genetic risk factors tended to accumulate in some individuals, causing a pathology (23). For example, Farrer and colleagues (2003) identified three independent loci on chromosomes 9, 10, and 12 determining Alzheimer's

disease in an Israeli-Arab community with a high consanguinity rate and a familial structure resembling the one found in Sudanese families (29). Moreover, Al-Mubarak and colleagues (2017) found at least two likely pathogenic variants in the majority of probands with attention deficit hyperactivity disorder (ADHD) in Saudi Arabia, leading to the conclusion that ADHD should be considered as a complex disorder (30).

ADGRVI loss of function variants have been reported to cause the rare form of AR Usher syndrome Type IIC (OMIM #605472) (31), characterized by retinitis pigmentosa and mild-to-moderate sensorineural hearing loss. However, the phenotype of the *Frings* mouse model carrying the homozygous c.6835delG (p.Val2250*) variant in *Mass1* (the mice orthologous gene of *ADGRVI*) consisted in generalized auditory-induced seizures (18) Up to now, four studies have suggested the involvement of *ADGRVI* in human epilepsy. The p.Ser2652* variant was identified at the heterozygous state in two patients with febrile and afebrile seizures of a Japanese pedigree (32). In addition, the p.Glu3215Lys variant in *ADGRVI* was reported in a patient with focal epilepsy and sudden unexpected death in epilepsy (SUDEP)(20). More recently, a study of 95 isolated cases with various myoclonic epilepsies showed a higher than average proportion of ultra-rare missense *ADGRVI* variants: five variants were identified in two patients with Lennox-Gastaut syndrome from a cohort of 46 patients with developmental or epileptic encephalopathies and in two patients from a group of eight with unclassified epilepsies, as well as in two JME patients from a cohort of 41 with GGE (Figure 2) (21) Finally, the p.Asp680Gly variant was identified at the heterozygous state in a 20 months-old girl and her mother, both with febrile seizures (33).

A key remaining question is how *ADGRVI* variants can lead to such diverse disorders as Usher type IIC syndrome and epilepsy. The case of *FIG4* gene is an illustrative example, variants in this gene can cause very different AR disorders: (i) Charcot-Marie-Tooth 4J (CMT4J), a

peripheral neuropathy (34), (ii) Yunis-Varón syndrome (YVS), in which neurodegeneration and brain malformations are associated with cleidocranial dysplasia, digital anomalies, and early death (35), and (iii) temporo-occipital polymicrogyria with seizures and psychiatric features (36). The major distinction is that patients with YVS are homozygous or compound heterozygous for null variants of *FIG4*, thus exhibiting complete loss of function. In contrast, patients with (CMT4J) or polymicrogyria retain partial function of *FIG4*. This is also the case for patients with Usher syndrome type IIC, who carry at least one premature stop codon variant. In GGE patients, all variants are missense at the heterozygous state.

From a physio-pathological point of view, in the *Frings* mice, the absence of *ADGRV1* impaired the entry of *Cdhr23*- and *Cdhr15*-expressing interneuron precursors to the embryonic cortex, leading to a decreased number of parvalbumin interneurons in the auditory cortex (37). This can be related to the identification in family F1 of a novel variant in *KCNIP1* gene, which encodes a Kv channel interacting protein (KChIP) found in various neuronal cells and is highly expressed in parvalbumin-positive GABAergic interneurons (38). *KCNIP1* deletion in mice causes increased susceptibility to PTZ-induced seizures, reinforcing the hypothesis that *KCNIP1* might play pivotal roles in the GABAergic inhibitory system (24). These data suggested that both genes acted on interneurons, especially parvalbumin neurons, from their migration to the control of their excitability. In this context, we can hypothesize that both variants identified in *ADGRV1* and *KCNIP1* interact in parvalbumin neurons on the GABAergic pathway, provoking seizures. Functional validation of this hypothesis could be performed by introducing both variants in an animal model and monitoring the mutated lines by video-EEG. In conclusion, our study provides a statistically significant association between *ADGRV1* variants and familial GGE, highly supporting for the first time that *ADGRV1* is a susceptibility gene for CAE/JAE and GGE-TCS. Identifying a variant at a homozygous state in a more severely affected patient suggests a gene dosage effect.

Screening of *ADGRVI* in GGE families with other ethnic backgrounds is needed to know whether *ADGRVI* is a GGE susceptibility gene specific to the Sudanese population. In addition, whole genome sequencing (WGS) may permit identifying additional variants in non-coding region/neighboring regulator areas of *ADGRVI*.

Table 1. Description of the cohort.

Family ID	Average age of onset	Phenotype	Sampled individuals affected/non affected	Number of first degree affected relatives	Consanguinity (index case)	EEG (index case)
F1	19.6 Ys	GGE-TCS	3/3	2	N	Clusters of intermixed generalized SW.
F2	8.5 Ys	CAE/JAE	2/3	1	Y	Generalized very high amplitude 3Hz SW.
F3	7 Ys	JAE/ GGE-TCS	2/3	1	Y	High amplitude 3Hz SW complexes.
F4	14.5 Ys	GGE-TCS	2/3	1	Y	Short runs of generalized slow waves.
F5	5 Ys	GGE-TCS	2/3	1	Y	Generalized high amplitude SW.
F6	7.5 Ys	CAE	2/3	1	N	Very high amplitude 3Hz SW complexes.
F7	16.5 Ys	GGE-TCS /JME	4/9	1	Y	Jerks and short runs of single, befit & polyspike-slow waves.
F8	13.6 Ys	JME/ GGE-TCS	3/6	1	Y	Jerks and short runs of single, befit & polyspike-slow waves.
F9	32.5 Ys	CAE/ GGE-TCS	2/4	zero	Y	High amplitude generalized irregular slow waves in the theta range.
F10	1.5 Ys	GGE-TCS	2/3	1	N	Short runs of generalized mixed slow waves in theta range.
F11	6 Ys	GGE-TCS	2/3	1	N	Bursts of 3Hz spike SW complexes.

Results

F12	5.4 Ys	CAE/ GGE-TCS	5/8	1	Y	Bursts of 3Hz spike SW complexes.
F13	5 Ms	GGE-TCS	2/4	1	N	Generalized sharp waves, 4Hz background activity.
F14	16.4 Ys	GGE-TCS	5/4	1	Y	Paroxysms of generalized mixed sharp and slow waves.
F15	1.5 Ys	GGE-TCS	2/3	1	N	No detected epileptiform activity
F16	12.6 Ys	GGE-TCS	3/4	1	Y	Paroxysms of generalized mixed sharp and slow waves.
F17	23 Ys	GGE-TCS	3/9	zero	Y	Short & long runs of generalized SW delta range waves.
F18	5.5 Ys	GGE-TCS	2/3	1	Y	Generalized sharp waves, 4Hz background activity.
F19	10 Ys	GGE-TCS	4/3	3	Y	Generalized high amplitude SW waves.
F20	9.5 Ys	JME	2/3	1	Y	No detected epileptiform activity.

CAE: childhood absence epilepsy; GGE-TCS: genetic generalized epilepsy with tonic-clonic seizures alone; JME: juvenile myoclonic epilepsy; EEG: electroencephalography; F: family; Hz: Hertz; SW: spike and wave; Ys: years; Ms: months; Y: yes, N: no.

Table 2. Clinical data of the patients

Family ID	Patient No.	Sex	Age at Sz onset	Age at examination	Epilepsy type	Sz/week before treatment	Triggering stimulus	Neurological examination	Current therapy	Response to treatment	EEG	Cognitive level
F1	V-1	M	18y	29y	GG E-TCS	1-2 (1.5)	None	Normal	VPA	Responder §	NA	Good interaction with the family, efficient work on the farm
F1	V-2	M	23y	28y	GG E-TCS	0-1 (0.5)	Lack of sleep	Normal	VPA	Responder §	NA	Good interaction with the family, efficient work on the farm
F1	V-4	M	18y	22y	GG E-TCS	2 - 3 (2.5)	Lack of sleep	Normal	VPA	Poor sz control	Cluster s of intermi xed generali zed SW	Good interaction with the family, efficient work on the farm
F2	VI-3	F	12y	14y	JAE	42 – 70 (56)	Nothing specific	Normal	VPA	Responder	NA	Very good school performanc e and interaction with siblings and colleagues
F2	VI-4	F	5y	12y	CAE	56 to 70 (63)	Nothing specific	Normal	VPA	Responder	General ized very high amplitu de 3Hz SW	Very good school performance and interaction with siblings and colleagues

F3	IV-5	F	5y	26y	GG E- TCS	1 to 2 (1.5)	Lack of sleep	Normal	LTG	Poor sz control	NA	A housewife and mother with 2 kids. Good interaction with her family
F3	IV-9	F	9y	14y	JAE	77 to 140 (108.5)	Menstrua l cycle, TV	Normal	LTG	Responder	High amplitu de 3Hz SW comple xes	School performance mildly affected by the disease. Good interaction with siblings and friends
F4	IV-6	F	28y	30y	GG E- TCS	3 to 4 (3.5)	Lack of sleep Fatigue, Sun exposure	Normal	CBZ +VP A	Poor sz control	Short runs of generali zed slow waves	A housewife and a mother with excellent communication with family and neighbors
F4	IV- 10	M	7 M	16y	GG E- TCS	1 to 2(1.5)	lack of sleep	Normal	CBZ	Responder	NA	Good performance in studies, football coach in his village
F5	IV-3	M	9 y	11y	GG E- TCS	0-1 (0.5)	Nothing specific	Normal	VPA	Responder	High amplitu de SW comple xes	Good school performance
F5	IV-4	F	9 M	13 y	GG E- TCS	15-21 (18)	Nothing specific	Normal	VPA	Responder	NA	Poor school performance, anxiety

Sz: seizure, GGE-TCS: genetic generalized epilepsy with tonic–clonic seizures alone, JAE: Juvenile absence epilepsy, CAE: Childhood absence Epilepsy, VPA: Sodium Valproate, LTG: Lamotrigine, CBZ: Carbamazepine. SW: Spike-wave discharges. Sz/week are expressed as intervals and mean values are given in parenthesis. §indicates a bad compliance. M: Male, F: Female. NA: not available.

Table 3. ADGRVI/KCNIPI Variant description in the families.

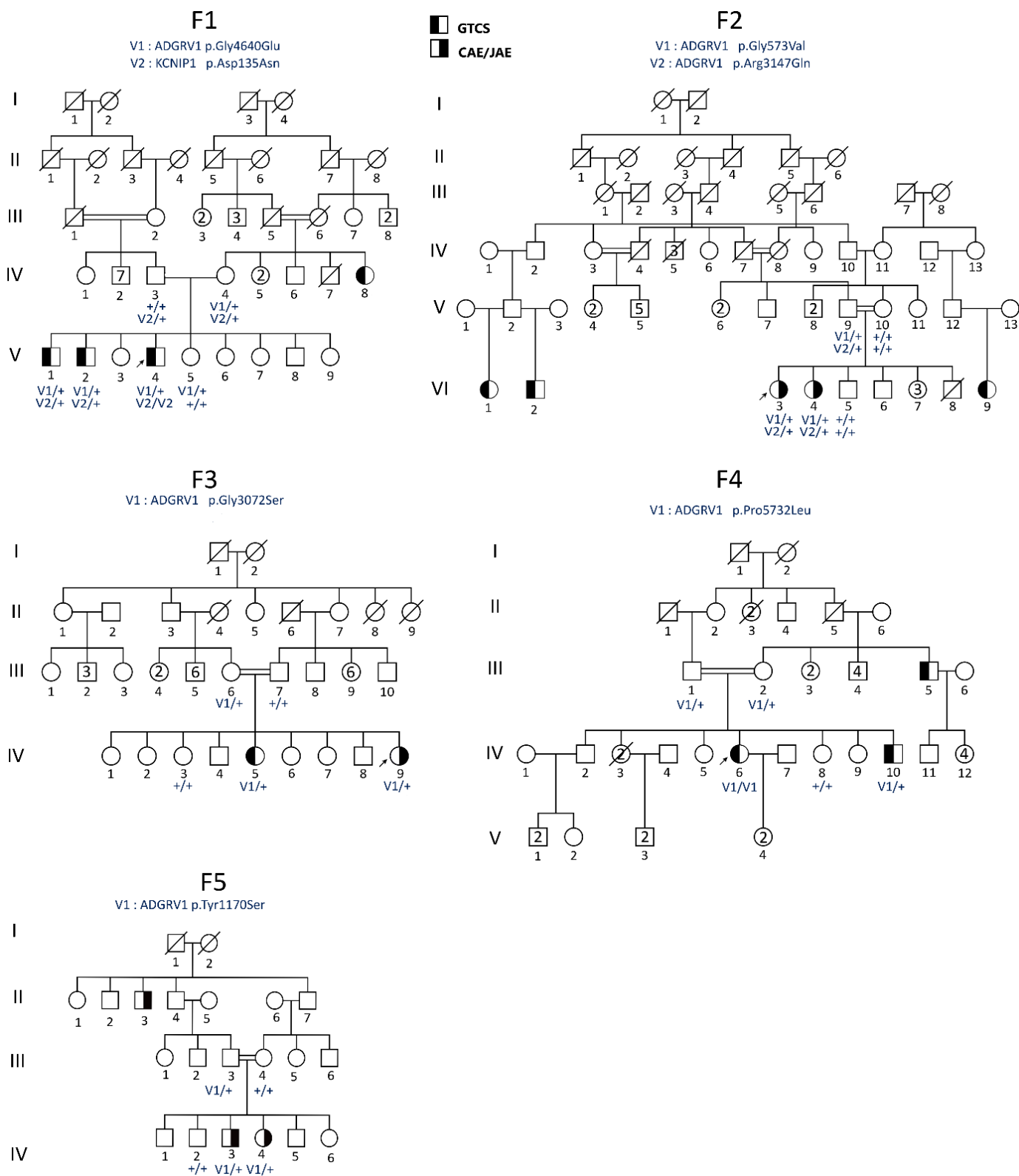
Gene	Family	Genomic position (hg19)	cDNA variant	Protein variant	rs-number	M-cap	CADD	Gnomad		GME (NEA+AP)	Sudanese Controls (n=119)	Domain
								Overall	Africans			
<i>ADGRVI</i>	F1	chr5:90085544 G>A	c.13919G> A	p.Gly4640Glu	rs72750470 6	D	29.4	0.00002	zero	0.003	2	Calx- beta 32
<i>ADGRVI</i>	F2	chr5:89925235 G>T	c.1718G> T	p.Gly573Val	rs20078956 3	D	24.9	0.0003	0.0007	zero	zero	-
<i>ADGRVI</i>	F2	chr5:90012539 G>A	c.9440G> A	p.Arg3147Gln	rs20079265 8	D	22.6	0.0003	0.0007	0.0009	zero	Calx- beta 22
<i>ADGRVI</i>	F3	chr5:90012313 G>A	c.9214G> A	p.Gly3072Ser	rs75756016 9	D	25.1	zero		zero	zero	Calx- beta2 2
<i>ADGRVI</i>	F4	chr5:90144629 C>T	c.17195C> T	p.Pro5732Leu	-	B	22.5	zero		zero	zero	-
<i>ADGRVI</i>	F5	chr5:89948255 A>G	c.3509A> C	p.Tyr1170Ser	rs18877287 5	D	24.2	0.0006	0.0001	0.0009	zero	Calx- beta9
<i>KCNIPI</i>	F1	chr5:170149717 G>A	c.403G>A	p.Asp135Asn	-	D	34	zero		zero	zero	-

Chr: chromosome, D: disease causing, B: benign. Protein domain annotation is from the Uniprot database

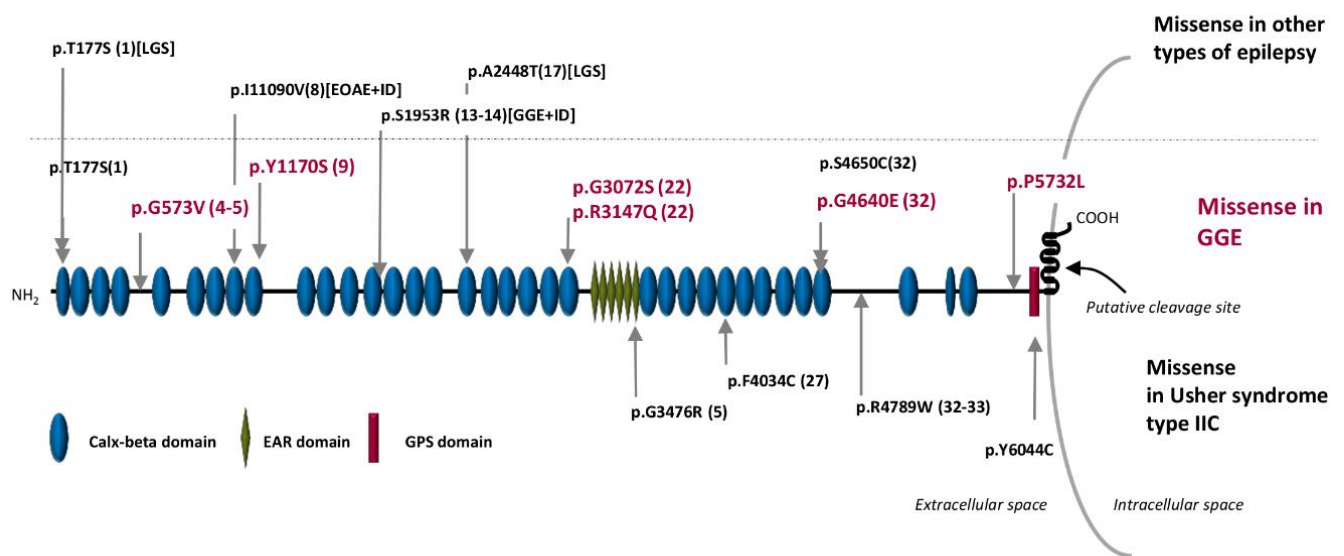
(<https://www.uniprot.org/>), Gnomad (<https://gnomad.broadinstitute.org/>) version v2.1.1 including all samples.

Refseq Transcript ADGRV1: NM_032119.3 , *KCNIPI* :NM 001278339.1.

Figure 1. Pedigrees of the Sudanese families (F1-F5) with *ADGRV1* variants.



Males are represented by squares and females by circles, the probands are shown by arrows and a diagonal line through the symbol denotes deceased subjects. Abbreviations: CAE, childhood absence epilepsy; GGE-TCS: GGE with tonic-clonic seizures alone. Genotypes are indicated below the tested individuals.

Figure 2. Schematic representation of the ADGRV1 protein structure.

In the upper part of the figure: in red the missense variants associated with GGE in Sudanese families (this publication) and in black, missense variants associated with other types of epilepsy in the Australian cohort reported by Myers and colleagues [21]. The dotted line separated the variants associated with GGE (bottom) from those associated with other types of epilepsy (up). In the bottom part of the figure are the rare pathogenic missense variants reported in Usher syndrome type IIC. The numbers inside brackets indicate the calx-beta domains sequential order. Abbreviations: GGE, Genetic generalized epilepsies, EOAE, early onset absence epilepsy, LGS, Lennox-Gastaut syndrome, ID, intellectual deficit.

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Declarations

Competing interests: None of the authors has any conflict of interest to disclose. We confirm we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Authors' contributions: MD contributed to the study's design, collection, analysis, and interpretation of the data. MSE and MA referred the patients and clinically evaluated them. EAA, FAE, WAA, OA, SG, and RA contributed to including patients and sample collection. JB contributed to the bioinformatics analysis and GA to the statistical tests, including segregation study. SBal and EAA contributed to the design of the figure. SBal, SB, LE, and EN critically reviewed the manuscript. AE contributed to supervising and revising the manuscript. EL designed, supervised, and obtained funding for the study. MD and EL wrote the manuscript. EL received funding from INSERM and ICM (France).

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Data Availability:

The data that support the findings of this study are available on request from the corresponding author

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Chapter 9: Genetic heterogeneity in familial forms of Genetic Generalized Epilepsy in Sudan (article in preparation)

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INTRODUCTION

Genetic variants impacting human traits, e.g. epilepsies, vary in their frequency and effect. Typically, an inverse relation exists between variant frequency and effect magnitude, where large-effect variants are rare, and small (but detectable) effect-variants are common. This link was first suggested by population genetic models, and later noted in data from association and sequencing studies in large cohorts.^{1}

Concepts of incomplete penetrance and variable expressivity have been described to make allowance to understand contributions of rare variants in more than one gene. In fact, monogenic diseases such as Braxton-Biedl syndrome, retinitis pigmentosa, nephronophthisis and many others were shown to be oligogenic. ^{2-4}

Genetic generalized epilepsy (GGE) including childhood absence epilepsy (CAE), juvenile absence epilepsy (JAE), juvenile myoclonic epilepsy (JME), and GGE with tonic-clonic seizures (TCS) (GGE-TCS) is genetically influenced with a two- to four-fold increased risk in the first-degree relatives of patients and the proportion of GGE patients with a positive family history (≥ 1 first-degree relative with epilepsy) is about 15% ^{5}. However, the large families in which GGE segregate are rare. In few families with autosomal dominant JME, variants have been identified in the *GABRA1* gene, encoding the $\alpha 1$ subunit of the GABAA receptor ^{6} or in the *EFHC1* gene, encoding the Myoclonin-1^{7,8}. However, the causative role of the *EFHC1* variants has been debated in different studies ^{9}. By combining genetic and electrophysiological approaches, rare coding variants in genes encoding subunits of the GABAA receptor, especially *GABRB2* and *GABRA5*, have been implicated in a cohort including American, European and Turkish sporadic patients with GGE ^{10}. Most studies have been performed in large international cohorts of sporadic cases with GGE by Whole Exome Sequencing (WES) studies. They supported the hypotheses that GGE (i) are genetically highly

heterogeneous, (ii) involve rare or ultra-rare variants and (iii) are determined by an oligogenic mode of inheritance {11}.

In these conditions, few genes including *SCN1A* and *SLC6A1* encoding the α 1 subunit of the voltage-gated sodium channel and a gamma-aminobutyric acid (GABA) transporter, respectively, have been reported as likely susceptibility genes for GGE {12}{13}. Recently, in Sudanese families with GGE, we reported rare missense variants of *ADGRV1* gene, which encodes the adhesion G protein-coupled receptor V1 {14}. While loss of function variants in *ADGRV1* are responsible for the rare autosomal recessive Usher IIC syndrome, the homozygous c.6835delG (p.Val2250*) variant in *Mass1* (the mouse orthologous gene of *ADGRV1*) causes generalized auditory-induced seizures in the *Frings* mouse {15}.

We performed further studies in the already reported cohort of 20 families by interpreting WES in 5 families with at least 3 patients with GGE and available DNA samples. Candidate susceptibility genes have been identified and screened in the proband's WES of the remaining families.

MATERIALS AND METHODS

Patients and Methods

Ethical approval

This study was prospectively reviewed and approved by the national health research ethics committee, Federal ministry of health, Sudan (1-4-18). Written informed consent was given by all participants.

Patient's cohort

Twenty Sudanese families with GGE were identified. In each family, we recruited the proband (n=20) along with all approachable affected family members (n=36) and available asymptomatic relatives (n=84). The total number of patients sampled was 56. Inclusion criteria for patients were clinical presentation of generalized epilepsies with a positive family history of epilepsy. Patients with focal seizures or epileptic encephalopathies were excluded. Patients were examined and diagnosed by the referring consultant neurologists/neuro-pediatricians, followed by a second standardized phenotyping by clinicians of the research team (EM, AM). Healthy relatives were examined to exclude subtle phenotypes. Electroencephalogram (EEG) was performed for at least one patient per family.

Whole Exome Sequencing

WES was performed in 40 affected members from F7, F8, F12, F14 and F19 families with at least 3 patients with available DNA, and at least the proband of the 13 remaining families. Sequencing libraries were prepared using the NimbleGen SeqCap EZ Exome v3 array (Nimblegen Inc., Madison, WI) and sequenced as 150-bp paired-end reads on the Nextseq 500 platform (Illumina, San Diego, CA, USA), at the iGenSeq sequencing platform at ICM (Paris). Reads were processed following a standard analysis pipeline at the Pitié-Salpêtrière University

Hospital. Overall sequence quality was assessed with FastQC v0.11.8, the reads were then aligned to the reference human genome sequence (hg19) using the Burrows-Wheeler Aligner BWA-mem v0.7.17, the alignment files were sorted and indexed using Samtools v1.9, and Sambamba v0.7.0 was used to flag duplicates. Variants were called using GATK Software v4.1.4 on Gencode v30 basic CDS targets. Multi-allelic variants were split and indels were normalized using vt 0.57721.

Variants prioritization and validation

Genetic filters were firstly applied to WES data. All variants with an allele frequency $\geq 1\%$, or not shared by all affected members within the family were excluded. Synonymous, missense, in-frame or frameshift indel as well as splicing site variants shared by all affected members of the family were systematically studied by the SpliceAI software. Candidate variants were prioritized based on: i) their frequency in the closest control populations with large available WES or WGS data, African controls extracted from GnomAD database (<https://gnomad.broadinstitute.org/>) and from GME variome database (<http://igm.ucsd.edu/gme/>), ii) their classification in ClinVar dataset (<http://www.ncbi.nlm.nih.gov/clinvar>), and iii) the pathogenicity prediction according to CADD v1.4 with a score ≥ 20 predicting the 1% most deleterious variants in the gene{16}, M-CAP {17} and REVEL {18}.

Candidate variants were then functionally prioritized by their expression profile in the cortex, ii) their already established links to epilepsy and iii) their involvement in neurodevelopment and synapse. They were validated and checked for segregation within the families by Sanger sequencing, according to standard procedures. They were also screened by Sanger sequencing in 119 Sudanese controls.

SNP microarrays

Thirty-five family members, including at least index cases were genotyped using 660W-Quad microarrays (Illumina, San Diego, CA, USA). Automated Illumina microarray experiments were performed according to the manufacturer's specifications. Image acquisition was performed using an iScan System (Illumina). Image analysis and automated CNV calling was performed using GenomeStudio v.2011.1 and CNVPartition v.3.1.6 with the default confidence threshold of 35. We also used a size threshold of 20 kb since CNVs <20 kb detected by 660W-Quad microarrays proved to be false positives. Identified CNVs were systematically compared with those present in the Database of Genomic Variants (DGV), excluding BAC-based studies, using an in-house bioinformatics pipeline, to assess their frequency in control populations. Only CNVs identical (breakpoints and copy number) to or totally included in those described in the DGV were considered; microrearrangements partially overlapping CNVs described in DGV or with a discordant copy number were considered to be novel. CNVs with a minor allele frequency $\geq 1\%$ in at least one study comprising ≥ 30 controls were excluded from further analysis. CNVs encompassing coding regions, and with a frequency $< 1\%$, were considered as possibly deleterious. CNV frequencies were compared with the Mann–Whitney or unilateral Fisher's exact tests. Candidate genes present in the CNVs were compared with those present in AutDB (<http://www.mindspec.org/autdb.html>).²⁰

RESULTS

Mode of inheritance in selected families

The families F7, F8, F12, F14, and F19 with at least 3 patients with available DNA were selected in order to search for candidate variants by comparing WES of patients within families. The clinical features of the affected members of these families are summarized in Table 1.

The inspection of the pedigrees allowed determining the mode of inheritance in some of these families. In F8, since only males are affected and females are asymptomatic carriers, the most probable transmission was firstly X-linked recessive and secondly autosomal dominant with incomplete penetrance (Figure 3). In F19, all affected members were issue from short consanguinity loops, suggesting an autosomal recessive mode of inheritance (Figure 2). In contrast, it was difficult to firmly determine the transmission of GGE in F7, F12, and F14. For example, F7 presented with two affected sibs with JME issue from related patients, who also had a second cousin with JME from *a priori* non-related parents and an uncle with a different GGE phenotype (GGE-TCS) (Figure 3). In F12, two patients were issued from different consanguinity loops but the 5 other affected members originated from unrelated parents and a vertical transmission by an asymptomatic carrier can be suspected in one branch (Figure 1).

Analysis of CNVs

The detection of rare CNVs was performed in the 20 index cases and 15 additional patients from F7, F8, F12, F14 and F19 families. The already published recurrent microdeletions at 15q11.2, 15q13.3 and 16p13.11 in GGE patients were not found in our cohort {19}. We identified 6 rare CNVs, 4 deletions and 2 duplications, which were shared by at least 2 families. Their sizes ranged from 75 to 224 000 bp. Among them, four were identified in more than two

families (Table 2). The limits of the duplication at 10q26 slightly varied from one family to another and were centered on the Chr10q26 (135508269-135290022) interval of 220 kb, including *Cen-CYP2E1-FRG2B-SYCE1-DUX4-DUX4L3-tel*. None of these genes were obvious candidate for GGE. The 5 remaining rearrangements were intragenic. Two were localized in introns of *SUMF1* or *KANK1* gene, with no predictable consequences. The 75 bp deletion in *IFNAR2* gene is localized within exon 1, which is untranslated. The intragenic duplication of exon 2 to 11 in *CES1* gene likely changed the translation of this gene. *CES1* is weakly expressed in brain and its function as a member of the carboxylesterase family cannot be related to epilepsy. The deletion of exon 1 and 2 of *CYP1B1* likely constituted a loss of function variant. However, only pathogenic variants in *CYP1B1* at the homozygous or compound heterozygous state caused primary open-angle glaucoma. Moreover, none of these identified CNV segregated with GGE in family F8, F12, F14 or F19 (table 2). In addition, none of the candidate genes included in our list (Table S2) were harbored in the regions with copy number variations.

Candidate variants detected in WES data

The candidate variants were defined as followed: i) variants shared by all tested patients of the family, ii) a MAF<1%, iii) with predictable consequences, including for missense a CADD Score ≥ 20 , and iii) in a gene already related to GGE (level 1) or to epilepsy (level 2) or expressed in the cortex (level 3).

For families F8, no candidate variants have been identified at the hemizygous state in X-linked gene. In contrast, our prioritization process retained 49 rare missense variants at the heterozygous state (table S3). Among them, the best candidate was the c.6482G>T (p.Arg2161Leu) in *CACNA1A* encoding the $\alpha 1$ subunit of the voltage-gated channel, which had already been involved in GGE {27} and epileptic encephalopathies {28}. In addition, a

spontaneous frameshift variant at homozygous state was shown responsible of the well-known mice model of absence epilepsy, the tottering mice {29}. This variant was predicted deleterious by CADD predictors with a score of 23. Its MAF in African GnomAD controls is 2.4×10^{-4} , in database GME 2×10^{-3} , and 3% in the 119 Sudanese controls.

In F19, a unique rare variant, c.13757C>T (p.Thr4586Met), in *FAT1* segregated at the homozygous state in all patients and was at the heterozygous state in one unaffected sib (IV-8). This variant is predicted deleterious by both M-CAP and CADD predictors with a score of 26. Its frequency is very low in AF GnomAD and GME NEA/PA and absent of the 238 Sudanese chromosome controls. The Bipoint-lodscore between this variant and the phenotype was 2.53 at $\theta=0.00$, supporting the involvement of *FAT1* in the phenotype. This variant is located in the extreme C-terminal domain of the protein interacting with the Scribble protein, which regulates dendritic spine development in association with NOS1AP {21}.

In F12, a WES was performed in 5 affected members including two patients from consanguineous marriages, leading to a very short list of candidate variants (Table S3): the c.63578G>A (p.Arg21193His) variant (CADD score=23.8) (plus the c.28299C>G (p.Asp9433Glu) but with a CADD score of 17.04) in the *TNN* gene (chr2:178,525,989-178,807,423 bp) and the c.310T>C (p.Ser104Pro) variant (CADD score=24.4) in the *SLC38A11* gene (chr2:164,898,329-164,955,486). These variants were at homozygous or heterozygote state in affected members. They were located in the same genomic interval of chromosome 2 and co-segregated in the family (Figure 1). Since both genes have not been related to epilepsy until now, it could be hypothesized that a variant in a gene between *SLC38A11* and *TNN* and not detected by SNPs microarrays or WES was responsible for the phenotype. The cluster of *SCN1A*, *SCN2A*, *SCN3A*, *SCN7A* and *SCN9A* gene encoding the α_1 , α_2 , α_3 , α_7 and α_9 subunits of the sodium voltage-gated channel, respectively, were localized very close to *SLC38A11*.(Figure 5)

In F14, since no candidate SNPs have been identified at the hemizygous or homozygous state, 32 missense variants at the heterozygous state fulfilled our genetic criteria. Among them, the c.1808G>A (p.Arg603His) variant (CADD score=25) was localized in the coding sequence of the *FBXO42* gene, which was recently reported in the top 3 of candidate genes for GGE by the Epi25 consortium (2021). This gene, which is highly expressed in the cortex in both development and post-natal period (Human Brain Transcriptome: <https://hbatlas.org/hbtd/images/wholeBrain/FBXO42.pdf>) encoded the F-Box Protein 42 acting as a protein-ubiquitin ligase. Variants have also been identified in other genes already related to epilepsy: c.712G>A (p.Glu238Lys) (CADD score=29.6) in *PIGG* (Phosphatidylinositol Glycan Anchor Biosynthesis Class G Protein) which is associated with an AR syndrome associating intellectual disability, hypotonia and seizures {22} and c.1993G>A (p.Val665Ile) (CADD score=22) in *LAMB1* encoding the laminin β 1 protein {23}. By Sanger sequencing, it was shown that both *FBXO42* and *PIGG* variant were identified in patient (V-11) (Figure 2) who was not included in the WES study.

In F7, no variants at the hemizygous or homozygous state fulfilled the selection criteria. No frameshift variants overcame the filters. 5 candidate missense variants have been selected by our algorithm, but none of them affected a gene previously related to epilepsy or seizures. In the 4 available patients of the family, the c.1441C>T (p.Pro481Ser) variant was identified in the *DCSH1* gene, which encodes the Dachshous cadherin related-1 protein also called Protocadherin 16 (*PCDH16*). Two Protocadherins, 7 and 19, have already been implicated in GGE and in female-restricted epilepsy and mental retardation (EFMR) {24} respectively. Moreover, *DCSH1/PCDH16* was a receptor of *FAT4*, belonging to the same protein family than *FAT1* gene incriminated in F19. The 3 patients with JME shared 7 additional candidate variants. The (p.Ser251Gly) in *ASTN2* encoding Astrotactin2 and the c.437C>G (p.Ala146Gly) in

CAMSAP1 represented the best candidate by function. Astrotactin2 regulates the surface expression of Astrotactin1 in glial-guided neuronal migration and modulates synapse strength in post-migratory neurons by trafficking and degradation of surface proteins. It is one of the 200 top genes identified in the epi25 consortium studies {24}{25} *CAMSAP1* plays a key role in the neuronal polarity by regulating the number of microtubules {26}.

Search for additional rare variants in candidate gene

For each gene with at least one candidate variant, additional variants were searched for in WES of probands in the 19 remaining families. We used the same filter criteria as those applied in WES process. The segregation of each variant was established after determining the genotype of family members by Sanger sequencing and indicated on the pedigrees (Figure 3). The retained variants are listed in table 3.

Additional variants were identified neither for *CACNA1A* nor for *FAT1* nor for *SCL38A11* selected in F8, F12, and F14 respectively. In contrast, in F7, additional variants were identified in *DCHS1* in family F18, and 1 in *ASTN2* in family F8. In family F8, the pArg898Gln variant in *ASTN2* segregated in the two JME patients but not in the affected sib with GTCS, the same condition than in F7.

DISCUSSION

We applied an integrative approach combining CNV detection and identification of candidate genes by WES in a cohort of 20 multigenerational families from Sudan with likely monogenic inheritance or at least clustering of patients. We took advantage of the particular familial structure in tribes or closed communities of the Sudanese population, associating endogamy and founder effects. Moreover, the size of siblings in rural areas of Sudan facilitates the identification of families with many affected members.

In the families, F8 and F14, for which a mode of transmission was determined with confidence, candidate genes segregated with GGE: *CACNA1A* in F8 and both *FBXO42* and *PIGG* in F14, which were already related to GGE {27}{28}.

In the F19, the perfect segregation with GGE of the likely pathogenic *FAT1* variant, c.13757C>T (p.Thr4586Met), with a lodscore of 2.53 at $\theta=0.00$ highly suggested the responsibility of *FAT1* in the phenotype. Frameshift, splicing and missense variants in *FAT1* were first described in nephropathic syndrome, tubular ectasia and haematuria {30}. Moreover, missense variants were identified in neurological diseases as fascioscapulohumeral-like dystrophy and autosomal dominant spinocerebellar ataxia (Figure 4) {31}. Ware and colleagues {32} reported a patient with epileptic encephalopathy including myoclonic jerks, focal motor seizures at 2 years, hypotonia, delayed visual maturation and severe intellectual disability, who was compound heterozygous for the ultra-rare c.8626G>C (p.Asp2876His) and c.7655A>G p.(Glu2552Gly) variants in *FAT1*. The c.13757C>T (p.Thr4586Met) variant in family F19 was in the highly conserved C-terminal 4584-HTEV-STOP codon motif which interacted with the scribble pathway playing a role in the neurite outgrowth {33}{34}{35}. From our knowledge, no missense variant has been reported in this C-terminal motif.

The functions of these genes are very different, from channels to component of proteasome. However, we identified likely pathogenic variants in gene encoding transmembrane proteins with structural and signal transduction function, especially at synapse in this Sudanese Cohort: *DCSH1* encoding the Protocadherin 16 {36}, *FAT1* an atypical Cadherin and *ADGRV1* an adhesion G protein-receptor (Dahawi et al., in press). This finding might be specific of the Sudan population. However, *ADGRV1* was reported in 2 JME patients in an Australian cohort {14}.

In order to identify susceptibility genes in complex diseases, studies on familial forms are complementary to those on large cohorts of sporadic cases. The large families with GGE are rare, explaining why the studies on familial forms are infrequent. The main advantage of familial cohorts is the possibility to study the segregation of identified variants with the phenotype. In a recent publication, Huguet and colleagues (2021) estimated that about 10 000 genes modulated cognitive ability in humans {36}. If the number of genes controlling firing of neurons is in the same order of magnitude, we can understand why no genes have been statistically involved until now by the international Epi25 consortium, which already performed WES in 4,453 GGE patients from different origins versus 8,364 matched controls. The target of 4,453 GGE patients tested was set in order to overcome the problem of genetic heterogeneity in GGE. Because of the relative homogeneity of the ethnic background of the Sudanese cohort, we could expect to diminish the genetic heterogeneity of GGE. In this way, we recently reported that susceptibility *ADGRV1* variants were frequent (25%) in Sudanese GGE families (Dahawi *et al.*).

Good candidate genes have been identified in families with a recognizable inheritance as in families F8, F12 and F19. Even they may interact with others genes, their variants segregated with GGE in AD (with incomplete penetrance) or recessive transmission. However, in F8, the

frequency of the *CACNA1A* variant is high (3%) in the Sudanese controls, raising the question of its responsibility in the disease or of the relevance of the variant frequency threshold used ($MAF < 1\%$). No additional variants have been identified for *FAT1* in the Sudanese cohort. GGE due to variants with a relatively strong effect might be very rare as shown for *GABRA1*-related JME, regardless of the studied population.

In family F7, the rare candidate variants segregated with GGE following a non-conventional transmission. They were always transmitted by an asymptomatic parent. We could hypothesize that the combination of variants in different genes, transmitted by each asymptomatic parent, determined the phenotype. This hypothesis was highly supported by Shirley and his colleagues in ALS {38}{39} and Artem Kim et al. in Holoprocencephaly {40}. The family F7 can be considered as a tribe with founder effects and deep endogamy. Several variants could circulate in the tribe till they were transmitted together in the same family member, who developed GGE. Since the 3 JME patients received the 3 variants in *DCSH1*, *ASTN2* and *CAMPS1*, 8/9 at-risk individuals, for whom it lacked at least one variant, did not develop JME (Figure 3). The hypothesis H_0 of independence of the two variables, genotype with 3 or < 3 variants, and occurrence of JME, could be rejected (Fischer's exact test, $p = 1.8 \cdot 10^{-2} < 0.05$). Despite the low number of tested individuals, there is a statistically significant association between the co-occurrence of the 3 variants and JME in F7. In the general population, the frequency of this association of variants should be much rarer, most often occurring in rare sporadic cases. It is interesting to note that in F8, *ASTN2* variant was also present only in the two patients with JME and not in the affected sib with GTCS (Figure 3). These data made *ASTN2* a good susceptibility candidate variant for JME, added to the fact that *ASTN2* is on the top 200 genes in the epi25 consortium study.

In Sudan, epilepsy, even GGE, which is a relatively mild phenotype, remains a taboo, especially in rural areas. This is why all identified patients could not be examined and sampled in the collected families. We expect that communication on advancement of our work will encourage family members to participate to future studies. The relatively small size of our cohort can represent a limitation that weakens the reliability of our results. This argument is common to the rare studies based on familial forms with human complex diseases including GEE. Another limitation of our approach is that we only analyzed the untranslated, translated and flanking splicing regions of genes, missing variants localized in noncoding or in far regulatory regions. Whole genome sequencing might be the appropriate technique to explore our hypothesis of a cryptic susceptibility variant in the *SLC38A11-TTN* region in family 12.

Conclusion

Our approach on familial form of GGE is complementary to large-scale collaborative consortia studies of sporadic cases. Both strategies incriminated similar genes such as *ASTN2* or *FBXO42*. The fact, that these candidate genes were identified by two different strategies in different populations, was an argument in favor of their role as susceptibility variants in GGE. The identification of *FAT1* as a probable susceptibility gene for GGE, points out with *DCHS1* a new pathway. In addition, our study reinforces the hypothesis that GGE is mainly oligogenic.

Figure1. Segregation of *SLC38A11*, *TNN* in F12

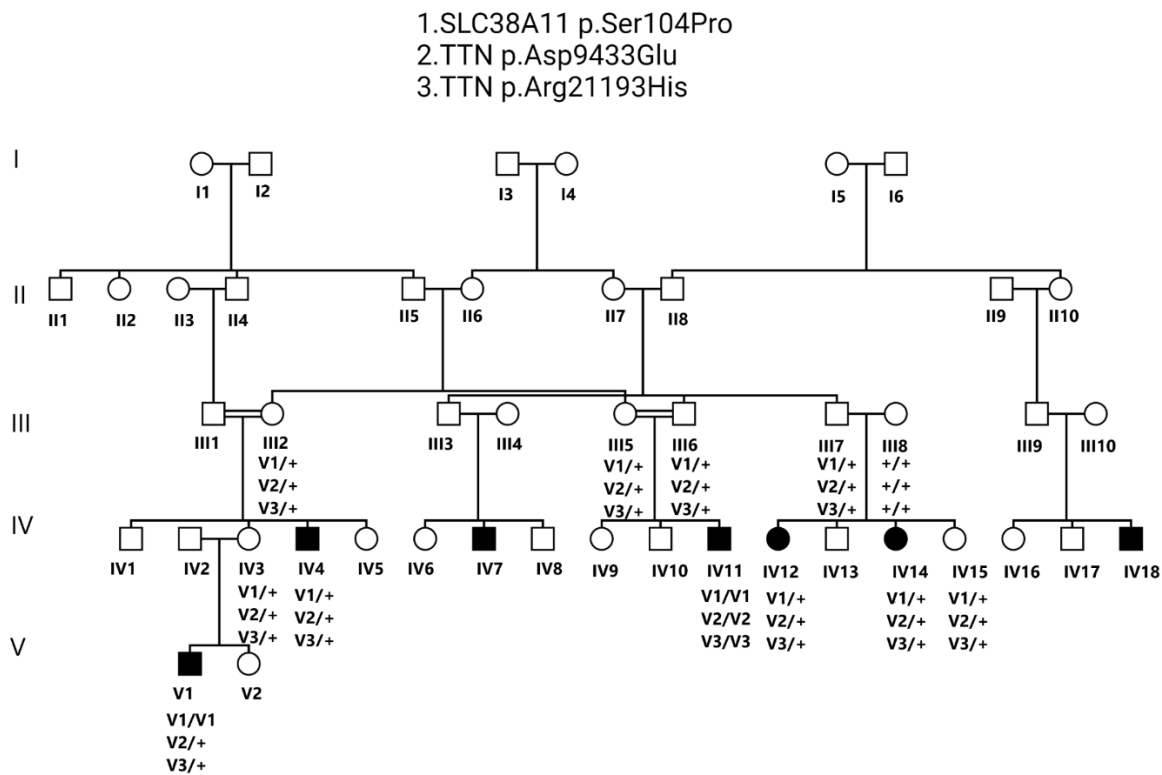


Figure 2. Segregation of *FBXO42* and *FAT1* in F14, F19

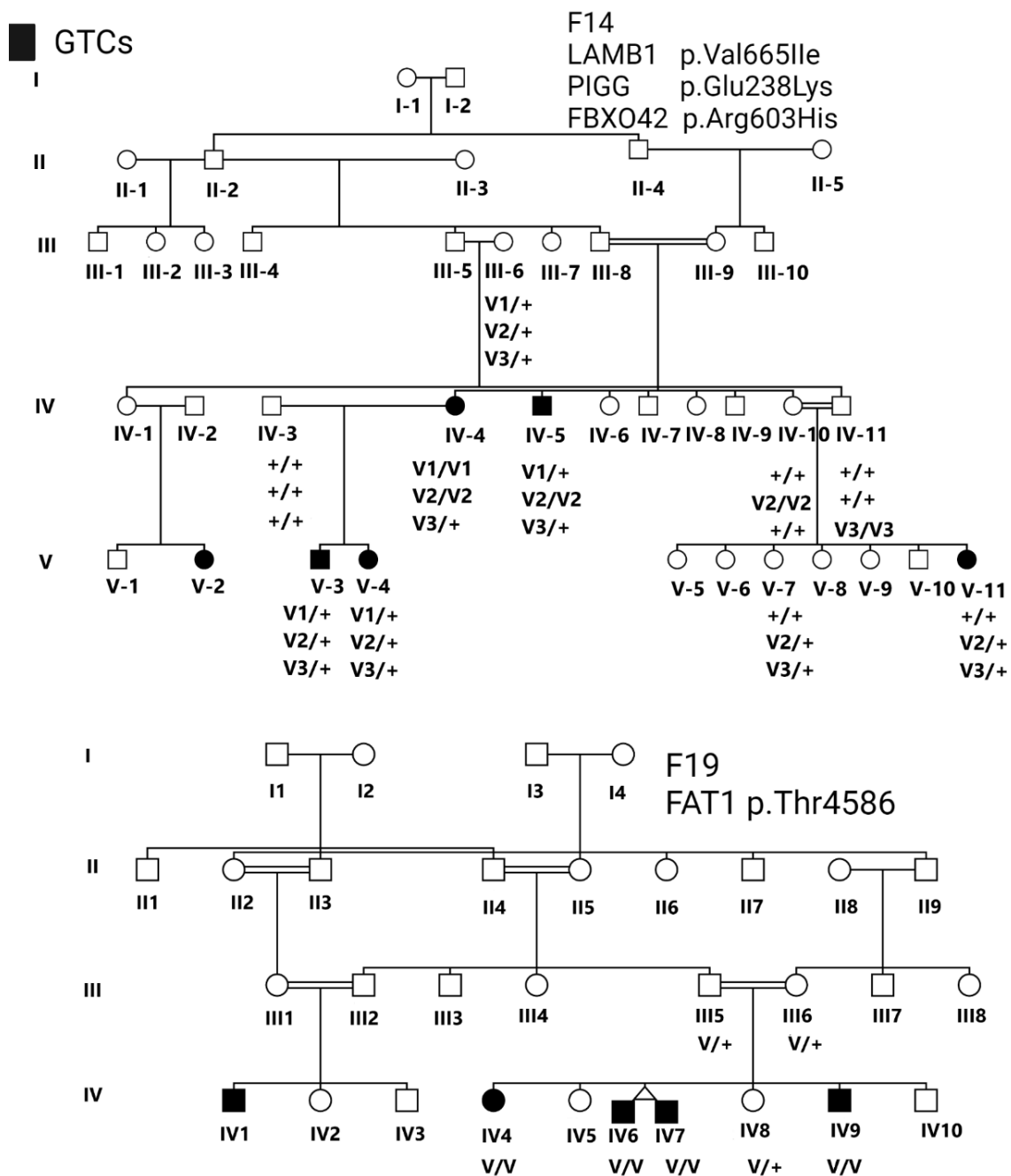


Figure 3. Segregation of *DCHS1*, *ASTN2* in F7, F8, F18.

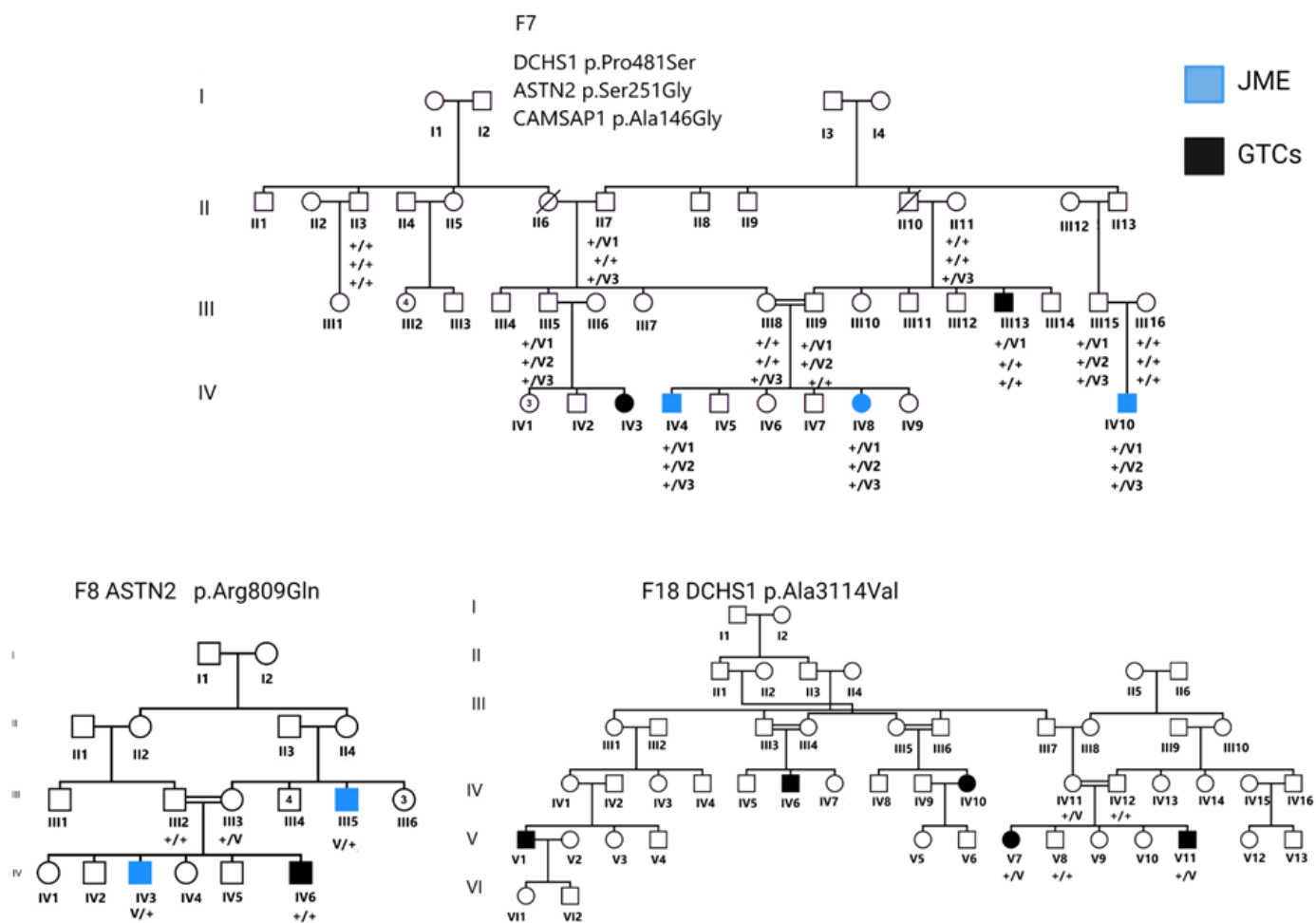
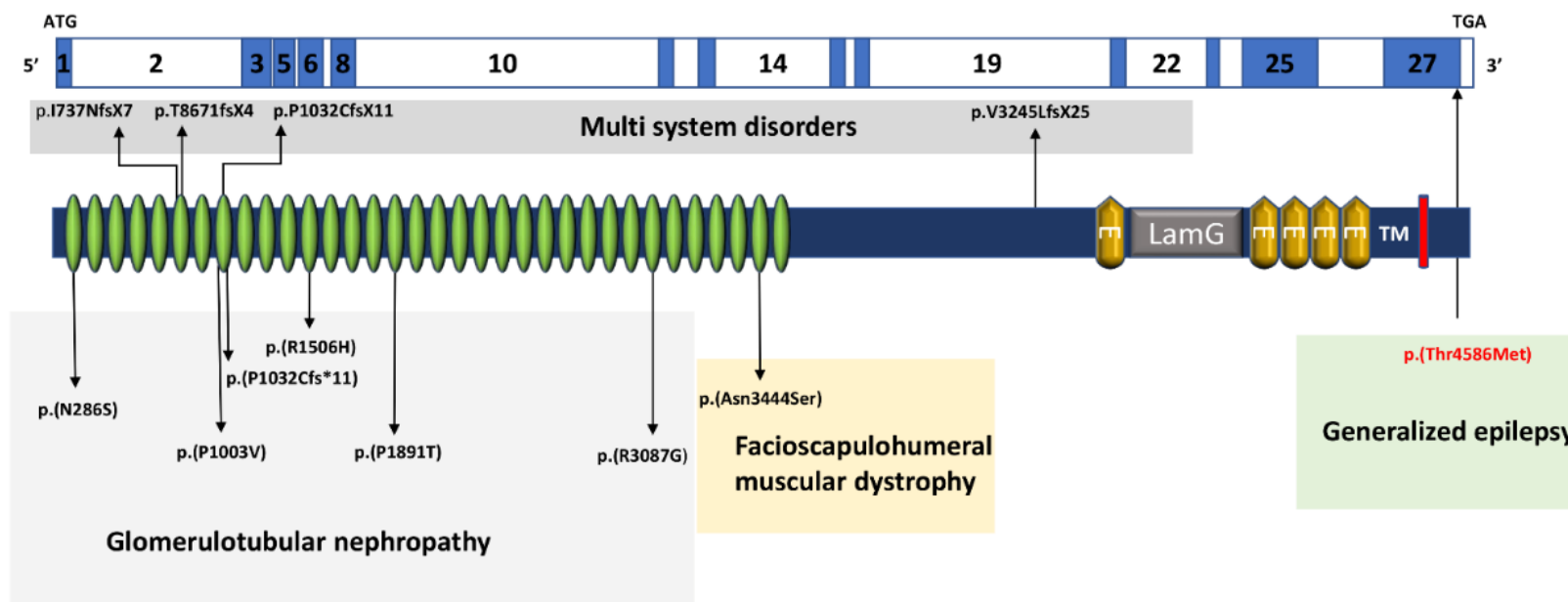


Figure 4. *FAT1* involvement in different diseases

FAT1 has 27 exons. Positions of start codon (ATG) and stop codon (TGA) are shown. Protein domains are depicted by coloured bars in relation to encoding exon positions. FAT1 contains 33 cadherin domains (CA), a laminin G domain (LamG) and five epidermal growth factor (EGF)-like repeat domains (brown) in its extracellular region, followed by a transmembrane region (dark blue bar) and a C-terminal cytoplasmic domain containing a PTB-like motif (red bar) with a PDZ-binding motif (-HTEV). Mutations were shown for different diseases in which FAT1 is involved: glomerulotubular nephropathy, Facioscapulohumeral muscular dystrophy, Generalized epilepsy.

Table 1. Clinical data of the different families.

Family ID	Patient No.	Sex	Age at seizure onset	Age at examination	Epilepsy type	Sz/week before treatment	Triggering stimulus	Neurological examination	current therapy	Response to treatment
F7	IV10	M	20Y	26Y	JME	Unspecified	None	Normal	Na valproate	Responder
F7	III13	M	9Y	37Y	GGE-TCS	0-1	Lack of sleep/Fatigue	Normal	Carbamazepine	Responder
F7	IV4	M	20y	23y	JME	0_1	None	Finger deformity	Carbamazepine	Responder
F7	IV8	F	15y	16y	JME	2_3	None	Normal	Na valproate	Responder
F12	IV11	M	6 Y	7 Y	CAE	14_21	None	Normal	Na valproate	Responder
F12	V1	M	5M	6 Y	GGE-TCS	1_2	None	Normal	Na valproate	Responder
F12	IV4	M	3Y	11Y	GGE-TCS	7_8	None	Normal	Na valproate	Responder
F12	IV12	F	5Y	7Y	GGE-TCS	More than 14 times	None	Normal	Na valproate	Responder
F12	IV14	F	12Y	16Y	CAE/GGE-TCS	0_1	None	Normal	Na valproate	Responder
F14	IV5	M	10 Y	52Y	GGE-TCS	5_7	TV/lack of sleep	Normal	Na valproate	Responder

F14	V3	M	Birth	21 y	GGE-TCS	3_5	Watching TV, Looking at sun light	Normal	Na valproate plus Levitracitam	Poor sz control
F14	V4	F	10 y	19 y	GGE-TCS	10_14	Watching TV/sitting in front of the computer	Normal	Na valproate	Responder
F14	IV4	F	10 y	46 y	GGE-TCS	8_14	Watching TV	Normal	Na valproate	Responder
F14	V11	F	16Y	20Y	GGE-TCS	3_4	Sun exposure_ mental or physical stress	Normal	Na valproate	Responder
F19	IV9	M	20 Y	24 Y	GGE-TCS	2_3	Excessive activity, fatigue	Mild motor deficit in the upper and lower limbs, mild cerebellar ataxia.	Na valproate	Responder
F19	IV4	F	4 Y	10 Y	GGE-TCS	0_1	Lack of sleep	Normal	Na valproate	Responder
F19	IV6	M	8 Y	8 Y	GGE-TCS	0_1	Lack of sleep	Normal	Na valproate	Responder
F19	IV7	M	8 Y	8 Y	GGE-TCS	0_1	Nothing specific	Normal	Na valproate	Responder

Table 2. CNVs in the different families.

Locus	Type of CNV	Interval	Size (bp)	Statut	Gene	Families	Segregation with GGE (tested Family)
2p22.2	del	38301915_38303095	1180	het	<i>Exon 1 and 2 of CYP1B1</i>	F7; F16	No (F7; F16)
3p26	del	4103926_4328131	224,205	het	<i>Intron 8 of SUMF1</i>	F6; F14	No (F14)
9p24.3	del	517563_527180	9617	het/hom	<i>Intron 3 of KANK1</i>	F7; F8; F12; F15; F17	No (F7; F8; F12; F14; F17)
10q26	dup	135508269-135290022	218,247	het	<i>Cen-CYP2E1-FRG2B-SYCE1-DUX4-DUX4L3-tel</i>	F6; F4; F16; F17; F5	No (F17)
16q12.2	dup	55843252_55864452	21,200	het	<i>Exon 2-11 of CES1</i>	F20; F16; F19; F18	No (F16; F19)
21q22.11	del	34602246_34602321	75	het	<i>Exon 1* of IFNAR2</i>	F6; F7; F8; F14	No (F7; F8; F14)

Table 3. Genetic data of the different genes from families F19,F14,F7,F18,F12

Gene	Family	Genomic position (hg19)	cDNA variant	Protein variant	rs-number	M-cap	CADD	Gnomad		GME (NEA+AP)	Sudanese Controls (n=119)	Domain
								Overall	Africans			
FAT1	F19	4 :1875097 56-G-A	c.13757 C>T	p.(Thr4586 Met)	rs376922855	D	26	0.00005	0.00004	Not reported	Zero	Not in an identifiable domain
FBXO42	F14	1 :1657751 1C>T	c.1808G >A	p.Arg603His	rs761599198	D	25	0.00002	0.00008	0.0009	0.004 (1 control)	Not in an identifiable domain
CAMSAP1	F7	9 :1387736 27G>C	c.437C> G	p.Ala146Gly	rs186863224	B	22	0.00007	0.0005	0.002	0.008 (2 controls)	
DCHS1	F7	11 :666140 4G>A	c.1441C >T	P.pro481Ser	rs140464593	B	21.9	0.00001	0.00008	Zero	0.008 (2 controls)	Cadherin 5
DCHS1	F18	11:6643566 G>A	c.9341C >T	p.Ala3114 Val	rs776120615	B	19.33	0.000007	0	Not reported	Zero	Not in an identifiable domain
ASTN2	F7	9:11924968 7 T>C	C.751A> G	p.ser251Gly	rs140483683	B	23.9	0.00009	0.001	Not reported	Zero	EGF-Like3
ASTN2	F8	9 :1194913 18 C>T	c.2426G >A	p.Arg809Gln	rs766074874	D	24.3	0.00003	zero	Not reported	0.004 (1 control)	Not in an identifiable domain
SLC38A11	F12	2:16579601 9A>G	c.310T> C	p.Ser104Pro	rs138491284	B	22.6	0.0003	0.0002	0.006	0.012 (3 controls)	-no domains found
CACNA1A	F8	19: 13320188	c.6482G >T	p.Arg2161 Leu	rs572722130	B	23	6.10 ⁻⁵	0.00024	0.002	0.03 (7 controls)	Not in an identified domain

Declarations

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Code availability: Not applicable.

Authors' contributions: MD contributed to the design of the study, collection, analysis, and interpretation of most data. MSE and MA referred the patients and clinically evaluated them. EAA, FAE, WAA, OA, SG and RA contributed to the inclusion of patients and sample collection. JMDSA contributed to the interpretation of WES, TC to the interpretation of SNPs microarrays and JB to the bioinformatics analyses. SBal and EAA contributed to the figures design. SBal, SB, LA and EN critically reviewed the manuscript. AE contributed to supervising and revising the manuscript. EL designed, supervised and obtained funding for the study. MD and EL wrote the manuscript.

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Consent to participate: Written informed consent was given by all participants.

Consent for publication: Written informed consent was given by all participants.

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Chapter 10: Results *C. elegans* part (work in progress)

Our lab has identified novel candidate genes causing CAE in a Tunisian family namely KIF13-B (kinesin-like protein) and IGF1-R (insulin like growth factor 1 receptor). These two genes segregated in an autosomal recessive manner in four siblings with CAE (see **Table 5**, **Figure 16**). Pedigree showing the Tunisian four affected siblings with the segregation of the 2 genes KIF13B and IGF1R.

Orange circles or squares represent sibs with CAE, Hatched circle mother with GTCS and “+” relatives with abnormal EEG profiles but no seizures (**Figure 16**). In this chapter, the main aim is to demonstrate and investigate their responsibility in GGE, using a *C. elegans* model.

A recent study showed that *KIF-13B* is involved in *Sonic hedgehog* (Shh) signaling ([Schou et al., 2017](#)), a pathway known to influence neurogenesis and neural patterning during the development of the CNS. Studies describe that a dysregulation of Shh signaling in the brain may lead to neurological disorders, among which is epilepsy ([Patel, Tomar, Sharma, Mahindroo, & Udayabanu, 2017](#)).

IGF1-R (*daf-2* ortholog in *C. elegans*) is another interesting gene related to lifespan extension in humans ([Leal et al., 2011](#)). Extended longevity in humans is associated with a distinct transcriptomic signature in the cerebral cortex, characterized by downregulation of genes related to neural excitation and synaptic function. In parallel, the worm longevity can be dynamically regulated by the excitatory-inhibitory balance of neural circuits.

It was shown that in *daf-2* mutated worms, reduced signaling is associated with longevity. Of note, when REST, a transcription factor, is upregulated with extended longevity, it represses excitation-related genes. Loss of function mutations in the *C. elegans* REST orthologs, *spr-3* and *spr-4*, elevates neural excitation and reduce the lifespan of long-lived *daf-2* mutants ([Zullo et al., 2019](#)).

Our aim is to study the effect of each gene separately and the combined effects of both variants in the *C. elegans* model.

Pt No.	Sex	Age at onset	Age at examination	Neuro-psychological evaluation	Language	EEG/BT	AED compliance
IV-12	M	8Y	19Y	Mild attention deficit	dysL, dysO	N/UT	NO
IV-16	F	5Y	13Y	Memory and concentration deficits	dysL, dysC, dysO	N/UT	YES
IV-15	F	5Y	10Y	Mild attention deficit, memory disorder	dysL, dysO	GSWD 3Hz/UT	YES
IV-19	M	3Y	8Y	Mild attention deficit	dysL, dysC	GSWD 3Hz/UT	YES

Table 5. Clinical description of CAE in the Tunisian family patients.

DysL: dyslexia, dysC: dyscalculia, dysO: dysorthographia; N: normal UT: under treatment.

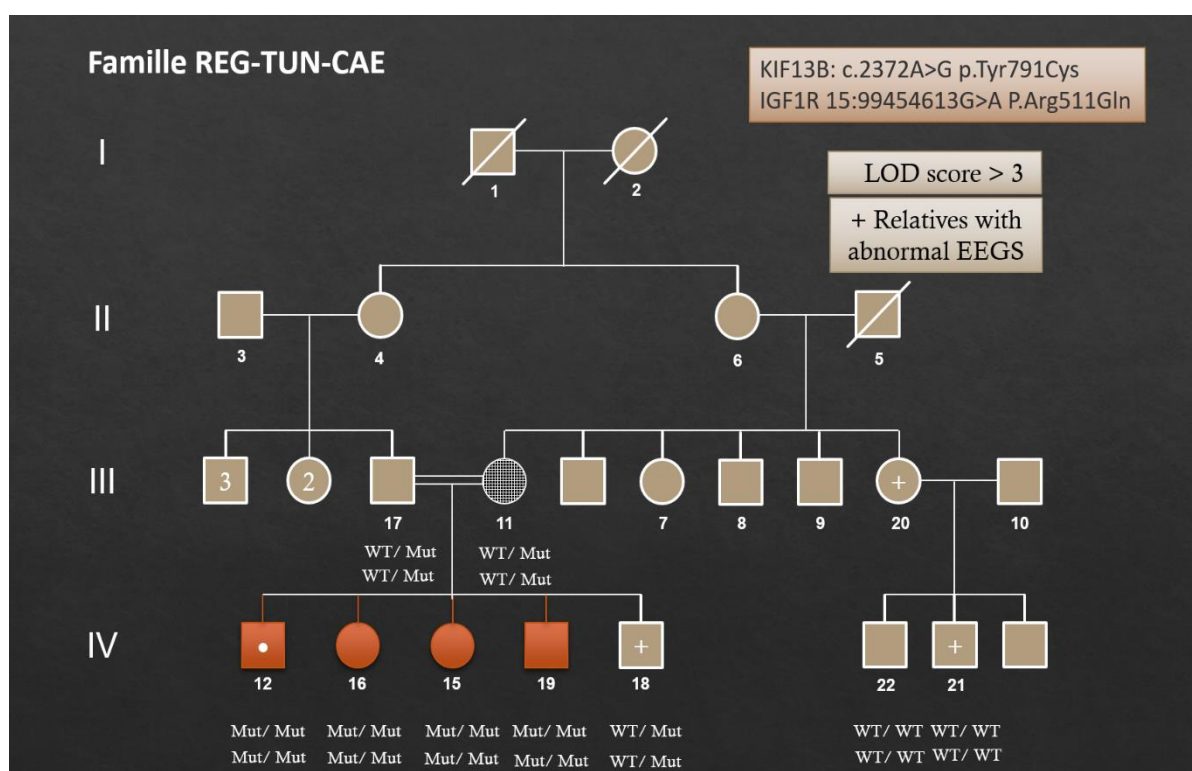


Figure 16. Pedigree showing the Tunisian four affected siblings with the segregation of the 2 genes *KIF13B* and *IGF1R*.

Orange circles or squares represent sibs with CAE, Hatched circle mother with GTCS and “+” relatives with abnormal EEG profiles but no seizures.

To be able to use PTZ to induce worm convulsions, we first verified that the administration of this selective GABA receptor antagonist does not cause neuronal loss in *C. elegans* GABAergic neurons, the most affected in generalized epilepsies. To do so, we used *unc-25::gfp* worms expressing the GFP fluorescent reporters specifically in the GABAergic neurons, in order to monitor their number/presence. The worms were synchronized and grown in an agar medium at 25°C. Young adult worms were exposed to increasing PTZ concentrations (0, 50, 300 and 600 mM). **Figure 17** shows that none of the PTZ concentration affected the number of neurons. Thus, PTZ administration is not toxic to GABAergic neurons.

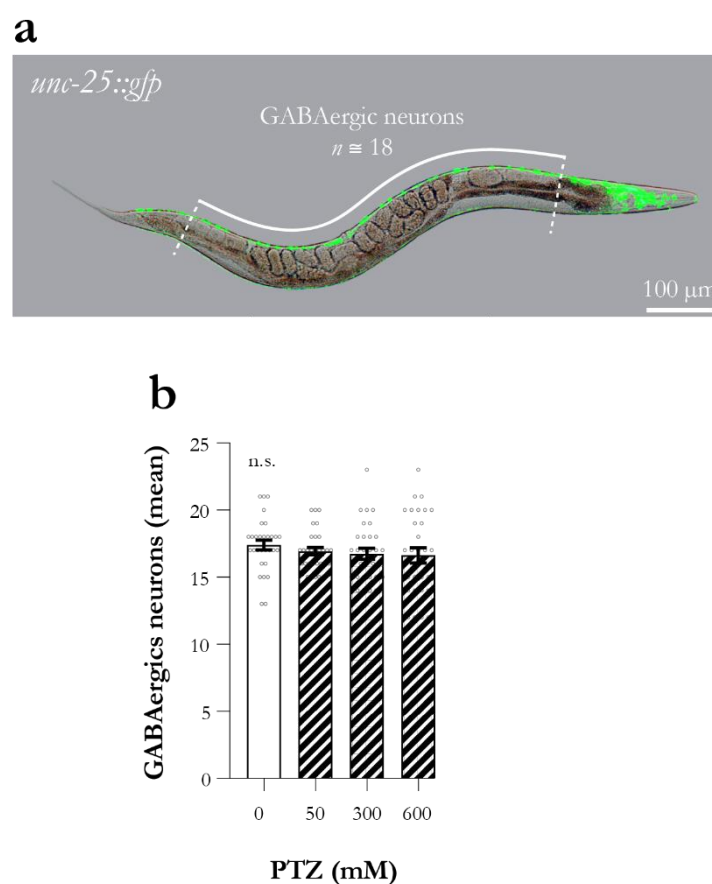


Figure 17. PTZ administration does not affect GABAergic neurons' integrity.

a. Brightfield and fluorescent imaging of *unc-25::gfp* worm. The GFP reporter labels GABAergic neurons among the ventral cord ($n \cong 18$). Scale: 100 μ m.

b. Young adult *unc-25::gfp* worms were exposed in liquid media to the indicated concentrations of PTZ for 5 minutes and were fixed by PFA 4%. Number of GABAergic neurons among the ventral cord ($n \cong 18$) were quantified with a fluorescent microscope at high magnification (objective x63). Data represents the mean \pm s.e.m; $N = 10$ with 3 independent experiments. Statistical analyses were performed with an ordinary one-way ANOVA, *Dunnett's* multiple comparisons test, untreated line versus all experimental conditions; *n.s.* is non-significant.

In order to test and validate our candidate genes, we first defined the effect of altering genes that are known to induce a severe early epileptic phenotype in humans. The first gene is *STXBP1*, causing *Ohtahara* syndrome (also known as early infantile epileptic encephalopathy with suppression-burst) and West syndrome in humans (H Saitsu et al., 2011; Hirotomo Saitsu et al., 2008, 2010). More than 100 different *STXBP1* variants have been identified, including missense mutations, truncating mutations, and partial/whole gene deletions (Stamberger et al., 2016).

Another gene is *FOXG1* (Forkhead box G1). It belongs to a clinical entity termed “*FOXG1*-related encephalopathy” in which children with deletions or intragenic mutations of *FOXG1* have recognized clinical features like microcephaly, developmental delay, severe cognitive disabilities, early-onset dyskinesia and hyperkinetic movements, stereotypies, epilepsy, and cerebral malformations (L.-C. Wong et al., 2019). Both genes were silenced through RNAi, along with the novel candidate gene detected in the Tunisian epileptic siblings *KIF13B* (Kinesin Family Member 13B). *Unc* (uncoordinated) mutant lines were used as controls: they manifested either spontaneous seizures, like *unc-43* mutant line (Reiner, Newton, Tian, & Thomas, 1999), or a head bobbing phenotype for the *unc-49* mutant when exposed to PTZ (Williams, Locke, Braden, Caldwell, & Caldwell, 2004a). We incubated young adult worms with the PTZ at a final concentration of 7 mg/mL for 15 minutes at 20°C. To quantify the proportion of the convulsing worms, short videos of 30 seconds were taken and analyzed for head and tail velocity using ImageJ software. PTZ-treated worms silenced for the genes *STXBP1* and *FOXG1* significantly decreased the head and tail movements compared with PTZ-treated N2 worms in which the empty vector was used (L4440).

Importantly, silencing *KIF13B* gene significantly reduced the head and tail movements in the same range as the known genes causing epilepsy (**Figure 18**).

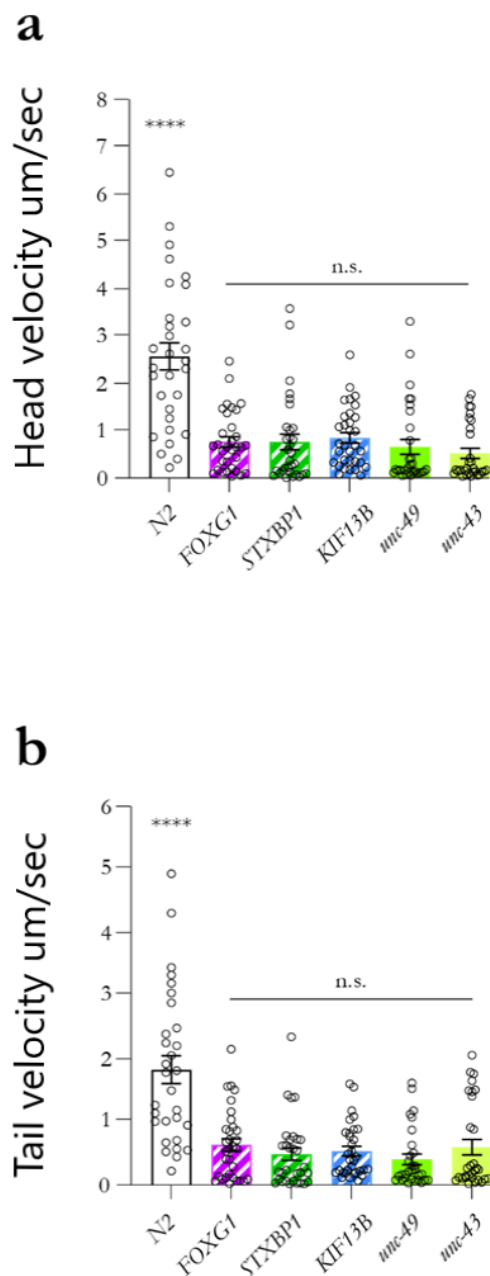


Figure 18 . Head and tail velocity of convulsing worms induced by RNAi gene invalidation.

Head (a) and tail (b) velocity of synchronized worms cultured on agar plates containing bacteria overexpressing selective dsRNA of *KIF13-B*, *FOXG1*, *STXBP1* genes, *unc-43* and *unc-49* mutant lines. Empty vector L4440 was used as a control on (N2) lines. Worms were then incubated for 15 minutes in 7 mg/mL PTZ. The incidence of the PTZ-induced seizure was measured as velocity of the head (A) and the tail (B) for each line in comparison with the empty control vector. Data represents the mean \pm s.e.m; N = 10 with 3 independent experiments. Statistical analyses were performed with an ordinary one-way ANOVA, Dunnett's multiple comparisons test, targeted gene with RNAi versus empty vector (L4440) and mutant lines (*unc-43* and *unc-49*); **** $p < 0.0001$; n.s. is non-significant.

In the previous figure, we used the knock-down method to test the whole gene, to study the role of the specific mutation identified in CAE patients; CRISPR-Cas9 gene editing method is used.

It allowed researchers to precisely edit any locus of their choosing, thereby facilitating an increased understanding of gene function. These edits can range from introducing premature stop codons into the *C. elegans* genome (e.g *rbm-3.2* gene) (Smith, Bergwell, Smith, Mathew, & Iyer, 2020), to unique point mutations that modulate the activity of potassium ion channels (Ben Soussia et al., 2019). We used a similar approach introducing two points mutations in two genes, (i) *KIF13B* and (ii) *IGF1R* (see materials and methods). Previous studies used thrashing behavioural tests to screen *STXBP1* pathogenicity in *C. elegans*, (Zhu et al., 2020) we applied the same test on synchronized young adult worms. They were grown on agar plates at 15°C, were moved to a liquid M9 medium and left to swim for at least one minute before starting to record the videos. Each point mutation was tested along with wild type referenced line (*N2*) and a corresponding knock-out line:

(i) *klp-Z19* line: the pz19 mutation corresponds to a single C-to-T point mutation (C3076T) in the cargo-binding tail of *klp-4*, that results in a premature stop codon (R1026Stop) (Monteiro et al., 2012)

(ii) *daf-2* (e1370: a temperature sensitive partial loss-of-function *daf-2* mutant (Apfeld & Kenyon, 1998). Interestingly, both targeted mutants showed a significant decrease of body bends compared to *N2* worms (Figure 19).

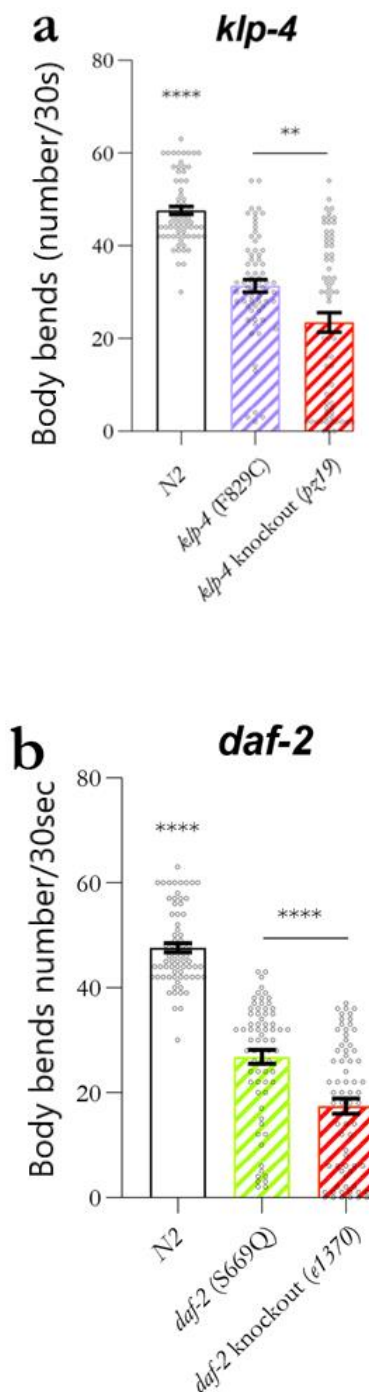


Figure 19. Mutant lines show significant defective locomotion.

The locomotion of young adults animals were quantified with thrashing assay test by counting the number of thrashes per 30 sec in liquid media. (a) The *klp-4* (F829C) mutant line and (b) the *daf-2* (S669Q) mutant line shows a significant defective locomotion in comparison with controls *N2*. Data represents the mean \pm s.e.m; $N = 20$ with 3 independent experiments. Statistical analyses were performed with an ordinary one-way ANOVA, *Dunnett's* multiple comparisons test, *N2* versus each line; **** $p < 0.0001$; *n.s.* is non-significant.

From an oligogenic perspective, the possibility of a genetic interactive/accumulative effect between these two genes was further investigated by the generation of a double mutant carrying both mutant alleles. Both our single targeted mutants and the double mutant *klp-4* (F829C) x *daf-2* (S669Q) showed significantly defective locomotion ($p < 0.0001$) compared to N2 (Figure 20). However, there was no statistical difference between the single mutants and the double mutant *klp-4* (F829C) x *daf-2* (S669Q).

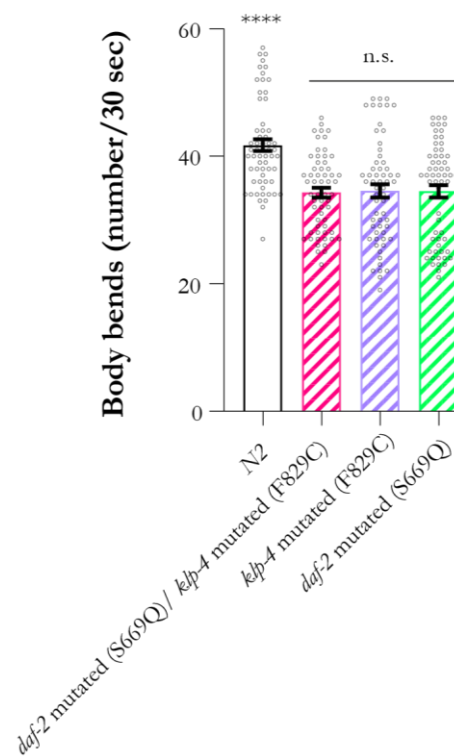


Figure 20. Locomotion velocity analysis of transgenic animals.

The locomotion of young adults animals were quantified with thrashing assay test by counting the number of thrashes per 30 sec in liquid media. The *klp-4* (F829C) mutant line, the *daf-2* (S669Q) mutant line and the double cross mutant (*klp-4* F829C / *daf-2* S669Q) show a significant defective locomotion in comparison with controls N2. Data represents the mean \pm s.e.m; $N = 20$ with 3 independent experiments. Statistical analyses were performed with an ordinary one-way ANOVA, *Dunnnett's* multiple comparisons test, double mutant line versus each line; **** $p < 0.0001$; n.s. is non-significant.

Though GABA is not known to be a neurotransmitter within the pharyngeal microcircuit (Franks, Holden-Dye, Bull, Luedtke, & Walker, 2006), GABAergic signaling has been reported to modulate pumping. We tested it in young adult worms on agar plates with bacteria.

The pumping frequency for each worm was calculated by the number of pumps over the total observation time (30 seconds) (Figure 21). Preliminary results showed a decrease in the frequency compared to wild type worms. Of note, this experiment was performed once, and further evaluation is ongoing. Figure 21 A (*klp-4* mutant F829C) compared to wild type N2 and a *klp-pz19* knockout line and Figure 21 B (*daf-2* mutant S669Q) compared to wild type N2 and *daf-2* knock out line (*e1370*).

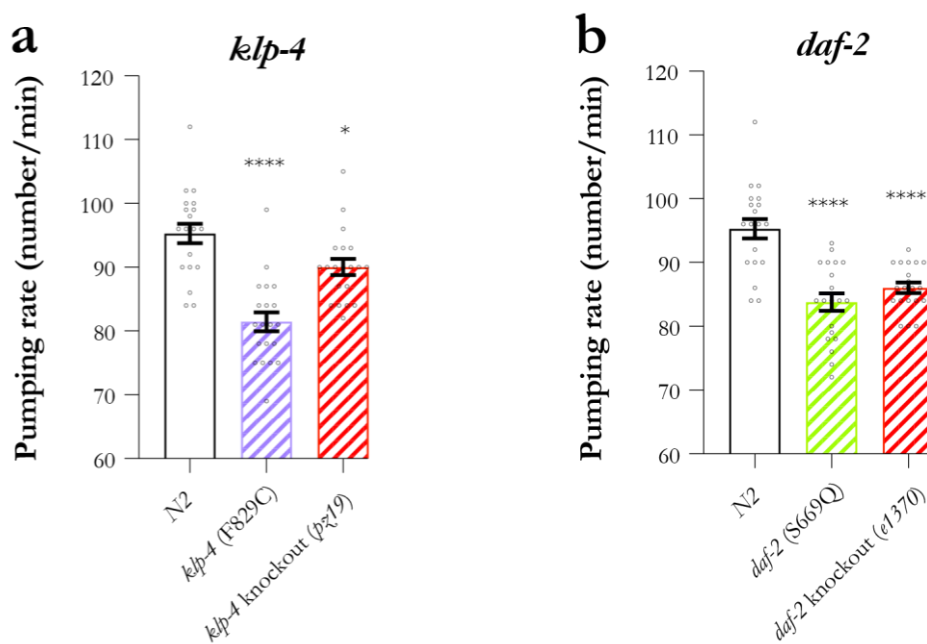


Figure 21. Pharynx activity is altered in the targeted mutants.

Pumping rate young adults *klp-4* mutant (a) or *daf-2* mutants (b) compared with N2 lines. The frequency of the pharyngeal pumping of young adults animals were calculated by the number of pumps in 30 sec.

Data represents the mean \pm s.e.m; N = 20 with 1 independent experiment. Statistical analyses were performed with an ordinary one-way ANOVA, Dunnett's multiple comparisons test, *klp-4* (F829C) was compared to N2 and *klp-4* (*pz19* mutant); *daf-2* (S669C) was compared to N2 and *daf-2* mutant (*e1370*); * $p < 0.05$ and **** $p < 0.0001$.

We focused on studying the effect of the targeted mutants GABAergic neurons. Ten wild type *unc-25::gfp* hermaphrodite males (generated by heat shock) were crossed with a single homozygotic *daf-2* (S669Q). A successful cross was assessed by GFP detection under the microscope. F1 virgin-GFP hermaphrodites were selected and were transferred to fresh NGM petri dishes and were left to produce progeny for 24 hours before being removed from the plates. F2 worms were separated in fresh NGM plates after GFP verification. From each cross the mutant *daf-2* (S669Q)^{GFP} was identified and the wild type correspondent *daf-2* control^{GFP} by PCR, same approach was done for *klp-4*. Neuronal count was done for each gene and compared with its corresponding control. There is no statistical difference of GABAergic neurons number between mutant and control animals, suggesting that the mutants do not induce GABAergic neuronal loss (**Figure 22**).

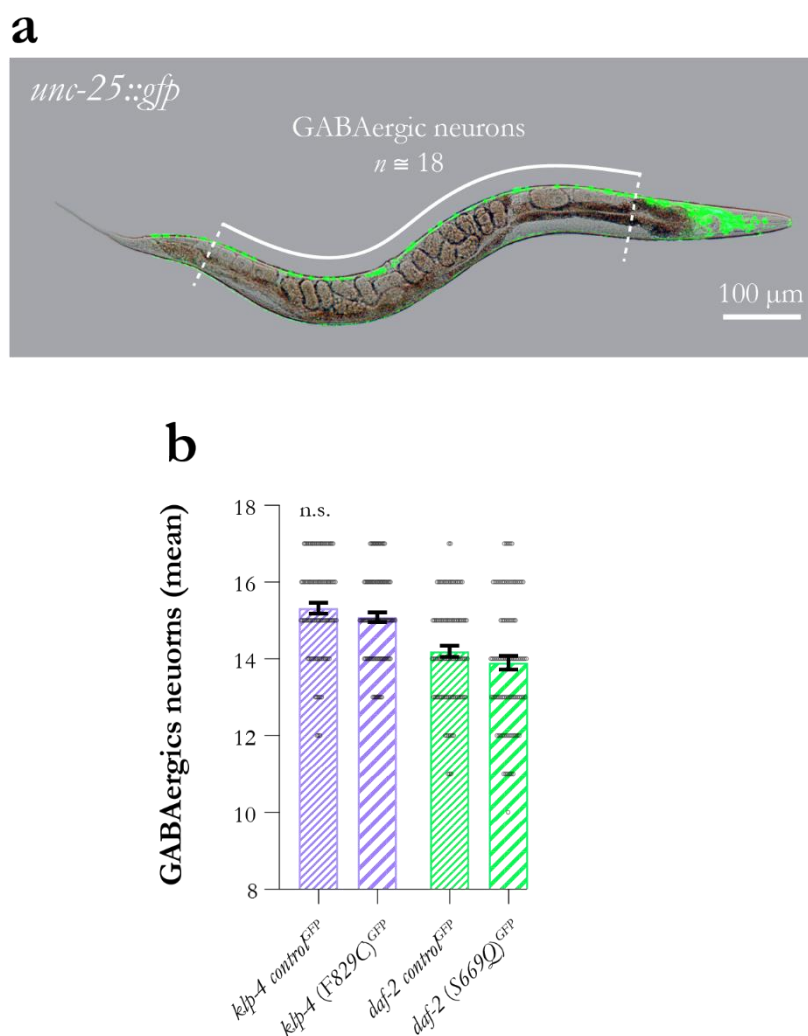


Figure 22. The mutations in *klp-4* (F829C) and *daf-2* (S669Q) genes does not affect GABAergic neurons integrity.

(legend on next page)

(a) Brightfield and fluorescent imaging of *unc-25::gfp* worm. The GFP reporter labels GABAergic neurons among the ventral cord ($n \cong 18$). Scale: 100 μm .

(b) Mean number of GABAergic neurons of PTZ-treated *unc-25::gfp* worms mutated (*klp-4* (F829C); *daf-2* (S669C)) or not (*klp-4* control^{GFP}; *daf-2* control^{GFP}) for *klp-4* and *daf-2* genes. Data represents the mean \pm s.e.m; $N = 30$ with 3 independent experiments. Statistical analyses were performed with an ordinary one-way ANOVA, *Dunnett's* multiple comparisons test, *klp-4* (F829C) versus *klp-4* control^{GFP}; *daf-2* (S669C) versus *daf-2* control^{GFP}; *n.s.* is non-significant.



Discussion

Chapter 11: Discussion

To our knowledge, in the human genetic era using WES, very few studies on familial forms of GGE have been performed (Landoulsi et al., 2018b). In contrast, studies on large cohorts of sporadic cases were published by international consortiums (A. S. Allen et al., 2017, Motelow et al., 2021). Little is known about the reasons of this discrepancy. We can hypothesize that large families with many affected members are very few, due to the low frequency of monogenic forms. Our work was strengthened by the recruitment of Sudanese families including up to 5 affected members, allowing an intra-familial approach already applied in monogenic diseases. This strategy gets around the problem of great genetic heterogeneity of GGE. It is an important call to replace strategies applied in the international research context and tunnel it to address specific questions.

I. From mono-to oligo/polygenic forms of GGE

In our research, we distinguished two types of pedigrees: (i) those with a recognizable monogenic mode of inheritance and (ii) those with a familial aggregation but without a defined transmission. For the former, solid candidate genes can be identified. For family F19, since only consanguineous sibs on a unique generation were affected, an AR inheritance was very likely. A candidate variant in *FAT1* was found at the homozygous state in patients with a statistically significant lodscore (publication 2). In the same way, for family F14, despite the consanguinity loops, the vertical transmission of the disease in one branch was in favor of an AD inheritance (with incomplete penetrance). A missense variant in *FBXO42*, a gene recently suspected by the Epi25 consortium (2021) (Motelow et al., 2021), segregated in all tested patients. These results were in favor of an AD inheritance with incomplete penetrance in family F14. These forms seem to be rare since no additional candidate variants in these genes have been identified in other Sudanese families. This is the case of genes which were shown responsible for AD GGE. Until now, only two genes have been reported: *GABRA1* encoding the $\alpha 1$ sub-unit of the GABA_A receptor, the Ala322Asp missense variant was identified at the heterozygote state, in a large French-Canadian family including 10 patients with JME, using a gene candidate approach after having mapped the disease on 5q34 chromosome. An *in vitro* electrophysiology study confirmed the functional consequences of the variant on

the GABA-evoked current. Since 2001, only 6 likely pathogenic variants (4 missense, 1 splicing variants and 1 deletion) in *GABRA1* have been reported in HGMD (July 2021) for AD JME and 5 (3 missense, 1 splicing variant and 1 insertion) for GGE. In addition, 52 other variants have been associated with unclassified epilepsy or epileptic encephalopathy, including West syndrome. The situation is more complex for the *EFHC1* gene encoding the EF-Hand domain (C-Terminal)-containing protein 1 (Myoclonin). Five likely pathogenic variants have been found in 6 unrelated families with JME from Mexican or European American origin with a linkage to 6p12-p11 (EJM1) (Suzuki et al., 2004). Both heterozygous and homozygous *EFHC1*-null mice developed frequent spontaneous myoclonus. Consecutive functional studies showed that Myoclonin increased R-type Ca^{2+} currents (Suzuki et al., 2004) interacts with microtubules to regulate cell division and cortical development (de Nijs et al., 2009), and that, it is not expressed in neurons but in choroid plexus and ependymal cells. This suggests that *EFHC1*-related JME could be a motile ciliopathy (Suzuki, Inoue, & Yamakawa, 2020). However, the responsibility of *EFHC1* in JME has been debated until now. Gonsales and colleagues (2020) showed that *EFHC1* variants identified in JME patients cannot be unequivocally classified regarding their impact on disease and hypothesized that they are not a monogenic cause of JME (Gonsales et al., 2020). Subaran and colleagues also reported pathogenic variants in control populations and hypothesized that some *EFHC1* variants may play a pathogenic role in JME only when introduced into specific genetic backgrounds. These hypotheses are at the frontier between mono- and oligogenic inheritance and will be discussed further in the context of the Sudanese population.

For human diseases as GGE, oligo/polygenism is the most probable mode of inheritance when the vast majority of patients are sporadic cases (Berkovic & Neale, 2019), only 15% of patients with GGE have a first to second-degree affected relative. In familial forms, oligogenism can be also suspected when a monogenic inheritance does not perfectly fit with the segregation of the disease in pedigrees. In the Sudanese cohort, this condition is frequent with affected members dispersed within large families combining consanguinity loops and vertical transmission of the disease.

The oligogenic hypothesis was reinforced by genetic findings of a statistical significance. Rare missense variants in *ADGRV1* were statistically associated with GGE in 5 among the 20 Sudanese families (publication 1, Figure 1). However, their segregation with the phenotype

did not correspond to any defined monogenic inheritance. Each variant was carried at the heterozygote state by affected sibs, who received it from one of the two asymptomatic parents. This result leads to the hypothesis that GGE could be determined by one *ADGRV1* variant and at least another variant in a different gene, this was also a conclusion of another Australian study on *ADGRV1* (K. A. Myers et al., 2018). This oligogenic hypothesis has long been formulated. In 1992, Janz and colleagues asked the question of environmental and genetic factors interacting with the unique locus identified at that time on chromosome 6p in familial form of JME (T. Sander, Bockenkamp, et al., 1997).

The family F7 particularly reflects this condition (publication 2). Three interesting candidate variants, in *DCHS1*, *ASTN2* or *CAMSAP1* have been transmitted together in the 3 patients with JME. In contrast, at least one of these variants was missing in 8/9 at-risk members without JME, especially their parents. Despite the weak number of F12 members, there was a statistically significant association between the presence of the 3 variants and the occurrence of JME in the family (publication 2). This pedigree should be enlarged in order to strengthen this conclusion.

II. From rare to frequent variant and back: the place of the studies of GGE familial forms

In our work, we collected familial forms of GGE in the Sudanese population. We then applied the current strategy of WES to identify candidate genes as it has been done for monogenic diseases. We selected non-silent and rare (minimum allele frequency (MAF) < 1%) variants shared by affected members of each family with at least 3 available patients. The results of our study have to be placed in the context of the efforts of the international community to identify the genetic factors of GGE.

The hypothesis of polygenic inheritance for GGE led to the question of the characteristics of the susceptibility variants compared to those of causative variants in monogenic forms, especially their frequency in the control populations. The successive approaches used to identify the genetic factors determining GGE could identify either common (MAF ≥ 1%) or rare (MAF ≤ 1%) / ultra-rare (MAF < 1/10⁴) variants.

Candidate-gene approach (CG) was the first strategy used to search for susceptibility genes in GGE. CG was applied on both families and sporadic cases. CG was sometimes combined with functional studies including cellular biology experiments or *in vitro* electrophysiology. The candidate genes mostly encoded subunits of voltage (calcium or potassium channels) or neurotransmitters (GABA_A receptor) gated ion channels (**Table 6**). The Sanger technique was used to detect variants in index cases and to study their segregation with the phenotype when families were available. In these studies, authors searched for rare variants, often absent in the control population. The maximum number of unrelated patients included in such a study was 455 (Neubauer et al., 2008b). For 9 among the 14 reported genes, the results were statistically significant but not generally replicated (**Table 6**). However, some of them were confirmed by another strategy (see next paragraph). Only two genes were incriminated for CAE twice, ie. in two different populations: *CACNA1H* encoding the calcium voltage-gated channel subunit $\alpha 1H$ in Australian population (Heron et al., 2007) and Chinese populations (Chen et al., 2003), and *GABRB3* encoding the $\alpha 3$ sub-unit of the GABA_A receptor, in Austrian (Urak et al., 2006) or Central/South American (Tanaka et al., 2008a) populations. Moreover, the two genes, *KCNQ2* and *KCNQ3*, both encoding two Q-type potassium voltage-gated channels were implicated in a cohort of 455 German sporadic cases with GGE (Neubauer et al., 2008a).

This era concluded by the work of the Jeff Noebels's group, who used massive parallel Sanger sequencing to screen the coding sequence of 237 genes encoding subunits of ion voltage- or neurotransmitter-gated channels. They examined exonic variants in 152 individuals with idiopathic epilepsy and 139 healthy, neurologically unaffected adults of similar age and ethnic backgrounds (Klassen et al., 2011). Unfortunately, they did not identify any variants, which were significantly more frequent in patients compared to controls. They concluded "that individuals with epilepsy typically carry more than one mutation in known human epilepsy genes".

The Genome-Wide Association Studies (GWAS) followed CG (**Table 6**). They were performed on large cohorts of sporadic cases versus controls matched for age, gender and ethnic backgrounds. GWAS was considered as the reference method to dissect the genetic components of complex diseases using SNPs with high MAF ($\geq 5\%$). GWAS studies are based on the principle that many common variants ($\sim 30\text{--}40\%$ MAF in the general population) confer

low risk for disease (relative risks ~ 1.1). GWAS used neutral variants as markers of the undetected disease mutation. Since all GWAS used only 2 different SNP microarrays, the 370K or 1M SNPs Illumina^R BeadChip, meta-analyses can be considered. However, this approach required a large number of unrelated patients and controls. Therefore, the two published GWAS meta-analyses for GGE were performed by international research consortia. The first work was reported in 2012 by the EPICURE European consortium. The authors carried out a two-stage GWAS including 3020 patients with GGEs, including 1434 patients Genetic AEs (GAE) and 1134 with JME versus 3954 controls of European ancestry. Genome-wide significant associations were found for GGEs at 2p16.1 and 17q21.32 close to the *VRK2* (Vaccinia-Related Kinase 2) and *PNPO* (pyridoxamine-phosphate oxidase) gene, respectively. When taking into account GGE subtypes, significant associations were obtained for GAEs at 2q22.3 and at 1q43 for JME, near *ZEB2* (zinc finger homeobox 1b) and *CHRM3* (cholinergic receptor muscarinic 3) gene, respectively ([Consortium et al., 2012](#)).

The second meta-analyses collected data from 12 previously published or unpublished genetic cohort studies from EPICURE, EPIGEN, Philadelphia (PA, USA), the Imperial-Liverpool-Melbourne Collaboration, GenEpa and Hong Kong (China). The cohort of epileptic patients (n=8696) included patients with GGE (n=2606) or focal epilepsy (FE=5310) or unclassified epilepsy (UE=780) versus 5736 controls. In the all-epilepsy analysis, two loci were identified with genome-wide significance ($p < 1.66 \times 10^{-8}$). The first signal was located at 2q24.3 close to *SCN1A* and the second at 4p15.1, including the 3' end of the protocadherin gene, *PCDH7*. For GGE, the authors detected a statistically significant association at 2p16.1 previously reported by the EPICURE consortium with the two high-ranking candidate *VRK2* and *FANCL* gene. We must underline that 1440 GGE patients came from the EPICURE consortium. However, after removing EPICURE cases, this genetic association remained statistically significant. In contrast, the 17q21 locus (*PNPO* as candidate gene) reported by EPICURE for GGE was not confirmed. ([Epilepsy-austin@unimelb.edu.au, 2014](#)).

In the Sudanese GGE families, we did not identify high-ranking variants neither in *VRK2* nor *FANCL* coding sequence, which is not surprising: GWAS can localize the susceptibility variants, which are common and with a weak effect on the disease while WES in familial forms identify rare to ultra-rare variants with a higher impact on the phenotype.

These rare to ultra-rare variants were searched for in the WES era (**Table 7**). In a collaborative study, the Epi4K consortium and the Epilepsy Phenome/Genome Project firstly performed a

WES case-control study on a cohort of 641 familial forms of GGE versus 3877 controls (table 2) (A. S. Allen et al., 2017). More recently, many groups working on genetics of epilepsy were gathered in the Epi25 international consortium granted by the National Institute of Health (NIH). They organized a network collecting DNA samples and clinical data from patients with developmental/epileptic encephalopathy (DEE), focal epilepsy or GGE, living in different parts of the world. The Epi25 consortium published two case-control studies in 2019 (Berkovic & Neale, 2019) and in 2021 (Motelow et al., 2021). These studies differed in the number of tested patients, 3108 and 5048 with GGE respectively, and in the applied filters: in 2019, they took into account the impact of the mutational constraint of genes and in 2021, the intolerance of genic sub-regions using the missense intolerance ratio (MTR). These studies showed that: compared to controls, i) individuals with GGE carried an excess of ultra-rare, deleterious variants in constrained genes and in genes previously associated with epilepsy (Epi4k, Epi25 2019) and ii) inhibitory GABA_A receptor genes were enriched for missense variants in GGE patients. However, no single gene surpassed exome-wide significance among individuals with GGE (Table 2). Moreover, only two genes; *SLC6A1* and *GABRA1* are in the top 8 of both studies.

In our Sudanese series, we identified variants of the top 10 genes with suggestive association with *GGE* reported in two case-control studies: *FBXO42*, *ASTN2* genes by the Epi25 consortium (2021). Their identification by two orthogonal methods (WES case-control versus familial study) and in two different ethnic backgrounds (European/American versus Sudanese patients) reinforced their responsibility as susceptibility genes in GGE.

III. Consanguinity, endogamy and GGE in Sudan

In the previous chapter, extensive GWAS or WES case-control studies led to the conclusion that GGE were polygenic with a great genetic heterogeneity, making it difficult to identify a single gene with a statistically significant association with GGE. Testing more patients and controls will increase the statistical power of the next international studies but also the genetic heterogeneity of the cohorts. Therefore, a complementary approach consists of working on populations with a restricted ethnic background. This is the case of the Sudanese population, in which, we restricted our sampling to the central and North Eastern parts of the region.

In Sudan, strong genetic division was found between the populations from the North Eastern parts of the region (The Nubians, central Arab populations and Beja) and populations towards the West and South (Nilotes, Darfur and Kordofan populations). This divergence is mainly brought about by a large Eurasian ancestry component of the North East populations, likely driven by Middle Eastern groups migration (Hollfelder et al., 2017). Moreover, the high rate of consanguinity in Sudan- estimated to be up to 54%-brought about another element to be considered in genetics studies in this region (Mohamed et al., 2019).

Firstly, we selected families with frequent consanguineous patients to search for AR forms of GGE. We were surprised by the paucity of candidate variants at the homozygous or compound heterozygous state. Indeed, a likely pathogenic variant in *FAT1* gene was only found in F19, in which GGE segregated with a highly probable AR inheritance. In the Sudanese cohort, consanguinity did not favor AR forms of GGE.

Our main question was how endogamy can promote familial clustering of common polygenic traits. Once a rare functional variant enters a family, it can segregate in many family members, increasing the likelihood of detecting its effect; this warrants the use of large, multigenerational families in which many meioses occurred. The identification of rare variants associated with schizophrenia has proven challenging due to genetic heterogeneity, which was reduced in founder populations (Lencz et al., 2021). In F14, (p.Arg603His) variant of *FBXO42* segregated with GEE. However, it was not directly transmitted to V-4 by her mother IV-8 who had close affected relatives, but by her father, IV-9, through a large consanguinity loop. In such a family, the susceptibility variant could be transmitted by each related parent, potentially increasing the number of affected children.

In F12 (publication 2), the c.63578G>A (p.Arg21193His) variant in *SLC38A11* was transmitted to the GGE patient V-1 not only by her mother IV-3 but also by her father IV-4 who could not be directly related to the pedigree by a defined consanguinity loop (Publication 2- Figure 1). F19 could be considered as a tribe, close to an isolate, with likely founder effects. We can hypothesize that this structure increased the frequency of susceptibility alleles in this community.

Moreover, endogamy can facilitate the co-transmission of susceptibility variants determining a polygenic disease as we discussed in the first part "from mono- to oligo/polygenic forms of GGE". Various susceptibility variants from common ancestors can circulate in the

consanguinity loops with a high probability to be transmitted together in a given tribe member.

It must be noticed that a strong advantage of working on rural countries of Sudan is the size of siblings (mean number of sibs = 5 in our cohort), which increase the occurrence of inherited diseases, including those with complex inheritance. However, statistical validation and replication of susceptibility genes need large cohort of families. Moreover, since the identified variants are possibly unique to the families in which they were discovered, we do not have the certitude to find additional rare variants. In this condition, the biological validation will be important in interpreting results ([Gagnon, Roslin, & Lemire, 2011](#)).

IV. New candidate genes, new pathways

Mammalian brain development requires coordination between neural precursor proliferation, differentiation, and cellular organization to create the intricate neuronal networks of the adult brain. Communication between migrating neurons and their cellular substrates is directed by different cell-cell adhesion molecules, including the cadherin superfamily, with over 100 members expressed in the nervous system in vertebrates ([Martinez-Garay, 2020](#)).

We explored the role of our candidate genes, and we observed that most of them affect neuronal migration mediated by cadherins. I will detail briefly about each one of them in the following paragraph.

DCHS1, the gene encoding Dachshous Cadherin-Related 1 protein, along with *FAT4* disrupts cerebral cortical development through the hippo signalling pathway, leading to a recessive syndrome in humans that includes periventricular neuronal heterotopia. Thus, *DCHS1* is as a key regulator of mammalian neurogenesis ([Cappello et al., 2013](#)).

ASTNS, a member of the astrotactin gene family, on the other hand intervenes at another step of the migration: Glial-guided neuronal migration is a key step in the development of laminar architecture of cortical regions of the mammalian brain. *ASTN2*, and *ASTN1* are both identified as a neuron-glia ligands during CNS glial-guided migration. *ASTN2* works at the level of the plasma membrane to regulate the levels of *ASTN1*, while the release of neuronal adhesions to the glial fiber during neuronal locomotion involves the intracellular trafficking of *ASTN1* ([Wilson, Fryer, Fang, & Hatten, 2010](#)). Interestingly, another study demonstrates that neuron–

glia attachment and neuronal migration depend on glial expression of the neural cadherin N-Cadherin (CDH2) and that *ASTN1* promotes migration by a direct interaction with neuronal and glial CDH2 (Horn, Behesti, & Hatten, 2018) N-cadherin role in migration is demonstrated in (Figure 23).

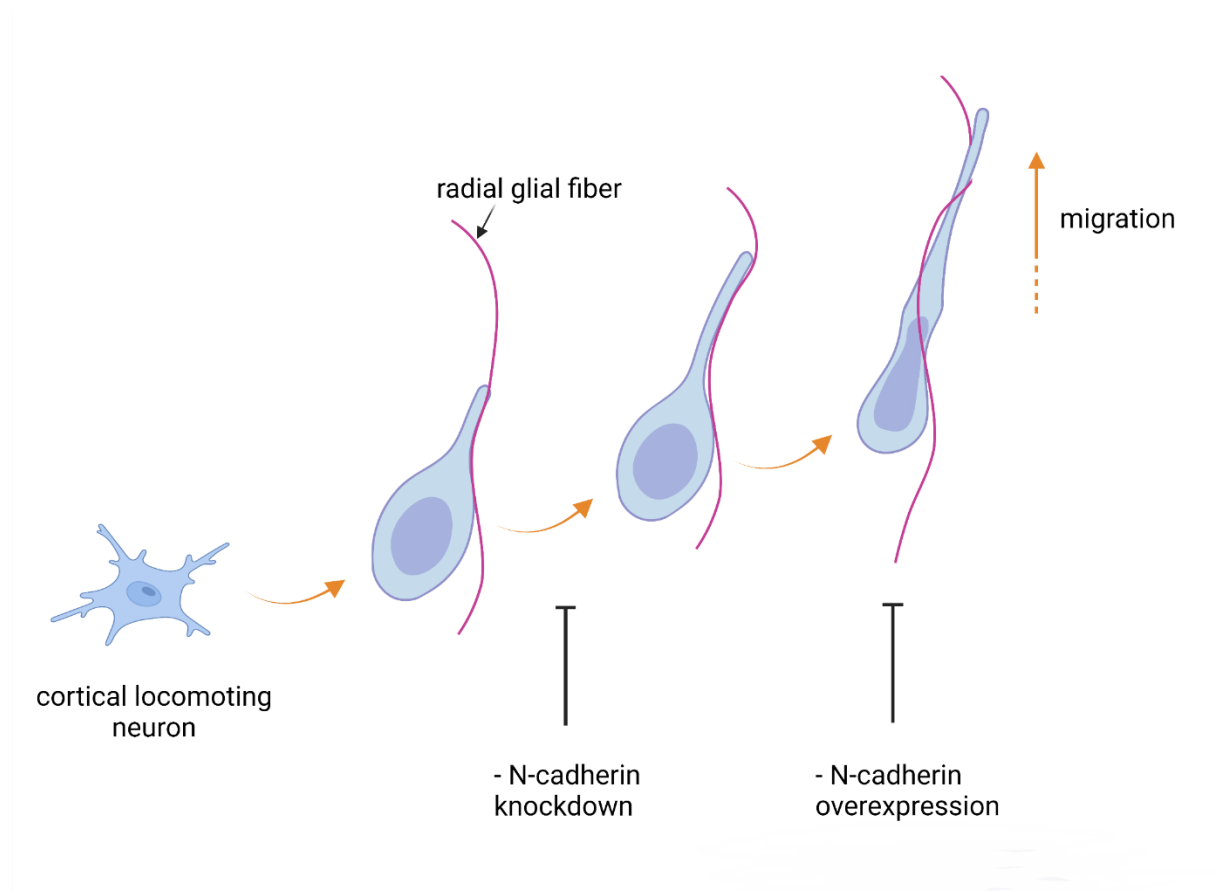


Figure 23. Multipolar cells form a thick leading process, retract the other processes and attach to the radial glial fibers in order to change into the locomoting neurons.

Knockdown of N-cadherin weakens the interaction between the polarized locomoting neurons and radial glial fibers. Subsequently, the locomoting neurons migrate toward the pial surface along the radial glial fibers. Increased levels of N-cadherin disturb the migration too. Adapted from (Martinez-Garay, 2020).

ADGRV1 was identified in 4 families of our cohort (see publication 1), its absence leads to impaired entry of cadherins *Cdhr23*- and *Cdhr15*- expressing interneuron precursors to the embryonic cortex, leading to a decreased number of parvalbumin interneurons in the auditory cortex (Libé-Philippot et al., 2017).

FAT1 is another atypical cadherin, it was shown that mutation of *Fat1* in mouse embryos causes defects in cranial neural tube closure, accompanied by an increase in the proliferation of cortical precursors and altered apical junctions, with perturbations in apical constriction and actin accumulation. Similarly, knockdown of *Fat1* in cortical precursors by in utero electroporation leads to over-proliferation of radial glial precursors. *Fat1* interacts with the related cadherin *Fat4* to regulate these processes. Moreover, *FAT1* regulates dendritic spine development in association with *NOS1AP* (Richier et al., 2010). Proteomic analysis shows that *Fat1* and *Fat4* bind different sets of actin-regulating and junctional proteins, and, as we mentioned above, *DCHS1* interacts with *FAT4*. These data support the interactive roles of all these cadherins in manifesting the different sub types of epilepsy (Badouel et al., 2015).

CAMSAP1, a microtubule minus-end binding protein, on the other hand acts mainly on neuronal polarization, migration, and cortical lamination. It was demonstrated that the microtubule-binding ability of *CAMSAP1* is critical for the protection of microtubule minus-ends and preservation of the “seeds” for microtubule regeneration, an ability regulated by *MARK2* (Zhou et al., 2020).

Little is known about *FBXO42* so far, in a drosophila female meiosis model, it is thought to be involved in post-translational modifications, phosphorylation and ubiquitination, cooperating to promote synaptonemal complex assembly (Barbosa et al., 2021).

V. *C. elegans* is worth considering in epilepsy studies

C. elegans is an attractive model for genetic studies, compared with traditional animal models, for different reasons among which are: a small body size, short lifespan, fully sequenced genome, more than 65% of genes associated with human disease (Shen, Yue, & Park, 2018), a transparent body allowing for microscopic visualization of gene expression patterns using reporter genes such as green fluorescent protein (GFP) (Boulin, Etchberger, & Hobert, 2006),

and, the availability of more than 3000 mutant strains at low cost at the Caenorhabditis Genetics Center (University of Minnesota, MN, USA) ([Stiernagle, 2006b](#)). Moreover, different methods are available to manipulate gene activity; RNA interference (RNAi), a form of gene silencing induced by double-stranded RNA (dsRNA), has been used as a screening tool. It works through inducing transcriptional or post-transcriptional gene silencing without altering the organism's DNA ([Grishok, 2005](#)).

Added to that is the revolutionized effect of the CRISPR-Cas9 technique in the field. RNA-guided endonucleases are used to create double-strand breaks (DSBs), and DNA templates containing the edits are used to repair the DSBs. Furthermore, innovations to improve editing efficiency are introduced, such as direct injection of CRISPR-Cas9 ribonucleo-protein complexes and linear DNAs with short homology arms as repair templates with no need for cloning or selection. Point mutations, insertions, deletions, and gene replacements can be applied using the same experimental pipeline in 4 days ([Paix, Folkmann, & Seydoux, 2017](#)).

The combination of these techniques – RNAi and CRISPR-Cas9 – among others, and the complete genome sequence information of *C. elegans* allows single gene targeting in this model. We used both methods to show the effect of gene silencing in known genes causing epilepsy like *STXBP1*, *FOYG1*, and novel genes as *KIF13B* identified in our lab. We went further and introduced point mutations in two novel genes causing absence epilepsy in a Tunisian family: *KIF13B*, *IGF1R*. We combined structural and behavioral tests to direct us towards answering the validity of the detected variant pathogenicity.

VI. *C. elegans* will help in answering the question of oligogenicity in GGE.

As shown in our genetic data, GGE is compatible with an oligogenic mode of inheritance. They are most likely the result of an additive/interactive pattern of multiple susceptibility genes contributing to the disease. However and although every year several genetic associations are reported, most of them lack biological/functional validation, adding to the high cost, in terms of money and time, of creating novel genetically modifiable mutant models for each gene (Bessa, Maciel, & Rodrigues, 2013).

The definitive proof of concept to study epilepsy on *C. elegans* is to confirm that the epileptic-like phenotype results from the abnormal synchronous activity of specific neurons (e.g., GABAergic neurons, the most explored in epilepsy). Observing the *C. elegans* locomotor circuit is thus the first step, as it shows its vitality for studying seizure susceptibility. Indeed, cholinergic innervation excites muscles to contract alternately on the ventral or dorsal side; and it simultaneously activates GABAergic inhibition to relax muscles on the opposite side. Thus, identifying the effect of a mutant through the locomotive system could give an early clue about its pathogenicity.

We went through different steps in our study: (i) silencing known and novel genes causing epilepsy, (ii) we induced seizures through PTZ, and (iii) we measured the velocity of the convulsing worms (head and tail) (see **Figure 18**) We went further and applied CRISPR-Cas9 technique to knock in 2 point mutations in both susceptibility genes we identified (KIF13B and IGF1R). In a recent study in *C. elegans*, this technique showed the conserved role played by a single amino acid position (TM2.6), located in the second transmembrane domain of two-pore domain potassium (K2P) channels, in increasing channel activity for all vertebrate K2P channels (Ben Soussia et al., 2019). We then applied a simple, rapid phenotypical analysis (thrashing test) to facilitate reproducibility and rapidity of screening and to detect the pathogenicity of each variant separately. We reported a statistical difference between the mutated lines and the wild-type young adult worms (N2) (**Figure 19**). The pathogenicity of different genetic variants (using thrashing test) was done on previous studies on *STXBP1* using CRISPR-Cas9 technique. Thrashing and electrophysiological studies of pharyngeal pumping were combined in these studies with promising results (Zhu et al., 2020). Due to the unavailability of the electrophysiological facility in the lab so far, we simply started testing

pharyngeal pumping (ongoing). Pharyngeal muscle contraction in *C. elegans* has an intrinsic myogenic capacity (Trojanowski, Raizen, & Fang-Yen, 2016), yet, the rhythmic high-frequency contractions seen during feeding require synaptic transmission via the pharyngeal nervous system (Raizen & Avery, 1994). Although GABA is not known to be a neurotransmitter within the pharyngeal microcircuit (Franks et al., 2006), GABAergic signaling has been reported to modulate pumping (Dallière et al., 2016). Given that GABA receptors are not present in the pharyngeal neurons, an unexpected result in a recent study showed that the electropharyngeogram (EPG) phenotype is even more marked in *unc-25* and *unc-49* than glutamatergic mutants like *eat-4* (with a very extended pump duration and absence of P peaks) and thus identifying a previously unreported GABAergic extrapharyngeal regulation of pharyngeal physiology (Calahorro, Keefe, Dillon, Holden-Dye, & O'Connor, 2018).

From the oligogenic frame of mind, we relied on this model since we believe that variants can have an additive/interactive effect. *C. elegans* allowed testing the accumulative/interactive effect of different variants in ciliopathies with its known oligogenic background. Ciliopathies like Nephronophthisis (NPHP -characterized by cystic kidney disease and retinal degeneration)- and Meckel–Gruber syndrome (MKS), a lethal gestational condition with skeletal abnormalities, cystic kidneys, and CNS malformation- are good examples (Hildebrandt, Benzing, & Katsanis, 2011). In these disorders, it was unexpectedly found that mutations within the same gene can cause either disorder (NPHP or MKS). Eight previously reported human *NPHP4* missense mutations were assessed in *C. elegans* to evaluate potential interactions with mutations in MKS complex genes, *mksr-2* and *mksr-1*. Six out of eight *nphp-4* mutations analyzed alter ciliary function, and three of these modify the severity of the phenotypes caused by disruption of *mksr-2* and *mksr-1*. Foraging and dye-filling defects phenotypes were studied as a reflection of the protein function in these studies. Multiple lines were created with double mutants to evaluate the pathogenic potential and the rescue effect of these variants (Masyukova, Winkelbauer, Williams, Pieczynski, & Yoder, 2011).

We crossed the two mutated lines creating the double mutant (*klp-4*- F829C + *daf-2*- S669Q). We compared the behavioral patterns of these lines, again with a statistical significance compared with the wild type. However, we could not detect a behavioral significance between each mutant separately and the double mutants. To explain these observations, we hypothesize that this could be related to the age of the animals in which we need to explore

the phenotype as the accumulative effect could be more evident in earlier stages of development, also screening other behavioral patterns including other neuronal systems can give us a clearer perspective.

Structurally, we aimed at looking at the GABAergic neurons as they are the most affected in epilepsy. We crossed our mutated lines with lines expressing the GFP fluorescent reporter in the GABAergic neurons to detect if there is any loss of neurons in these lines. We did not notice any statistical difference, directing us to other questions like vesicle distribution, for example. A comparable model is Lissencephaly, a nervous system disorder characterized by a "smooth brain" lacking convolutions or gyri due to abnormal neuronal migration and poor survival of cortical neurons during development, in which patients have intractable epilepsy. It can be caused by mutations in the TUBA1A, LIS1, ARX, DCX, and RELN genes ([Dobyns, Reiner, Carrozzo, & Ledbetter, 1993](#); [Kitamura et al., 2002](#); [Kumar et al., 2010](#); [Pilz et al., 1998](#)) among others. *C. elegans* *lis-1* mutants can be lethal to worms, and survivors present marked seizure susceptibility when exposed to PTZ. No significant defects were reported in neuronal architecture, but severe presynaptic defects in GABAergic vesicle distribution were found in these mutants ([Williams et al., 2004a](#)).

There are still many questions to be addressed to conclude about the exact biomolecular role of the variants and the nature of their interactive pattern if there are any. However, the transparency of the worm with the GFP-tagged lines will allow us to detect the tiny changes at the level of the dendrites/axons of a specific group of neurons. On the other hand, combining pharyngeal electrophysiology studies and calcium imaging techniques will shorten the gap in explaining the physiological patterns.

C. elegans does not have a brain, yet, it is the only organism whose neuronal wiring diagram has been described. It provides unique genetic tools and shows well-described phenotypes of seizures. Showing the pathogenic potential of point mutations involved in absence epilepsy, as in our results, opens a door for more studies in the field.

Table 6. Candidate gene approach.

Genes	Locus	Phenotype	Methods	Population	Statistical Significance (Significant threshold); OR*	Year of publication	Reference	Geographic origin
<i>GRIK1</i>	21q21.3	JAE	CG	20 F	p= 0.004	1997	(T. Sander, Hildmann, et al., 1997)	German or Austrian
<i>GABRB3</i>	15q12	CAE	CG	45 F	p = 0.012	1999	(Urak et al., 2006)	Austrian
<i>CACNB4</i>	2q23.3	IGE(JME) + Atx	CG	90 F	ns	2000	(Escayg et al., 2000)	Northern European
<i>GABRG2</i>	5q34	CAE; GEFS+	PC + Ephi	1 F	LS=6.06 at $\alpha=0.00$	2001	(R H Wallace et al., 2001)	UK
<i>JRK</i>	8q24.3	CAE	CG	97 IC/F	ns	2001	(Moore et al., 2001)	Caucasian
<i>GABRA1</i>	5q34	CAE, JME	PC +Ephi	1 F	LS=3.1 at $\alpha=0.00$	2002	(Cossette et al., 2002)	French canadian
<i>CACNA1H</i>	16p13.3	CAE	CG	118 IC	na	2003	(Chen et al., 2003)	Chinese Han
<i>GABRD</i>	1p36.33	IGE	CG + EPhi	72 IC	ns	2004	(Dibbens et al., 2004)	Australian
<i>GJD2 (CX36)</i>	15q14	JME	CG	29 F	p=0.03	2004	(Mas et al., 2004)	European
<i>ME2</i>	18q21.2	JME; JAE; GTC	CG	156 IC 108F	p=0.001	2005	(Greenberg et al., 2005)	European
<i>CACNA1H</i>	16p13.3	CAE	CG + Ephi	240 IC	ns	2007	(Heron et al., 2007)	Australian
<i>CACNA1G</i>	17q21.33	IGE	CG	123 IC	ns	2007	(B. Singh et al., 2007)	Japanese and Hispanic
<i>CASR</i>	3q21.1	IGE; JME; FE	PC	1 F 96 IC	LS=3.05 at $\alpha=0.00$	2008	(Kapoor et al., 2008)	South Indian
<i>GABRB3</i>	15q12	CAE	CG + BC+ Ephi	48 F	na	2008	(Tanaka et al., 2008b)	Mexico, Honduras, Salvador
<i>KCNQ2</i>	20q13.33	IGE	CG	455 IC	p = 0.008	2008	(Neubauer et al., 2008b)	German
<i>KCNQ3</i>	8q24.22	IGE	CG	455 IC	p = 0.004	2008	(Neubauer et al., 2008b)	German
<i>(CHRM3)</i>	1q43	JME	GWAS (MA)	1134 IC	p= 4.14 10 ⁻⁸ (<5.0×10 ⁻⁸) OR=	2012	(Consortium et al., 2012)	European
<i>(MAST4)</i>	5q12.3	JME	GWAS (MA)	1134 IC	ns	2012	(Consortium et al., 2012)	European
<i>(SCN1A)</i>	2q24.3	JME	GWAS (MA)	1134 IC	ns (5.0×10 ⁻⁸)	2012	(Consortium et al., 2012)	European
<i>(GPC5)</i>	13q31.3	CAE	NPL/PL	235 F	HLOD=5.02; $\alpha=0.22$	2012	(Leu et al., 2012)	European

<i>(SLC4A3)</i>	2q25	JME	NPL/PL	118 F	LOD _{np} =3.43	2012	(Leu et al., 2012)	European
<i>(ZEB2)</i>	2q22.3	CAE	GWAS (MA)	1434 IC	p=9.10 ⁻⁹ (5.0×10 ⁻⁸) OR=	2012	(Consortium et al., 2012)	European
<i>NIPA2</i>	15q11.2	CAE	CG + CNVD	380 IC	p = 0.043	2012	(Y. Jiang et al., 2012)	Chinese Han
<i>GLUT1-SLC2A1</i>	1p34.2	IGE	CG + BC	504 IC	p=0.02	2012	(Arsov et al., 2012)	Australia and Israel
<i>(PNPO)</i>	17q21.3 2	GGE	GWAS (MA)	2606 IC	p=9.3.10 ⁻⁹ (5.0×10 ⁻⁸) OR=0.77	2012	(Consortium et al., 2012)	European
<i>(VRK2)</i>	2p16.1	GGE	GWAS (MA)	2606 IC	p=2.5.10 ⁻⁹ (5.0×10 ⁻⁸) OR=0.81	2012	(Consortium et al., 2012)	European
<i>(FANCL)</i>	2p16.1	GGE	GWAS (MA)	2606 IC	p=9.99 × 10 ⁻⁹ (1.66 × 10 ⁻⁸) OR=	2014	(Epilepsy-austin@uni melb.edu.au , 2014)	European
<i>(PCDH7)</i>	4p15.1	GGE, FE, UE	GWAS (MA)	8696 IC	p=5.44 × 10 ⁻⁹ (1.66 × 10 ⁻⁸) OR=	2014	(Epilepsy-austin@uni melb.edu.au , 2014)	European
<i>(SCN1A)</i>	2q24.3	GGE, FE, UE	GWAS (MA)	8696 IC	p=8.71 × 10 ⁻¹⁰ (1.66 × 10 ⁻⁸) OR=	2014	(Epilepsy-austin@uni melb.edu.au , 2014)	European
<i>(MMP8)</i>	11q22.2	GGE	GWAS (MA)	2606 IC	ns	2014	(Epilepsy-austin@uni melb.edu.au , 2014)	European
<i>GPHN</i>	14q23.3	IGE	CNVD + BC	3725 IC	ns	2014	(Dejanovic et al., 2014)	North-European German
<i>SLC12A5-KCC2</i>	20q13.1 2	IGE	CG + Ephi	380 IC	p=7.50 × 10 ⁻³	2014	(Kahle et al., 2014)	Canadian
<i>NRXN1</i>	2p16.3	GGE	CNVD	1366 IC	na	2015	(Lal et al., 2015)	European
<i>PCDH7</i>	4p15.1	GGE	CNVD	1366 IC	na	2015	(Lal et al., 2015)	European
<i>RBFOX1</i>	16p13.3	GGE	CNVD	1366 IC	na	2015	(Lal et al., 2015)	European
<i>GABRA2</i>	4p12	GGE	WES (CG) + Ephi	152 F	na	2018		European
<i>GABRA5</i>	15q12	GGE	WES (CG) + Ephi	152 F	na	2018		European

Table 7. International WES studies.

WES Studies (GGE)	Title	Top Genes	p value= ; OR=	Significant threshold	Geographic origin	Patients/controls
Epi4K & Epilepsy Phenome/Genome Project Lancet Neurol, 2017	Ultra-rare genetic variation in common epilepsies: a case-control sequencing study	<i>CACNA1B</i> <i>KEAP1</i> <i>COPB1</i> <i>KCNQ2</i> <i>SLC9A2</i> <i>GABRG2</i> <i>ATP1A3</i> <i>SCN1A</i>	1.7 10 ⁻⁵ 5.6 10 ⁻⁵ 2.2 10 ⁻⁴ 4 10 ⁻⁴ 4 10 ⁻⁴ 9.2 10 ⁻⁴ 9.2 10 ⁻⁴ 1.3 10 ⁻³	<8.9 10 ⁻⁷	EA	640 F* /3877 *affected relative up 3rd degree
Epi 25 collaborative AJHG, 2019	Ultra-Rare Genetic Variation in the Epilepsies: A Whole-Exome Sequencing Study of 17,606 Individuals	<i>CACNA1G</i> <i>EE1FA2</i> <i>UNC79</i> <i>GABRG2</i> <i>ALDH4A1</i> <i>SLC6A1</i> <i>RC3H2</i> <i>GABRA1</i> <i>DNAJC13</i> <i>ZBTB2</i>	2.5 10 ⁻⁴ ; 9.1 3.8 10 ⁻⁴ ; 32 6.2 10 ⁻⁴ ; 19 6.2 10 ⁻⁴ ; 19 1.4 10 ⁻³ ; Inf 2 10 ⁻³ ; 7.3 2 10 ⁻³ ; 16.3 2.2 10 ⁻³ ; 9.5 5.2 10 ⁻³ ; Inf 5.2 10 ⁻³ ; Inf	<6.2 10 ⁻⁷	WW (EO)	3108 SC / 8300
Epi25 collaborative AJHG, 2021	Sub-genic intolerance, ClinVar, and the epilepsies: A whole-exome sequencing study of 29,165 individuals			<1.6 10 ⁻⁷	WW (EO)	5048 / 14100

<i>SLC6A1</i>	2.1 10 ⁻⁶ ; 16.6
<i>SCN1A</i>	3.4 10 ⁻⁵ ; 3.4
<i>FBXO42</i>	4.5 10 ⁻⁴ ; 13.6
<i>DAW1</i>	6.2 10 ⁻⁴ ; 27.2
<i>GRIN2A</i>	8.6 10 ⁻⁴ ; 18.8
<i>GABRA1</i>	1.4 10 ⁻³ ; 5.6
<i>KCNK18</i>	1.6 10 ⁻³ ; Inf
<i>ZNF324B</i>	1.7 10 ⁻³ ; Inf



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Abstract in English

Genetic generalized epilepsy (GGE) is a common form of epilepsy, including childhood absence epilepsy, juvenile absence epilepsy, juvenile myoclonic epilepsy, and GGE with tonic-clonic seizures alone. Since large families with GGE are rare in western countries, most studies were performed in American and European cohorts of sporadic cases. They suggested that susceptibility genes for GGE are numerous and their variants rare, challenging their identification. They also underlined that the inheritance of GGE is likely oligogenic. Up to now, only few genes including *GABRA1* and *SCN1A* have been reported as susceptibility genes for GGE.

In this project, our aim was to study familial forms of GGEs from Sudan as a different approach to answer the question of their genetic determination. We identified for the first time *ADGRV1* ultra rare/rare variants in 5 of 20 (25%) Sudanese families with GGE. In these families, there was a statistically significant association between GGE and *ADGRV1* variants proposing its oligogenic contribution to GGE. We identified a novel gene, *FAT1*, encoding an atypical cadherin involved in neurite outgrowth, in a family with an autosomal recessive form of Generalized tonic-clonic epilepsy. Novel candidate genes, *DCHS1*, *ASTN2* and *FBXO42*, were also identified in large families with GGE.

In parallel, we explored *C. elegans* as an animal model for functional validation of candidate genes in epilepsy. We performed knockdown and knock-in techniques on known and novel genes responsible for epilepsy. Behavioral tests showed statistical difference between mutant lines compared to controls. We also explored the oligogenic hypothesis in the worm by testing double mutants.

Our project paved the way in answering the oligogenicity puzzle in GGEs and the use of *C. elegans* as the first functional screening of candidate genes for epilepsy.

Key words: Genetic generalized epilepsy, *ADGRV1*, *FAT1*, *DCHS1*, *ASTN2*, oligogenic, *C. elegans*

Résumé en français

L'épilepsie généralisée génétique (EGG) est une forme courante d'épilepsie, qui comprend l'épilepsie-absence infantile, l'épilepsie-absence juvénile, l'épilepsie myoclonique juvénile et l'EGG avec crises tonico-cloniques seules. Les grandes familles avec EGG étant rares dans les pays occidentaux, la plupart des études ont été réalisées sur des cas sporadiques de cohortes américaines et européennes. Ces études ont suggéré qu'il existait de nombreux gènes de susceptibilité à l'EGG et que leurs variants étaient rares, ce qui rend difficile leur identification. Il a également été souligné que l'hérédité de l'EGG est probablement oligogénique. Jusqu'à présent, seuls quelques gènes, dont *GABRA1* et *SCN1A*, ont été identifiés comme gènes de susceptibilité à l'EGG.

Dans ce projet, notre objectif était d'étudier les formes familiales d'EGG au Soudan, comme approche complémentaire pour identifier leur déterminisme génétique. Nous avons identifié pour la première fois des variants ultra rares/rare d'*ADGRV1* dans 5 des 20 familles soudanaises (25%) atteintes d'EGG. Dans ces familles, il y avait une association statistiquement significative entre l'EGG et les variants d'*ADGRV1*, montrant qu'*ADGRV1* était un gène de susceptibilité à l'EGG. Nous avons aussi identifié un nouveau gène, *FAT1*, codant pour une cadherine atypique impliquée dans la croissance des neurites, dans une famille présentant une forme autosomique récessive d'EGG avec crises tonico-cloniques. De plus, de nouveaux gènes candidats, *DCHS1*, *ASTN2* et *FBXO42*, ont été identifiés dans de grandes familles atteintes d'EGG.

En parallèle, nous avons exploré le nématode *C. elegans* comme modèle animal pour la validation fonctionnelle de gènes candidats à l'épilepsie. Nous avons effectué des techniques de knock-down et knock-in sur des gènes connus et nouveaux, responsables de l'épilepsie. Les tests comportementaux ont montré une différence statistique entre les lignées mutantes et témoins. Nous avons également exploré l'hypothèse oligogénique chez le nématode, en testant des doubles mutants.

Notre projet a ouvert la voie pour répondre à l'énigme de l'oligogénicité dans les EGG ainsi qu'à l'utilisation de *C. elegans* comme premier criblage fonctionnel de gènes candidats de l'épilepsie.

Mots-clés: Epilepsie généralisée génétique, *ADGRV1*, *FAT1*, *DCHS1*, *ASTN2*, oligogénique, *C. elegans*