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THÈSE DE DOCTORAT

Exploration du profil clinique et génétique des patients atteints de schizophrénie précoce et de leurs apparentés au 1^{er} degré : un protocole d'étude familiale et multicentrique en population française

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du grade de **docteur en Sciences
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Protocole GenAuDiss

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RESUME

La schizophrénie précoce (SP) est une forme rare, sévère et neurodéveloppementale de schizophrénie débutant avant l'âge de 18 ans. Afin de mieux comprendre les bases génétiques complexes de ce trouble nous avons développé un projet pilote avec pour objectif principal de caractériser sur le plan clinique et génétique les patients atteints de SP. Compte tenu du chevauchement clinique et génétique de la SP avec les autres troubles neurodéveloppementaux, dont le Trouble du Spectre de l'Autisme (TSA) et le Trouble Déficit de l'Attention Hyperactivité (TDAH), nous avons porté une attention particulière aux gènes impliqués dans le neurodéveloppement.

Il s'agit d'une étude multicentrique conduite d'avril 2014 à mai 2023. Les critères d'inclusion sont: âge de 7 à 22 ans, un diagnostic de SP (K-SADS-PL DSM-5) avec des symptômes autistiques prémorbides (ADI-R 0-5 ans) et un $QI > 40$; les parents et les frères et sœurs sont inclus. L'exploration du profil clinique a été effectuée à l'aide d'outils standardisés (KSADS-PL, PANSS) et a compris des évaluations neurocognitives (WISC-V/WAIS-IV). L'exploration génétique (approche hiérarchisée) a compris une recherche de l'X-Fragile, la réalisation d'un CGH-array puis, en cas de négativité des examens précédents, la réalisation d'un séquençage de l'ADN (exome) en trio. Nous avons procédé à la priorisation des gènes en combinant de multiples outils bio-informatiques.

20 sujets ont été inclus: 15 Garçons et 5 filles. L'âge moyen de début du trouble était de 8,90 ans (+/- 2,30). Les comorbidités psychiatriques étaient le TDAH (15/20 patients), les troubles anxieux (14/20) et le TSA (13/20). Le QI moyen était de 70,26 (+/-18,09). Un retard de langage et une rupture scolaire étaient notés chez 18/20 patients. L'affection somatique principale était l'asthme (15/20 patients). Sur le plan génétique, nous rapportons chez une patiente (ségrégation familiale) une duplication 10q26.3 (324 Kb) comprenant une partie du gène *INPP5A*. Nous avons montré que son homologue de la drosophile *5Ptase1* est spécifiquement exprimée dans le système nerveux central. Enfin, grâce aux séquençages de l'ADN (9 exomes en trio ; mère, père, enfant soit 27 sujets) et aux outils bio-informatiques, nous avons identifié la présence de variants dans les gènes appartenant aux voies de signalisation Wnt, cadhérine et cholécystokinine.

Ainsi, nous avons mis en évidence chez nos patients atteints de SP une grande hétérogénéité clinique avec des comorbidités psychiatriques et des atteintes neurodéveloppementales systématiquement associées. *INPP5A* est un gène exprimé dans le cerveau (humain, souris et drosophile), très conservé entre espèces et qui code une InsP3 5-phosphatase dont les produits d'hydrolyse mobilisent le calcium intracellulaire, essentiel pour la morphogenèse des épines dendritiques dans les neurones. L'altération de ce processus, par dysrégulation de la voie de signalisation InsP3/ Ca^{2+} , est retrouvée à la fois dans la schizophrénie et le TSA, renforçant le lien entre ces troubles. De plus, nous avons effectué la première description de l'implication potentielle de voies de signalisation Wnt, Cadhérine et Cholécystokinine dans la SP. L'implication de ces différentes voies de signalisation décrite de manière variable dans d'autres troubles neurodéveloppementaux et/ou psychiatriques souligne l'hétérogénéité génétique de ce trouble. Élucider les mécanismes moléculaires de la SP et ouvrir la voie à une intervention thérapeutique spécifique nécessitera d'effectuer systématiquement et à grande échelle : 1) le diagnostic syndromique précis de SP avec un âge exact de début de la maladie et le phénotype neurodéveloppemental associé ; 2) une évaluation génétique hiérarchisée allant jusqu'au séquençage du génome et 3) le partage des données à l'échelle internationale avec la constitution de bases de données spécifiques, comparables, génétiques et moléculaires corrélées aux phénotypes de précision des différentes formes de SP.

Mots-clés : psychiatrie de l'enfant et de l'adolescent, schizophrénie précoce, trouble du spectre de l'autisme, TDAH, CNV, SNV, séquençage de l'exome, INPP5A, troubles neurodéveloppementaux.

ABSTRACT

Early-onset schizophrenia (EOS) is a rare, severe and neurodevelopmental form of schizophrenia beginning before the age of 18. In order to better understand the complex genetic basis of this disorder, we have developed a pilot project with the main objective of clinically and genetically characterize EOS patients presenting additional neurodevelopmental disabilities. Given the clinical and genetic overlap of EOS with other neurodevelopmental disorders, including Autism Spectrum Disorder (ASD) and Attention Deficit Hyperactivity Disorder (ADHD), we paid particular attention to the genes involved in neurodevelopment.

This is a multi-center study carried out from April 2014 to May 2023 in a paediatric population. Inclusion criteria are: age 7-22 years, a diagnosis of EOS (K-SADS-PL DSM-5) with premorbid autistic symptoms (ADI-R 0-5 years) and IQ > 40; parents and siblings are included. Clinical profile explorations are performed using standardized tools (KSADS-PL and PANSS) and included neurocognitive assessments (WISC-V/WAIS-IV), the search for psychiatric co-morbidities, neurodevelopmental disorders and associated extracerebral somatic pathologies. The exploration of the genetic profile consists in identifying genetic mutations by a hierarchical approach searching for Fragile-X Syndrome (PCR), CGH-array and, in case of negativity of the previous examinations, DNA sequencing (exome) in trio (mother, father, child). Finally; we proceed to the prioritization of genes by combining multiple bioinformatics tools.

20 subjects were included: 15 boys and 5 girls. The mean age of onset of the disorder was 8.90 years (+/-2.30). Psychiatric comorbidities (DSM-5) were ADHD (15/20 patients), anxiety disorders (14/20) and ASD (13/20). The mean IQ was 70.26 (+/-18.09). Language delay and school disruption were noticed in 18/20 patients. The main associated somatic condition was asthma (15/20 patients). Genetically, we report a 10q26.3 324 kb microduplication in one patient (with familial segregation), encompassing part of the *INPP5A* gene. We have shown that its homologue *5PtaseI* is specifically expressed in the *Drosophila* central nervous system. Furthermore, we have identified, through DNA sequencing of 9 exomes of patients in trio (27 subjects with mother, father and child) and bioinformatics tools, the presence of variants in genes belonging to the Wnt, cadherin and cholecystokinin signaling pathways.

In our EOS patients, we have shown a large clinical heterogeneity with psychiatric co-morbidities and neurodevelopmental disorders systematically associated. *INPP5A* is expressed in the brain (human, mouse and *Drosophila*), is highly conserved between species and encodes a InsP3 5-phosphatase whose hydrolysis products mobilize intracellular calcium, essential for the morphogenesis of dendritic spines in neurons. The alteration of this process, by the InsP3/Ca²⁺ signaling pathway, is found in both schizophrenia and ASD, strengthening the link between these disorders. In addition, we have made the first description of the potential involvement of the Wnt, Cadherin and Cholecystokinin signaling pathways in EOS. The already described involvement of these different pathways in other neurodevelopmental and/or psychiatric disorders underlines the genetic heterogeneity of this disorder. Therefore, elucidating the molecular mechanisms of EOS and paving the way for specific therapeutic interventions will require systematic and large-scale: 1) definition of the precise syndromic diagnosis of EOS with an exact age of onset and determination of the premorbid neurodevelopmental phenotype, psychiatric comorbidities and associated extracerebral somatic pathologies; 2) genetic evaluation using a hierarchical approach up to whole genome sequencing; 3) data sharing between teams on an international scale with the constitution of specific, comparable, genetic, and molecular databases correlated to the precise phenotypes of the different forms of EOS.

Keywords: child and adolescent psychiatry, childhood onset schizophrenia, autism spectrum disorder, ADHD, CNV, SNV, exome sequencing, INPP5A, neurodevelopmental disorders.

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Les étudiants,

Les patients.

INTRODUCTION

Bien que les schizophrénies très précoces (STP) et précoces (SP) soient des formes rares et sévères de schizophrénie débutant respectivement avant l'âge de 13 et 18 ans (Driver *et al.*, 2020), la symptomatologie clinique utilisée pour les décrire est identique à celle des formes de début adulte (DSM-5, APA, 2013). Pourtant, un taux plus élevé d'atteintes neurodéveloppementales associées [telles que la déficience intellectuelle (DI), le Trouble Hyperactivité avec Déficit de l'Attention (TDAH), des symptômes évocateurs de Trouble du Spectre de l'Autisme (TSA), un trouble de la communication ou un trouble moteur] est observé dans la SP par rapport aux formes de début adulte (Driver *et al.*, 2020) et n'est pas pris en compte dans les classifications internationales. De plus, si leur présence n'a pas de valeur diagnostique particulière à l'heure actuelle, ces anomalies neurodéveloppementales sont corrélées à la sévérité du pronostic de ces patients (Driver *et al.*, 2020). Par convention, dans ce travail, nous emploierons le terme de STP pour les formes débutant avant l'âge de 13 ans et de SP pour les toutes les formes débutant avant 18 ans, c'est-à-dire incluant les STP.

Sur le plan épidémiologique, les cas de STP sont rares, puisqu'on estime généralement que moins d'un enfant sur 10 000 en est atteint [incidence généralement acceptée de 0,04% selon le National Institute of Mental Health (NIMH), 25 fois moins fréquente que chez l'adulte ; Nicolson and Rapoport, 1999; Driver *et al.*, 2020], avec une légère prédominance de garçons (Ordóñez *et al.*, 2016).

En France, dans la région PACA, le Service Universitaire de Psychiatrie de l'Enfant et de l'Adolescent (SUPEA) de Nice a mené une étude multicentrique translationnelle (financement P.H.R.C.) avec pour objectif d'estimer la prévalence de la SP dans une population d'enfants pris en charge dans des structures sanitaires ou médico-sociales. Dans cette population, une proportion notable de 8,9% d'enfants, avec une SP, a été mise en

évidence lors des évaluations psychiatriques et neurocognitives standardisées (Dor-Nedonsel *et al.*, 2020). Ce résultat souligne le probable sous-diagnostic de ce trouble. C'est dans ce contexte, où notre équipe effectuait à la fois une pratique clinique de terrain approfondie dans le champ de la SP et une analyse concomitante méticuleuse de la littérature scientifique, que nous avons développé un projet pilote. Il s'agissait d'étendre l'étude de cette population à de nouveaux patients et de s'intéresser également aux aspects génétiques de leur trouble. Nous avons ainsi développé le protocole GenAuDiss qui est une étude familiale, multicentrique, clinique et génétique s'intéressant aux patients atteints de SP.

Si le modèle neurodevelopmental de la schizophrénie (Rapoport *et al.*, 2012) permet de comprendre, en recherche, le chevauchement diagnostique avec d'autres troubles neurodéveloppementaux comme le TDAH ou le TSA, cela reste, en pratique de terrain, une source de confusion diagnostique d'autant plus importante que le trouble débute précocement. Pour illustrer ce chevauchement, il est intéressant de constater que 27% de patients atteints de STP (cohorte Nord-Américaine du NIMH ; Rapoport *et al.* 2009) et 41% de patients atteints de SP (séries de cas du SUPEA de Nice ; Dor-Nedonsel *et al.*, 2020) présentaient des symptômes autistiques prémorbides, avant l'émergence schizophrénique. Les liens entre autisme et schizophrénie sont complexes et nous renvoient aux origines de la discipline psychiatrique. Si l'apport du modèle neurodéveloppemental et l'intérêt croissant pour les formes à début précoce de schizophrénie enrichissent la discussion actuelle autour des liens entre ces conditions, le sens que présente l'expression symptomatique psychiatrique, à un moment donné du développement d'un enfant atteint de troubles neurodéveloppementaux, continue d'être une source d'interrogation.

Sur le plan étiologique, l'abord génétique de la schizophrénie est une idée déjà ancienne (études des jumeaux, haute héritabilité supérieure à 80%), mais notre compréhension de l'architecture génétique des formes précoces évolue (et se complexifie)

avec les innovations technologiques récentes (Forsyth *et al.*, 2020). Nous savions déjà que plus de 10% des patients atteints de STP présenteraient des anomalies cytogénétiques, contre moins d'1% dans la population générale, avec au premier plan les variations du nombre de copie des gènes (CNVs) dont les syndromes de délétion 22q11.21 (Nicolson *et al.*, 1999 ; Addington *et al.*, 2009). Cependant, ces macrolésions génomiques ne sont ni nécessaires ni suffisantes à expliquer l'émergence du trouble et la plupart des CNVs ne sont pas spécifiques de la schizophrénie mais retrouvés également dans d'autres troubles neurodéveloppementaux comme le TSA. Les facteurs environnementaux sont également à prendre en compte en interaction avec les anomalies génétiques et se répartissent globalement en facteurs périnataux, infectieux, sociaux, toxiques (Brown, 2011) et épigénétiques (Kebir *et al.*, 2018). Plus récemment, de nouvelles mutations non héritées (*de novo*), appelées polymorphismes d'un seul nucléotide (SNPs), ont également été impliquées dans la genèse de la STP en utilisant le séquençage de l'ADN à haut débit (Ambalavanan *et al.*, 2016). L'influence de ces SNPs sur des gènes liés au Chromosome X chez le sujet de sexe masculin a également été suggérée (Ambalavanan *et al.*, 2019).

Ainsi, la STP, comme la schizophrénie à début plus tardif à l'adolescence, semblent être des troubles d'origine multifactorielle aux mécanismes moléculaires complexes (génétiques et épigénétiques), incomplètement connus à ce jour et dont certains restent soumis à controverse, comme ceux décrits récemment dans le modèle omnigénique de la schizophrénie (Weinberger, 2019).

Le premier chapitre de ce travail est une revue systématique de littérature concernant les mutations, à l'échelle du génome, retrouvées chez des patients atteints de STP. Un soin particulier a été pris pour décrire, chaque fois qu'ils étaient disponibles, les phénotypes associés à ces mutations.

CHAPITRE 1 : Revue de littérature sur les aspects génétiques de la schizophrénie très précoce et les phénotypes associés

1.1 Rationnel

Malgré une attention croissante pour l'architecture génétique complexe de la schizophrénie, aucune revue de littérature ni base de données disponibles à notre connaissance ne permettaient de consulter l'ensemble des mutations existantes au niveau de l'ADN pour les STP. Seule la revue de Robert F. Asarnow (PhD, Department of Psychiatry and Biobehavioral Sciences, UCLA) en 2013 (actualisée en 2020), abordait la question de la génétique de la SP de manière narrative (non systématique) et sous le prisme de la schizophrénie adulte (Asarnow and Forsyth, 2013; Forsyth and Asarnow, 2020). Face à ce constat, nous avons effectué une étude méticuleuse de la littérature indexée, selon la méthode Prisma, sur l'ensemble des mutations, au niveau de l'ADN, décrites chez des patients atteints de STP, sans limitations de date ni de langage. 205 articles ont été évalués et 36 inclus. Les résultats ont été publiés dans la revue *Frontiers in Genetics* (rang SIGAPS B, 5years-IF 4.06) avec, parmi les relecteurs, le Professeur Guy Rouleau de l'Université Mc Gill au Canada.

1.2 Article publié dans la revue *Frontiers in Genetics*

Childhood-Onset Schizophrenia: a systematic overview of its genetic causes from classical studies to the genomic era

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1.2.1 Abstract

Childhood-onset schizophrenia (COS), a very rare and severe chronic psychiatric condition, is defined by an onset of positive symptoms (delusions, hallucinations, disorganized speech or behavior) before the age of 13. COS is associated with other neurodevelopmental disorders such as Autism Spectrum Disorder (ASD) and Attention Deficit and Hyperactivity Disorder (ADHD). Copy Number Variations (CNVs) represent well documented neurodevelopmental disorder risk factors and, recently, *de novo* Single Nucleotide Variations (SNVs) in genes involved in brain development have also been implicated in the complex genetic architecture of COS. Here, we aim to review the genetic changes (CNVs and SNVs) reported for COS, going from previous studies to the whole genome sequencing era. We carried out a systematic review search in PubMed using the keywords "childhood(early)-onset schizophrenia(psychosis)" and "genetic(s) or gene(s) or genomic(s)" without language and date limitations. The main inclusion criteria are COS (onset before 13 years old) and all

changes/variations at the DNA level (CNVs or SNVs). Thirty-six studies out of 205 met the inclusion criteria. Cytogenetic abnormalities (n=72, including 66 CNVs) were identified in 16 autosomes and two sex chromosomes (X, Y), some with a higher frequency and clinical significance than others (e.g., 2p16.3, 3q29, 15q13.3, 22q11.21 deletions; 2p25.3, 3p25.3 and 16p11.2 duplications). Thirty-one single nucleotide mutations in genes principally involved in brain development and/or function have been found on 12 autosomes and on the X chromosome. We also describe five SNVs in X-linked genes inherited from a healthy mother, arguing for the X-linked recessive inheritance hypothesis. Moreover, *ATPIA3* (19q13.2) is the only gene carrying more than one SNV in more than one patient, making it a strong candidate for COS. Mutations were distributed on various chromosomes illustrating the genetic heterogeneity of COS. More than 90% of CNVs involved in COS are also involved in ASD, supporting the idea that there may be genetic overlap between these disorders. Different mutations associated with COS are probably still unknown, and pathogenesis might also be explained by the association of different genetic variations (two or more CNVs or CNVs and SNVs) as well as association with early acquired brain lesions such as infection, hypoxia or early childhood trauma.

1.2.2 Introduction

Childhood-onset schizophrenia (COS) is a rare (< 1/40,000) and severe chronic psychiatric condition that shares with adult-onset schizophrenia (AOS) positive symptoms (delusions, hallucinations and disorganized speech or behavior), but presents an early onset (before the age of 13) (Burd and Kerbeshian, 1987; Nicolson and Rapoport, 1999). It remains considered by many authors as an early and severe variant of AOS (Nicolson and Rapoport, 1999; Biswas et al., 2006). In COS, neurodevelopmental abnormalities (deficits in cognition,

communication or neuromotor impairments) and premorbid dysfunction are more frequent compared with AOS (Vourdas et al., 2003) and a clinical overlap exists with other neurodevelopmental disorders: 28% of patients with COS in the US cohort of the National Institute of Mental Health Child Psychiatry Branch met criteria for comorbid autism spectrum disorder (ASD) (Rapoport et al., 2009). In addition, more than 80% of children with schizophrenia or schizoaffective disorder present comorbid attention deficit and hyperactivity disorder (ADHD) (Ross et al., 2006). Few genetic studies of COS were reported, due to the very low prevalence ($<1/40,000$) (Burd and Kerbeshian, 1987) and to nosographic difficulties, which made it hard to obtain a consensual clinical definition of this disorder and to carry out etiological studies (Maier, 1999; Gochman et al., 2011). Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) classification provides recent clarification in this area with schizophrenia no longer excluding the diagnosis of ASD (Petty et al., 1984; American Psychiatric Association, 2013). Thus, clinical overlap between COS and ASD is now formally accepted. Surprisingly, DSM-5 still does not recognize the existence of COS, which therefore remains considered an adult clinical presentation (AOS) (American Psychiatric Association, 2013). Indeed, COS is a very rare complex disorder related to other neurodevelopmental disorders, and it represents a real challenge for clinical diagnosis with, to date, no objective test based on genetics (Petty et al., 1984). However, a high heritability rate of COS ($> 80\%$) has been suggested in early adoption/twin studies (Kallmann and Roth, 1956) and has been confirmed by familial aggregation studies (Asarnow and Forsyth, 2013). To determine the etiology of COS, it is indispensable to start by reviewing the publications that have linked COS to DNA changes.

Macro-lesional cytogenetic abnormalities such as copy number variations (CNVs), including the 22q11.21 deletion, are more frequent in COS than in AOS [10.6% of patients

with COS (DSM-III-R, onset <13 years) vs. 2–5%, in AOS, $p < 0.0001$]. These anomalies in the general population would concern only 0.86% of newborns (Nicolson et al., 1999).

Recently, Next Generation Sequencing (NGS) or “high throughput sequencing” allowed, with unprecedented scalability and speed, to determine the DNA sequence of a given individual. This tool opened up new perspectives to understand complex neurodevelopmental disorders, with particular attention to *de novo* single nucleotide variations (SNVs) occurring in genes involved in brain development (Veltman and Brunner, 2012). Only one study used whole exome sequencing (WES), a NGS method, in a cohort of patients with COS. This study identified 20 *de novo* variants in 17 COS probands (rate: 1.17) in genes previously linked to neuronal function or to psychiatric disorders (Ambalavanan et al., 2016). These arguments (phenotypic overlap with other neurodevelopmental disorders, high heritability, disease-related CNVs, and *de novo* SNV rates) strongly support the neurodevelopmental and genetic bases of COS (Rapoport et al., 2012). In this context, the main aim of this study is to describe the COS genomic variation (CNVs and SNVs) in the scientific literature to identify interesting genes or genetic pathways in both clinical practice and research.

1.2.3 Methods

We carried out a systematic review of the MEDLINE database accessible *via* the search engine PubMed (www.ncbi.nlm.nih.gov/pubmed/) with the following key words: “childhood-onset schizophrenia” or “childhood-onset psychosis” or “early-onset schizophrenia” or “early-onset psychosis” and “genetics” or “genetic” or “gene” or “genes” or “genomic” or “genomics.” Our search terms were not limited by language or date of publication and were manually reviewed. According to inclusion criteria, we considered all genomic changes occurring in COS patients (age of onset before 13). We excluded all

abnormalities at RNA or protein levels (regardless the age of onset). Genomic variations were classified based on cytogenetic position (**Table 1**) and candidate gene names (**Table 2**).

All CNVs were manually annotated using the University of California Santa Cruz (UCSC) Genome Browser (UCSC Mar. 2006 (NCBI36/hg18 or NCBI37/hg19) assembly; <http://genome.ucsc.edu/>). Regarding their type (gain or loss), their size, their genomic content, and making comparisons with external databases, we ranked each CNV as “pathogenic,” “uncertain clinical significance” or “benign” (according to the American College of Medical Genetics standards and guidelines for interpretation and reporting of postnatal constitutional copy number variants). For each CNV, we checked on the Simons Foundation Autism Research Initiative (SFARI) gene database (autism/genetic database, <http://sfari.org>) which CNV involved in COS was also involved in ASD. For each gene, we checked on the Phenocarta Database (<https://gemma.msl.ubc.ca/home.html>) the evidence linking genes to phenotypes of neurodevelopmental disorders (**Figure 1**, Venn diagram). Phenotypes were systematically described, if available.

The selection took place before September 2018. At this time, 36 articles (1994 to 2018) out of 205 (1982 to 2018) met the inclusion criteria. Article reviewing process, including selection and exclusion, is summarized in a PRISMA flow diagram (**Figure 2**). Two articles were added after the freezing of the inclusion process (41; 52). Mutations were identified in 21 chromosomes. The results were ranked either in ascending order of their chromosomal position for structural variants (cytogenetic abnormalities) (**Table 1**) or in alphabetical order according to their gene name for genetic variants (lesions at gene level) (**Table 2**).

1.2.4 Results

I) Cytogenetic Abnormalities Associated With COS

The following cytogenetic abnormalities (n = 72, including 66 CNVs) were identified in 16 autosomes (1, 2, 3, 5, 6, 7, 8, 9, 10, 15, 16, 17, 18, 19, 20, and 22) and two sex chromosomes (X and Y) of 46 patients (17–40). The results are ranked in ascending order of their chromosomal position (Table 1) and are summarized in a genomic map (Figure 3).

1) CNVs

a) Deletions (CNVs) from 15Kb to 43Mb: 2p16.3, 2q31.2-q31.3, 3p12.2-p12.1, 3q29, 6p22.31, 9p24.2, 10q22.3, 15q11.2, 15q13.3, 16p12.1, 16p13.11, 16q24.1, 19p12, 20p12.1, 22q11.21, Xp22.31, Xq24-ter, and Xq23-q28;

b) Duplications (CNVs) from 120Kb to 17Mb: 1q21.3, 2p14, 2p25.3, 3p21.31, 3p25.3, 5q12.3, 7p13, 7q11.21-q11.22, 8p22, 8q11.23, 8q24.3, 10p11.23, 10p13, 15q11-q13, 15q13.3, 15q26.2–26.3, 16p11.2, 16p13.11, 16q22.2-ter, 16q22.3-q24.3, 16q23.3, 17q21.31, 18p11.31-p11.23, 18q22.1, 22q13.32, Xp22.31, Xq28, and Yq11.221.

Only six CNVs (9%) of our study are described in COS only: del2q31.2-q31.3 (smaller than the CNV described in ASD), del3p12.2-p12.1 (described in ASD as a duplication), delXq24-ter (larger than the CNV described in ASD), dup7p13 (described in ASD as a deletion), dup16q22.2-ter (larger than the CNV described in ASD), and dup16q22.3-q24.3 (larger than the CNV described in ASD).

In an on-site case series and literature review focusing on Childhood-Onset Schizophrenia Spectrum Disorders (SSDs; larger spectrum than COS), Giannitelli et al. (2018) showed that some CNVs, previously un-described in COS patients, are associated to childhood psychosis: 1q21.1 deletion, 1q21.1 duplication, Williams-Beuren region (7q11.23) duplication and 16p11.2 deletion (Giannitelli et al., 2018).

The phenotypes of only 15 out of the 46 patients were fully described (33%). The neurodevelopmental disorders that have been presented are: motor impairments (fine or growth milestones delay, coordination disability or tics) in 11/15 patients, language retardation in 7/15 patients, intellectual disability (IQ < 70) in two patients, and ASD in five patients [(including one Pervasive Developmental Disorder-Not Otherwise Specified (PDD-NOS)]. Social impairment was present in six other patients with COS and ADHD in three patients. Inattention impairment was specified in only one patient. The psychiatric comorbidities that have been highlighted are: two cases with mood disorders (major depressive disorder or dysthymia) and two cases with anxiety disorders. The somatic comorbidities detected are: dysmorphia in four cases and epilepsy in two cases. Sporadic cases of hypospadias, ureteric reflux, congenital ichthyosis, Chiari type 1, or celiac disease were also described.

2) Other DNA Lesions

a) Aneuploidy

One case of Trisomy X (47XXX) and two cases of Turner syndrome (45X0), one atypical form (46,X,del(X)(q24-ter) and one with mosaicism 46,X,i(X)(q10)(22%)/45,X(78%) (Eckstrand et al., 2008; Addington and Rapoport, 2009);

b) Uniparental and Segmental isoDisomy (iUSD)

In isodisomy, both copies of a chromosomal set are inherited from one parent (the mother or the father). An iUSD on 5q32-ter (35Mb) was described in a patient with COS (Eckstrand et al., 2008; Addington and Rapoport, 2009; Seal et al., 2006);

c) Translocation 1, 7: p22q22 (Gordon et al., 1994; Nicolson et al., 1999; Yan et al., 2000; Eckstrand et al., 2008; Idol et al., 2008; Addington and Rapoport, 2009)

d) Trinucleotide Repeat Expansions

i) CGG Expansions: Although a link between Fragile X syndrome (FXS) and COS has never been reported, Vantalon et al. (2005) described a 1.5 Kb expansion and complete methylation of the CpG island upstream of FMR1 in a 9 years-old girl with COS, dysmorphia, learning and social impairments, and mild mental retardation (Vantalon et al., 2005). This abnormality is inherited from the mother who carries an FXS premutation. Interestingly, instead of being unaffected or weakly affected as most patients carrying a premutation, the mother presents schizotypal personality. It seems that the severity of the schizophrenic spectrum disorder, which affects both mother and daughter with gradual severity, is linked to the CGG expansion degree. Effect of the hazard could not be excluded in this case (Vantalon et al., 2005).

ii) CAG/CTG Expansion: a longer repeat expansion on chromosome 18 was found in a COS cohort and in a male subclass, with a significant p-value especially for the males (0.036 and 0.002, respectively; Wilcoxon–Mann–Whitney U test) (Burgess et al., 1998).

e) Genetic Syndromes

In their recent review, Giannitelli et al. (2018) showed that some genetic syndromes, previously un-described in COS, are associated to childhood-onset SSDs: juvenile Huntington disease, Prader-Willi syndrome, Steinert myotonia, Ondine syndrome, Rubinstein-Taybi syndrome, and GLUT1 deficiency syndrome (Giannitelli et al., 2018).

II) Genes Associated With COS

In total, 32 candidate genes have been described on 12 autosomes (1, 2, 3, 6, 7, 8, 11, 13, 15, 17, 19, and 22) and 1 sex chromosome (X) (**Tables 1 and 2**) through the following studies (Addington et al., 2004; Sekizawa et al., 2004; Gornick et al., 2005; Addington et al., 2005;

Addington et al., 2007; Pakhomova et al., 2010; Addington et al., 2011; Raznahan et al., 2011; Smedemark-Margulies et al., 2016; Chaumette et al., 2018; Ambalavanan et al., 2019):

1) Genetic Association Studies (**Table 2**)

a. Family-Based “Transmission Disequilibrium Test” Studies (From 2004 to 2007)

Transmission disequilibrium test, a family-based association test for the presence of genetic linkage between a genetic marker and a trait, was used to describe the following candidate genes: *DAOA*, *DAOA-AS1*, *DTNBPI*, *GADI*, and *NRG1*. The Linkage Disequilibrium Analyses for Quantitative and Discrete Traits (QTDT) program was used to make statistical analysis (p-values).

i) *DAOA* and *DAOA-AS1* (Addington et al., 2004): three Single Nucleotide Polymorphisms (SNPs) are associated with COS (rs1935058, rs3916967, and rs2391191 (p = 0.5, 0.015, 0.3, respectively)). The most significant SNP (p = 0.015) is rs3916967 (genomic sequence reference: NG_012694.1:g.4133T > C);

ii) *DTNBPI* (Gornick et al., 2005): one SNP, rs11558324 (NG_009309.1:g.5154A > G), is associated with COS (p = 0.014) and two two-marker haplotypes (containing rs11558324) are also associated with COS (p = 0.021, 0.008),

iii) *GAD 1* (Addington et al., 2005): three four-marker haplotypes are associated with COS (p = 0.005);

iv) *NRG1* (Addington et al., 2007): individual markers show association with COS (rs35753505, rs2881272, 420M9-1395 (microsatellite), and rs327417, with p-value between 0.009 and 0.05). The most significant SNP (p = 0.009) is rs327417 (NG_012005.2:g.341913G > A). Further, several novel four-marker haplotypes are associated with COS (lowest p = 0.0004).

b. Population-Based “Case vs. Control” Studies (From 2004 to 2011)

A polymorphism (VAL66MET) in the *BDNF* (11p13) gene was associated with COS in a 65 patient cohort (10.5 ± 3.7 years old at onset) vs. 111 controls ($p = 0.03$; χ^2 test) (Pakhomova et al., 2010). A mutation (VAL158MET) in *COMT* (22q11.21) that increases protein activity levels in the brain accelerated adolescent cortical thinning (MRI findings) in both schizophrenia probands and their siblings (with resolution after a certain age for siblings), illustrating the influence of dopaminergic disruption on brain cortical maturation. Authors analyzed data from an 83 COS patient cohort vs. 208 healthy controls (Raznahan et al., 2011). A mutation (A218C) in the *TPHI* (11p15.3-p14) gene ($p = 0.0058$; χ^2 test) is described in 51 patients (early adolescent onset cohort before 16 years old) vs. 148 controls (Sekizawa et al., 2004).

2) Gene Sequencing Studies (2011 to Present)

Finally, five studies by DNA sequencing (candidate genes or whole exome sequencing) have allowed identifying the following 18 mutations in 11 genes (Addington et al., 2011; Ambalavanan et al., 2016; Smedemark-Margulies et al., 2016; Chaumette et al., 2018; Ambalavanan et al., 2019).

i. Six SNVs inherited from a healthy mother:

- Five X-linked recessive variants: *LUZP4* (arg278fs*10), *OPHN1* (met461val), *PCDH19* (leu1022ile), *RPS6KA3* (arg723his), and *UPF3B* gene (gln228fsX18) that also segregates in the sibling.
- One SNV (ala813val) in *ATPIA3* (a boy with onset at 10 years old and co-morbid ASD).

ii. Three SNVs inherited (missenses variants) in the *FXYP* gene family. *FXYP1* (arg90cys), *FXYP6-FXYP2* (val101ala), and *FXYP6* (gly73arg) genes. Only the patient with the mutation in *FXYP1* presents a co-morbid ASD (Asperger's disorder).

iii. Nine *de novo* SNVs in the following genes: *ATPIA3* (val129met; asp801asn; glu815lys), *GPR153* (arg73cys), *GTF2IRD1* (arg357cys), *ITGA6* (glu1063del), *RYR2* (glu746tyr), *SEZ6* (thr229_thr231del), and *TTBK1* (arg258gln).

The ATPase Na⁺/K⁺ transporting Alpha-3 Polypeptide (*ATPIA3* gene) encodes the alpha-3 catalytic subunit of the Na⁺/K⁺-ATPase transmembrane ion pump mapping at 19q12-q13.2 (Harley et al., 1988). The *ATPIA3* isoform is exclusively expressed in neurons of various brain regions, including the basal ganglia, hippocampus, and cerebellum (summary by Rosewich et al., 2012). Mutations in this gene have been associated with a spectrum of disorders depending on the domain they affect in the corresponding protein. The majority of mutations associated with rapid-onset dystonia parkinsonism, or dystonia-12 (*DYT12*), were located in exons 8 and 14 whereas those with alternating hemiplegia of childhood-2 (AHC2) were located in exons 17 and 18 and in general they seem to affect transmembrane and functional domains, being the most severe dysfunctions. By genetic analysis of clinical data from 155 patients with AHC2, 132 confirmed to have *ATPIA3* mutations. Among those with AHC2, the most frequent mutations were D801N (in 43%), E815K (in 16%) and G947R (in 11%). E815K was associated with a severe phenotype, with greater intellectual and motor disability; D801N appeared to confer a milder phenotype and G947R correlated with the most favorable prognosis. For those with epilepsy, the age at seizure onset was earlier for patients with the E815K or G947R mutations than for those with the D801N mutation (Panagiotakaki et al., 2015). In 10 patients from three unrelated families with cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss (CAPOS; 601338) (Demos et al., 2014), the same heterozygous missense mutation in the *ATPIA3* gene was identified (E818K; OMIM 182350.0014).

The G Protein-coupled Receptor 153 (*GPR153*) gene, located on 1p36.31, belongs to the large rhodopsin (RHO; OMIM 180380) family of GPCRs (Gloriam et al., 2005) and

shows a highest similarity to serotonin receptors, (Gloriam et al., 2005). Furthermore, knockdown of *GPR153* in mice showed reduction in food intake and increased anxiety according to the elevated plus Maze test (Sreedharan et al., 2011).

The InTeGrin Alpha-6 (*ITGA6*) gene is located on 2q31.1 (Hogervorst et al., 1991). While functional absence of *ITGA6* has been associated with epidermolysis bullosa (Hogervorst et al., 1991; Georges-Labouesse et al., 1996), a few works addressed the role of *ITGA6* in neurons. Alpha-6 integrin was initially reported to be involved in neural migration (Yao et al., 2018). In addition, recent data suggested that $\alpha 6$ and $\beta 1$ integrins may play a role in mediating Schwann cell interactions with axons and promote axonal regeneration (Chang et al., 2018).

The Ryanodine Receptor 2 (*RYR2*) gene—located on chromosome 1 between q42.1 et q43—encodes a calcium channel that is located in the sarcoplasmic reticulum and is the major source of calcium required for cardiac muscle excitation-contraction coupling. (Bhuiyan et al., 2007). *Ryr2*^{-/-} mice die at approximately embryonic day 10 with morphologic abnormalities in the heart tube. Ca²⁺ signaling has been associated with ASD (Kabir et al., 2016; Stephenson et al., 2017; Castagnola et al., 2018) and with other psychiatric and neurological diseases (Heyes et al., 2015). It is not surprising that *RYR2* was linked to ASD by genetic studies (Lu and Cantor, 2012; Soueid et al., 2016; Chen et al., 2017). However, it is very interesting to underline that an SNP in this gene was associated with ASD in families with only affected males in contrast with those with affected females (Lu and Cantor, 2012) suggesting that *RYR2* is a sex-related genetic factor for ASD.

The SEiZure-related 6 (*SEZ6*) gene is located on the 17q11.2 chromosome. *Sez6* types 1 and 2 have an N-terminal signal sequence, followed by a threonine-rich region, a Short Consensus Repeat (SCR), a CUB-like domain, a second SCR, a second CUB-like domain, three tandem SCRs, a transmembrane domain, and a cytoplasmic C-terminal tail. They differ

only in the region between the last SCR and the transmembrane domain. *SEZ6* was predicted to be involved in neuronal maturation and plasticity (Miyazaki et al., 2006). Recently mutations and altered expression of this gene have been associated with Alzheimer's and Niemann-Pick disease (Causevic et al., 2018; Paracchini et al., 2018).

The Tau Tubulin Kinase 2 (*TTBK2*) gene, located on 15q15.2, encodes a member of the casein kinase (CK1) group of eukaryotic protein kinases. *TTBK1* has been implicated in Alzheimer's disease (OMIM 104300) and in neurofibrillary tangles formation (Sato et al., 2006). Mutations in this gene also cause spinocerebellar ataxia 11 (SCA11; 604432). SCA11 is a pure progressive cerebellar ataxia that has been linked to 15q14-q21 (Worth et al., 1999; Houlden et al., 2007). In an 8-generation English family they found a one-base insertion in the *TTBK2* gene creating a premature stop codon and a truncation of the normal protein (OMIM 611695.0001). In a second family of Pakistani ancestry, a different mutation was found (OMIM 611695.0002). Goetz et al. (2012) concluded that *TTBK2* is required for removal of CP110 for the initiation of ciliogenesis (Goetz et al., 2012).

3) Candidate Genes From Cytogenetic Studies (**Table 1**)

Interesting candidate genes deleted, duplicated, or truncated by the CNVs have also been found in cytogenetic studies (see above). These genes have brain expression and are mostly described in other neurodevelopmental or psychiatric disorders (**Figure 1**). Nine genes are described as putative COS-causing genes: *CHRNA7* (15q13.3), *DGCR6* (22q11.2), *FMR1* (Xq27.3), *MYTIL* (2p25.3), *NRXN1* (2p16.3), *PRODH* (22q11.2), *PTK2* (8q24.3), *STS* (Xp22.31), and *SRGAP3* (3p25.3) (Yan et al., 1998; Nicolson et al., 1999; Usiskin et al., 1999; Liu et al., 2002; Sporn et al., 2004; Vantalon et al., 2005; Eckstrand et al., 2008; Walsh et al., 2008; Addington and Rapoport, 2009; Rapoport et al., 2009; Wilson et al., 2011; Lee et al., 2012; Ahn et al., 2014; Duong et al., 2015; Zhou et al., 2016; Malik et al., 2017).

1.2.5 Discussion

COS is a neurodevelopmental disorder with several degrees of complexity (clinical and genetic heterogeneity). Clinically, getting the diagnostic is very challenging (severe disorder, comorbidities, and association with other neurodevelopmental disorders) (Gochman et al., 2011). The clinical overlap with ASD is well documented and in our study we found a co-morbidity rate (33%) nearly equal to the National Institute of Mental Health (NIMH) COS cohort rate (28%) (Rapoport et al., 2009). The genetic overlap with ASD is also well documented and we show that 91% of described CNVs are also described in ASD (*SFARI*). In the literature, we found only 20% of COS patients with co-morbid ADHD vs. 84% according to Ross et al. and we hypothesize that this trouble was under-diagnosed in schizophrenia studies (Ross et al., 2006). All intellectual, motor, communication, and learning impairments are also frequently observed in COS (Ross et al., 2006; Nicolson et al., 1999). Psychiatric comorbidities were rarely described (two cases of mood disorders and two cases of anxiety disorders), which was an unexpected outcome given the published literature (Ross et al., 2006). Here, we highlight that only one-third of the full phenotypes associated with the mutations published in the literature are described, which constitutes a significant loss of information for researchers. Therefore, it appears fundamental to carry out preliminary work before genetic testing: perform a rigorous and homogeneous phenotypic characterization using International Classification of Disease (*ICD-10* and *DSM-5*) with standardized and internationally validated psychiatric categorical assessments and in accordance with medical history (including perinatology), biography (with significant life event and trauma), and environmental factors (such as toxic exposure).

COS is characterized by a complex genetic architecture with both inherited and *de novo* mutations distributed in almost all chromosomes. Most of the genes causing COS are

unknown yet. It is interesting that, the few that have been already proposed (see before) are involved both in neurodevelopmental and neurodegenerative disorders such as Parkinson, Alzheimer, or ataxia. Moreover, schizophrenia has been shown to have complex genetic traits with high polygenic risk (Ahn et al., 2016). Thus, a second hit (or more), in addition to CNV, is probably essential to explain the phenotypes. It includes *de novo* SNVs, other CNVs and/ or environmental factors (e.g., trauma at early childhood, central nervous system infections or injuries) (Davis et al., 2016). At the interplay between genetic and environmental factors, epigenetics opens new perspectives to understand biological mechanisms of psychosis. In fact, recent findings suggest that pangenomic methylation changes during adolescence accompany conversion to psychosis (Kebir et al., 2018). In clinical practice, as suggested by Szego et al. for ASD (Szego and Zawati, 2016), it would seem useful to propose to COS patients genetic sequencing instead or in addition to microarrays (Anagnostou et al., 2014; Soden et al., 2014) to improve genetic testing and to allow *de novo* SNV detection.

In research, the major challenge of the upcoming years will be the analysis of big data from NGS (prioritization and interpretation of DNA variations) (Richards et al., 2015) and the experimental validation of putative mutations. Sharing data with other teams around the world will be helpful to unravel the molecular pathology of COS and its underlying causes, paving the way for an early therapeutic intervention.

Table 2 : Genomic microlesions (including single nucleotide variations) in COS patients with their localisation, lenght and type of inheritance. When available, phenotypes (sex, age of onset, other neurodevelopmental disorders and comorbidities) are described.

Gene name	Gene localisation	SNV ID number / mutation (protein level)	Population / Phenotypes	Inheritance	p-value	Bibliography	
FAMILY BASED ASSOCIATION STUDY							
DAOA (G72) / DAOA-AS1 (G30)	13q33.2	rs1935058, rs3916967, rs2391191	n=64 (53 COS trios, 11 COS dyads)	–	0,015 to 0,5	43	
DTNBP1	6p22.3	rs11558324	n=92 (73 COS + PDD-NOS trios, 19 COS + PDD-NOS dyads)	–	0,014	44	
GAD1	2q31.1	rs3749034, rs2270335, rs2241165	n=66 (55 COS + PDD-NOS trios, 11 COS + PDD-NOS dyads)	–	0,005	45	
NRG1	8p12	rs35753505, rs2881272, rs327417	n=70 (59 COS + PDD-NOS trios, 11 COS + PDD-NOS dyads)	–	0,009 to 0,05	46	
CASE CONTROL STUDY							
BDNF	11p13	val66met	65 patients (10.5 ± 3.7y) vs 111 controls	–	0,03	47	
COMT	22q11.21	val158met	83 patients (<13 y) vs 208 controls	–	–	48	
TPH1	11p15.3-p14	ala218cys	51 patients (<16 y) vs 148 controls	–	0,0058	49	
GENES SEQUENCING (candidate genes or full exome)							
ATP1A3	19q13.2	val129met	Male, onset : 6 y, motor delay	de novo	–	50	
		asp801asn	Male, onset : 10 y, ASD, dysmorphia, motor, intellectual and learning delays, Recurrent MDD		–		
		glu815lys	Male, onset : 12 y, motor and communication impairments, dysmorphia, ASD		–		
		ala813val	Male, onset : 10 y, ASD, motor and intellectual delays	mother	–		
FXD1	19q13.12	arg90cys	Male, onset : 7 y, Asperger's disorder	inherited	–	51	
FXD6-FXD2	11q23.3	val101ala	Male, onset : 13 y		–		
FXD6	11q23.3	gly73arg	Female, onset : 12 y	de novo	–		15
GPR153	1p36.31	arg73cys	Male, onset : 12 y		–		
GTF2IRD1	7q11.23	arg357cys	Female, onset : 12 y	de novo	–	15	
ITGA6	2q31.1	glu1063del	Female, onset : 12 y		–		
LUZP4	Xq23	arg278fs*10	–	mother	–		52
OPHN1	Xq12	met461val	IQ 88, PDDNOS		–		
PCDH19	Xq22.1	leu1022ile	–		–		
RPS6KA3	Xp22.12	arg723his	IQ 64, PDDNOS		–		
RYR2	1q43	glu746tyr	Male, onset : 8 y, PDDNOS, Separation anxiety disorder, Asperger's disorder	de novo	–	15	
SEZ6	17q11.2	thr229_thr231del	Male, onset : 11 y		–		
TTBK1	6p21.1	arg258gln	Male, onset : 7 y, Asperger's disorder		–		
UPF3B	Xq24	gln228fsX18	Male, 10 y, ADHD, PDDNOS, ASD	mother	–		53

Abreviation :

ADHD = Attention Deficit Hyperactivity Disorder
ASD = Autism Spectrum Disorder
CNV = Copy number variation
COS = Childhood Onset Schizophrenia
DEL = Deletion
DUP = Duplication
MDD = Major Depressive Disorder
PDD-NOS = pervasive developmental disorder not otherwise specified
SNV = Single Nucleotide Variation
Y = years

Figure 1: Venn diagram: evidence for genetic overlap in neurodevelopmental disorders (Phenocarta Database)

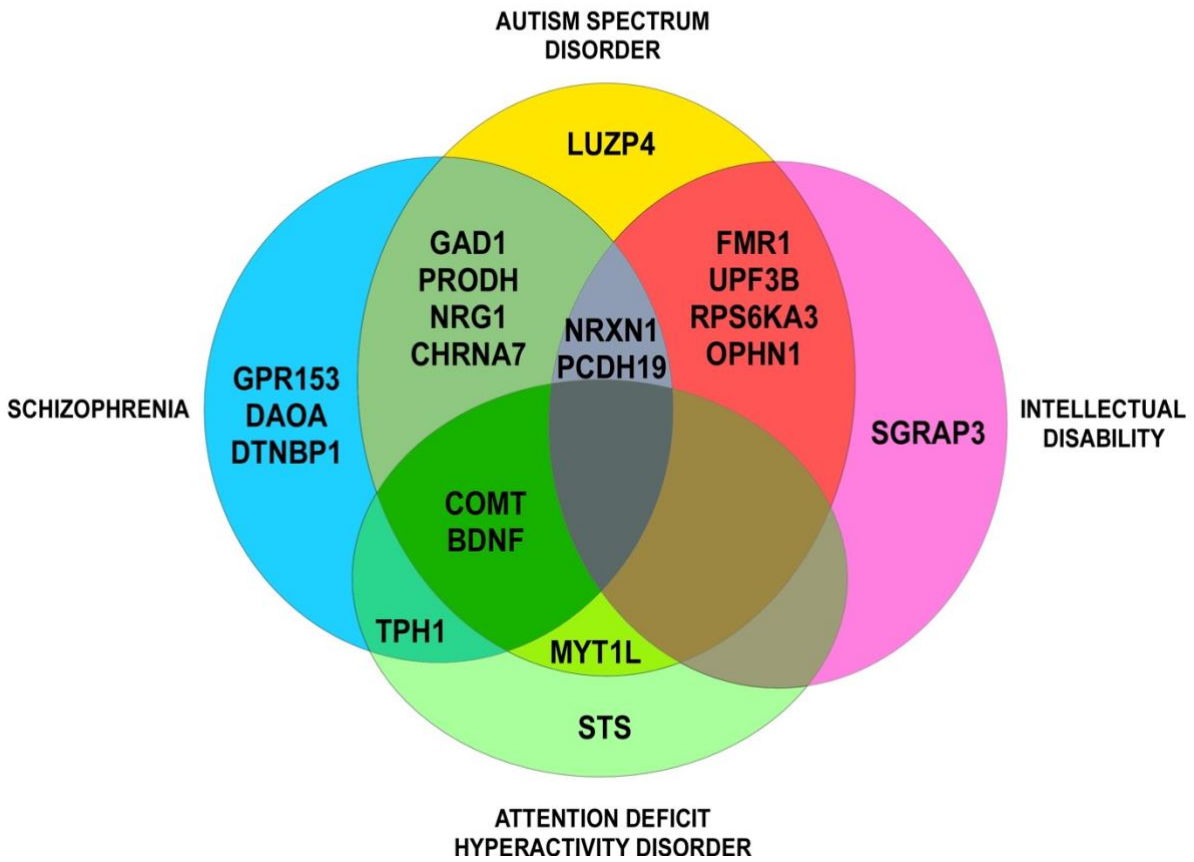


Figure 2: PRISMA Flowchart diagram

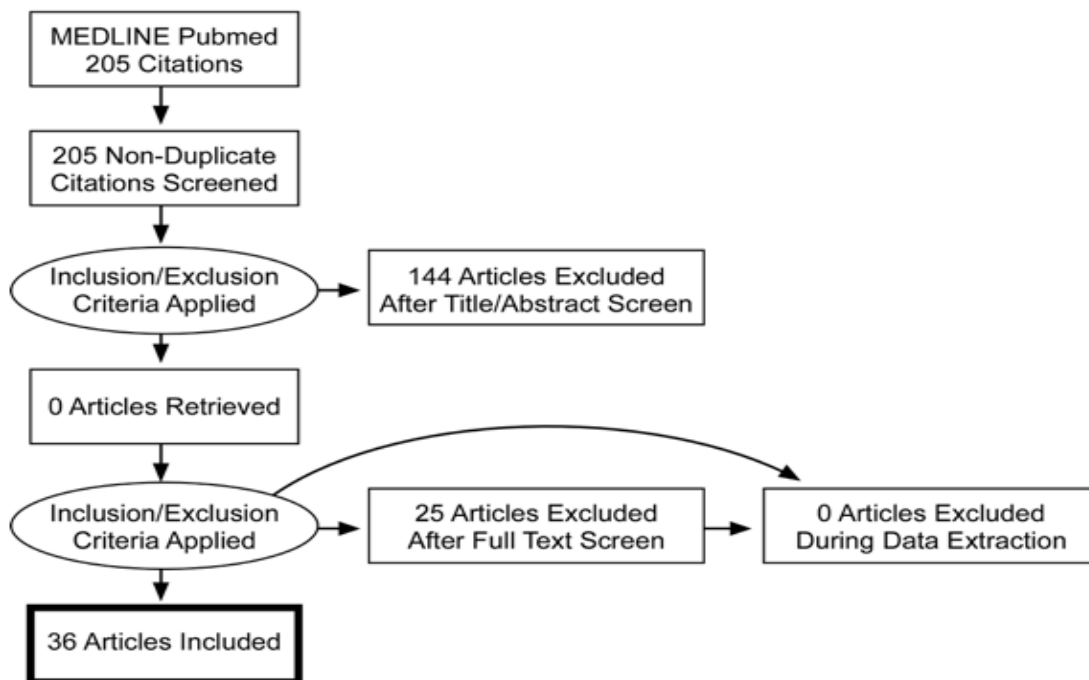
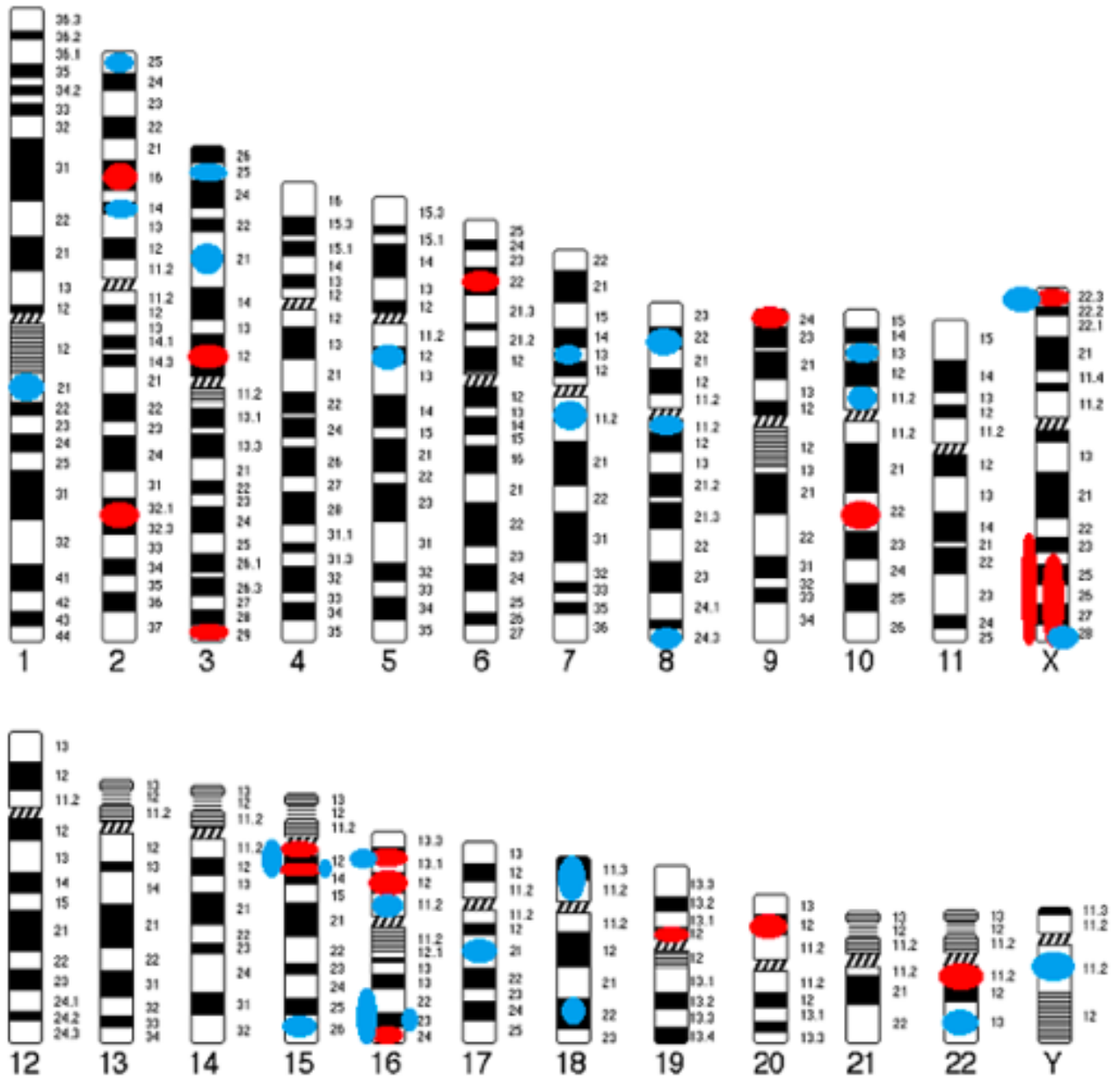


Figure 3 : Genomic map of CNVs identified in COS . Circles indicate their position and color represents type of abnormality (Red circle=deletion ; Blue circle=duplication)



CHAPITRE 2 : Hypothèses et objectifs

2.1 Hypothèses

Sur le plan clinique, la SP présente un large spectre d'expression clinique qui se distingue de la schizophrénie à début adulte par l'atteinte neurodéveloppementale plus fréquente et plus sévère (Driver et al., 2020). Il existe par ailleurs peu de données sur les comorbidités psychiatriques spécifiques aux STP (Ross *et al.*, 2006) et pas d'études à notre connaissance ciblant les pathologies organiques extracérébrales associées. De plus, comme vous l'avons montré dans le chapitre 1, les mutations génomiques décrites dans la littérature scientifique (études génétiques) ne sont associées à un phénotype précis que dans un tiers des cas (Fernandez *et al.*, 2019), ce qui représente une perte de données majeure pour la comparaison des données entre équipes qui est nécessaire à la compréhension des mécanismes moléculaires sous-jacents. Il n'est donc pas possible à l'heure actuelle de déterminer le phénotype de précision de ce trouble en fonction de l'âge de début de la maladie. Cependant, les différences observées sur le plan clinique entre les formes de début précoce et les formes de début à la fin de l'adolescence ou à l'âge adulte nous permettent de formuler **l'hypothèse qu'il pourrait exister, au sein même des SP, un regroupement syndromique des différentes atteintes associées en fonction de l'âge de début du trouble.**

Sur le plan génétique, l'étude des formes à début précoce de maladies débutant habituellement à des âges plus élevés présente un intérêt majeur dans la compréhension des mécanismes étiologiques sous-jacents à ces maladies. La maladie d'Alzheimer et le cancer du sein illustrent bien ce principe. En effet, l'étude de leurs formes précoces (rares) a permis des avancés diagnostiques et thérapeutiques majeures qui ont été applicables aux formes plus fréquentes débutant plus tardivement (Farmer *et al.*, 2005 ; Bertam and Tanzi, 2008). Ce

rationnel nous a permis d'envisager la SP comme un modèle d'étude prometteur des troubles neurodéveloppementaux à expression psychiatrique dans l'optique de mettre en évidence des anomalies génétiques. De plus, compte-tenu des données acquises, les SP devraient présenter des mutations génomiques à la fois macro-lésionnelles (anomalies cytogénétiques ou CNVs) et micro-lésionnelles (SNPs) à la fois héritées et *de novo*, rendant compte de la grande complexité de ses mécanismes étiopathogéniques. **Nous formulons l'hypothèse que l'étude des formes précoces de schizophrénie nous permettra de mettre en évidence des gènes ou des réseaux de gènes candidats impliqués dans la pathogenèse avec une probabilité plus importante si l'âge de début de la maladie est précoce et qu'il existe une atteinte neurodéveloppementale associée.**

2.2 Objectifs

Afin de mieux comprendre l'expression clinique et les bases génétiques et moléculaires de la SP, nous avons développé un projet pilote avec pour objectif principal de **caractériser sur le plan clinique (phénotype de précision) et génétique (approche hiérarchisée) les patients atteints de formes précoces et neurodéveloppementales (symptômes autistiques prémorbides associés) de schizophrénie et leurs apparentés au 1^{er} degré (étude familiale).**

CHAPITRE 3 : Méthodes

3.1 Article publié dans la revue *BMJOpen*

Exploration and characterization of the phenotypic and genetic profiles of patients with early onset schizophrenia associated with autism spectrum disorder and their first-degree relatives: a French multicenter case-series study protocol (GenAuDiss)

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3.1.1 Abstract

Introduction: Early-onset Schizophrenia (EOS) is a rare and severe condition. A higher rate of neurodevelopmental abnormalities, such as intellectual or communication impairments as well as attention deficit hyperactivity disorder, is observed in EOS compared to adult-onset schizophrenia. Early signs of autism spectrum disorders (ASD) are present in about 30% of patients. Genetic abnormalities, including copy number variations, are frequent in neurodevelopmental disorders and have been associated to ASD pathophysiology. Implicated genes encode proteins involved in brain development, synapses morphology and plasticity and neurogenesis. In addition, an increasing number of genetic abnormalities are shared by EOS and ASD, underlying the neurodevelopmental hypothesis of EOS.

The main objective of our study is to identify disease-causing genetic mutations in a cohort of patients affected by both EOS and ASD. Special attention will be paid to genes involved in neurodevelopmental pathways.

Methods and analysis: We describe a multi-centric study in a paediatric population. The study started in April 2014. Inclusion criteria are: age 7 to 22 years, diagnosis of EOS with co-morbid ASD and IQ above 50; Parents and siblings are also enrolled. We perform psychiatric assessments (MINI, K-SADS-PL, PANSS, SANS) together with neurocognitive evaluations (IQ, TMT A/B, verbal fluency). Then, we study variants of the coding part of DNA (exome), using next generation sequencing (NGS) process on trio (mother, father, child). Bio-informatics tools (RVIS, PolyPhen-2) are used to prioritize disease-causing mutations in candidate genes. The inclusion period will end in November 2019.

Ethics and dissemination: The study protocol was approved by the local Ethic Committee and by the French National Agency for Medicines and Health Products Safety. All patients signed informed consent upon enrolment in the study. Results of the present study should help

to unravel the molecular pathology of EOS, paving the way for an early therapeutic intervention.

Trial registration number: NCT02565524

3.1.2 Strengths and limitations of this study

- University Child Psychiatry Department has recognized specialists in rare and severe disorders such as Early-Onset Schizophrenia (EOS).
- Innovative clinical research protocol in the field of child psychiatry associating psychiatric assessments, neuropsychological explorations and DNA next generation sequencing of patients as well as their parents and siblings.
- Collaboration with research may provide new understanding of the molecular mechanisms of the condition and could provide biomarkers for diagnosis and treatment.
- Clinical research very challenging in EOS patients and families (severity of illness and impact on individual and family life), with limited sample size (rare condition, regional enrolment) and difficulties in involving both parents (separation, conflict, death...).
- Exome analysis may miss causative mutations in non-coding part of the genome.

3.1.3 Introduction

Early-onset Schizophrenia (EOS) is a rare (1/5000 to 1/20000) and severe chronic psychiatric condition defined by an onset of schizophrenia positive symptoms (delusions, hallucinations, and disorganized speech or behavior) before the age of 18 (Häfner and Nowotny, 1995;

Okkels et al., 2013). Onset before 13 years of age is called Childhood-Onset Schizophrenia (COS) (Nicolson and Rapoport, 1999; Gochman et al., 2011).

A higher rate of neurodevelopmental abnormalities is observed in EOS compared to Adult-Onset Schizophrenia (AOS), and patients typically have frequent deficits in cognition, communication or neuromotor impairments associated with attention deficit, hyperactivity disorder (ADHD) or autism spectrum disorder (ASD) (Vourdas et al., 2003; Rapoport et al., 2009; Ross et al., 2006). Furthermore, 28% of patients with COS in the US cohort of the National Institute of Mental Health Child Psychiatry Branch met criteria for comorbid ASD (Rapoport et al., 2009), and more than 80% of children with schizophrenia or schizoaffective disorder might present comorbid ADHD (Ross et al., 2006). In addition, EOS (including COS) is a complex disorder related to other neurodevelopmental disorders, and it represents a real challenge for clinical diagnosis (Gochman et al., 2011) with, to date, no objective test based on genetics. Therefore, it is critical to conduct a rigorous and homogeneous phenotypic characterization by using international classifications such as ICD or DSM [World Health Organization, 1992; American Psychiatric Association, 2000; American Psychiatric Association, 2013) and by using standardized and internationally validated psychiatric categorical assessments.

A few genetic studies of EOS are reported, due to the very low prevalence (Häfner and Nowotny, 1995; Okkels et al., 2013) and to the nosographic difficulties which made it difficult to obtain a consensual clinical definition of this disorder and to carry out etiological studies. Interestingly, DSM-5 classification provides clarification in this area with schizophrenia no longer excluding the diagnosis of ASD and formalizing the overlap between these two disorders. On the other hand, DSM-5 does not recognize the specificities of EOS compared to adult onset schizophrenia (American Psychiatric Association, 2013).

Submicroscopic cytogenetic abnormalities, such as disease-related Copy Number Variations (CNVs), are more frequent in COS than in AOS (about 11% [Nicolson et al., 1999; Ahn et al., 2014] vs 2-5%, $p < 0.0001$ (Ahn et al., 2014)). A high heritability rate of COS (>80%) has also been suggested in early adoption/twin studies and has been confirmed by familial aggregation studies [Sullivan et al., 2003; Asarnow and Forsyth, 2013]. The fact that genome-wide association studies (GWASs) failed to yield a genome-wide significant result in AOS patients appears to argue in favor of a polygenic basis of EOS with hundreds of genes with small individual effects being involved (Purcell et al., 2009). In EOS pathogeny, recent studies also demonstrated the involvement of mutations in *NRXN1* (2p16.3) and *UPF3B* (Xq24), crucial for development and function of the brain (Ahn et al., 2014; Addington et al., 2011).

Next generation sequencing (NGS) is a process to determine the entire DNA sequence of a given individual with unprecedented throughput, scalability and speed. Whole-exome sequencing (WES), a NGS method, corresponds to an exhaustive analysis of the exome (the coding part of the genome) thus helping to understand complex neurodevelopmental disorders such as EOS (Veltman and Brunner, 2012). To our knowledge, until now, only two studies using WES have been published in the field of EOS. SMEDEMARK-MARGULIES and colleagues described *de novo* heterozygous mutation (c.385G>A) in the *ATPIA3* gene (19q13.2) (Smedemark-Margulies et al., 2016). Another cohort study using WES identified 20 *de novo* variants in 17 COS probands (rate: 1,17) in genes previously linked to neuronal function or to psychiatric disorders (Ambalavanan et al., 2016). These arguments (phenotypic overlap with other neurodevelopmental disorders, high heritability, disease-related CNVs and *de novo* SNPs rates) strongly support the neurodevelopmental basis of schizophrenia (Rapoport et al., 2012) particularly of the early onset subclass. In addition, our

trio approach, both at the clinical and genetic level, will help to better understand transgenerational inheritance and to differentiate *de novo* mutations from inherited ones.

3.1.4 Methods and analysis

Objectives

The main objective of this study is to identify disease causing mutations primarily in genes involved in neurodevelopmental pathways in a cohort of patients affected by EOS.

The secondary objectives are the characterization of the clinical and neurocognitive profiles of patients and their first-degree relatives.

Trial design

GenAuDiss is a translational multicenter study. Patients with EOS were initially recruited through the 2011-2013 Interregional Hospital Clinical Research Program (NCT01512641) whose main goal was to estimate the prevalence of dissociative disorders in a population of children in child psychiatry care or medico-education structures. A total of six patients and 14 first-degree relatives have been enrolled from this completed program. In addition, an enrolment in the present study is offered to in- and out-patients corresponding to the inclusion criteria of the investigator, Child and Adolescent Psychiatry (CAP) centers, as well as their first-degree relatives. Participants are included either directly at the study sites or after referral by child and adolescent psychiatrists of the Provence-Alpes-Côte d'Azur (PACA) region.

The investigator centers of the GenAuDiss are the Children's Hospitals of Nice CHU-Lenval, the Marseille University Hospital, the Hospital La Fontonne (Antibes) and the Hospital Bonnet (Frejus). To date, 13 patients and 31 first-degree relatives (44 subjects in total) have been included in the GenAuDiss study protocol. At inclusion (V1), clinical assessments (both

psychiatric and neurocognitive) are performed in patients as well as first-degree relatives (parents and siblings). Blood test are performed in a second visit (V2) within six months. Study procedure, visits and inclusion criteria are presented in table 1.

Inclusion criteria

The study is proposed to children and adolescents presenting EOS with co-morbid ASD, and their first-degree relatives.

Major inclusion criteria for patients:

- Age of 7 to 22 years;
- Diagnosis of early-onset schizophrenia (onset before age 18), diagnosed by the semi-structured interview *Kiddie Schedule for Affective Disorders and Schizophrenia - Present and Lifetime Version* (K-SADS-PL) psychosis section;
- Diagnosis of Autism Spectrum Disorder (ASD) (*Autism Diagnostic interview-Revised* (ADI-R));
- Intelligence quotient (IQ) ≥ 50 at *Wechsler Intelligence Scale for Children* (WISC IV, abridged version).

Major inclusion criteria for parents and siblings:

- First degree biological relationship (no adoption) with the index patient.

Exclusion criteria

- Refusal or withdrawal of consent;
- Children without verbal language;
- Adults protected by law (relatives).

Measures

Quantitative and qualitative measures are assessed during the study (summarized in Table 1):

- Clinical parameters from medical history (including pregnancy, birth, etc.) and biographic parameters, types and dates of significant life events including trauma and environmental exposures (*e. g.*, drugs and substances);
- Clinical parameters from physical examination (*e. g.*, body weight, BMI, arterial pressure);
- Semistructured interviews to assess main diagnosis at inclusion (K-SADS-PL (Kaufman et al., 1997), ADI-R (Lord et al., 1994), MINI (Hergueta et al., 1998; Lecrubier et al., 1998));
- Clinical heteroassessments with specific rating scales (WISC IV (Wechsler, 2004) or V (Wechsler, 2014), PANSS (Kay, 1987), SANS (Andreasen, 1989) TMTA, TMTB, Verbal fluency);
- Self-report questionnaires (Cloninger's TCI 226 [Cloninger et al., 1994; Pélissolo et al., 1997], Baron Cohen's AQ (Baron-Cohen et al., 2001));
- Genetic analysis (including DNA testing for fragile-X syndrome, high resolution karyotype, DNA CNVs from CGH array, DNA SNPs from exome sequencing).

Table 1: GenAuDiss study procedure and evaluation criteria

PATIENT	V0: Selection	V1: Inclusion	V2
Inclusion criteria	+		
Informed consent	+		
Medical history + genealogic tree		+	
Psychiatric evaluation			
K-SADS-PL psychotic disorders	+		
ADI-R	+		
K-SADS-PL (full) (DSM IV/5)		+	
SANS		+	
PANSS		+	
Neuropsychological evaluation			
WISC IV (abridged version)	+		
IQ (full) * ¹		+	
Verbal fluency		+	
TMT A/TMT B		+	
Genetic testing			
Blood tests			+

SIBLINGS/PARENTS	V0: selection	V1: Inclusion	V2
Inclusion criteria	+		
Informed consent	+		
Medical history		+	
Psychiatric evaluation			
K-SADS-PL (full version) or MINI/MINI-S (DSM IV/5) * ²		+	
AQ		+	
TCI 226 (parents only)		+	
Neuropsychological evaluation			
IQ (abridged version) * ¹		+	
Genetic testing			
Blood tests			+

*Clinical tools are selected dependent on patient's age

1) Neurocognitive tools: < 7: WPPSI; 7-16: WISC; > 16: WAIS

2) Psychiatric tools: < 17: K-SADS-PL; > 16: MINI

ADI-R: Autism Diagnostic Interview - Revised

AQ: Autism Quotient

IQ: Intelligence Quotient

K-SADS-PL: Kiddie Schedule for Affective Disorders and Schizophrenia -Present and Lifetime Version

MINI-S: Mini International Neuropsychiatric Interview - Simplified

PANSS: Positive and Negative Syndrome Scale

SANS: Scale for the Assessment of Negative Symptoms

TCI: Temperament and Character Inventory

TMT: Trail Making Test

WAIS: Wechsler Adult Intelligence Scale

WISC: Wechsler Intelligence Scale for Children

WPPSI: Wechsler Preschool and Primary Scale of Intelligence

Data collection process

Each investigator at study sites is responsible for collecting and entering data on the CRF paperwork at the time of the visit and for realizing quality check of the data and regulation requirements. Results will be manually entered in the database by the clinical research assistants at the main investigator study center. In order to avoid missing data or filling errors, a double-check will be carried out before freezing of the database. Data extraction and analysis of the database will be performed by the Department of Clinical Research and Innovation (DRCI) of the Nice University Hospital.

Statistical analysis

Statistical analysis will be conducted by the DRCI of the Nice University Hospital. A descriptive analysis of all collected parameters will be performed. For continuous variables (*e. g.*, age, weight), indicators such as the mean, SD and range values will be calculated. Categorical data will be presented by means of frequency (n, %). Changes in the parameters and in the distribution of categorical variables from baseline to follow-up points will be tested with univariate analysis using appropriate testing according to the type of variable and distribution. In addition, multivariate analysis will be carried out to investigate putative relationships between variables. Level of significance for the p value was fixed at <0.05.

Outcomes

The main objective is to expand the repertoire of the genetic abnormalities associated to EOS phenotypes by performing:

- WES on trio (mother, father and child);

- Bioinformatics analysis and variant prioritization using validated tools (RVIS and Polyphen2) (Petrovski et al., 2013; Adzhubei et al., 2010) and strategies in accordance with international guidelines (Richards et al., 2015).

The secondary objectives of the GenAuDiss study are the characterization of the clinical and neurocognitive profile of patients and their first-degree relatives.

Clinical profile:

- K-SADS-PL for DSM IV-TR / DSM-5 co-morbid psychiatric diagnosis of patients and minor siblings;
- MINI assessments for DSM IV-TR / DSM 5 psychiatric diagnosis of parents and major siblings;
- Evaluation of the intensity of positive and negative symptoms, general psychopathology and subtypes of schizophrenia using PANSS/SANS;
- Personality dimensional test using computerized version of TCI 226 (Cloninger) in parents;
- Autistic traits using AQ (Baron-Cohen) in siblings and parents.

Neurocognitive profile:

- Evaluation of executive and attentional functions of patients using Trail Making Test (TMT) A and B and verbal fluency;
- Wechsler Intelligence Scale for Children (WISC) IV or V, full version in patient and WISC IV abridged version in minor siblings; Wechsler Adult Intelligence Scale-III (WAIS III) in a short form in adult siblings and parents (Wechsler, 1997; Grégoire and Wierzbicki, 2009).

Patient and Public Involvement

Results will systematically be transmitted with patients and legal caregivers. Results will also be discussed with the referent psychiatrist of child psychiatry care and/or medico-education structures. Patients have not been directly evolved in the study design. Nevertheless, our clinical experience and collaboration with families and care structures helped us to design this study, especially the clinical evaluation protocol.

3.1.5 Discussion

This project is based on a translational cross-sectional study protocol that aims to gain further understanding of the pathogenic molecular mechanisms of EOS. In fact, even if genomics is more and more often used for the diagnosis of human diseases including psychiatry (Ginsburg and Willard, 2009), the pathophysiology of EOS still needs to be elucidated. Importantly, to date, contrary to other neuropsychiatric pathologies (*e.g.*, Alzheimer's disease) (Bettens et al., 2013) only two studies describe the WES genetic approach in EOS patients (Smedemark-Margulies et al., 2016; Ambalavanan et al., 2016).

Furthermore, the known genetic heterogeneity of adult onset schizophrenia spectrum disorders imposes to build up models that consider the interplay between several factors. In fact, the "two-hit" hypothesis proposes to consider the impact of at least two genetic or clinical factors (CNV + CNV, CNV + SPN or CNV + epigenetic impact of trauma, etc.) (Bayer et al., 1999).

Perspectives

Mutated genes will be classified according to their pathogenic level, and the most likely disease causing candidate mutations will be studied by a research team expert in neuroscience (Laboratory "RNA Metabolism and Neurodevelopmental Disorders", **Dr. Barbara Bardoni**,

IPMC, CNRS, France). Further study of gene expression levels and *in vivo* cell activity in animal models will help predict the impact of the mutation on the protein structure and function. Finally, better understanding of EOS pathophysiology should help to develop objective diagnosis tools, early intervention and targeted treatment strategies.

Limitations of the study

EOS is a rare (1/5000 to 1/20000) (Häfner and Nowotny, 1995; Okkels et al., 2013) and severe mental disorder with a major impact on patients, families and care givers. The follow-up as well as inclusions in research protocols are therefore very challenging and need an experienced multidisciplinary team. Social precariousness, geographical distance from the hospital, and difficulties of reach both parents (*e. g.*, because of separation, conflict or death) might be significant obstacles limiting sample size.

Ethics and dissemination

The study protocol was approved by the Local Ethics Committee ‘Sud Méditerranée V’ (number 14.002) and authorized by the French National Agency for Medicines and Health Products Safety (ANSM 2013-A01699-36). All patients, their parents and siblings signed informed consent upon enrolment in the study. The GenAuDiss study was funded by Monaa (Monaco Against Autism), the Conseil Général des Alpes-Maritimes (CG-06), FRM (*Fondation pour la Recherche Médicale*, ING20140129004) and by ANR (*Agence Nationale de la Recherche*, ANR-11-LABX-0028-01). It is an ongoing study with the inclusion period starting in April 2014 and ending in November 2019. It has been registered on ClinicalTrials.gov (NCT02565524).

CHAPITRE 4 : Résultats

A ce jour (étude en cours jusqu'en mai 2023), 30 participants pédiatriques avec SP sont inclus : 10 filles et 20 garçons. L'âge d'onset moyen de la SP est de 10,27 ans (+/- 3.23) et 23/30 patients sont atteints de STP. Le QI moyen est de 74.90 (+/- 20.35). Tous les patients présentent des symptômes autistiques prémorbides à degré variable (critère d'inclusion ADI-R 0-5 ans, au moins une aire positive) et 23/30 patients présentent un TDAH comorbide à la K-SADS-PL.

Les apparentés du 1^{er} degré représentent 67 membres de la famille (soit un total de 97 participants en incluant les 30 enfants et adolescents atteints de SP).

Concernant l'analyse génétique, elle a fait l'objet d'une approche hiérarchisée avec réalisation :

- d'une recherche de l'X-Fragile : négatives
- et d'un Caryotype Haute Résolution (CHR) : négatifs
- ou d'un Caryotype Moléculaire de type CGH-Array : positifs pour 2 patients présentant les anomalies suivantes :

- le patient 6 : duplication 10q26.3 (cas clinique publié dans la revue *American Journal of Medical Genetics Part A*)

- le patient 27 : duplications 13q34 et p24,3

- et d'un séquençage de l'Exome en Trio pour les patients 11, 14, 16, 19, 23, 24, 25 et 26 (séries de cas soumise à la revue *Genes*) qui présentaient tous une recherche de l'X-Fragile et un CHR et/ou un CGH-Array négatifs.

Le tableau 1 ci-dessous présente une vue d'ensemble des patients inclus ainsi que les patients dont les aspects cliniques et/ou génétiques ont fait l'objet d'une publication dans une revue scientifique de niveau international avec comité de lecture.

Tableau 1 : Patients inclus dans le protocole GenAuDiss au 1^{er} février 2021 (n=30)

Patient	Sexe	Âge d'onset	QI	TSA	TDAH	Données publiées
1	M	12	65	++++		Phénotype
2	M	8	62	++++	+	Phénotype
3	M	10	70	++++	+	Phénotype
4	M	7	87	+	+	Phénotype
5	M	12	65	+++	+	Phénotype
6	F	12	74	+++	+	Phénotype / Dup10q26.3
7	M	6	60	++++	+	Phénotype
8	M	10	69	++++	+	Phénotype
9	F	5	53	++++		Phénotype
10	M	10	121	+++		Phénotype
11	F	8	73	++++	+	Phénotype / Exome
12	M	12	52	+++	+	Phénotype
13	M	7	104	++++	+	Phénotype
14	M	7	49	++++	+	Phénotype / Exome
15	F	7	69	++++	+	Phénotype
16	F	12	72	++++	+	Phénotype / Exome
17	M	8	47	++	+	Phénotype / Exome
18	M	7	94	++++	+	Phénotype
19	M	11	97	++++	+	Phénotype / Exome
20	M	7	105	++++	+	Phénotype
21	F	17	96	++++		-
22	F	16	105	+		-
23	M	14	40	+	+	Phénotype / Exome
24	F	8	73	+	+	Phénotype / Exome
25	F	13	69	+++	+	Phénotype / Exome
26	M	16	47	++++	+	Phénotype / Exome
27	F	13	76	+++	+	-
28	M	13	88	++++		-
29	M	12	70	+++		-
30	M	8	95	++++	+	-

4.1 Phénotype clinique (atteintes neurodéveloppementales, comorbidités psychiatriques et pathologies extracérébrales associées) des patients atteints de STP

4.1.1 Rationnel

Nous avons inclus les 20 premiers patients consécutifs (et 31 parents soit 51 sujets au total) du protocole GenAuDiss présentant une STP (début du trouble < 13 ans). Notre objectif était d'effectuer un phénotypage clinique de précision comportant l'âge de début de la maladie, les atteintes neurodéveloppementales, les comorbidités psychiatriques et les pathologies extracérébrales associées. Nous avons recherché les associations statistiquement significatives entre l'âge de début de la maladie et les différentes variables. Nous n'avons pas pu mettre en évidence de profil type en fonction de l'âge mais une grande **hétérogénéité clinique**. Une discussion sur les liens entre TSA et STP a été effectuée. L'article a été publié dans la revue *Canadian Journal of Psychiatry / Revue Canadienne de Psychiatrie* (Rang SIGAPS B, IF 2018 : 4.08).

4.1.2 Article publié dans la revue *Canadian Journal of Psychiatry*

**Atteintes neurodéveloppementales, comorbidités psychiatriques et pathologies associées
chez des patients atteints de schizophrénie très précoce avec symptômes autistiques
prémorbides**

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4.1.3 Résumé

Introduction : La schizophrénie très précoce (STP) est une forme rare (1/40000), grave et neurodéveloppementale de schizophrénie débutant avant 13 ans. Les comorbidités et atteintes associées spécifiques des STP étant peu étudiées, l'objectif de notre étude a été de les évaluer sur le plan psychiatrique, neurodéveloppemental et somatique.

Méthode : Il s'agit d'une étude ancillaire du protocole GenAuDiss. Un entretien psychiatrique standardisé (K-SADS-PL DSM5) et un bilan neuropsychologique (WISC-V/WAIS-IV) ont été effectués chez les patients atteints de STP ainsi qu'une anamnèse concernant la grossesse, la périnatalité, le développement, la biographie et les antécédents médicaux et psychiatriques, personnels et familiaux.

Résultats : 20 sujets ont été inclus. L'âge moyen de début du trouble était de 8,90 ans (+/- 2,30). Les comorbidités psychiatriques (DSM5) étaient le Trouble Déficitaire de l'Attention avec Hyperactivité (15/20 patients), les troubles anxieux (14/20) et le Trouble du Spectre de l'Autisme (13/20). Le QI moyen était de 70,26 (+/-18,09). Un retard de langage et une rupture de parcours scolaire étaient notés chez 18/20 patients. Enfin, l'affection somatique principale associée était l'asthme (15/20 patients).

Discussion : Nous avons mis en évidence chez nos patients atteints de STP une fréquence élevée de comorbidités dont au moins un trouble psychiatrique systématique. Or, bien que la schizophrénie infantile soit une pathologie de pronostic sévère impactant le patient, sa famille

et la société, sa prise en charge demeure essentiellement symptomatique. En pratique clinique, il apparaît nécessaire de rechercher systématiquement ces comorbidités et de les prendre en charge pour améliorer la qualité globale des soins.

4.1.4 Introduction

La schizophrénie très précoce (STP) est une pathologie rare (incidence généralement acceptée de 0,04% selon le National Institute of Mental Health (NIMH), 25 fois moins fréquente que chez l'adulte) (Driver et al., 2020). Elle est définie cliniquement par l'apparition de symptômes positifs (délires, hallucinations, discours et comportements désorganisés) avant l'âge de 13 ans et reste considérée sous l'angle de la schizophrénie adulte dans le DSM-5 de l'Association Américaine de Psychiatrie (APA, 2013).

L'applicabilité des critères diagnostiques DSM de la schizophrénie adulte aux formes de début précoce a été démontrée pour la première fois par Maziade *et al.*, (1996) avec la version III-R du DSM. Dans cette étude, les auteurs ont également démontré la stabilité longitudinale du diagnostic de STP depuis l'enfance jusqu'à l'âge adulte, constituant un argument en faveur de la validité diagnostique à long-terme de la STP (Maziade *et al.*, 1996).

Sur le plan de l'expression clinique, comme chez l'adulte, la STP est exceptionnellement un diagnostic isolé et les comorbidités psychiatriques sont fréquentes, en particulier le trouble déficitaire de l'attention avec hyperactivité (TDAH), le trouble du spectre de l'autisme (TSA) ou les troubles anxieux (Ross et al., 2006; Bermanzohn et al., 2000).

La majorité des travaux rapporte un taux plus élevé d'anomalies du neurodéveloppement chez les patients atteints de STP que dans la schizophrénie débutant à l'âge adulte, suggérant que la gravité de ces déficits pourrait en réalité représenter un

phénotype prémorbide (Vourdas et al., 2003).

Sur le plan génétique, la schizophrénie est caractérisée par une architecture génétique complexe, présentant des mutations à la fois héritées et *de novo*. Il s'agit d'une affection hautement héritable (>80%) (Sullivan et al., 2003). La STP partage avec la schizophrénie de l'adulte ce support génétique avec au premier plan les variations du nombre de copies des gènes (CNVs), dont les classiques syndromes de délétion 22q11.21 (Forsyth and Asarnow, 2020). Pour autant, la plupart des patients présentant une STP et qui bénéficieront d'une investigation génétique de 1^{ère} intention, en présence d'une atteinte associée, aura un résultat négatif. Cette investigation de 1^{ère} intention comprend la recherche de l'X-Fragile (en cas de TSA associé) et/ou un caryotype standard ou moléculaire de type CGH-ARRAY (en cas de déficience intellectuelle associée). En effet, soit les mutations sont indétectables par ces méthodes (en particulier les Single Nucleotide Variations), soit les gènes impliqués dans la STP sont encore inconnus. Parmi les gènes connus, une majorité semble être partagée avec les autres troubles neurodéveloppementaux dont le trouble du spectre de l'autisme (TSA) (chevauchement génétique > 90%) (Fernandez et al., 2019).

Ainsi, la STP, comme la schizophrénie à début plus tardif à l'adolescence ou à l'âge adulte, semble être un trouble d'origine multifactorielle aux mécanismes moléculaires complexes (génétiques et épigénétiques), incomplètement connus à ce jour et dont certains restent soumis à controverse, comme ceux décrits récemment dans le modèle omnigénique de la schizophrénie (Weinberger, 2019).

Par ailleurs, de nombreuses données multimodales (biologiques, génétiques et en neuroimagerie) plaident en faveur du modèle neurodéveloppemental de la schizophrénie très précoce (Rapoport et al., 2012), même si, à ce jour, peu de travaux étiologiques spécifiques sur cette population de patients atteints de formes précoces ont été effectués. Ceci s'explique par la très faible prévalence du trouble par rapport aux formes débutant à l'âge adulte et par

les difficultés nosographiques historiques qui ont longtemps rendu difficile l'obtention d'une définition clinique consensuelle des schizophrénies débutant avant l'âge de 13 ans.

Dans une démarche d'approche globale du patient pédiatrique, il nous a paru pertinent d'explorer également les atteintes somatiques associées à ce trouble. Or, aucune étude n'a recherché spécifiquement, à notre connaissance, les atteintes organiques extra-cérébrales présentées par les patients atteints de STP.

La STP se présente donc comme un trouble grave, neurodéveloppemental, comorbide sur le plan psychiatrique et dont la fréquence des atteintes organiques extra-cérébrales associées est peu étudiée. Par ailleurs, l'âge de début de la maladie apparaît comme une variable déterminante du pronostic sans toutefois permettre, à notre connaissance, de prédire les atteintes psychiatrique et somatique associées à la STP.

L'objectif principal de cette étude a donc été d'évaluer les atteintes neurodéveloppementales et cliniques (psychiatrique et somatique) des patients atteints d'une STP. L'objectif secondaire a été de rechercher une corrélation, positive ou négative, entre l'âge de début de la maladie et les données soio-démographiques, la présence/l'absence des atteintes neurodéveloppementales, des diagnostics psychiatriques comorbides associés et des pathologies organiques extra-cérébrales associées.

4.1.5 Méthodes

Il s'agit d'une étude ancillaire du protocole GenAuDiss (NCT02565524) faisant suite au Programme Hospitalier de Recherche Clinique Interrégional (PHRC) 2011 (NCT01512641) du Service Universitaire de Psychiatrie de l'Enfant et Adolescent de Nice (SUPEA).

Initialement, le SUPEA de Nice a mené une étude multicentrique translationnelle avec pour objectif d'estimer la prévalence des troubles du spectre schizophrénique dans une

population d'enfants pris en charge dans des structures sanitaires ou médico-sociales. Dans cette population, une proportion notable de 8,9% d'enfants avec un trouble du spectre schizophrénique a été mise en évidence lors des évaluations psychiatriques et neurocognitives (Dor-Nedonsel et al., 2020). Ces patients atteints de STP ont été inclus de manière prospective dans le protocole GenAuDiss qui est une étude de recherche biomédicale avec évaluation clinique (psychiatrique et neuropsychologique) et génétique (analyse de l'exome par séquençage de l'ADN à haut débit) du patient et ses apparentés du premier degré (parents et fratries) (Fernandez et al., 2018). Ce protocole (GenAuDiss) est en cours (fin des inclusions en mai 2023). L'actuelle étude ancillaire clinique a analysé spécifiquement les atteintes neurodéveloppementales, les comorbidités et les pathologies associées des patients atteints de STP inclus de manière consécutive dans le protocole GenAuDiss.

Les critères d'inclusion dans l'étude étaient un patient avec un diagnostic de STP (K-SADS-PL) et de TSA (ADI-R positive à l'évaluation passée, pour la période 0-5 ans), âgé de 7 à 22 ans et un quotient intellectuel (QI) supérieur ou égal à 40 selon l'échelle de Wechsler.

Dans un premier temps nous avons recueilli les informations concernant l'anamnèse du patient, ses antécédents psychiatriques et somatiques, l'histoire de son développement (statur pondéral, psychomoteur, affectif) ainsi que réalisé un arbre généalogique de la famille et recueilli les éléments biographiques (migration, traumatisme dans la petite enfance, données sociodémographiques actuelles). Une analyse précise des renseignements sur la prise en charge spécialisée paramédicale a également été effectuée : bilan orthophonique, bilan psychomoteur et bilan neuropsychologique antérieurs si existants. Les informations concernant les acquisitions précoces dans les domaines de la motricité globale, la communication et les apprentissages ainsi que l'histoire médicale précoce ont également été recherchées. Ces données nous ont permis d'apprécier l'évolution globale de l'enfant depuis la période périnatale au jour de l'évaluation.

Dans un deuxième temps nous avons effectué les passations des différentes échelles standardisées d'évaluations cliniques (K-SADS-PL avant 16 ans ou MINI-S après 16 ans et ADI-R) et neurocognitives des patients et des parents (WISC-V avant 16 ans ou WAIS-IV après 16 ans) (Thümmler and Askenazy, 2018; Sheehan et al., 1998; Wechsler, 1949; Wechsler, 1955).

La présentation des résultats pour les variables continues a été faite avec le calcul de leur moyenne et de leur écart-type (+/-).

Enfin, nous avons recherché une éventuelle relation entre l'âge de début de la maladie et les comorbidités retrouvées par l'analyse des corrélations, positives ou négatives, entre ces variables. Les effets du sexe ou du groupe d'âge d'onset (+/- 8 ans) ont été testés par un test-t de student pour les variables continues et par un test Fisher ou Chi2 pour les variables catégorielles.

4.1.6 Résultats

- Étude familiale et caractéristiques sociodémographiques

Les résultats de l'étude familiale (profil clinique, psychiatrique et cognitif des parents de patients atteints de STP) sont présentés dans l'annexe 1.

L'intensité de la symptomatologie positive, négative et globale du trouble des patients (scores et sous-scores de la PANSS) est présentée dans l'annexe 2.

Les résultats concernant les caractéristiques sociodémographiques sont présentés dans le Tableau 1.

20 sujets ont été inclus de manière prospective dans l'étude. Cet échantillon est composé de 15 garçons et 5 filles. Les patients avaient un âge moyen de 13,60 ans (+/-3,66) au moment des évaluations, avec un âge moyen de début de la maladie de 8,90 ans (+/-2,34).

- Les comorbidités psychiatriques (K-SADS-PL - DSM-5)

Les résultats concernant les comorbidités psychiatriques (DSM-5) sont présentés dans le Tableau 2.

Tous les patients de l'échantillon présentaient au moins un diagnostic comorbide ou associé, psychiatrique ou somatique. Les sujets avaient en moyenne 4,50 (+/-4,36) diagnostics psychiatriques associés.

Le TDAH était le trouble comorbide le plus fréquemment retrouvé, présent chez 15/20 patients évalués.

Nous avons également mis en évidence un ou plusieurs troubles anxieux associés chez 14 sujets sur 20 (dont 11 sur 14 avaient au moins deux troubles anxieux associés). Les troubles phobiques étaient les plus fréquemment retrouvés (près d'un tiers des troubles anxieux).

Un TSA était mis en évidence chez 13/20 sujets de notre échantillon à l'évaluation diagnostique actuelle (K-SADS-PL) tandis que tous les patients avaient une ADI-R positive (critère d'inclusion) concernant la période 0-5 ans.

Enfin, 12 sur les 20 patients inclus présentaient des troubles de l'humeur dont les plus fréquemment retrouvés étaient les troubles disruptifs avec dysrégulation émotionnelle, présents chez sept patients sur 12.

- Les atteintes neurodéveloppementales

Les résultats concernant les atteintes neurodéveloppementales sont présentés dans le Tableau 2.

Concernant le développement de l'intelligence (évalué à lors du bilan cognitif à l'aide des échelles de Weschler), un QI < -2DS (soit <70), c'est-à-dire une déficience intellectuelle, a été mis en évidence chez 10/20 patients. La moyenne du QI total de tous les sujets était de 70,26 (+/- 18,09).

Un trouble de type retard de langage a été retrouvé chez 18/20 des patients et était le trouble de la communication le plus fréquent. Une rupture dans le parcours scolaire habituel a été notée chez 90% (18/20) des patients avant le diplôme national du Brevet Français (soit avant la fin de la 9^{ème} année de scolarité), avec une orientation médico-sociale.

Un trouble moteur de type retard de motricité globale était retrouvé chez 5/20 des patients de notre population.

-Les affections somatiques associées

Les résultats concernant les affections somatiques sont présentés dans le Tableau 3.

Des antécédents médicaux et/ou chirurgicaux étaient présents chez 17/20 patients. Nous avons constaté une fréquence élevée d'antécédents de pathologies respiratoires telles que l'asthme ou les bronchiolites dans la petite enfance. Nous avons observé huit patients ayant présenté une crise d'asthme qui a nécessité une prise en charge médicale au cours de leur vie et sept patients ayant présenté au moins trois épisodes de bronchiolite (équivalent asthme du nourrisson) soit au total 15 sujets sur 20.

Des antécédents de pathologies oto-rhino-laryngologiques (ORL) étaient retrouvés chez cinq patients de notre échantillon.

Concernant les antécédents chirurgicaux, les plus fréquents dans notre échantillon étaient les adénoïdectomies (six patients sur 20) et amygdalectomies (cinq patients sur 20). Ces chirurgies étaient réalisées dans la plupart des cas comme traitement au long cours de pathologies respiratoires ou ORL chroniques ou récidivantes.

- Corrélations entre l'âge de début de la STP et les autres variables

Les résultats des corrélations en termes de données sociodémographiques, d'atteintes neurodéveloppementales, de comorbidités psychiatriques et de pathologies organiques non neurologiques associées sont présentées dans le tableau 4.

Nous avons mis en évidence qu'il existait une corrélation positive entre l'augmentation de l'âge des patients au moment du diagnostic et le nombre de diagnostics de troubles anxieux ($r^2=0,34$, $p=0,007$). Ce résultat (corrélation positive entre nombre de diagnostics de trouble anxieux et âge de début de la maladie) reste significatif ($t=-2,97$, $p=0,008$) en comparant deux groupes d'âge de début de la maladie différents (+/- 8 ans) comprenant 11 sujets pour le groupe « 8 ans et moins » et 9 sujets pour le groupe « plus de 8 ans ».

4.1.7 Discussion

Sur le plan sociodémographique, le sex-ratio en faveur des garçons (3 :1) paraît cohérent compte-tenu des atteintes neurodéveloppementales présentées par ces patients (May et al., 2019). Cependant, une étude récente concernant spécifiquement des patients atteints de STP ne mettait pas en évidence de différence significative en termes de sex-ratio (Ordóñez et al., 2016). Sur notre échantillon limité, cet effet peut être dû au hasard.

La littérature scientifique concernant les comorbidités des patients atteints de schizophrénie est conséquente. Néanmoins, les travaux concernant spécifiquement les formes à début très précoce (< 13 ans) sont encore très rares (Ross et al., 2006). En accord avec la principale étude traitant spécifiquement des comorbidités psychiatriques de la STP, nous avons retrouvé le plus fréquemment un TDAH, des troubles anxieux, un TSA et des troubles de l'humeur (Ross et al., 2006). Il est à noter qu'aucun enfant de cet échantillon n'avait d'antécédents d'abus ou de dépendance à la nicotine, à l'alcool ou à d'autres substances, comorbidités pourtant fréquentes dans les schizophrénies à début adulte (Batel, 2000).

A la K-SADS-PL, seulement 13/20 patients présentaient une symptomatologie de type TSA à l'évaluation diagnostique actuelle tandis que tous les patients avaient une ADI-R

positive (critère d'inclusion) concernant la période 0-5 ans. Cette différence (13/20 patients avec diagnostic actuel de TSA vs 20/20 patients avec symptômes autistiques avant 5 ans) peut être expliquée soit par une diminution de la symptomatologie autistique au cours du temps (optimal outcome selon Fein *et al.*, 2013) soit correspondre à une différence de sensibilité des différents outils pour l'évaluation de ces deux périodes ou encore peut être expliquée par un biais de rappel pour la période la plus ancienne. Il est donc possible qu'un sur-diagnostic ait été effectué avec l'ADI-R (qui retrouvait 20/20 diagnostics « passés » de TSA). L'ADI-R est un outil validé dans l'évaluation clinique des patients atteints de TSA et est considéré comme un gold standard (Zwaigenbaum and Penner, 2018). Barlati *et al.*, (2018) ont utilisé cet outil (ainsi que l'ADOS) dans une cohorte de 75 patients atteints de schizophrénie et ont mis en évidence un sous-groupe de patients schizophrènes avec une comorbidité autistique. L'étude des liens complexes entre ces deux conditions fait l'objet d'une attention croissante et l'utilisation et la création d'outils spécifiques de l'évaluation des symptômes autistiques chez un patient avec schizophrénie est à encourager (comme par exemple la PAUSS selon Pina-Camacho *et al.*, 2020). Des études de validation restent néanmoins nécessaires. Ainsi, à l'heure actuelle, l'usage de l'ADI-R chez un patient atteint de STP nous paraît valide sur le plan scientifique pour évaluer les symptômes autistiques précoces (atteinte neurodéveloppementale) mais insuffisant pour poser, seul, le diagnostic de TSA (Reaven et al., 2008). Les résultats de l'ADI-R dans son usage isolé, rétrospectif et en particuliers en contexte de trouble du spectre schizophrénique doivent être interprétés avec prudence. Nous pouvons donc considérer les signes autistiques précoces de nos patients comme un phénotype prémorbide d'un trouble schizophrénique à venir. La raison pour laquelle 28% des patients (selon la cohorte du NIMH, Rapoport et al., 2009) et 13/20 patients de notre étude présenteront toujours des symptômes évocateurs d'un TSA (et pas simplement des

symptômes autistiques qui tendent à s'atténuer voire disparaître après l'émergence de la STP) reste inconnue à ce jour.

Les autres atteintes neurodéveloppementales mises en évidence étaient les troubles de la communication (retard de langage dans 90% des cas) constatés par les familles et/ou l'orthophoniste. Une difficulté dans les apprentissages était présente dans 90% des cas avec le plus souvent une rupture dans le parcours scolaire classique antérieur au diagnostic de STP. Ces résultats, concordants avec ceux précédemment décrits dans la littérature (Ross et al. 2006; Nicolson and Rapoport, 1999), permettent de suggérer aux professionnels de santé d'exercer une vigilance accrue lorsqu'un enfant présente une atteinte neurodéveloppementale quel que soit le domaine (par exemple : communication, apprentissage, intelligence). Il n'est cependant pas possible, à l'heure actuelle, de définir un profil type de trouble du neurodéveloppement à risque de développer une STP, faute de marqueurs cliniques suffisamment spécifiques. En effet, la majorité des patients présentant une atteinte neurodéveloppementale ne développera pas une STP (Rutter and Mawhood, 1991).

Ainsi, la nature neurodéveloppementale de la STP et les atteintes neurodéveloppementales associées rendent ce diagnostic hétérogène, complexe et difficile à poser. Selon Gochman *et al.* (2011) on peut parler de « défi diagnostique » qui impose une grande rigueur méthodologique dans son approche diagnostique. En pratique clinique, l'utilisation d'outils diagnostiques standardisés comme la K-SADS-PL (Thümmler and Askenazy, 2018) est nécessaire pour confirmer le diagnostic de STP mais également pour orienter vers un diagnostic différentiel et rechercher les diagnostics associés. Cette évaluation devrait être effectuée en systématique par des professionnels expérimentés. De plus, la réalisation d'un bilan cognitif au plus tôt du début des troubles neurodéveloppementaux permettrait de suivre l'évolution du trouble et son retentissement tout au long de l'évolution de la maladie.

Concernant les pathologies somatiques associées à notre population de sujets atteints de STP, elles étaient principalement respiratoires (asthme et bronchiolites à répétition) pour 15/20 sujets (75%) ce qui est supérieur à la variation de prévalence de l'asthme entre 12,3 et 13,4% qui est décrite en population pédiatrique générale (Delmas et al., 2009). Cet effet peut être dû au hasard, faute de groupe contrôle et compte tenu du nombre restreint de patients inclus. Cela pourrait être également lié à la fréquence plus importante des pathologies respiratoires dans les populations au plus faible niveau socio-économique (Williams et al., 2009). En effet, selon Green *et al.*, (1992), 89% d'enfants atteints de STP étaient issus des classes sociales les plus pauvres. Chez l'adulte, plusieurs études réalisées tendent à démontrer l'existence d'un lien entre l'asthme et l'augmentation du risque de schizophrénie pour les patients. Une étude effectuée sur une base de données de 2002 suggérait, dans une population de patients schizophrènes adultes, un risque relatif de 1,3 de développer un asthme concomitant (Chen et al., 2009). Cependant ce résultat plaide en défaveur d'un impact neurodéveloppemental de l'inflammation des voies respiratoires, compte tenu de l'émergence des troubles schizophréniques à l'âge adulte alors que l'asthme débute dans l'enfance. Une seconde étude réalisée en 2012 vient confirmer ces résultats en démontrant une association entre l'asthme chez les patients et un risque accru de développer une schizophrénie adulte de 1,59 (Pedersen et al., 2012). Ainsi, le lien entre schizophrénie à début adulte et inflammation de l'appareil respiratoire est simplement suggéré par ces études, il n'existe actuellement dans la littérature aucunes données l'affirmant et aucunes données évaluant le lien entre ces pathologies respiratoires, d'origine inflammatoire, et le risque potentiel de développer une STP.

Concernant les corrélations entre l'âge de début de la maladie et les variables étudiées, seule la présence d'un trouble anxieux était corrélée positivement à l'âge de début de la maladie. Jerrel *et al.*, (2017) ont également mis en évidence une proportion plus importante de

patients présentant une comorbidité anxieuse quand l'âge de début de la schizophrénie est plus élevé, dans une cohorte de 613 patients atteints de schizophrénie précoce (âge de début inférieur à 18 ans). Ce résultat concordant attire notre attention sur l'association fréquente entre un trouble anxieux et les formes à débuts précoces et très précoces de schizophrénie. Il constitue un argument pour la recherche systématique de la comorbidité anxiété afin de favoriser la mise en place d'actions thérapeutiques spécifiques.

4.1.8 Conclusion

Les comorbidités de la schizophrénie sont présentes chez la majorité des patients et particulièrement chez ceux atteints d'une forme à début très précoce. La nécessité de diagnostiquer et traiter ces comorbidités devrait s'intégrer dans une démarche diagnostique et thérapeutique systématisée s'appuyant sur des outils standardisés et validés comme la K-SADS-PL (DSM-5). Cela permettra d'améliorer la prise en charge globale, le fonctionnement et la qualité de vie de ces patients. La STP est un trouble neurodéveloppemental avec un large spectre d'expression symptomatique psychiatrique (hétérogénéité clinique) mais également somatique. Des recherches cliniques prospectives et translationnelles devraient être conçues avec pour objectif de rechercher une association syndromique qui ne se limiterait pas à l'expression psychiatrique de ce trouble.

Limitations

La recherche clinique est très difficile chez les patients atteints de STP et auprès de leurs familles qui sont très impactées du fait de la gravité de la maladie et ses répercussions sur leur enfant et la vie de la famille en général.

La taille de l'échantillon est limitée du fait de la rareté de la maladie.

Enfin, le recueil des antécédents somatiques et des troubles respiratoires (comme l'asthme en particulier) ont été effectué par un interrogatoire libre. Un questionnaire d'évaluation des atteintes inflammatoires d'organes (digestifs, respiratoires, articulaires par exemple) aurait permis une évaluation plus systématisée et fiable de ces antécédents. Le même constat est à faire pour les allergies dont le recueil a été déclaratif et à la lecture du carnet de santé mais sans grille de recueil systématisée. Enfin, les comorbidités somatiques métaboliques (évaluations clinico-biologiques) comme le diabète ou les dyslipidémies n'ont pas été recherchées spécifiquement. Leur présence, souvent imputée aux effets indésirables des traitements antipsychotiques, pourrait également s'intégrer dans un cadre syndromique ou en association à une dysimmunité.

4.1.9 Figures et suppléments

Tableau 1 : Caractéristiques sociodémographiques des patients atteints de STP

Patient	Âge	Sexe	Âge de début de la maladie	Situation parentale	Lieu de résidence
1	13	M	12	séparés	urbain
2	17	M	8	séparés	urbain
3	12	M	10	séparés	urbain
4	7	M	7	mariés	rural
5	18	M	12	séparés	urbain
6	18	F	12	séparés	urbain
7	14	M	6	séparés	urbain
8	11	M	10	séparés	urbain
9	14	F	5	mariés	urbain
10	11	M	10	séparés	rural
11	10	F	8	mariés	rural
12	18	M	12	mariés	rural
13	12	M	7	séparés	urbain
14	18	M	7	séparés	rural
15	16	F	7	séparés	rural
16	12	F	12	séparés	urbain
17	20	M	8	mariés	urbain
18	8	M	7	séparés	rural
19	11	M	11	séparés	rural
20	12	M	7	séparés	rural
<i>Moyenne</i>	<i>13,60</i>		<i>8,90</i>		
<i>Ecart-type</i>	<i>3,66</i>		<i>2,34</i>		
<i>Total</i>		<i>15 M / 5 F</i>		<i>15 séparés 5 mariés</i>	<i>11 urbains 9 ruraux</i>

STP : Schizophrénie très précoce

F : Féminin

M : Masculin

Âges exprimés en années

Tableau 2 : Récapitulatif des diagnostics psychiatriques (K-SADS-PL DSM-5) et des atteintes neurodéveloppementales chez les patients atteints de STP

Diagnostics et atteintes associés	Garçons n = 15	Filles n = 5	Total n = 20
Trouble déficitaire de l'attention/Hyperactivité	11	4	15/20
Troubles anxieux	11	3	14/20
Trouble panique	0	1	1
Agoraphobie	5	1	6
Anxiété de séparation	3	2	5
Anxiété sociale/Mutisme sélectif	5	2	7
Phobie spécifique	9	1	10
Anxiété généralisée	3	2	5
Trouble obsessionnel compulsif	4	1	5
Troubles du spectre de l'autisme	8	5	13/20
Troubles de l'humeur	9	3	12/20
Dépression	3	2	5
Episode suicidaire	0	1	1
Manie/Hypomanie	0	1	1
Trouble disruptif avec dysrégulation émotionnelle	7	0	7
Troubles disruptifs du contrôle des impulsions et des conduites	8	2	10/20
Trouble oppositionnel avec provocation	6	1	7
Trouble des conduites	4	2	6
Tics	2	0	2
Troubles du contrôle sphinctérien	3	1	4/20
Enurésie	3	1	4
Encoprésie	0	0	0
Atteintes neurodéveloppementales	15	5	20/20
Troubles moteurs	3	2	5
Troubles du langage	14	4	18
Trouble des apprentissages	13	5	18
Trouble du développement intellectuel	8	2	10

Tableau 3 : Antécédents médicaux-chirurgicaux des patients atteints de STP	
	Patients n = 20
Antécédents respiratoires	15/20
Asthme	7
Bronchiolites (Asthme du nourrisson)	8
Antécédents chirurgicaux	15/20
Amygdalectomie	5
Adénoïdectomie	6
Appendicectomie	1
Hernie inguinale	1
Posthœctomie	2
Antécédents oto-rhino-laryngologiques	5/20
Otites séro-muqueuses	4
Angines	1

Tableau 4 : Corrélations entre l'âge de début de la schizophrénie très précoce et les autres variables

	ADM	AN	QI	TA	TH	AS	TR
Âge de début de la maladie (ADM)	1	0,27	0,07	0,63	0,36	0,26	0,18
Atteintes neurodéveloppementales (AN)		1	0,33	0,36	0,02	0,2	0,15
Quotient intellectuel (QI)			1	0,07	0,2	0,06	0,01
Trouble Anxieux (TA)				1	0,28	0,29	0,34
Trouble de l'Humeur (TH)					1	0,02	0,12
Antécédents somatiques (AS)						1	0,04
Trouble respiratoire (TR)							1

Légende :

QI = Quotient intellectuel

Gras : p=.002

Annexe 1 : Profil clinique (psychiatrique et cognitif) des parents de patients atteints de schizophrénie très précoce

Patient	Âge	Sexe	Âge de début de la	Mère				Père			
				Âge à la naissance	QI	Diagnosics psychiatriques (MINI)	Ancédents personnels	Âge à la naissance	QI	Diagnosics psychiatriques (MINI)	Ancédents personnels
1	13	M	12	25	66	Aucun	Proctocolite	35	75	Aucun	Aucun
2	17	M	8	27	84	Aucun	Insuffisance rénale, polyarthrite rhumatoïde, HTA	-	-	-	-
3	12	M	10	25	72	EDM	Aucun	39	69	EDM, TSPT, AG, Addiction au tabac	Aucun
4	7	M	7	31	59	EDM, Boulimie, TSPT, TUS	Hépatite B	35	72	TDAH	Aucun
5	18	M	12	30	103	EDM, TDAH, AS	Aucun	27	-	Aucun	Fructosémie congénitale
6	18	F	12	25	108	Episode maniaque, Risque suicidaire, Agoraphobie, TP, TSPT, Trouble psychotique, Boulimie	Asthme	39	-	-	-
7	14	M	6	29	88	-	-	25	-	-	-
8	11	M	10	34	-	-	-	61	-	Aucun	Tumeur cérébrale
9	14	F	5	32	69	Aucun	Troubles du sommeil	40	-	Aucun	Asthme, Dyslipidémie, Diabète de type 2
10	11	M	10	26	95	Aucun	Aucun	35	-	-	-
11	10	F	8	26	89	Agoraphobie, TP, TDAH, Trouble psychotique	Troubles auditifs	35	-	-	-
12	18	M	12	33	81	EDM, Risque suicidaire, Episode hypomaniaque	Maladie de Lyme, Sclérose En Plaques	34	105	EDM, AG	Diabète de type 2
13	12	M	7	26	103	TDAH	Aucun	30	123	Episode hypomaniaque, TUS	Aucun
14	18	M	7	34	103	EDM, Risque Suicidaire, Agoraphobie, TP, Boulimie, AG	Asthme	40	116	Agoraphobie, Trouble psychotique	Asthme, Guillain-Barré
15	16	F	7	34	86	EDM	Aucun	32	114	EDM, Risque suicidaire, AG, TP, Phobie sociale, TUA	Aucun
16	12	F	12	40	53	EDM, TP, TSPT, TUA	Diabète gestationnel	-	-	Aucun	Diabète
17	20	M	8	26	59	Personnalité antisociale, Trouble de l'humeur, Risque suicidaire, TSPT, Trouble psychotique, Addiction au tabac	Aucun	24	92	EDM, Addiction aux jeux vidéos et au tabac	Aucun
18	8	M	7	32	97	Aucun	Discopathie dégénérative, asthme, SAOS, tremblements essentiels	-	-	-	-
19	11	M	11	24	92	EDM, Episode maniaque, Trouble bipolaire de type 2, TP, AG, TDAH, Trouble psychotique bref	Aucun	31	86	TUS	Diabète
20	12	M	7	28	86	EDM, AG	Aucun	37	-	-	-

Annexe 2 : Scores aux échelles positive, négative et de psychopathologie générale à la PANSS							
Patient	Âge	Sexe	Âge de début de la maladie	Echelle positive	Echelle négative	Echelle psychopathologique générale	Total
1	13	M	12	17	22	37	76**
2	17	M	8	28	24	49	101***
3	12	M	10	27	27	45	99***
4	7	M	7	9	15	31	55
5	18	M	12	23	22	57	102***
6	18	F	12	10	20	29	59*
7	14	M	6	16	23	25	64*
8	11	M	10	21	27	41	89**
9	14	F	5	9	19	34	62*
10	11	M	10	11	9	25	45
11	10	F	8	25	18	52	95***
12	18	M	12	21	37	60	118****
13	12	M	7	7	14	24	45
14	18	M	7	16	10	33	59*
15	16	F	7	36	32	77	145****
16	12	F	12	20	26	39	85**
17	20	M	8	21	17	39	77**
18	8	M	7	30	28	56	114****
19	11	M	11	17	25	45	87**
20	12	M	7	21	23	40	84**

- * = score supérieur à 58 ; individu légèrement malade
- ** = score supérieur à 75 ; individu modérément malade
- *** = score supérieur à 95 ; individu nettement malade
- **** = score supérieur à 115 ; individu sévèrement malade

4.2 Génétique

4.2.1 Cas clinique (Patiente 6) : Duplication 10q26.3 et gène *INPP5A*

4.2.1.1 Rationnel

Au cours de l'évaluation génétique par approche hiérarchisée nous avons obtenu un résultat positif pour la patiente 6 au CGH-array. Cette patiente présente une STP (onset à 12 ans) avec des signes autistiques prémorbides (4 aires positives à l'ADI-R 0-5 ans) et d'autres atteintes neurodéveloppementales. L'anomalie retrouvée était une microduplication de 324 KDa sur le bras long du Chromosome 10. Après vérification du segment génomique concerné, nous avons observé la présence de 3 gènes dont un seul avec une expression cérébrale et codant une protéine : le gène *INPP5A*. Également présent dans le cerveau de la souris (sa délétion entraîne une ataxie, Yang *et al.*, 2015), nous avons montré que l'homologue d'*INPP5A* chez la drosophile (*5PtaseI*) est exprimé dans son système nerveux central (résultat original). Cet argument, associé à sa conservation inter-espèces et à la voie de signalisation dans laquelle ce gène est impliqué, nous permet de supposer qu'il jouerait un rôle significatif dans la pathogénèse de la STP de la patiente.

4.2 .1.2 Article publié dans la revue *American Journal of Medical*

Genetics Part A

A novel microduplication in *INPP5A* segregates with Schizophrenia Spectrum Disorder in the family of a patient with both Childhood Onset Schizophrenia and Autism Spectrum Disorder

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Equal contribution

4.2.1.3 Abstract

Childhood-Onset Schizophrenia (COS) is a very rare and severe psychiatric disorder defined by adult schizophrenia symptoms occurring before the age of 13. We report a microduplication in the 10q26.3 region including part of the *Inositol Polyphosphate-5-Phosphatase A (INPP5A)* gene that segregates with Schizophrenia Spectrum Disorders (SSDs) in the family of a female patient affected by both COS and Autism Spectrum Disorder (ASD). Phenotyping and genotyping (including CGH-array) were performed for mother, healthy sister and affected child according to the GenAuDiss protocol (NCT02565524). The duplication size is 324 kb and is present in a patient with COS and in her mother with SSD, but not in the patient's healthy sister. *INPP5A* encodes a membrane-associated 43kDa type I inositol 1,4,5-trisphosphate (InsP3) 5-phosphatase. This protein is found both in mouse and

human brains and we found that its *Drosophila* homologue *5PtaseI* is specifically expressed in the central nervous system. Hydrolyzed products from InsP3 5-phosphatases mobilize intracellular calcium, which is relevant for dendritic spine morphogenesis in neurons, altered in both schizophrenia and ASD. These may constitute arguments in favor of this gene alteration in the pathophysiology of COS.

4.2.1.3 Introduction

Schizophrenia spectrum disorders (SSDs) include schizophrenia, other psychotic disorders and schizotypal (personality) disorder (Association AP, 2013). Childhood-Onset Schizophrenia (COS) is a very rare and severe form of schizophrenia defined by Adult-Onset Schizophrenia (AOS) symptoms occurring before the age of 13 according to the Diagnostic and Statistical Manual of mental disorders 5th edition (DSM-5): disorganized speech and behavior, hallucinations and delusions (Association AP, 2013; Nicolson & Rapoport, 1999). This disorder is highly heterogenic at both clinical and genetic levels. As in other neurodevelopmental disorders, the pathophysiology of COS involves genetic factors as well as environmental factors damaging the brain (e.g., hypoxemia, early childhood trauma or maternal-fetal infections) (Rapoport et al., 2012). The respective responsibility of each of its causes remains unknown (Davis et al., 2016). The genetic architecture of COS is complex and involves rare and common DNA changes: copy Number Variations (CNVs) and Single Nucleotide Polymorphisms (SNPs) and variations (SNVs), both inherited and de novo. (Ahn et al., 2014; Ahn et al., 2016; Ambalavanan et al., 2016; Fernandez et al., 2019). At a metabolic level, many different pathways have been implicated in the pathogenesis of schizophrenia and alterations in the phosphoinositide signaling pathway leading to changes in Ca²⁺ signaling play a major role (Berridge, 2016). According to the N-Methyl-DAspartate

Receptor (NMDAR) hypo-function hypothesis of schizophrenia pathogenesis, the decline in Ca²⁺ signaling resulting from a decrease in NMDAR activity is responsible for the instability of the GABAergic inhibitory neurons (Timms et al., 2013). These neurons express receptors that activate the phosphoinositide signaling pathway acting to increase the Ca²⁺ signal and maintaining an excitatory/ inhibitory balance (Berridge, 2016).

We present the description of a microduplication in the 10q region including part of the Inositol Polyphosphate-5-Phosphatase A (INPP5A; HGNC: 6076/Entrez Gene ID: 3632/OMIM: 600106/ Ensembl: ENSG00000068383) gene that segregates with SSD in the family of a patient affected by both COS and Autism Spectrum Disorder (ASD). We also show the expression of the INPP5A Drosophila homologue in the central nervous system, supporting its role in the pathogenesis of COS.

4.2.1.4 Materials and Methods

GenAuDiss protocol

GenAuDiss is a translational multicentric study (NCT02565524). Patients with COS or Early-Onset-Schizophrenia (EOS) (with symptoms occurring before the age of 18) were initially recruited through the 2011–2013 Interregional Hospital Clinical Research Program (NCT01512641) whose main goal was to estimate the prevalence of COS and EOS in a population of children and adolescents in child psychiatry care or medical-educational structures (Dor-Nedonsel et al., 2020; Fernandez et al., 2018). In addition, an enrolment in the present study is offered to inpatients and outpatients of Child and Adolescent Psychiatry (CAP) centers, as well as their first-degree relatives. Participants are included either directly at the study sites or after referral by child or adolescent psychiatrists of the Provence-Alpes-Côte d'Azur (PACA, France) region. At inclusion (V1), clinical assessments (both psychiatric

and neurocognitive) are performed in patients as well as in first-degree relatives (parents and siblings). Blood tests are performed in a second visit (V2) within 6 months. The study is proposed to children and adolescents presenting COS or EOS with comorbid ASD and to their first-degree relatives.

Major inclusion criteria for patients:

- 7–22 years of age;
- Diagnosis of COS (onset before age 13) or EOS (onset before age 18) by the semi-structured interview Kiddie Schedule for Affective Disorders and Schizophrenia—Present and Lifetime Version (K-SADS-PL) psychosis section;
- Diagnosis of ASD by Autism Diagnostic Interview-Revised (ADI-R);
- $IQ \geq 40$ at Wechsler Intelligence Scale for Children (WISC-V).

Major inclusion criteria for parents and siblings:

- First-degree biological relationship (no adoption) with the index patient.

The main objective of the GenAuDiss protocol is to identify disease-causing genetic mutations in a cohort of patients affected by both COS or EOS and ASD.

Phenotyping

The propositus is an inpatient at the time of the inclusion process. Quantitative and qualitative measures were assessed during hospitalization:

- Medical history (including pregnancy, birth and later development) and biographic parameters, types and dates of significant life events including trauma and environmental exposures (*e. g.*, drugs and substances);
- Clinical parameters from physical examination (*e. g.*, body weight, body mass index and arterial pressure);

- Semistructured interviews to assess main diagnosis at inclusion (KSADS-PL, ADI-R, and Mini International Neuropsychiatric Interview [MINI]) (Kaufman et al., 1997; Lord et al., 1994; Sheehan et al., 1998a; Sheehan et al., 1998a);
- Clinical heteroassessments with specific rating scales (WISC-V, Positive and Negative Syndrome Scale [PANSS], Scale for the Assessment of Negative Symptoms [SANS], Trail Making Test [TMT] A, TMT B, and verbal fluency) (Andreasen, 1989; Kay et al., 1987; Tombaugh, 2004; Wechsler, 2014);
- Self-report questionnaires (Cloninger's Temperament and Character Inventory [TCI] 226 and Baron Cohen's Autism Quotient [AQ]) (Baron-Cohen et al., 2001; Cloninger et al., 1994; Pélissolo et al., 1997).

Genotyping

The genetic analysis strategy includes DNA testing for Fragile-X Syndrome by PCR FRAXA (Todorov et al., 2010), high-resolution karyotype or DNA CNV research by CGH array from both parents, siblings and patient. A quantitative PCR (LightCycler® 480 SYBR Green I Master) is made for gene detection and quantification if a putative gene is found to be deleted, duplicated or truncated by a CNV.

In situ hybridization in Drosophila

Drosophila embryos were dechorionated in 50% bleach, fixed in 4% formaldehyde, devitellinized in 100% methanol, and stored in 100% ethanol. The *5PtaseI* anti-sense probe was made from the Drosophila Genomics Resource Center (DGRC) SD05875 cDNA linearized with EcoRI and transcribed with T7 polymerase using the Riboprobe Combination System kit (Promega, Madison, WI) and the DIG RNA Labeling Mix (Roche, Indianapolis,

IN). Embryos were stained using the TSA Plus Biotin System (Perkin Elmer, Waltham, MA) according to Tevy *et al.*, 2014.

4.2.1.5 Results

The GenAuDiss protocol [11], a French multicentric study designed to identify disease-causing genetic mutations in a cohort of patients affected by COS or EOS and ASD, allowed us to identify a novel interesting CNV in a COS proband: a ~324 kb duplication including a portion of the *INPP5A* gene. Mitchell *et al.* (1996) used fluorescence *in situ* hybridization to map the gene on chromosome 10 (10q26.3). The duplication was inherited from the mother diagnosed with psychotic disorder that is included in SSD. The healthy sister of the patient does not carry this duplication (Figure 1). Unfortunately, the father died at patient's early childhood, is not available for genetic testing and we do not have any psychiatric information about him.

Medical history and developmental features

The proband, a 17-year-old girl, was diagnosed with COS around age 12. She was born at 41 weeks of gestation, by caesarian section. Her birthweight was 3500 g, she measured 51 cm of length and 36 cm of head circumference. Apgar score was normal (10-10). She presents a psychotraumatic family history with the violent father's death murdered at her early childhood (before one year old). Delayed motor milestones were noticed (walking after 24 months) with also communication disorders (first words at three and a half years and stuttering), intellectual disability (IQ = 74) and learning impairments. She was admitted in a medical-social care center since the age of seven years, because of behavioral symptoms

including tantrums and poor interactions with other children. At age 12, she presented a delusion of persecution, visual hallucinations and depressive mood and was diagnosed with COS by two independent child psychiatrists. She was treated by a monotherapy of antipsychotic (Risperidone) during five years with a good response on delusion, but mood lability and depressive symptoms remained. At the age of 14, she made a first suicide attempt by drug ingestion. During hospitalization in an adolescent psychiatry unit, a switch to Olanzapine allowed a durable mood stabilization with also a good response for positive symptoms (delusions and hallucinations).

Clinical work-up

At the age of 17, she was hospitalized again for a relapse of suicidal behavior with associated mood-congruent psychotic features. Neurological and general examinations were normal, including EEG, cerebral MRI, standard blood analysis, and metabolic screening.

Somatic features

The patient has been and is presently suffering from moderate asthma (no long-term control medications needed), primary enuresis and primary amenorrhea (first periods at the age of 17) without associated drug-induced hyperprolactinemia.

Clinical Genetics

Fragile-X Syndrome testing by PCR FRAXA was negative. High-resolution karyotype was not carried out because we first performed a molecular karyotype (Array CGH).

Array CGH analysis shows an interstitial 324 kb duplication in the chromosomal region 10q26.3, inherited from the mother (Figure 2A, B) that was confirmed by Quantitative PCR (qPCR). Three genes are contained in this microduplication: *AF086154*, *LINC02870* and *INPP5A*. Only one gene (*INPP5A*) is expressed in the brain, The other two genes have unknown function and no data about the encoded proteins are available (Figure 2C). Only one

portion of *INPP5A* is duplicated in this interval (transcript ENST00000368593.7: from exon 1/13 to 12/13 with truncation in intron 12/12; transcript ENST00000368594.8: from exon 1/16 to 12/16 with truncation in intron 12/15; transcript ENST00000342652.6 : from exon 1/13 to 9/13 with truncation in intron 9/12).

Family study

The patient's mother was evaluated with MINI-S and was diagnosed with psychotic disorder, mood/anxiety disorders (suicide ideation without suicide attempt) and Post-Traumatic Stress Disorder (PTSD). The AQ was high (30/50) and revealed autistic traits, near the cut-off (32/50) for Asperger's syndrome diagnosis. The TCI personality test revealed also a probable personality disorder (cooperation <-2 SD). Finally, IQ was normal around 108. In her medical history, she felt sad very regularly but had never seen a psychiatrist and had never benefited from a psychotropic drug. The patient's sister was diagnosed with tobacco use disorder and past social phobia. Her AQ score was negative for autistic traits (16/50) and no personality disorder was diagnosed. Her medical history was insignificant.

Central nervous system expression in Drosophila

The human *INPP5A* gene has one *Drosophila* homologue called *5PtaseI* (Seeds et al., 2004). The two proteins present 46% identity and 63% similarity. No *5PtaseI* mutant is currently available and its expression pattern has not been reported. To determine whether the function of *INPP5A* is conserved, we carried out *in situ* hybridization on *Drosophila* embryos. At stages 15 and 16, *5PtaseI* is strongly expressed in the brain and in the ventral nerve cord (Appendix 1). This result reinforces the role of *INPP5A* in nervous system development.

4.2.1.6 Discussion

Our case report is the description of a patient with COS associated with neurodevelopmental disorders (mental retardation, learning disabilities and ASD) and carrying a 10q26.3 microduplication in the *INPP5A* gene. According to the large Decipher database (<https://decipher.sanger.ac.uk/>), most gain and loss of function CNVs encompassing *INPP5* are predicted to be pathogenic in the general population. In the patient's family, the genetic testing of first degree relatives (Figure 1) revealed a familial segregation of the mutation where both mother and patient with SSD carry this 10q26.3 CNV microduplication while the 22 year-old sister in good mental health (with typical development) does not carry it.

While the proband's mother psychotic disorder evolved since adolescence (15 years), the proband has an earlier, very severe and neurodevelopmental form of schizophrenia. We can speculate that their different phenotypes are underpinned by the effects of a variable combination of additional hits (e.g., a rare genomic mutation, a common variant or an epigenetic effect of early childhood trauma).

INPP5A encodes a membrane-associated 43 kDa type I inositol 1,4,5-trisphosphate (InsP3) 5-phosphatase that hydrolyzes Ins(1,4,5) P3 and Ins(1,3,4,5)P4 to generate Ins(1,4)P2 and Ins(1,3,4)P3, respectively (Laxminarayan et al., 1994; Ooms et al., 2009). InsP3 5-phosphatases hydrolyze Ins(1,4,5)P3, which mobilizes intracellular calcium and acts as a second messenger mediating cell responses to various stimulations (Berridge, 2016). Primary and modulatory actions of the InsP3/Ca²⁺ pathway have been implicated in psychiatric disorders (ASD, schizophrenia and bipolar disorder) and in major human diseases affecting the brain (Alzheimer's, Huntington's, amyotrophic lateral sclerosis, epilepsy, and spinocerebellar ataxia) or other tissues (e.g., cardiac diseases) (Berridge, 2016). This protein is found in both mouse (Yang et al., 2015; <http://mouse.brain-map.org/gene/show/84380>) and human brains (De Smedt et al., 1994;

<https://www.genecards.org/cgi-bin/carddisp.pl?gene=INPP5A#expression>].

Phosphoinositides are phospholipids participating in membrane remodeling and all the processes linked to it, like cytoskeletal remodeling, cell signaling cascades, ion channel activity, and membrane traffic (Volpatti et al., 2019). At a molecular level, INPP5A appears to regulate intracellular Ca²⁺ through modulation of Ins(1,4,5)P₃ levels, which is relevant for dendritic spine morphogenesis in neurons (Billcliff & Lowe, 2014; Speed et al., 1999; Windhorst et al., 2012). Importantly, dendritic spine morphology and number are altered in both schizophrenia (Glantz & Lewis, 2000) and ASD (Hutsler & Zhang, 2010). These may constitute arguments in favor of the role of this gene alteration in the pathophysiology of COS.

It would be interesting to explore the levels, in both mother and daughter, of the truncated InsP₃ 5-phosphatase that we hypothesize is generated by the microduplication. Indeed, the presence of an abnormal InsP₃ 5-phosphatase would strongly suggest a dominantnegative effect. However, due the inaccessibility of the tissues where the InsP₃ 5-phosphatase is expressed, this important information is lacking.

Lithium, a classic mood stabilizer in clinical practice, reduces the formation of inositol (by inositol monophosphatase inhibition) and seems to be a therapeutic target of this pathway (Berridge, 2016). Reduced brain inositol in rats treated by lithium was first shown more than 50 years ago (Allison & Stewart, 1971). To explain the clinical effects of lithium, Berridge et al., (1989) introduced the inositol depletion hypothesis, which postulates a reduction in the supply of inositol lipids required for signaling in cells within the central nervous system (CNS) (therapeutic action) or in the developing embryo (teratogenic action) (Berridge et al., 1989). More recently, a growing literature about the neuroprotective action of lithium in various CNS disorders

including neurodegenerative disorders is emerging (Chiu & Chuang, 2011). However, despite a suggested link between the alteration in the inositol pathway (INPP5A microduplication) and the diagnosis of our patient, the therapeutic effects of lithium on COS and other forms of schizophrenia remain unknown and should be specifically studied.

In mice, the loss of function of *Inpp5A* (presenting 90.21% similarity with human INPP5A) causes ataxia and cerebellar Purkinje cell degeneration (Yang et al., 2015). In humans, INPP5A has been shown to play a role in the development and progression of cutaneous squamous cell carcinoma and to have a prognostic value in these diseases (Cumsky et al., 2019; Sekulic et al., 2010). Although strong proof of the involvement of the brain-expressed INPP5A gene in human psychiatric disorders is lacking, to our knowledge, two SNVs in INPP5A (rs751524528: p.A80V and rs775793924: p.R168W) were described in human psychiatric disorders. rs751524528 (p.A80V) is a benign non-synonymous mutation (according to Polyphen2) in a 31-year-old female affected by adult-onset schizoaffective disorder (bipolar type) without developmental delays, mental retardation, and learning disabilities (Xu et al., 2011). rs775793924 (p.R168W) is a rare SNV defined by authors as a «non-synonymous damaging strict and disruptive » variant and was found in a multiplex family with subjects affected by bipolar disorder or SSD with no phenotypic description (Ganesh et al., 2019). Finally, an epigenome-wide significant ($p < 1.7 \times 10^{-8}$) association for phonemic verbal fluency (cg12507869, $p = 2.5 \times 10^{-9}$) was found in an epigenome-wide association study. This CpG site is located in INPP5A, but the top SNP (rs113565688) for this site explains only 1.2% of the variance in methylation and rs113565688 was not correlated with alterations in cognitive functions in a recent GWAS. Thus, in this case, the environmental influence in INPP5A methylation in the cognitive alteration seems predominant over the genetic contribution (Marioni et al., 2018). In *Drosophila*, all the genes of the phosphoinositol pathway are present except for the I(1,3,4)P3 5/6 kinase (Seeds et al.,

2004). The INPP5A homologue 5PtaseI is among the *Drosophila* genes with high probability of having a synaptic function (Obregón et al., 2015) and is a robust suppressor of Ataxin-3 toxicity in the eye (Voßfeldt et al., 2012). 5PtaseI is a modifier of toxicity brought up by expression of expanded PolyQ Ataxin-3 present in Spinocerebellar Ataxia 3 (SCA3) patients (Voßfeldt et al., 2012). This was found through a deep RNAi screen in the eye and shown to involve protein turnover/ quality control, nuclear import/export and mRNA transport/editing/ translation.

In *Drosophila*, the INPP5A homologue is a crucial enzyme in the phosphoinositide pathway. We found that 5PtaseI is expressed exclusively in the CNS of embryos. This original finding suggests that INPP5A is a gene involved in CNS development. We can propose that the fly may be an excellent model to study the role of INPP5A/5PtaseI in normal and pathological development of the nervous system.

In clinical practice, as suggested by Szego et al. for ASD (Szego & Ma'n, 2016), it would seem useful to propose to COS patients systematic whole exome/genome sequencing instead of or in addition to microarrays to improve genetic testing, allow SNV detection and consequently end the “diagnostic Odyssey” (Soden et al., 2014). In research, the major challenge of the upcoming years will be the analysis of big data from next generation sequencing (prioritization and interpretation of DNA variations) (Richards et al., 2015) and the experimental validation of putative mutations in animal models. Sharing patients' data among teams around the world will be helpful to unravel the molecular pathophysiology of COS and ASD and their underlying causes, paving the way for an early therapeutic intervention.

4.2.1.7 Figures and appendix

Figure 1: Genealogical tree of the patient's family

The proband is a 17-year-old female with COS (black dot).

Schizophrenia spectrum disorder status: affected (red); unaffected (green); unknown (white)

CNV 10q26.3 status: DUP + = positive; DUP - = negative

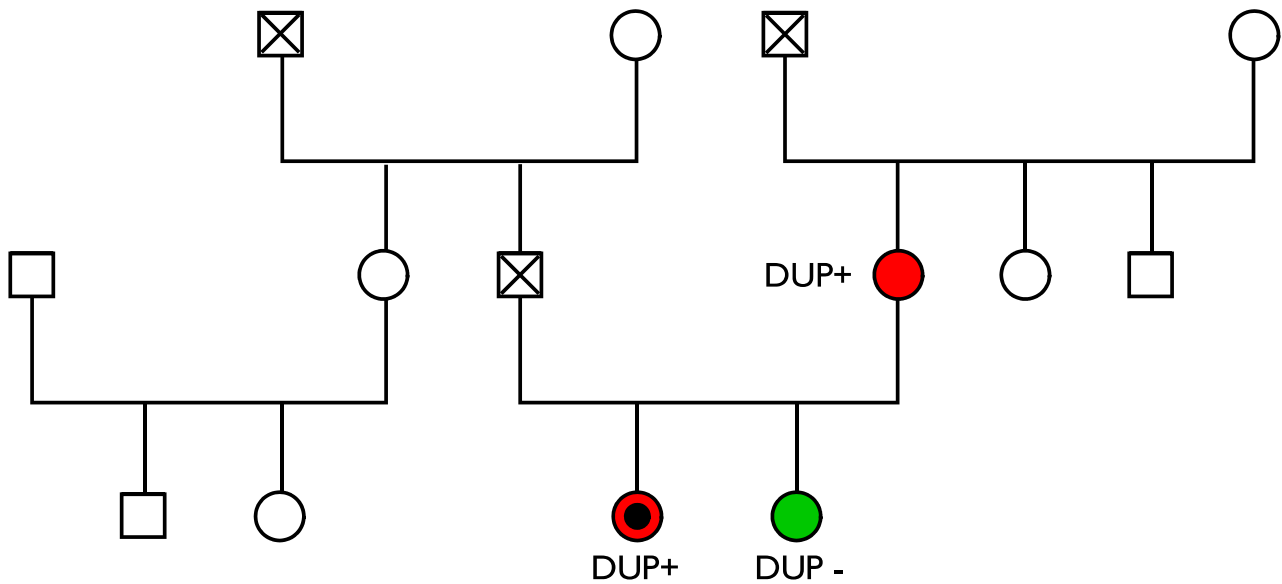


Figure 2: Mapping of a chromosome 10 microduplication «chr10:134,258,887-134,582,804»

(A) Schematic representation of chromosome 10 according to NCBI build 37 (human genome 19).

(B) Known RefSeq Genes in the duplicated chromosomal area.

(C) Summary of coding genes, encoded proteins, corresponding OMIM reference and brain expression (RPKM mean score) included in the microduplication.

OMIM : www.omim.org;

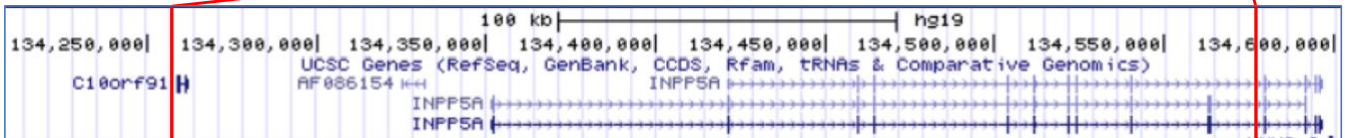
NCBI GENE : www.ncbi.nlm.nih.gov/gene

Figure 2

A



B

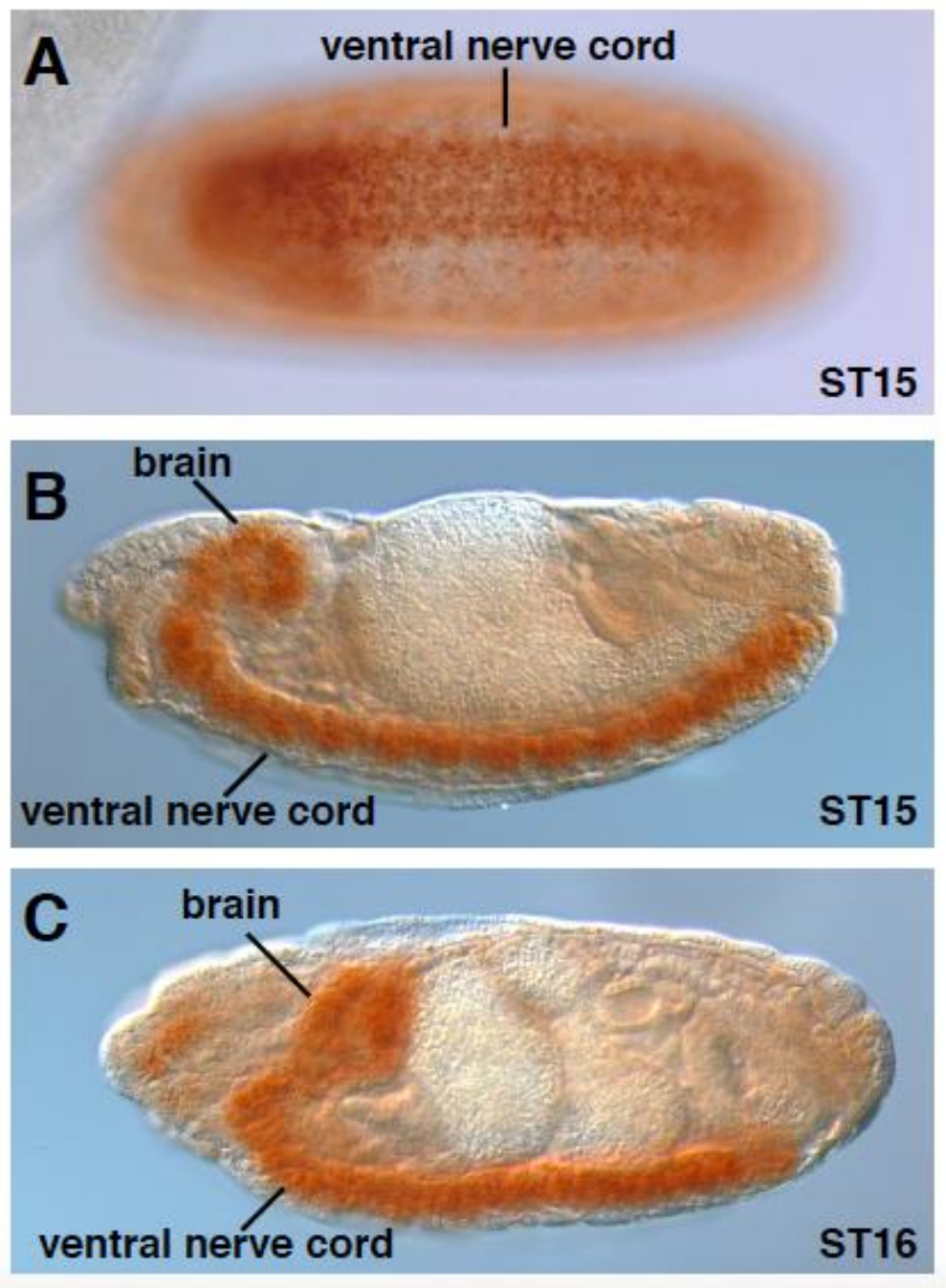


C

CODING GENE	ENCODED PROTEIN	FUNCTION (NCBI - gene)	OMIM	BRAIN EXPRESSION (RPKM)
INPP5A	InsP3 5-phosphatase	mobilizes intracellular calcium and acts as a second messenger mediating cell responses to various stimulation	600106	3.5
AF086154	-	unknown	-	0.045
LINC02870	unknown	unknown	-	0

Appendix 1. *5PtaseI* expression in *Drosophila* embryos. *5PtaseI* expression patterns in embryos detected by *in situ* hybridization with TSA amplification followed by histochemistry.

(A) Ventral view of a stage 15 embryo. Strong expression is observed in the ventral nerve cord. (B) Lateral view of a stage 15 embryo. Strong expression is observed in the brain and in the ventral nerve cord. (C) Lateral view of a stage 16 embryo. Strong expression is observed in the brain and in the ventral nerve cord. In all panels, the anterior end is to the left.



4.2.2 Série de cas : Apport du séquençage de l'ADN (exome)

4.2.2.1 Rationnel

Cette étude présente les résultats du séquençage de la partie codante de l'ADN (exome) pour 9 patients et leurs 2 parents (soit 27 sujets). La difficulté principale a été de disposer des deux parents (Trio) car un nombre significatif des familles que nous avons évalué dans le cadre du protocole GenAuDiss étaient fragiles, précaires, isolées et avec des parents séparés. Les 9 patients dont nous avons pu séquencer l'ADN avaient tous une recherche de l'X-Fragile négative et un Caryotype Haute résolution et/ou un CGH-array sans anomalies. Les résultats ont été soumis à la revue *American Journal of Medical Genetics Part B* (Rang SIGAPS C).

4.2.2.2 Article soumis à *American Journal of Medical Genetics part B*

A pilot study on Early-Onset Schizophrenia reveals the implication of Wnt, cadherin and cholecystokinin receptor signaling in its pathophysiology

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4.2.2.2.1

Abstract

Early-Onset Schizophrenia (EOS) is a very rare mental disorder that is a form of schizophrenia occurring before the age of 18. EOS is a brain disease marked by an early onset of positive and negative symptoms of psychosis that impact development and cognitive functioning. Clinical manifestations commonly include premorbid features of Autism Spectrum Disorder (ASD), attention deficits, Intellectual Deficiency (ID), neurodevelopmental delays, and behavioral disturbance. After the onset of psychotic symptoms, other neuropsychiatric comorbidities are also common, including obsessive-compulsive disorder, major depressive disorder, expressive and receptive language disorders, auditory processing and executive functioning deficits. With the purpose to better gain insight into the genetic bases of this disorder, we developed a pilot project performing whole exome sequencing of nine trios affected by EOS, ASD and mild ID. We carried out gene prioritization by combining multiple bioinformatic tools allowing us to identify the main pathways that could underpin the neurodevelopmental phenotypes of these patients. Indeed, we identified the presence of variants in genes belonging to the Wnt, cadherin and cholecystokinin receptor signaling pathways.

Early -Onset Schizophrenia (EOS) is a very rare form of schizophrenia (SCZ) affecting children before the age of 18 (Grover et al., 2019). Very early-onset schizophrenia (also called childhood-onset schizophrenia) is the rarest form of SCZ (less than 1 out of 10,000 individuals) and occurs before the age of 13 (Grover et al., 2019). According to the Diagnostic and Diagnostic and Statistical Manual of mental disorders 5th edition (DSM-5, 2020), SCZ patients need to exhibit at least one of the following positive symptoms: delusions (firm convictions based on fake information that cannot be changed when confronting reality), hallucinations (perception without a real external stimulus) or disorganized speech (*e.g.*, changing from one subject to another). Negative symptoms include lack of interest in social activities and interactions, reduction in showing feelings, low self-motivation, poverty of the speech content, and anhedonia (DSM-5, 2020). The diagnosis of EOS is based on the same criteria as in patients diagnosed with Adult Onset Schizophrenia (AOS), with a good long-term diagnostic stability (Maziade et al., 1996). Before the introduction of DSM-III, the diagnostic criteria for EOS were not well established (Forsyth et al., 2020). In addition, the occurrence of EOS is usually preceded by a period of normal development. Sometimes, atypical interests and beliefs can be confused with social deficits typical of Autism Spectrum Disorder (ASD) patients. Indeed, around 27% of EOS patients, before manifestation of the first psychotic symptoms, meet criteria for ASD (Driver et al., 2020). Hallucinations and delusions are traits that can help distinguish EOS patients from ASD (DMS-5, 2020). Similar to other neurodevelopmental disorders, EOS is characterized by genetic heterogeneity (Fernandez et al., 2019). Genetic studies of EOS patients suggest that rare Copy Number Variations (CNVs) are more common in EOS than in AOS. Some of these rare CNVs are also associated with ASD and Intellectual Disability (ID) (Addington and Rapoport, 2009;

Fernandez et al., 2019; Fernandez, Drozd, Thümmeler, et al., 2021). EOS is thought to have higher familial transmission and higher prevalence of rare allelic variants than AOS and was associated with common polymorphisms in *Glutamate decarboxylase 1 (GAD1)*, *dysbindin-1 (DTNBP1)*, *Neuregulin 1 (NRG1)*, *G72/G30*, and *Brain- derived neurotrophic factor (BDNF)* (Fernandez et al., 2019). Genome-Wide Association Studies (GWAS) of AOS patients involving more than 16,161 individuals did not indicate significant changes. For this reason and due to the low prevalence of EOS, GWAS on EOS patients have not yet been performed (Forsyth et al., 2020). Additionally, only in one third of genetic studies related to EOS, the full phenotype of patients is described (Fernandez et al., 2019).

Due to the importance to define the genetic bases of EOS, we performed a pilot study carrying out Whole Exome Sequencing (WES) on nine EOS patients and their parents. The main objective is to identify disease-causing mutations primarily in genes involved in neurodevelopmental pathways in these patients. We carried out gene prioritization by the following multiple bioinformatic tools: Residual Variation Intolerance Score (RVIS) (Petrovski et al., 2015), Gene Damage Index (GDI) (Itan et al., 2015), STRING (Franceschini et al., 2013), IntegraGen tools, VarElect, DatabasE of genomiC variation (Stelzer et al., 2016) and Phenotype in Humans using Ensembl Resources (DECIPHER), and Protein ANalysis THrough Evolutionary Relationships (PANTHER) (Mi et al., 2020). This allowed us to identify pathways that could underpin the neurodevelopmental phenotypes of these patients and that could be useful to understand the pathophysiology of these disorders paving the way for future therapeutic interventions.

4.2.2.2.3

Methods

Trio recruitment and clinical evaluations

Patients with EOS were initially recruited through the 2011-2013 Interregional Hospital Clinical Research Program (NCT01512641) whose main goal was to estimate the prevalence of EOS in a population of children in child psychiatric care or medico-educational structures (Dor-Nedonsel et al., 2020). In addition, an enrolment in the GenAuDiss protocol (NCT02565524) study was offered to in- and out-patients, to Child and Adolescent Psychiatry (CAP) centers, as well as to their first-degree relatives (Fernandez et al., 2018; Fernandez, Pasquet-Levy, Laure, et al., 2021). Participants were included either directly at the study sites or after referral by child and adolescent psychiatrists of the Provence-Alpes-Côte d'Azur (PACA) region of south of France. At inclusion (V1), clinical assessments (both psychiatric and neurocognitive) were performed in patients as well as first-degree relatives (parents and siblings). The following quantitative and qualitative measures were assessed during the study: i) Clinical parameters from medical history (including pregnancy and birth) and biographic parameters, types and dates of significant life events including trauma and environmental exposures (*e. g.*, drugs and substances); ii) Clinical parameters from physical examination (*e. g.*, body weight, BMI and arterial pressure); iii) Semistructured interviews to assess main the diagnosis at the time of inclusion (K-SADS-PL, ADI-R and MINI); iv) Clinical heteroassessments with specific neurocognitive rating scales (WISC-V, TMTA, TMTB, and Verbal fluency) and specific psychiatric rating scales (PANSS and SANS); self-report questionnaires (Cloninger TCI 226 to assess personality trouble and Baron Cohen AQ to assess autistic traits). In Table I, all the main phenotypes are recapitulated.

Sample collection

Blood samples of trios (patients and their parents) were collected by the staff of the University Children's Hospital of Nice (France). Samples were anonymized and DNA from blood was extracted using the GenElute Mammalian Genomic DNA Miniprep Kit (SIGMA

Cat. No. G1N70). First, we performed current testing (including DNA testing for Fragile-X Syndrome, high-resolution karyotype and CGH-array). Then, in case of negative results from this step, we performed WES on trios. Isolated DNA samples were sent for WES analysis to IntegraGen SA (Evry, France). Targeted exome sequencing included library preparation, exome capture, sequencing, and final pair-end sequencing on Illumina HiSeq4000 as paired-end 75 bp reads. Base calling was performed according to the IntegraGen software with default parameters. Sequence reads were mapped to the human genome build (hg19/GRCh37). Sequencing data were further subjected to bioinformatic analysis.

Bioinformatic analysis

Gene prioritization was performed by combining multiple bioinformatic tools we describe here. **RVIS** is based on the assumption that genes with common genetic functional variations are more tolerant and the variations occurring in these genes are common (higher RVIS score) and are unlikely to be disease-causing. On the other hand, genes with lower genetic variation (lower score) are considered to be intolerant and have high chances to be disease-causing. The RVIS percentile refers to the percentage of intolerant genes among which the gene of interest is found (Petrovski et al., 2015). **GDI** predicts if a given gene is prone to harbor disease-causing variants. The index evolved from the observation that more than a half of rare variants found in protein coding sequences are located in 2% of the genes (Itan et al., 2015). **STRING** is a protein-protein interaction database that selects data based on experiments (co-crystallization, co-purification and genetic interaction), databases (pathways and protein complexes), textmining (names of proteins that are often mentioned together), co-occurrence of gene patterns across genomes, and coexpression (Franceschini et al., 2013). **IntegraGen tools** such as ERIS and SIRIUS enable WES analysis allowing to follow the family transmission of variants, predict the impact of variants and get access to external databases like 1000Genomes, ExAC and prediction programs like **SIFT** and **PolyPhen**. **VarElect** is a

tool that enables gene prioritization according to the disease and/or phenotype association. It also includes access to tools such as RVIS and GDI (Stelzer et al., 2016). **DECIPHER** is a database that combines multiple tools to facilitate evaluation of the impact of a given variant (Chatzimichali et al., 2015). The **PANTHER** classification system facilitates protein and gene categorization according to family and subfamily classes, molecular function, biological process, and pathways (Mi et al., 2020). **MutationTaster** enables to estimate the pathogenic potential of DNA sequence variants (Schwarz et al., 2014).

Statistical Analyses

The two tails Mann-Whitney Test was performed for all the analyses. Data are presented in the form of violin plots. Statistical analyses were performed using the Prism Software 7 and 8 versions (GraphPad Software, Inc.).

Ethic Statement

The study protocol was approved by the Local Ethics Committee ‘Sud Méditerranée V’ (number 14.002) and authorized by the French National Agency for Medicines and Health Products Safety (ANSM 2013-A01699-36). All patients signed a document authorizing the analyses described in this study. Collection of patients was authorized for a precise period (April 2014 - November 2021) and it was registered on ClinicalTrials.gov (NCT02565524).

4.2.2.2.4

Results

***De novo* variant analysis**

As described in the Material and Methods section, we carried out WES on patients belonging to nine unrelated families whose genealogic trees are described in Figures 1-3.

Previous data suggest that there is a difference between the RVIS and GDI scores of inherited and *de novo* mutations. *De novo* mutations are thought to occur more frequently in genes that are more prone to harbor disease-causing variants and have less common variation (Ambalavanan et al., 2016). We compared the RVIS and GDI scores of inherited and *de novo* mutations, but statistical analysis with the two tails Mann-Whitney Test did not reveal statistical differences between these two groups (Fig. 4A, 4B). Even when we considered only missense *de novo* mutations, we did not obtain the same pattern as in previous studies (Table 1; Fig. 4). Actually, more missense variants occurred in genes that were predicted to have more common variation (Supplementary Table I), which is opposite to what was found by other groups (Ambalavanan et al., 2016).

In order to find a link between trios, we created a Venn Diagram with the *de novo* variants identified in families 1-9 (Supplementary Fig. 1). We focused on exon, exon plus splice intron, missense, nonsense plus splice exon, frameshift, in frame deletion/insertion, and splice acceptor variants. Due to splice variant identification, some changes were localized in the intronic part of the genes. We focused on *de novo* variants in genes that were common for at least three families from our dataset (Supplementary Table 2).

In families 1-5 and 7, we identified many *de novo* changes in *Zinc Finger Protein 717* (*ZNF717*) that is a transcriptional regulator coding the KRuppel-Associated Box (KRAB) zinc-finger protein. According to the DECIPHER database, *ZNF717* was found within the CNV region in five patients suffering from ID and in two patients with ASD. All reference SNPs (rs2918517, rs3009004, rs78135954, rs80214016, rs74357986, rs77110669, rs79138891, rs73843014, rs75737034, rs150497643, rs149076283, rs77378861, and rs776210532) do not have ClinVar reference. Most altered alleles have low frequency or data

are not available. GDI predicts that changes within the *ZNF717* gene have moderate chances to be disease-causing. In 2018, a *de novo* variant in *ZNF717* was reported as a mutation potentially associated with growth retardation and ID (Diao et al., 2018). Variants in *Mucin 6 (MUC6)* were identified in families 1-5. *MUC6* is actually one of the most frequently mutated genes in the general population and are susceptible to false positive results in next generation sequencing <http://massgenomics.org/2013/06/ngs-false-positives.html> (Itan et al., 2015). However, evidence of late-onset Alzheimer disease (LOAD)-associated genetic polymorphism within an exon of Mucin 6 (*MUC6*) and immediately downstream from the Adaptor Related Protein Complex 2 Subunit Alpha 2 (*AP2A2*) have been reported (Katsumata et al., 2020).

Variants of the *FSHD Region Gene 1 (FRG1)* gene were found in Families 1-3. *FRG1* (located in 4q35) codes a splicing factor and was implicated in FascioScapuloHumeral muscular Dystrophy (FSHD) as a modifier gene (De Simone et al., 2017, Jagannathan et al., 2019). 4p/4q CNVs of variable sizes have been associated with several different psychiatric findings and developmental disability. It was shown that the prevalence of independent deletions at 4p/4q involves *PIGG*, *TRIML2* and *FRG1*. This suggests a possible implication of *FRG1* in neurodevelopmental disorders (Novo-Filho et al., 2016). This gene is also susceptible of false positive results <http://massgenomics.org/2013/06/ngs-false-positives.html>

Another variant shared by families 1-3 is *Poly(A) Binding Protein Cytoplasmic 1 (PABPC1)*. This gene was shown to be upregulated in male patients affected by SCZ (Qin et al., 2016). According to GDI prediction, *PABPC1* has moderate chances to harbor disease-causing mutations. On the other hand, RVIS predicts that this gene has less common genetic variation.

Variants in *Aggrecan (ACAN)* were found in families 2, 3 and 4. The variant found in family 4 (rs12899191) turned out to have a high frequency in the general population

[G=0.27107 (3538/13052, GnomAD)] and is not reported in the ClinVar database. DECIPHER analysis shows that this gene is extremely intolerant to loss-of-function variation (Burd and Kerbeshian, 1987, gocham et al., 2011). *ACAN* was shown to be downregulated in the brain cortex region of SCZ individuals (Pietersen et al., 2014). Both RVIS and GDI predict this gene as highly mutated which means that there is little probability to be a disease-causing gene. A same variant in *RNA-Binding Motif Protein, X-Linked-Like-3 (RBMXL3)*, an RNA binding protein with elevated recurrent mutation rate in neuroblastoma (Lee et al., 2020), was found in families 1, 3 and 4. The DECIPHER database provides reports of two patients with abnormalities in the nervous system who carry variants in the *RBMXL3* gene. MutationTaster shows that deletions in this gene might be disease-causing. GDI places this gene among ones that have moderate chances to harbor disease-causing mutations. The RVIS database does not contain information about this gene. In order to group the genes harboring *de novo* variants according to their association with certain diseases or phenotypes, we carried out analysis with the VarElect tool. As a query, we used the following phenotypes: SCZ, ASD, ID, and neurodevelopmental disorders. We concentrated on the first 10 best scored genes (Table 2).

A missense mutation in the *Potassium Voltage-Gated Channel Subfamily B Member 1 (KCNB1)* gene was found in a patient of family 6. This mutation is predicted by the IntegraGen tool to have a moderate impact. SIFT and PolyPhen predict this change as deleterious (score 0) and probably damaging (score 0,999), respectively. MutationTaster indicates this change as disease-causing. *KCNB1* is mainly linked to Early Infantile Epileptic Encephalopathy (de Kovel et al., 2017). The proband was, apart from EOS, diagnosed with idiopathic epilepsy, thus, this mutation identified in *KCNB1* may be associated with the epileptic phenotype of the patient.

The second gene predicted by VarElect is *Major Histocompatibility Complex, Class II, DR Beta 1 (HLA-DRB1)*. A missense variant (rs201929247) in this gene was identified in family 4. MutationTaster predicts this variant to be of polymorphic nature. Moreover, this variant turns out to be very frequent [A=0.147043 (14841/100930, ExAC)] and is not reported in ClinVar. According to GDI and RVIS predictions, variations in this gene are very common. For these reasons, its impact on the phenotype is very dubious.

A variant for the third predicted gene *Bromodomain PHD Finger Transcription Factor (BPTF)* was identified in family 8. *BPTF* is associated with a neurodevelopmental disorder characterized by ID, dysmorphic facies and distal limb anomalies (Midro et al., 2019). The IntegraGen tool predicts the impact of this change (in-frame insertion) as moderate. GDI analysis suggests that *BPTF* is among moderately-damaging genes. RVIS predicts this gene as a gene of less common variation.

A missense variant identified in *HECT And RLD Domain Containing E3 Ubiquitin Protein Ligase 2 (HERC2)* was found in family 1. Mutations in *HERC2* were associated with developmental delay with Angelman-like features (including strong ID) (Harlalka et al., 2013). This variant (rs146883683) is known to have high frequency [G=0.11394 (9547/83792, ExAC)] and is not reported in the ClinVar database. Moreover, MutationTaster predicts this variant as polymorphism.

A missense variant in *Cystathionine Beta-Synthase (CBS)* was identified in family 3. According to ClinVar, the variant rs5742905 is associated with homocystinuria. Interestingly, this disorder is frequently accompanied by ID and *CBS* is one of the genes associated with Down Syndrome (Pogribna et al., 2001, Marechal et al., 2019). MutationTaster predicts this change as disease-causing. GDI analysis suggests that *CBS* is among moderately damaging genes and RVIS predicts this gene as a gene of less common variation.

An insertion in *Neurogenic Locus Notch Homolog Protein 4 (NOTCH4)* was identified in family 4. MutationTaster predicts this variant as a polymorphism. Interestingly, polymorphic variants in this gene were previously associated with SCZ and schizoaffective disorder (Ujike et al., 2001, Zhang et al., 2015). Both GDI and RVIS predictions indicate that variants in this gene are not prone to be disease-causing.

A missense variant in *Folate Hydrolase 1 (FOLH1)* was found in family 3. This variant (rs75111588) is frequent in the general population [A=0.187153 (20786/111064, ExAC)]. Hence, it is unlikely that this variant is responsible for the phenotype of the patient.

A missense variant in *Filaggrin (FLG)* was identified in family 5. It turned out to be frequent in the general population [G=0.08408 (1087/12928, GO-ESP; G=0.117 (70/600, NorthernSweden)], which suggests that this variant does not impact the phenotype. FLG mutations were associated with atopic dermatitis increased ischemic stroke risk in the general population (Varbo et al., 2017).

An in-frame deletion in *Achaete-Scute Family BHLH Transcription Factor 1 (ASCLI)* was identified in family 6. The IntegraGen tool predicts this change to have a moderate impact. GDI places this gene among the genes with moderate chances to harbor disease-causing variants. *ASCLI* promotes neural differentiation (Castro et al., 2011) and its level is more elevated during neural differentiation in murin *Fmr1*-null embryonal stem cells compared with WT cell line (Khalfallah et al., 2017). According to the genomAD browser this variant is quite frequent (delGCA=0.01150; 340/29556). MutationTaster predicts this change as potential polymorphism.

In family 6, we also identified a frameshift variant in *Zinc Finger Protein 148 (ZNF148)*. IntegraGen predictions indicate high impact of this variant. Mutations in *ZNF148* were associated with Global Developmental delay, Absent or hypoplastic Corpus Callosum and dysmorphic Facies (GDACCF). Apart from this disorder, the phosphorylation level of

ZNF148 was shown to be decreased in SCZ patients, but its overall level was unchanged (Jaros et al., 2012).

In general, the VarElect analysis has provided a further insight into genes that are already implicated in the pathogenesis of neurological disorders. We could find interesting variants in genes such as *ZNF148* and *KCNB1* that could impact the phenotype of the proband of family 6. We were able to exclude some variants of polymorphic nature that turned out to be common in the general population and that were not associated with any neurological disorder. We came across polymorphisms that could increase the risk of SCZ (*NOTCH4*). The VarElect analysis enables to extract variants that sometimes can be overlooked in general analyses (e.g., some polymorphisms that are frequently excluded from candidate genes).

Inherited variant analysis

We created a Venn Diagram for inherited variants to find genes that are common among at least three families (Supplementary Figure 3). We found shared inherited variants in the following genes: *ZNF717*, *FAM182B*, *LRP5L*, *HRNR*, *OR4C5*, *APOL3*, *BCLAF1*, *FLG*, *RP1L1*, *MUC6*, *HYDIN*, *PLEC*, *TTN*, *NRAP*, *ADAMTS8*, *SSPO*, *FAM104B*, *DAAM2*, *ZFH3*, and *AHNAK2*. According to GDI and RVIS predictions, most of these genes are less prone to harbor disease-causing mutations than the genes cited above (*de novo* variants) and belong to genes that have more variability.

We focused on genes that did not have RVIS references. In families 1 to 7, we found 17 missense variants, two frame-shifts and one insertion in *ZNF717*. This gene was previously found during *de novo* mutation analysis. Considering the high amount of variants found and the fact that the majority of them have references in SNPdb, variants in this gene may be very common and not prone to be disease-causing.

In families 3 and 4, we found variants in *Family With Sequence Similarity 182 Member B* (*FAM182B*). In family 4, we found a variant in the 5'-UTR and a missense variant.

In family 3, we found a variant in the 5'-UTR and one synonymous variant. This gene is predicted by GDI to have medium chances to harbor disease-causing mutations.

In families 1, 2 and 9, we identified variants in *Hornerin* (*HRNR*). All of them have references in SNPdb (rs79513582, rs76694305, rs61814943, and rs61814936) and have high frequencies in the general population.

We found some variants in *Olfactory Receptor Family 4 Subfamily C Member 5* (*OR4C5*): i) the same deletion (rs66829866) in families 1 and 3 that was reported in SNPdb and that is known to have a high frequency; ii) an insertion in family 1 (rs114053360) that has low frequency and no report in ClinVar; iii) an insertion in family 4. *OR4C5* is a gene predicted by GDI to be highly damaging.

We identified three variants in *Plectin* (*PLEC*). A missense variant in family 2 is predicted by PolyPhen2 to be benign (score 2). In family 4, we found a synonymous variant (rs113133985) described in SNPdb with low frequency. A missense variant in family 5 also turned out to have low frequency and no records in the ClinVar database. GDI was predicted to be high, whereas RVIS was very low.

Overall, all inherited variants that we found seem to have benign consequences.

Interaction and pathway analysis

We carried out interaction analysis with the STRING database. Due to the fact, that some mothers from our cohort have some mild symptoms associated with neurodevelopmental disorders, we decided to focus on the genes inherited from the mothers of the probands. We took into consideration all inherited genes regardless of their potential impact of the carrying variants. The analysis of the mutated genes that were inherited from the mother revealed that “the network among the genes subjected to the analysis had significantly more interactions than expected”. This means that these proteins have more interactions among themselves than what would be expected for a random set of proteins of similar size

drawn from the genome. Such an enrichment indicates that the proteins are at least partially biologically connected. Molecular functional enrichment in this network includes transferase activity [False Discovery Rate (FDR) = 0.00019], catalytic activity (acting on protein, FDR = 0.0011), adenylyl ribonucleotide binding (FDR = 0.0011), ATP-binding (FDR = 0.0011), and ubiquitin protein ligase activity (FDR = 0.0014). For instance, among the genes in the enriched pathways we came across POLA1, PARP1 and CDK5 (Full gene list in Supplementary Table). Interestingly, POLA1 was recently associated with X-Linked Intellectual Disability Associated with Severe Growth Retardation, Microcephaly, and Hypogonadism (Van Esch et al., 2019). Elevated levels of PARP1 have been associated with Down Syndrome which is characterized by intellectual disability (Salemi et al., 2013) and it was involved in the context of dyslexia and neuronal migration (Tapia-Paez et al., 2008). Dysregulations of the CDK5 activator are associated with SCZ (Engmann et al., 2011). Considering PFAM protein domains, enrichment was observed in protein kinase (FDR = 0.0081), protein tyrosine kinase (FDR = 0.0089), microtubule-binding (FDR = 0.0439), and kinesin motor domains (FDR = 0.0439).

We performed pathway analysis with PANTHER for altered genes that were inherited from the mother. We looked for genes that belong to the same pathways. We assigned each gene to the corresponding pathway according to PANTHER classification. Subsequently, a Venn diagram was created in order to see if the pathways co-occur in our cohort of families (Supplementary fig. 2). Functional classification analysis showed that all nine families share variants in genes belonging to the WNT and Cadherin pathways (Supplementary figure 3). In addition, families 1 to 8 share additionally the cholecystokinin receptor (CCKR) signaling pathway. Cholecystokinin activates the CCK1 receptor that belongs to G-protein-coupled receptors. Variants associated with these pathways by PANTHER comprise, among others:

CDH11, CDH3, CDH18, PCDH15, PCDHA4, FAT4, FAT2, NFATC2, WNT6, WNT11, and CTNNA3.

4.2.2.2.5

Discussion

In this pilot study, we carried out a WES analysis on a small group of patients and their parents with the final purpose to define the possibility that deregulated molecular pathways could affect the severe phenotype of the probands. Even if we cannot exclude the hypothesis that minor changes in these genes could contribute to the phenotypes of patients if they are co-occurring, the analysis of common genes for all the families' studies did not reveal any strong candidate gene. We reasoned that it could be interesting to analyze the interaction among deregulated pathways since genes associated with these signaling pathways may be of importance in the context the phenotype of the patients. This analysis does not aim at finding any particular disease-causing gene, but at the identification of processes and networks that could be crucial in the development of the SCZ/ASD/ID pathophysiology. Our analysis defined the following pathways as deregulated by the presence of variants in disorder-candidate genes that were never associated before to EOS: WNT, cadherin signaling and CCK₁ receptor signaling. The WNT pathway participates in many processes such as regulation of gene transcription, apoptosis, proliferation, cell migration, and cytoskeletal dynamics. It plays an important role in Central Nervous System (CNS) development, in particular, in the differentiation of neural progenitors, synaptogenesis, neuronal migration, and plasticity (Mulligan and Cheyette, 2017). Aberrations in WNT signaling are related to the pathogenesis of diverse neurodevelopmental diseases such as SCZ, ASD and BD (Mulligan and Cheyette, 2017). Indeed, genes such as the *APC Regulator of WNT Signaling Pathway* (*APC*), *chromodomain helicase DNA-binding protein 8* (*CHD8*), *Disrupted in schizophrenia*

1 (DISC1), Phosphatase And Tensin Homolog (PTEN), WNT Family Member 1 (WNT1), WNT2, WNT3, WNT7A, β -catenin (CTNNB1), Transcription Factor 4 (TCF4), and TCF7 have been linked to ASD. Wnt/ β -catenin pathway loci were identified in the genomic analysis of SCZ patients and some of them were suggested to be a risk factor for this disorder. Among them: *CHD8, CTNNB1, DISC1, TCF4, Dickkopf WNT Signaling Pathway Inhibitor 1 (DKK1), and DKK4* (Mulligan and Cheyette, 2017). It was shown that the mRNA expression levels of the WNT signaling members *Frizzled Class Receptor 7 (FZD7)* and *Nuclear Factor Of Activated T Cells 3 (NFATC3)* are increased in SCZ patient (Hoseth et al., 2018a; Hoseth et al., 2018b). Moreover, the plasma levels of Dickkopf-1 and Sclerostin are lower in SCZ patients (Hoseth et al., 2018a, Hoseth et al., 2018b). One of the best targets of Fragile X mental retardation Protein (FMRP) – whose absence causes the Fragile X Syndrome – is the mRNA of *Apc* (Maurin et al., 2018, Sawicka et al., 2019). It is interesting to underline that that some antipsychotic drugs used to treat patients affected by various forms of neurodevelopmental disorders (*e.g.*, haloperidol, clozapine, fluoxetine, and Ritalin) are WNT pathway modulators (Bae and Hong, 2018). Furthermore, lithium is known to be used in the treatment of ASD (Mintz and Hollenberg, 2019) and BD (Berk et al., 2017) and was proposed at the preclinical level for FXS, since several phenotypes of adult *Fmr1*-KO mice are rescued by lithium treatment (Liu and Smith, 2014). Lithium is known to act as an activator of the WNT pathway, suggesting that down-regulation of WNT signaling contributes to the pathophysiology of FXS and other forms of ASD (Liu and Smith, 2014).

The Cadherin signaling pathway, involving many cell adhesion molecules, plays a role in CNS development and is associated with WNT. Indeed, cadherins negatively regulate WNT signaling by sequestering β -catenin and play an important role in the regulation of β -catenin-dependent transcription (Howard et al., 2011). Cadherins participate in neural circuit formation, synapse development, differentiation of grey matter, neuronal migration, and spine

morphology. Cadherins that are present in the brain display spatio-temporal specific expression (Redies et al., 2012). GWAS studies revealed many genes involved in this pathway involved in neuropsychiatric diseases such as ASD, SCZ, epilepsy, bipolar disorder, and ID. Cadherins have been associated to brain disorders including ID, ASD and SCZ (Taylor et al., 2020; Ballaz, 2017).

The CCK₁ receptor is responsible for regulation of serotonin neuron activity and maintenance of proper dopamine levels in raphe nuclei (clusters of cell groups localized in the brainstem). It also influences hormone and neurotransmitter regulation in the hypothalamus. Due to its functions, CCK was suggested to be a target in the treatment of SCZ, mood disorders and drug addiction (Wang et al., 1985). Especially, the relation between CCK and dopaminergic circuits was proposed to play a role in the development of SCZ. The concentration of CCK was shown to be lowered in some brain regions of SCZ untreated patients, leading to the conclusion that CCK may display antipsychotic effects in sub-populations of SCZ patients. Lastly, antipsychotic drug treatment can boost CCK and CCKR levels (Curley and Lewis, 2012).

All the pathways we identified are known to be involved in ID and ASD as well as in SCZ, reinforcing the idea that a link exists among these disorders (Curley and Lewis, 2012; Zhang et al., 2014; Kwan et al., 2016; Hoseth et al., 2018a; Hoseth et al., 2018b) . To date, these pathways have not been described in the context of EOS. Nevertheless, the presence of numerous variants in genes belonging to these pathways suggests common genetic background among EOS and other neurological disorders such as SCZ, ASD and ID. The link between these disorders is not very surprising since epidemiological studies show co-occurrence of several neurological and psychiatric features in most neurodevelopmental disorders. This phenotypic overlap is also mirrored at the genetic and molecular levels and

should be viewed as a continuum of developmental brain dysfunction(s). In addition, if EOS and SCZ are caused by the same altered pathways we can speculate that the early onset may be related to environmental causes or it is caused by the presence of additional mutations in one or more genes that ”*per se*” is/are not causing a disease but worsen a pathology caused by other factors. Indeed, we have listed all the variants identified in the patients even if some of them should be considered with caution due to their high frequency of mutation.

For all these reasons, the accuracy of diagnoses seems to be a key point to be able to define the precise and complete phenotype of each patient and also, in the case of SCZ, the exact age of on-set of the disorder. In the future, this will allow comparison between the genetic and molecular bases of the various forms of SCZ depending on the age of onset.

It will be crucial to move now to larger cohortes of patients. However, we think that this pilot study is useful because it suggests that for these complex and severe phenotypes it will be useful to focus on familial analyses enlarging both phenotypic and genetic studies to other members of the family (even displaying only very mild phenotypes) with the final purpose to identify altered pathways amenable to therapeutic approaches.

Table 1. Prenatal, perinatal and neurodevelopmental phenotypes.

	Family 1	Family 2	Family 3	Family 4	Family 5	Family 6	Family 7	Family 8	Family 9
Pregnancy complications									
Maternal disorders	no	no	no	no	no	no	no	in vitro fertilization and embryo transfer	no
Drugs	no	no	no	no	no	no	no	no	no
Maternal smoking during pregnancy	no	yes	yes	no	yes	no	no	no	no
Birth characteristics									
Maternal age at birth of child (years)	21	32	32	33	26	29	34	33	34
Paternal age at birth of child (years)	24	35	40	32	24	51	32	34	40
Birth weight (grams)	3000	3020	1570	3500	2950	normal	3180	3280	normal
Multiple birth	no	no	no	no	no	no	no	twin	no
Method of delivery	vaginal birth	vaginal birth	vaginal birth	vaginal birth	vaginal birth assisted by vacuum extraction	vaginal birth	vaginal birth	vaginal birth	vaginal birth
Apgar score	10 10 10	10 10 10	10 10 10	10 10 10	09 10 10	10 10 10	10 10 10	08 10 10	10 10 10
Gastational age (weeks)	33	39	35	41	41	normal	40	39	normal
Perinatal conditions									
Infections	no	no	no	no	no	no	neonatal bronchiolitis	no	no
Digestive, endocrine or metabolic disorders	no	no	no	no	neonatal jaundice	no	no	no	no
Other perinatal conditions	no	no	no	no	maternal depression	no	no	single nuchal cord	no
Congenital malformations									
	no	no	no	no	no	no	no	no	no
Neurodevelopmental impairments									
Intellectual ability (IQ)	96	73	53	105	47	40	69	63	71
Communication	no	no	yes	no	yes	yes	yes	yes	yes
Motor	no	no	no	no	no	no	no	yes	yes
Learning	no	yes	yes	yes	yes	yes	yes	yes	yes
ADHD	no	yes	no	no	yes	yes	yes	yes	yes
ASD	yes	no	yes	no	yes	no	yes	yes	yes
Consanguinity									
	no	no	yes	no	no	no	no	no	no

Table 2. The first 10 best scored genes according to VarElect predictor. SCZ: Schizophrenia; ASD: Autism Spectrum Disorder; ID: Intellectual disability

Symbol	Gene name	Matched Phenotypes	Matched Phenotypes	LOG10(P)	Score	Average Disease Causing Likelihood
KCNB1	Potassium Voltage-Gated Channel Subfamily B Member 1	SCZ, ASD, ID	5	2,27	69,28	62,07%
HLA-DRB1	Major Histocompatibility Complex, Class II, DR Beta 1	SCZ, ASD, ID,	5	2,25	68,54	0,99%
BPTF	Bromodomain PHD Finger Transcription Factor	ASD, ID,	4	2,27	62,32	67,63%
HERC2	HECT And RLD Domain Containing E3 Ubiquitin Protein Ligase 2	SCZ, ASD, ID	5	2,1	59,22	56,87%
CBS	Cystathionine BetaSynthase	SCZ, ASD, ID	5	1,52	34,34	54,73%
NOTCH4	Notch Receptor 4	SCZ, ASD, ID	5	1,43	30,96	20,47%
FOLH1	Folate Hydrolase 1	SCZ, ASD, ID	4	1,19	19,64	30,23%
FLG	Filaggrin	ASD, ID	4	1,11	16,91	0,09%
ASCL1	Achaete-Scute Family BHLH Transcription Factor 1	SCZ, ASD, ID,	4	1,09	16,51	69,21%
ZNF148	Zinc Finger Protein 148	ID	3	1,16	16,04	92,24%

Figure 1. Schema of the genealogical trees of Family 1, Family 2 and Family 3. The proband of each family is indicated with a black dot.

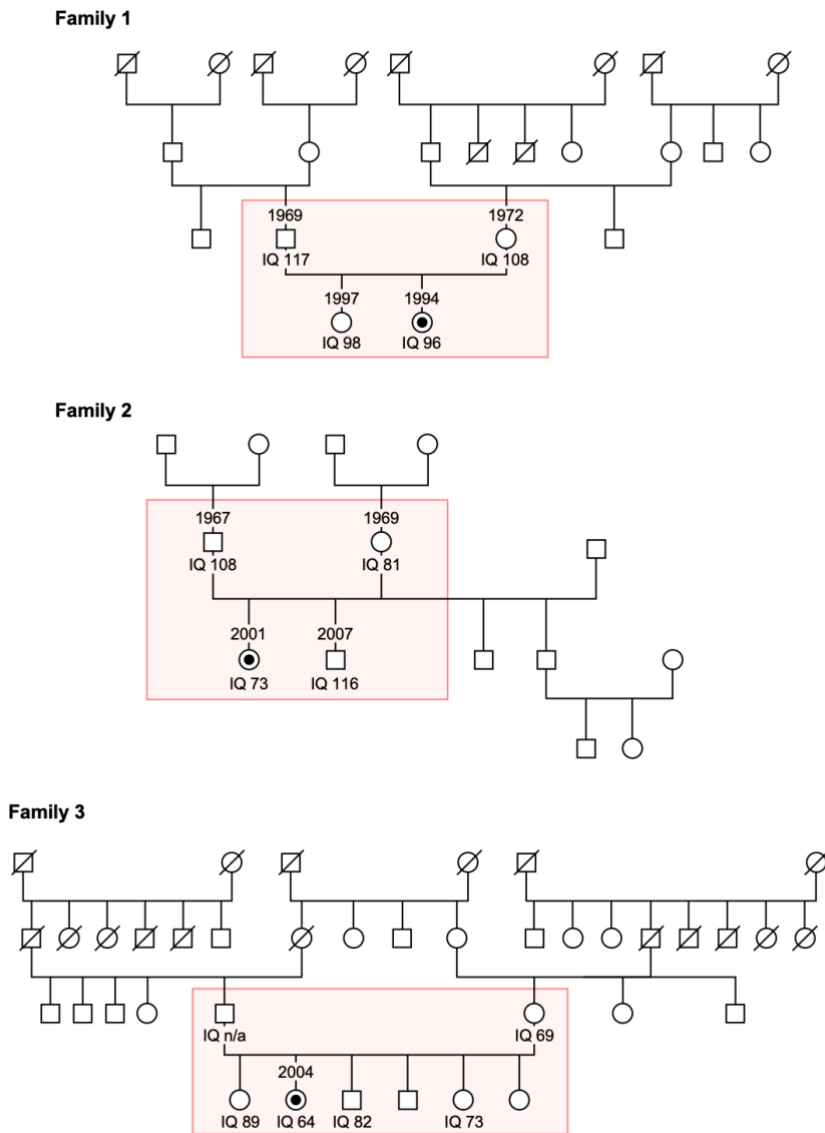
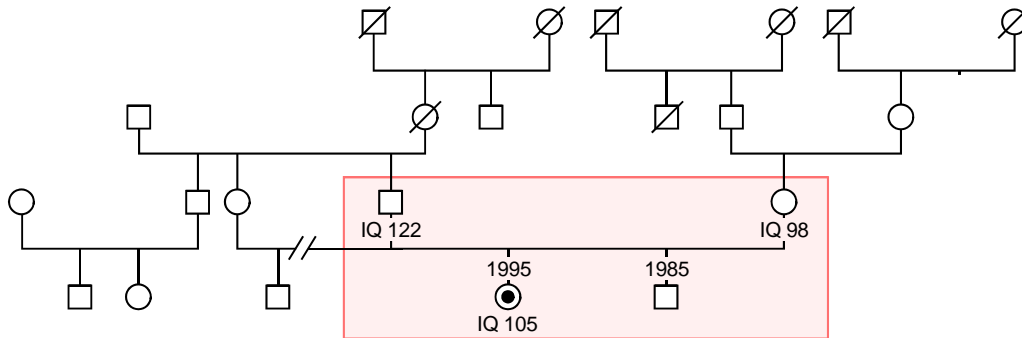
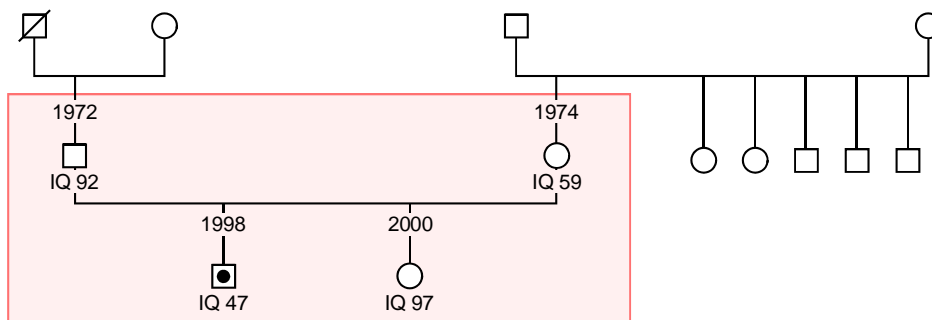


Figure 2. Schema of the genealogical trees of Family 4, Family 5 and Family 6. The proband of each family is indicated with a black dot.

Family 4



Family 5



Family 6

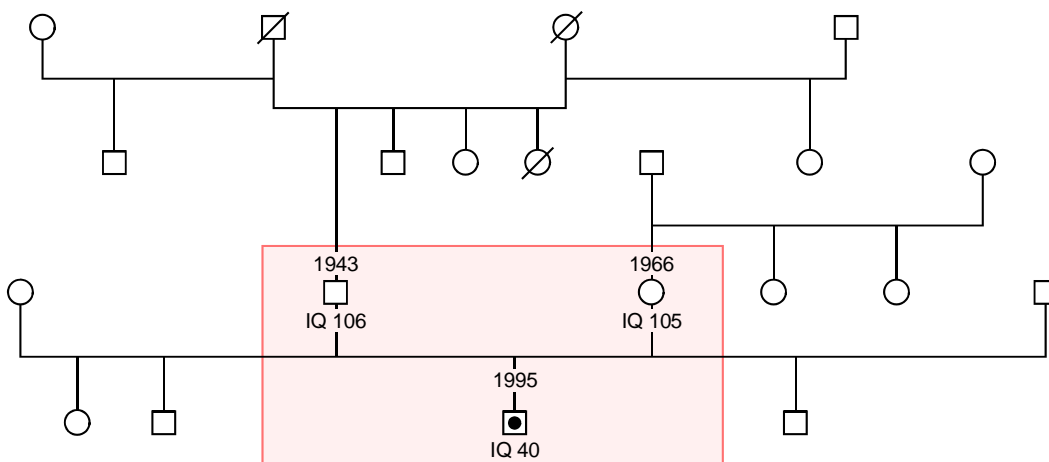


Figure 3. Schema of the genealogical trees of Family 7, Family 8 and Family 9. The proband of each family is indicated with a black dot.

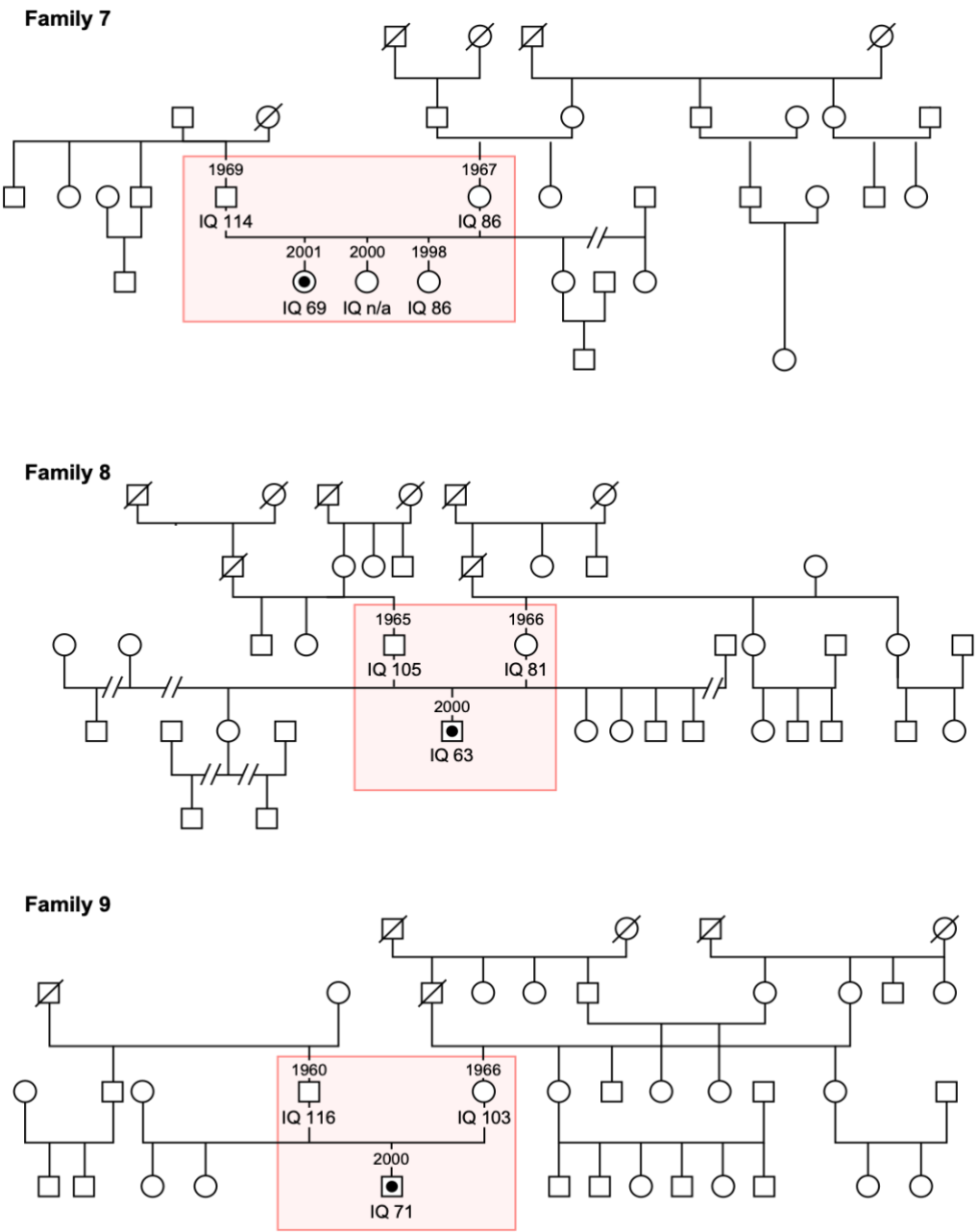
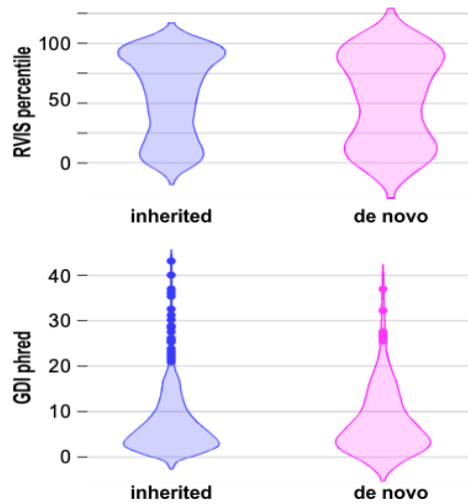
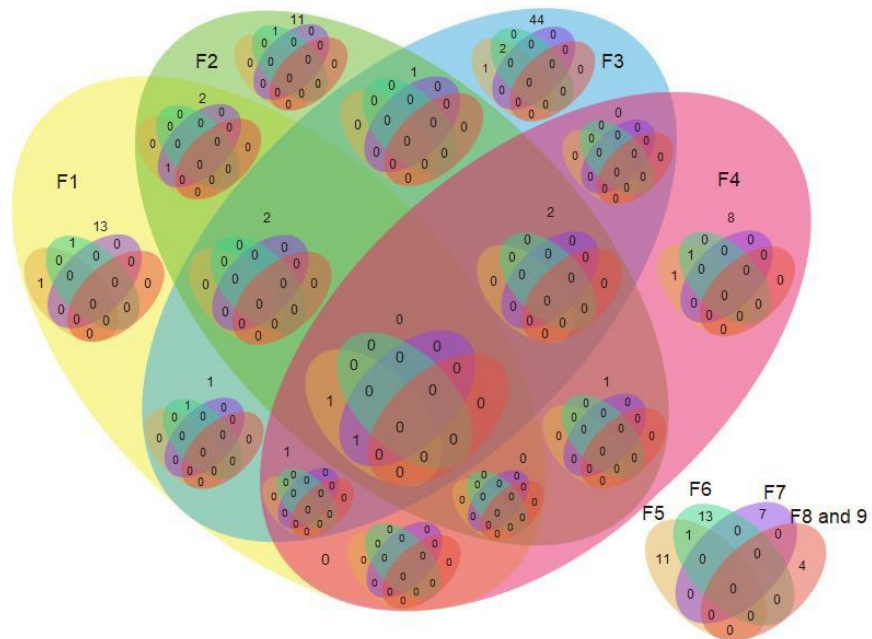


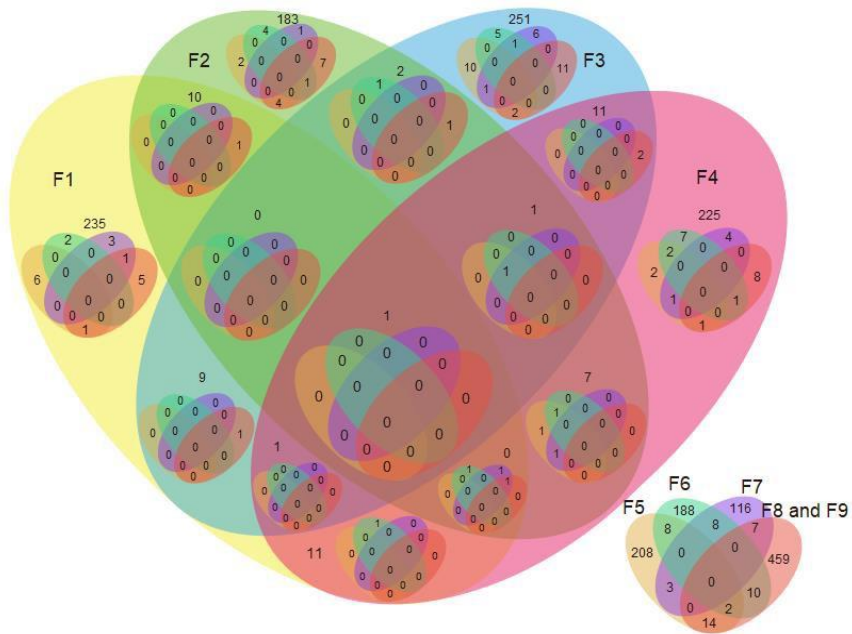
Figure 4. Violin plots for RVIS and GDI. Upper panel. Violin plots showing the RVIS percentile. Lower percentile indicates more “intolerant” variants. Statistical analysis with the two-tails Mann Whitney test did not reveal statistical differences between inherited and *de novo* mutations. Lower Panel. Violin plot showing the GDI phred. Lower GDI values indicate genes more prone to harbour disease-causing mutations. Statistical analysis with the two-tails Mann Whitney test did not reveal statistical differences between inherited and *de novo* mutations.



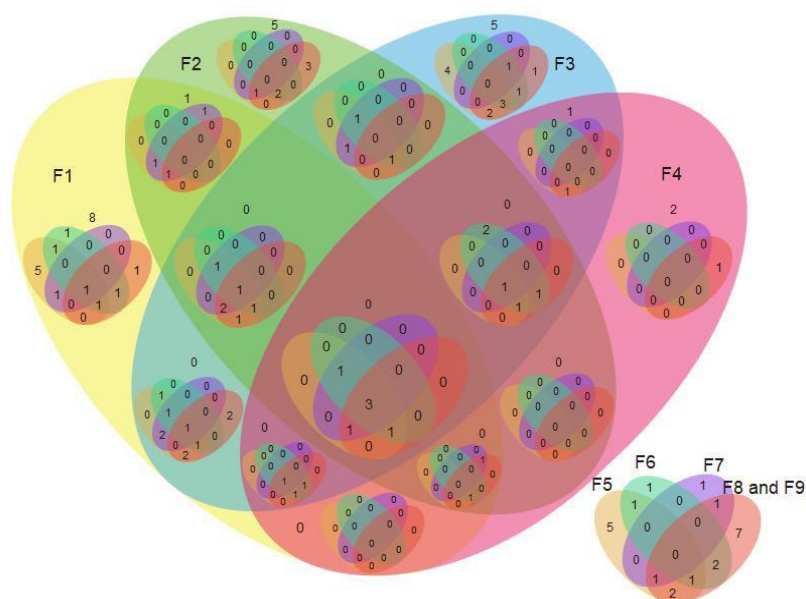
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Table I. All de novo missense variants.

Chr	Position	Gene	Ref seq	Protein variation	Variation type	rs
chr1	>89449483"	RBMXL1	NM_019610	K9N	missense	rs74100106
chr1	>117146504"	IGSF3	NM_001007237	R456C	missense	rs61786577
chr2	>96617102"	ENSG00000174501	ENST00000528268	E420K	missense	
chr3	>75786942"	ZNF717	NM_001290209	R561I	missense	rs2918517
chr3	>75788158"	ZNF717	NM_001290209	L156V	missense	rs3009004
chr7	>100551035"	MUC3A	NM_005960	S539T	missense	rs62483695
chr8	>101721839"	PABPC1	NM_002568	V365L	missense	
chr11	>1017498"	MUC6	NM_005961	S1768T	missense	rs67507057
chr11	>1017746"	MUC6	NM_005961	L1685F	missense	rs78848170
chr15	>28447562"	HERC2	NM_004667	S2471P	missense	
chr16	>24788455"	TNRC6A	NM_014494	P122Q	missense	
chr17	>39346622"	KRTAP9-1	NM_001190460	C162S	missense	rs79470847
chr19	>56283297"	RFPL4AL1	NM_001277397	D43N	missense	rs75204738
chr1	>117660667"	TRIM45	NM_025188	L404P	missense	
chr1	>145281656"	ENSG00000213240	NM_203458	T196S	missense	rs4649852
chr3	>75786687"	ZNF717	NM_001290208	L696H	missense	rs80214016
chr3	>75787199"	ZNF717	NM_001290209	H475Q	missense	
chr6	>30996959"	MUC22	NM_001198815	E1251K	missense	rs115182197
chr6	>32714125"	HLA-DQA2	NM_020056	Q241R	missense	
chr7	>72413581"	POM121	NM_172020	V752I	missense	rs71554687

chr7	>72413593"	POM121	NM_001257190	Y756H	missense	rs71554688
chr7	>100550795"	MUC3A	NM_005960	T459I	missense	
chr9	>33385656"	AQP7	NM_001170	Q245L	missense	
chr11	>1018419"	MUC6	NM_005961	T1461I	missense	
chr11	>48387237"	ENSG00000176540	ENST00000319813	V261I	missense	rs72898877
chr15	>89399701"	ACAN	NM_001135	D1295E	missense	
chr2	>96604606"	ENSG00000174501	ENST00000456556	G535V	missense	rs80350011
chr3	>75786211"	ENSG00000227124	NM_001290209	S805P	missense	rs77715040
chr3	>75786759"	ENSG00000227124	NM_001290208	K672R	missense	rs75759940
chr3	>75788068"	ZNF717	NM_001128223	F236V	missense	rs74357986
chr3	>75788199"	ENSG00000227124	NM_001128223	H192R	missense	rs77110669
chr5	>156479571"	HAVCR1	NM_012206	M158I	missense	rs75340804
chr7	>72081774"	ENSG00000254184	ENST00000435769	C557R	missense	rs3015854
chr7	>72413581"	POM121	NM_172020	V752I	missense	rs71554687
chr7	>72413593"	POM121	NM_001257190	Y756H	missense	rs71554688
chr7	>100677279"	MUC17	NM_001040105	S861T	missense	rs76184171
chr7	>100677285"	MUC17	NM_001040105	G863E	missense	rs74852422
chr7	>100678481"	MUC17	NM_001040105	T1262S	missense	rs73168389
chr7	>100678560"	MUC17	NM_001040105	T1288K	missense	rs77199586
chr7	>100678568"	MUC17	NM_001040105	T1291S	missense	rs75492258
chr7	>100682624"	MUC17	NM_001040105	M2643L	missense	
chr11	>1017084"	MUC6	NM_005961	P1906L	missense	rs34649796
chr11	>49208267"	FOLH1	NM_001014986	R190W	missense	rs75111588
chr12	>11183722"	TAS2R31	NM_176885	F71L	missense	rs78562467
chr14	>60074085"	RTN1	NM_206852	A63T	missense	
chr15	>89399758"	ACAN	NM_013227	D1314E	missense	
chr17	>39274364"	KRTAP4-11	NM_033059	R68S	missense	rs425784
chr21	>11049596"	BAGE2	NM_182482	C102Y	missense	rs28571918
chr21	>44483184"	CBS	NM_001178008	I278T	missense	rs5742905
chr1	>17263214"	CROCC	NM_014675	R347W	missense	
chr3	>75786252"	ZNF717	NM_001290208	P841H	missense	rs79138891
chr3	>75787405"	ZNF717	NM_001290209	G407R	missense	rs73843014
chr3	>75787620"	ENSG00000227124	NM_001290208	H385R	missense	rs75737034
chr5	>40853031"	CARD6	NM_032587	R533C	missense	
chr6	>32549588"	HLA-DRB1	NM_002124	S133L	missense	rs117994013
chr6	>32714164"	ENSG00000237541	NM_020056	L254P	missense	rs115121776
chr11	>1017498"	MUC6	NM_005961	S1768T	missense	rs67507057
chr15	>89400023"	ACAN	NM_013227	T1403A	missense	rs12899191
chr16	>70954691"	HYDIN	NM_001270974	K2530E	missense	rs1798528
chr19	>44891043"	ZNF285	NM_001291491	P300Q	missense	rs77661661
chr1	>152278856"	FLG	NM_002016.1	S2836R	missense	rs11582087
chr10	>47087078"	NPY4R	NM_005972.5	A99S	missense	
chr17	>39471763"	KRTAP17-1	NM_031964.1	G47D	missense	rs74252500
chr19	>55105722"	LILRA1	NM_001278318.1	V5L	missense	
chr19	>55358686"	KIR2DS4	NM_012314.3	K247N	Missense	rs112697729
chrX	>55172659"	FAM104B	NM_138362.3	I69T	missense	rs5018687
chr4	>191003044"	DUX4L4	NM_001177376.2	R258W	missense	rs370569001
chr10	>46967616"	SYT15	NM_031912.4	S154L	missense	rs74128855

chr16	>70891640"	HYDIN	NM_001270974.1	K4088R	missense	rs1774416
chr16	>70908771"	HYDIN	NM_001270974.1	P3537A	missense	
chr16	>70926334"	HYDIN	NM_001270974.1	T3116R	missense	rs1774423
chr16	>90095620"	C16orf3	NM_001214.3	V44A	missense	rs61740023
chr19	>17397483"	ANKLE1	NM_001278444.1	V639L	missense	rs367668712 &rs60338123
chr20	>47991056"	KCNB1	NM_004975.2	S347R	missense	
chr3	>75787081"	ZNF717	NM_001128223.1	C565S	missense	rs77378861
chr11	>1267825"	MUC5B	NM_002458.2	A3239P	missense	rs200304875
chr11	>1267852"	MUC5B	NM_002458.2	A3248T	missense	rs201342042
chr11	>1856582"	SYT8	NM_138567.3	C65G	missense	
chr19	>9271435"	ZNF317	NM_020933.4	R372C	missense	

Supplementary Table 2. De novo variants in genes common for at least three families.

Chr	position	Gene	Ref seq	Protein variant	Variant type	Rs
Fam 1						
chr3	>75786942"	ZNF717	NM_001290209	R561I	missense	rs2918517
chr3	>75788158"	ZNF717	NM_001290209	L156V	missense	rs3009004
Fam2						
chr3	>75786175"	ZNF717	NM_001290208	E867*	nonsense	rs78135954
chr3	>75786687"	ZNF717	NM_001290208	L696H	missense	rs80214016
chr3	>75787199"	ZNF717	NM_001290209	H475Q	missense	
Fam3						
chr3	>75788068"	ZNF717	NM_001128223	F236V	missense	rs74357986
chr3	>75788199"	ZNF717	NM_001128223	H192R	missense	rs77110669
Fam4						
chr3	>75786252"	ZNF717	NM_001290208	P841H	missense	rs79138891
chr3	>75787405"	ZNF717	NM_001290209	G407R	missense	rs73843014
chr3	>75787620"	ZNF717	NM_001290208	H385R	missense	rs75737034
Fam5						
chr3	>75786041"	ZNF717	NM_001128223.1	S911X		rs77747132&rs150497643
					frameshift	
Fam7						
chr3	>75786035"	ZNF717	NM_001128223.1	F915FX	frameshift	rs149076283
chr3	>75787081"	ZNF717	NM_001128223.1	C565S	missense	rs77378861
chr3	>75787266"	ZNF717	NM_001128223.1	TG504TX	frameshift	rs776210532
chr3	>75788403"	ZNF717	NM_001128223.1	T124NX	frameshift	
Fam1						
chr11	>1017444"	MUC6	NM_005961		Exon	
chr11	>1017498"	MUC6	NM_005961	S1768T	missense	
chr11	>1017746"	MUC6	NM_005961	L1685F	missense	rs78848170
Fam2						
chr11	>1016989"	MUC6	NM_005961		Exon	
chr11	>1018419"	MUC6	NM_005961	T1461I	missense	
Fam3						
chr11	>1017084"	MUC6	NM_005961	P1906L	missense	rs34649796
Fam4						
chr11	>1017498"	MUC6	NM_005961	S1768T	missense	
Fam5						
chr11	>1017443"	MUC6	NM_005961.2	TT1786T	Inframe deletion	
RBMXL3						
Fam1						
chrX	>114425105"	RBMXL3	NM_001145346		exon deletion	
Fam3						
chrX	>114425105"	RBMXL3	NM_001145346		exon deletion	
Fam4						
chrX	>114425105"	RBMXL3	NM_001145346		exon deletion	
ACAN						
Fam2						
chr15	>89399701"	ACAN	NM_001135	D1295E	missense	

Fam3						
chr15	>89399758"	ACAN	NM_013227	D1314E	missense	
Fam4						
chr15	>89400023"	ACAN	NM_013227	T1403A	missense	rs12899191
PABPC1						
Fam1						
chr8	>101721839"	PABPC1	NM_002568	V365L	missense	
Fam2						
chr8	>101721933"	PABPC1	NM_002568		exon deletion	
Fam3						
chr8	>101719035"	PABPC1	NM_002568		intron+splice intron	
FRG1						
Fam1						
chr4	>190878556"	FRG1	NM_004477		Exon deletion	
Fm2						
chr4	>190864422"	FRG1	NM_004477		exon insertion	
Fam3						
chr4	>190862999"	FRG1	NM_004477		intron deletion	

CHAPITRE 5 : Discussion

5.1 Hétérogénéité clinique

Nous avons mis en évidence chez nos patients atteints de SP des comorbidités psychiatriques et des atteintes neurodéveloppementales systématiquement associées. Ceci souligne la grande **hétérogénéité clinique** de ce trouble et le fait que le diagnostic de SP n'est jamais un diagnostic isolé. Nous n'avons pas pu mettre en évidence une organisation syndromique des atteintes en fonctions de l'âge de début de la maladie en dehors de la variable anxiété qui est plus présente quand l'âge de début du trouble augmente. Ceci s'explique probablement par la faible taille de notre échantillon. Une autre explication serait que nous n'avons pas pu mettre en évidence d'association syndromique au sein des SP car elle n'existe pas ou que les marqueurs cliniques dont nous disposons ne sont pas suffisamment discriminants pour mettre en évidence une organisation syndromique des symptômes en fonction de l'âge de début du trouble.

Devant la complexité du tableau clinique présenté par ces patients et le faible nombre de données publiées existantes, il apparaît indispensable que les patients bénéficient de la détermination de la nature exacte de leur trouble selon une procédure diagnostique qui devrait comprendre :

- La **confirmation du diagnostic** par une échelle validée et standardisée (K-SADS-PL, DSM5) effectuée par des professionnels formés et **la recherche des comorbidités psychiatriques** systématiques (trouble anxieux et de l'humeur par exemple).

- La **datation exacte du début de l'émergence schizophrénique**. Il est préférable de considérer les signes positifs (délire, hallucinations et comportement désorganisé) plutôt que les signes négatifs, plus difficiles à diagnostiquer, plus insidieux et s'inscrivant souvent dans des manifestations prodromiques difficiles à dater précisément.

- Le **phénotypage des atteintes neurodéveloppementales associées**. Les professionnels paramédicaux ont leur place dans cette étape, mais la coordination et la contextualisation de ces résultats au trouble et au stade de développement du patient doivent rester une démarche médicale. Cette étape doit comprendre :

-Un bilan neuropsychologique. Cette étape est utile pour la prise en charge du patient (orientation et pour cibler les interventions de remédiation cognitive par exemple) mais elle est également importante dans un but diagnostique (diagnostic différentiel avec les psychoses affectives notamment, Kendell and Jablensky, 2003). En effet, dans la schizophrénie, l'atteinte neurodéveloppementale des processus cognitifs est un symptôme noyau et ne doit pas être considérée comme un processus secondaire et consécutif à l'évolution de la maladie (Weinberger and Gallhofer, 1997).

La STP illustre bien que l'atteinte cognitive n'est pas un déclin (ni un prodrome) de la maladie mais une atteinte prémorbide à considérer comme un arrêt prématuré (ou un ralentissement) dans le processus normal de développement (Bedwell *et al.*, 1999). Le bilan neuropsychologique, qui ne se contentera pas du seul QI, permettra également d'évaluer les processus connus pour être altérés dans les STP (et de façon plus importante que dans les formes à début adulte) : l'attention, la mémoire de travail et les fonctions exécutives (Biswas *et al.*, 2006).

- Un bilan psychomoteur pour objectiver un trouble du développement moteur (de la coordination motrice ou une dyspraxie par exemple).

- Un bilan orthophonique pour objectiver un trouble de la communication (retard de langage et trouble de la pragmatique du langage par exemple, de Boer *et al.*, 2020) ou des apprentissages (lecture, écriture et calcul) en lien avec un bilan scolaire.

- Un bilan multidisciplinaire en **Centre Ressource Autisme (CRA)**, pour les cas complexes (ou une synthèse sur dossier ou en format RCP) apportera des éléments

de discussion importants concernant la symptomatologie autistique, fréquemment associée.

- **La recherche des pathologies somatiques associées** doit être faite durant un entretien médical minutieux (si possible utilisant des grilles critériées) à la recherche des pathologies d'organes. Dans un premier temps, cette démarche est nécessaire pour améliorer la qualité globale des soins du patient porteur de trouble mental. Ensuite, cela permettra de faire émerger certaines associations étonnamment fréquentes (nous avons montré que 75% des patients avec STP étaient porteurs d'un asthme) sans que ces associations ne soient suffisamment étudiées pour qu'un lien ne puisse être mis en évidence.

A noter que cette partie de la discussion traite des pathologies somatiques associées et pas du bilan initial de diagnostic différentiel ou étiologique à effectuer lors de l'émergence schizophrénique. Ce bilan doit faire intervenir un médecin pédiatre ou neuropédiatre et comprend un bilan neurosensoriel (auditif et ophtalmologique), un examen clinique à la recherche d'étiologies génétiques connues (taches café au lait, dysmorphie faciale, insuffisance vélaire, anomalies de l'examen cardio-vasculaire, évolution du périmètre crânien,...) et la réalisation d'examens paracliniques motivés par les hypothèses cliniques consécutives à l'examen physique (IRM cérébrale non injectée, EEG de veille et +/- ponction lombaire avec analyse du liquide céphalo-rachidien). La réalisation d'examens de seconde intention (bilan génétique ciblé, de psychose auto-immune ou neurométabolique) doit faire l'objet d'une prescription documentée et être sous-tendue par des hypothèses diagnostiques précises.

5.2 Hétérogénéité génétique

Compte tenu de la grande variété des gènes, des mécanismes lésionnels et des voies de signalisation moléculaires atteintes, nous avons choisi une méthode **d'exploration génétique hiérarchisée**. L'association clinique avec des symptômes autistiques prémorbides nous a fait rechercher systématiquement un **X-Fragile**. Aucun de nos patients testés à ce jour n'en était porteur. En cas de positivité, nous aurions considéré cette anomalie comme responsable du tableau clinique et posé un diagnostic différentiel de TSA secondaire. Il est intéressant de noter que notre revue systématique de littérature (Fernandez *et al.*, 2019) nous a permis d'identifier un cas publié de STP (fille de 9 ans) avec un X-Fragile positif (Vantalou *et al.*, 2004). De plus la mère de la patiente présentait également un trouble su spectre de la schizophrénie (trouble de la personnalité schizotypique) mais qui diffère de la schizophrénie (et particuliers de la STP), entre autre, par la sévérité de l'atteinte qui est inférieure. Dans ce cas, il semblerait que l'amplification générationnelle mère-fille, qui concerne habituellement le nombre de triplets CGG dans le syndrome X-Fragile, se soit manifestée dans le cadre de l'atteinte psychiatrique avec une atteinte graduelle et augmentée au fil des générations (d'un trouble de la personnalité schizotypique chez la mère à une STP chez la fille). Cet exemple, où le phénotype était parfaitement décrit, n'est pas généralisable mais illustre la grande complexité des chevauchements cliniques et génétiques entre TSA et STP.

Ensuite, la stratégie diagnostique a consisté en la réalisation d'un **caryotype moléculaire (CGH-Array)** et nous a permis de mettre en évidence le rôle de *INPP5A* dans la STP du patient 6 (Fernandez *et al.*, 2021). Il s'agit d'un gène exprimé dans le cerveau (humain, souris et drosophile), très conservé entre espèces et qui code une InsP3 5-phosphatase dont les produits d'hydrolyse mobilisent le calcium intracellulaire, essentiel pour la morphogenèse des épines dendritiques dans les neurones. L'altération de ce processus (voie de signalisation InsP3/Ca²⁺), est retrouvée à la fois dans la schizophrénie et dans le TSA, renforçant la probabilité d'un lien entre ces troubles. Cette démonstration de pathogénicité

reste faible du fait que la ségrégation familiale est limitée en nombre de cas, que nous n'avons pas retrouvé cette anomalie [duplication totale ou partielle ou autre(s) mutation(s) sur *INPP5A*] chez un autre patient, que cette protéine ne présentait pas d'expression dans le sang (absence de données dans la littérature et échec des manœuvres expérimentales) et que nous n'avons pas pu accéder au tissu cérébral de la patiente (pour des raisons éthiques évidentes). Nous n'avons donc pas pu augmenter la force de notre démonstration par analyse de la protéine affectée, mais nous suggérons néanmoins de s'intéresser à la **voie de signalisation de l'inositol** dans la schizophrénie (au-delà des troubles de l'humeur comme la bipolarité dans lesquels cette association a été décrite) et en particuliers dans les formes de début précoce ainsi qu'à ses cibles thérapeutiques comme le lithium, y compris en dehors de son indication de régulateur de l'humeur. En application directe des résultats de la recherche, la patiente 6 bénéficie actuellement de ce traitement avec une remarquable efficacité sur les signes positifs (délires, hallucinations) malgré la diminution de l'Olanzapine pour un problème d'effet indésirable métabolique (hyperprolactinémie).

Enfin, la dernière étape dans la stratégie de diagnostic génétique était la réalisation **d'un séquençage de l'exome en trio**. Dans cette étude nous nous sommes concentrés sur l'analyse transversale de plusieurs familles car l'analyse séparée de chaque famille n'a pas montré de variants forts avec les outils bio-informatiques. Compte tenu du fait que les analyses des grandes cohortes en schizophrénie adulte (GWAS) n'ont pas montré de gènes candidats pertinents (Forsyth *et al.*, 2020), nos résultats ne sont pas surprenants. Nous avons donc étendu notre recherche aux mutations d'allure bénigne dans des gènes fréquemment mutés, sans les exclure de principe comme nous le ferions dans une approche classique par gènes candidats, mais en focalisant sur le fond génétique commun qu'ils partagent (voies de transmission communes). Cette approche nous a paru valable dans la mesure où, à l'heure actuelle, il est impossible d'exclure que les gènes sans pertinence phénotypique évidente

puissent jouer un rôle de gènes modificateurs ayant un impact, par exemple, sur l'âge d'apparition de la maladie. Nous avons ainsi effectué la première description de l'implication potentielle de voies de signalisation Wnt, Cadhérine et Cholécystokinine dans la SP. L'implication de ces différentes voies de signalisation, déjà décrite de manière variable dans d'autres troubles neurodéveloppementaux et/ou psychiatriques, souligne **l'hétérogénéité génétique** de ce trouble.

5.3 Intérêts du phénotypage, du géotypage et de la constitution de bases de données spécifiques et autres perspectives

L'équipe du Pr Judith Rapoport, de la branche pédopsychiatrique du NIMH dans la région de Washington aux USA, recommande une observation hospitalière à visée diagnostique de 2 à 3 semaines comportant une fenêtre pharmacologique, et souligne l'importance de l'expérience pratique des cliniciens et de réévaluations cliniques régulières pour faire face au « défi diagnostique » de la STP (Gochman *et al.*, 2011). En pratique, à défaut de pouvoir hospitaliser les patients suspects de STP et de pouvoir réaliser des fenêtres pharmacologiques selon la méthode Nord-Américaine, il est nécessaire d'organiser la coordination des différents bilans et examens, du parcours de soins et du diagnostic avec des professionnels dédiés au sein d'équipes hospitalo-universitaire avec en leur site un centre de compétences schizophrénie précoce ou un référent contact avec le centre de compétence le plus proche. C'est ce que nous avons organisé sur la région PACA (mission intersectorielle et interdépartementale) en se focalisant sur trois axes : l'évaluation diagnostique, le parcours de soin et la recherche.

Sur la question du diagnostic, nous allons poursuivre les inclusions dans le cadre du protocole GenAuDiss (mai 2023) et faire évoluer cette recherche d'une étude transversale

(séries de cas) vers la **constitution d'une cohorte** de patients. L'évaluation clinique des apparentés (parents, fratries) va également se poursuivre (résultats préliminaires actuellement en cours de traitement). Les inclusions seront poursuivies à Nice, avec les partenaires déjà impliqués au niveau régional (dont l'APHM de Marseille) et étendues à une collaboration européenne. Localement, nous allons mettre en place une unité d'évaluation des troubles neurodéveloppementaux à expression psychiatrique afin de pouvoir effectuer les évaluations multidisciplinaires ambulatoires actuelles en hôpital de jour pour les patients qui le nécessitent et bénéficier de la plateforme et son équipe pour faciliter la coordination des parcours de soin en lien avec les partenaires extérieurs (secteurs de pédopsychiatrie, secteur médico-social, aide sociale à l'enfance, éducation nationale...)

Sur le plan de la recherche, nous allons poursuivre la constitution de notre biobanque à ADN, de notre base de données cliniques avec le phénotype de précision des patients (évaluation initiale) et procéderons à une réévaluation clinique (diagnostic clinique et atteintes fonctionnelles) dans le cadre de la future cohorte. Enfin, une base de données génétiques avec la séquence ADN de ces patients sera mise en place en lien avec l'INRIA, car nous souhaitons dans un premier temps rendre accessible ces données anonymes (plateforme internet) puis, dans un second temps, développer une base de donnée internationale afin que le partage entre équipes de ces données et les annotations de la base soient facilités.

CHAPITRE 6 : Conclusion

La SP est un trouble rare, sévère et neurodéveloppemental qui présente une grande hétérogénéité clinique et génétique. Élucider les mécanismes moléculaires de la SP et ouvrir la voie à une intervention thérapeutique spécifique nécessitera d'effectuer systématiquement et à grande échelle :

1) le diagnostic syndromique précis de SP avec un âge de début de la maladie, une détermination du phénotype neurodéveloppemental prémorbide, des comorbidités psychiatriques et des atteintes somatiques extracérébrales associées ;

2) une évaluation génétique par une approche hiérarchisée allant jusqu'au séquençage du génome entier ;

3) le partage des données entre équipes à l'échelle internationale avec la constitution de bases de données spécifiques, comparables, génétiques et moléculaires corrélées aux phénotypes de précision des différentes formes de SP.

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APPENDICES

Les contributions à la recherche en dehors des publications dans des revues à comité de lecture, indexées et en premier auteur (autre rang, revue non indexée, poster...) sont listées dans les appendices :

Appendice 1 : Article : Early-Onset Schizophrenia in a paediatric population of French psychiatric and medico-social care centres: A cross sectional study

Appendice 2 : Article : Pharmacoresistance and side effects of antipsychotics associated with poor metabolizer phenotype for cytochrome P450 2D6 in a context of early-onset schizophrenia: A case report

Appendice 3 : Early Onset Schizophrenia: Studying the Links between Cognitive and Clinical Dimensions

Appendice 4 : Poster : Neurodevelopmental and childhood-onset schizophrenia in an adolescent presenting inherited 10q26.3 duplication

Appendice 1 : Article : Early-Onset Schizophrenia in a paediatric population of French psychiatric and medico-social care centres: A cross sectional study

PLOS ONE

RESEARCH ARTICLE

Early-Onset Schizophrenia in a paediatric population of French psychiatric and medico-social care centres: A cross sectional study

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Data Availability Statement: The data underlying the results presented in the study cannot be shared publicly and are available from "Fondation Lenval, 57 avenue californie, 06200, Nice, (+33492030326)". The request will be addressed to Mme Laure Gaëlle (+33492030326) or at laure.g@pediatrie-chu.lenval-nice.fr.

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Abstract

Background

Early-Onset Schizophrenia (EOS) is rare but severe mental health disorder in children and adolescents. Diagnosis of schizophrenia before the age of 18 years remains complex and challenging, especially in young children. In France, there are no recent reliable epidemiological data about the prevalence of EOS. The present study evaluates the EOS rate in a target clinical population of children and adolescents in psychiatric and medico-social care centres in the South-East of France.

Methods

Psychiatric and medico-social centres for children and adolescent in the geographical area have been contacted, and after receiving their agreement to participate in the study, eligible patients corresponding to inclusion criteria were selected based on patients' medical records. Main inclusion criteria were age 7 to 17 years and intelligence quotient > 35. EOS categorical diagnosis was assessed by Kiddie-SADS Present and Lifetime psychosis section.

Results

37 centres participated and 302 subjects have been included in the study. The main result was the categorical diagnosis of EOS in 27 subjects, corresponding to a rate of 8.9% in the study population. Half of the patients presented mild to moderate intellectual deficiency. Interestingly, only 2.3% had a diagnosis of schizophrenia spectrum disorder noted in their medical records before standardized assessment.

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PLOS ONE

Early-Onset Schizophrenia in a French paediatric population

had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Conclusions

The results of the study highlight the importance of using a standardized diagnostic tool for the diagnosis of schizophrenia in the paediatric population. In fact, EOS might be underdiagnosed in children and adolescents with neurodevelopmental disorders and subnormal cognitive functioning.

Trial registration

NCT01512641. Registered 19 January 2012; <https://clinicaltrials.gov/ct2/show/NCT01512641>

Appendice 2 : Article : Pharmacoresistance and side effects of antipsychotics associated with poor metabolizer phenotype for cytochrome P450 2D6 in a context of early-onset schizophrenia: A case report

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Cas clinique

Pharmacorésistance et effets secondaires des antipsychotiques associés à un phénotype métaboliseur lent pour le cytochrome P450 2D6 dans un contexte de schizophrénie très précoce : à propos d'un cas clinique



Pharmacoresistance and side effects of antipsychotics associated with poor metabolizer phenotype for cytochrome P450 2D6 in a context of early-onset schizophrenia: A case report

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INFORMATION

Historique de l'article :

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La schizophrénie à début précoce est une maladie chronique grave représentant un problème de santé public impactant la qualité de vie de nos patients. Elle nécessite souvent une prise en charge pharmacologique. Cependant, certains patients présentent une pharmacorésistance et/ou de nombreux effets indésirables [1,2]. Les antipsychotiques les plus fréquemment prescrits et ayant l'AMM en population pédiatrique, tels que la Risperidone et l'Aripiprazole, sont métabolisés par le cytochrome P450 2D6 [3,4]. Les anomalies génétiques fonctionnelles du CYP2D6 (p. ex. duplication, délétion, ou certains polymorphismes) définissent différents phénotypes : métaboliseurs ultrarapide (inefficacité), intermédiaire ou lent (effets indésirables) [5]. Ce cas clinique illustre l'impact d'une anomalie pharmacogénétique type phénotype métaboliseur lent pour CYP2D6 sur l'évolution et le retentissement d'une schizophrénie précoce traitée par antipsychotiques.

Observation

L'adolescente, âgée de quinze ans présente un diagnostic de schizophrénie très précoce. Elle est suivie en pédopsychiatrie depuis ses six ans dans un contexte de difficultés relationnelles avec ses pairs et présente un retard dans l'acquisition du langage. Dans les antécédents familiaux nous notons le placement judiciaire de la fratrie et un épisode dépressif caractérisé chez la grand-mère maternelle avec passages à l'acte suicidaires. À treize ans, la patiente est hospitalisée en pédopsychiatrie dans un contexte de syndrome délirant aigu, sans étiologie organique ni toxique, marquant une rupture avec l'état antérieur. Plusieurs traitements antipsychotiques sont successivement instaurés sans efficacité clinique (Risperidone, Aripiprazole, puis Quétiapine avec Loxapine). De plus, malgré une posologie adaptée, nous notons des effets secondaires invalidants neuromusculaires (syndrome extrapyramidal, dystonies), digestifs (constipation, hyperphagie et prise pondérale majeure) et endocriniens (aménorrhée, galactorrhée). Dans ce contexte, la symptomatologie s'aggrave, impliquant un retentissement socio-scolaire, familial et somatique important. Les

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hospitalisations sont itératives. La patiente présente un tableau clinique de schizophrénie avec syndrome délirant chronique polythématique (persécution, mystique et mégalomaniaque), de mécanismes multiples (hallucinatoire acoustico-verbal et cénesthésique, intuitif, et interprétatif). L'adhésion est totale, la participation affective intense allant jusqu'à des mises en danger en réponse à ses convictions délirantes. L'observation clinique retrouve également un syndrome dissociatif intellectuel (différence, barrages, rationalismes morbides), comportemental et affectif (sourires immotivés, bizarreries comportementales, ambivalence affective) et un syndrome déficitaire (isolement social, apragmatisme). Dans ce contexte de pharmacorésistance et effets indésirables, le génotypage du *CYP2D6* retrouve un polymorphisme associé à un phénotype métaboliseur lent (*4/*41), pouvant donc expliquer la mauvaise tolérance des antipsychotiques prescrits et partiellement en lien avec la pharmacorésistance constatée. Un traitement par amisulpride est décidé, évitant le métabolisme par *CYP2D6* (métabolisme rénal). Ce traitement est efficace sur la symptomatologie dissociative mais pas sur le syndrome délirant. Des effets secondaires sont rapportés, nécessitant une augmentation très progressive de la posologie de ce traitement. Un traitement par clozapine (métabolisme par *CYP1A2*, évitant *CYP2D6*) est alors instauré avec la surveillance nécessaire, permettant un apaisement du syndrome délirant. À trois mois et une posologie de 400 milligrammes par jour, le traitement est arrêté suite à des malaises avec hypotension artérielle et un syndrome extrapyramidal, imputables à un surdosage. Dans ce contexte, et de manière pluridisciplinaire une prise en charge par électroconvulsivothérapie (ECT) associée à un traitement par Olanzapine (métabolisé principalement par *CYP1A2*, possibilité d'administration IM) est décidée. Vingt-six séances d'ECT (en deux cures) sont effectuées, permettant un certain apaisement psychique et une sortie séquentielle d'hospitalisation, la patiente a alors quinze ans. Le traitement par olanzapine est finalement arrêté devant une hyperprolactinémie impliquant aménorrhée et galactorrhée. La clozapine est alors réintroduite avec surveillance étroite de la clozapinémie, en association avec l'amisulpride, permettant une relative stabilisation de la pathologie.

Discussion

Ce cas de schizophrénie très précoce sévère et résistante, présentant de nombreux effets indésirables, associés à un phénotype métaboliseur lent du *CYP2D6*, illustre l'intérêt d'une évaluation pharmacogénétique. L'analyse ayant permis de faire évoluer la prise

en charge médicamenteuse en évitant la prescription de molécules métabolisées par *CYP2D6*. Les traitements instaurés par la suite, peu ou non métabolisés par *CYP2D6* associés au traitement non pharmacologique (ECT) ont permis une stabilisation clinique avec un contrôle des effets secondaires. Cependant, la pharmacorésistance observée chez cette patiente ne peut uniquement être expliquée par l'anomalie pharmacogénétique. Le métabolisme individuel des médicaments est complexe et dépend aussi de celui de ses métabolites [6]. Il repose, au-delà du *CYP2D6*, sur d'autres gènes du *CYP450*, du transporteur de la molécule et du récepteur de l'organe cible [5]. Enfin, l'évolution des pathologies psychiatriques dépend aussi de prises en charge non médicamenteuses.

Ainsi, les anomalies pharmacogénétiques du *CYP450* pourraient concerner d'autres enfants ou adolescents présentant des pathologies mentales sévères et être en lien avec leur pharmacorésistance ou leur tolérance aux psychotropes. Des études complémentaires restent nécessaires pour étudier ce lien [7,8].

Déclaration de liens d'intérêts

Les auteurs déclarent ne pas avoir de liens d'intérêts.

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Appendice 3 : Early Onset Schizophrenia: Studying the Links between Cognitive and Clinical Dimensions

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ABSTRACT. Background. Early-onset schizophrenia (EOS), a rare and severe chronic psychiatric condition, is defined by an onset of schizophrenia symptoms before the age of 18. Core symptoms also include cognitive impairments. However, little is known about links between psychiatric symptoms of EOS and cognitive abilities. **Objective.** To explore the clinical and neurocognitive profiles of EOS patients and their links. **Method.** EOS patients have been phenotyped using standardized psychiatric assessments for DSM-5 diagnoses (K-SADS-PL) and for symptoms (PANSS and SANS), together with neurocognitive evaluations (IQ using WISC-IV, Trail Making Test A/B and verbal fluency). The study was approved by the ethical committee (ref.2011-A00787-34). **Results.** The EOS sample (n=27, 12.4 +/-3.2 years) presented hallucinations (83%), negative symptoms (70%) and delusion (59%). 81% of patients presented comorbidities such as anxiety disorders (33%), autism spectrum disorders (26%) and attention-deficit hyperactivity disorder (26%). Patients presented borderline intellectual deficiency (total IQ = 72.5 +/-4.7), with low performances in working memory subtest. We highlight a positive correlation between the IQ and intensity of positive symptoms (PANSS) and between the IQ and a first treatment being administered at an older age. We also highlight a negative correlation between the IQ and attention items of SANS.

Conclusion. Cognitive skills are correlated with symptom intensity in EOS patients. An older age of onset seems to be a protective factor for cognitive development. Further studies are needed to evaluate this link and the potential benefit of neuropsychological interventions such as cognitive remediation for the treatment of psychiatric symptoms in EOS

Appendice 4 : Poster : Neurodevelopmental and childhood-onset schizophrenia in an adolescent presenting inherited 10q26.3 duplication

EPA18-1458



Neurodevelopmental and childhood-onset schizophrenia in an adolescent presenting inherited 10q26.3 duplication

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Introduction: Childhood-onset Schizophrenia (COS), a rare and severe condition, is defined by an onset of schizophrenia positive symptoms (delusions, hallucinations, disorganized speech or behavior) before 13 years [1]. A higher rate of neurodevelopmental abnormalities is observed in COS compared to adult onset schizophrenia [2] and patients typically have learning, intellectual, communication or motor impairments associated with autism spectrum or attention deficit hyperactivity disorders. Cytogenetics abnormalities are also more frequent in COS [3], but the exact pathogenic mechanism remains unknown.

Case description:

1- Clinical presentation

- 17-year old girl
- 41 weeks of gestation, caesarian section, 3500 g, 51 cm, HC 36 cm, Apgar 10-10
- Psycho-traumatic family history with the violent father's death in early childhood of the patient (at 6 month-old)

Developmental features:

- Delayed **motor** milestones (walking after 24 months).
- **Communication disorders** (first words at 3 and a half years, stuttering)
- **Intellectual** disability (IQ = 74) and learning impairments
- Admission in a french medico-social sector since age of 7 years, cause of behavioral symptoms including tantrums and poor interactions with other children. At age of 12, she presented a delusion (persecution) and visual hallucinations. She was diagnosed with COS by a child psychiatrist. At the age of 14, she made a suicide attempt by drug ingestion. She was treated by RISPERONE during 5 years with a good response on delusion but mood lability and depressive symptoms remain.
- Normal clinical work-up (including EEG, cerebral MRI, standard blood analysis and metabolic screening)

Somatic features:

- **Asthma** (no Long-term control medications needed)
- Primary enuresis
- Primary amenorrhea (first periods at the age of 17) without hyperprolactinemia

2- Clinical Genetics

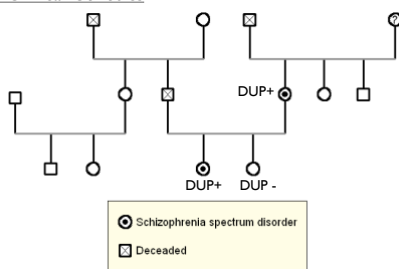


Fig. 1. Propositus is a 17-year-old female with COS (K-SADS-PL, DSM 5). Her mother was diagnosed with a psychotic disorder (MINI, DSM IV-TR). Her grandmother was not directly evaluated but after the mother's assessment she could possibly have been diagnosed with schizophrenia spectrum disorder (Schizoid personality ?). CNV 10q26.3 status : DUP + = positive ; DUP - = negative

Chromosomal microarray analysis: Inherited interstitial 324kb duplication in chromosomal region 10q26.3

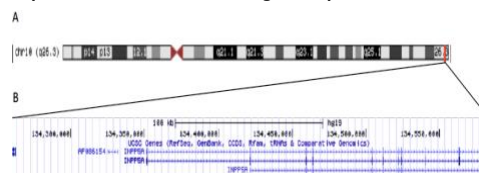


Fig. 2. Mapping of a chromosome 10 duplication (A) Schematic representation of chromosome 10 according to NCBI build 37 (human genome 19) (B) Known RefSeq Genes in the deleted chromosomal area

CODING GENE	ENCODED PROTEIN	FUNCTION (ncbi - gene)	OMIM	BRAIN EXPRESSION (RPKM)
INPP5A	InpP3 5-phosphatases	mobilize intracellular calcium and act as a second messenger mediating cell responses to various stimulation	600306	3.5
AF086154	unknown	unknown		0.045
C10orf91	Coding status : incertain	unknown		0

Fig. 3. Summary of coding genes, encoded proteins, corresponding OMIM reference and brain expression (RPKM mean score) included in the duplication

Discussion: Several clinical cases described autistic patients with a 10q26.3 CNV [4,5] but our case report is the first description of a patient with a 10q26.3 duplication associate to a childhood-onset schizophrenia with developmental delay. The genetic testing of first degree relatives (FIG. 1) revealed a familial segregation of the mutation where both mother and patient, with schizophrenia spectrum disorders, were positive for this CNV while good mental health sister of 22 years (with normal development) was negative. Thus, the mother's psychotic disorder (DSM-IV-TR) evolving since adolescence (15 years) while her daughter has an early and neurodevelopmental form of schizophrenia. A second "hit" [6] probably happened : another CNV ? A Single Nucleotide Polymorphism (SNP) ? The epigenetic impact of the violent father's death

This 324kb duplication leads to a gain of function in a phosphoinositide metabolism gene, with an over expression during aging (significantly more than development) in healthy persons in human prefrontal and dorsolateral brain's regions [7], both involved in neurodevelopmental disorder.

Conclusion: We reported a patient with a 324Kb duplication at 10q26.3 inherited from mother and associated with neurodevelopmental childhood-onset schizophrenia involving a phosphoinositide metabolic gene. The early and severe childhood psychotrauma in this case argues in favor of the schizophrenia "two-hit hypothesis"

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