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

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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 with 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 physiopathology. 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 multicentric study in a paediatric population. The study started in April 2014. Inclusion criteria are: age 7–22 years, diagnosis of EOS with comorbid ASD and IQ >50; Parents and siblings are also enrolled. We perform psychiatric assessments (Mini International Neuropsychiatric Interview, Kiddie Schedule for Affective Disorders and Schizophrenia -Present and Lifetime Version, Positive and Negative Syndrome Scale and Scale for the Assessment of Negative Symptoms) together with neurocognitive evaluations (IQ, Trail Making Test A/B and verbal fluency). Then, we study variants of the coding part of DNA (exome), using next-generation sequencing process on trio (mother, father and child). Bioinformatics tools (RVIS and PolyPhen-2) are used to prioritise 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

Strengths and limitations of this study

► University Child Psychiatry Department has recognised 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 and regional enrolment) and difficulties in involving both parents (separation, conflict, death and so on).
► Exome analysis may miss causative mutations in non-coding part of the genome.

National Agency for Medicines and Health Products Safety. All patients signed informed consent on 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 NCT0256552; Pre-results.

INTRODUCTION

Early-onset schizophrenia (EOS) is a rare (1/5000 to 1/20 000) and severe chronic psychiatric condition defined by an onset of schizophrenia-positive symptoms (delusions,

hallucinations and disorganised speech or behaviour) before the age of 18.[1 2] Onset before 13 years of age is called childhood-onset schizophrenia (COS).[3 4]

A higher rate of neurodevelopmental abnormalities is observed in EOS compared with 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).[5–7] 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,[6] and >80% of children with schizophrenia or schizoaffective disorder might present comorbid ADHD.[7] In addition, EOS (including COS) is a complex disorder related to other neurodevelopmental disorders, and it represents a real challenge for clinical diagnosis with,[4] to date, no objective test based on genetics. Therefore, it is critical to conduct a rigorous and homogeneous phenotypic characterisation by using international classifications such as International Classification of Diseases (ICD) or Diagnostic and Statistical Manual of Mental Disorders (DSM)[8–10] and by using standardised and internationally validated psychiatric categorical assessments.

A few genetic studies of EOS are reported, due to the very low prevalence[1 2] and to the nosographic difficulties which made it difficult to obtain a consensual clinical definition of this disorder and to carry out aetiological studies. Interestingly, DSM, Fifth Edition (DSM-5) classification provides clarification in this area with schizophrenia no longer excluding the diagnosis of ASD and formalising the overlap between these two disorders. On the other hand, DSM-5 does not recognise the specificities of EOS compared with adult onset schizophrenia.[10]

Submicroscopic cytogenetic abnormalities, such as disease-related copy number variations (CNVs), are more frequent in COS than in AOS (about 11%[11 12] vs 2%–5%, p<0.0001[12]). A high heritability rate of COS (>80%) has also been suggested in early adoption/twin studies and has been confirmed by familial aggregation studies.[13 14] The fact that genome-wide association studies failed to yield a genome-wide significant result in patients with AOS appears to argue in favour of a polygenic basis of EOS with hundreds of genes with small individual effects being involved.[15] 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.[12 16]

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.[17] 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 *ATP1A3* gene (19q13.2).[18] Another cohort study using WES identified 20 de novo variants in 17 COS probands (rate: 1,17)[19] in genes previously linked to neuronal function or to psychiatric disorders.[19] 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[20] 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.

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 characterisation of the clinical and neurocognitive profiles of patients and their first-degree relatives.

Trial design
GenAuDiss is a translational multicentre 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 6 patients and 14 first-degree relatives have been enrolled from this completed programme. In addition, an enrolment in the present study is offered to inpatients and outpatients corresponding to the inclusion criteria of the investigator, Child and Adolescent Psychiatry (CAP) centres, 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 centres 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 6 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 comorbid ASD and their first-degree relatives.

Major inclusion criteria for patients:
► Age of 7–22 years.

Table 1 GenAuDiss study procedure and evaluation criteria

Patient	V0: Selection	V1: Inclusion	V2
Inclusion criteria	+		
Informed consent	+		
Medical history+genealogical tree		+	
Psychiatric evaluation			
K-SADS-PL psychotic disorders	+		
ADI-R	+		
K-SADS-PL (full) (DSM 4/5)		+	
SANS		+	
PANSS		+	
Neuropsychological evaluation			
WISC-IV (abridged version)	+		
IQ (full)*†		+	
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 4/5)*‡		+	
AQ		+	
TCI 226 (parents only)		+	
Neuropsychological evaluation			
IQ (abridged version) *†		+	
Genetic testing			
Blood tests			+

*Clinical tools are selected dependent on patient's age.
†Neurocognitive tools: < 7: WPPSI; 7-16: WISC; > 16: WAIS.
‡Psychiatric tools: < 17: K-SADS-PL; > 16: MINI.
ADI-R, Autism Diagnostic Interview–Revised; AQ, Autism Quotient; IQ, intelligence quotient; DSM, Diagnostic and Statistical Manual of Mental Disorders; 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.

▶ Diagnosis of EOS (onset before age 18), diagnosed by the semistructured interview *Kiddie Schedule for Affective Disorders and Schizophrenia–Present and Lifetime Version* (K-SADS-PL) psychosis section.

▶ Diagnosis of ASD (*Autism Diagnostic Interview-Revised* (ADI-R).
▶ 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 (summarised in table 1):
▶ Clinical parameters from medical history (including pregnancy, birth and so on) and biographic parameters, types and dates of significant life events including trauma and environmental exposures (eg, drugs and substances).
▶ Clinical parameters from physical examination (eg, body weight, body mass index, arterial pressure).
▶ Semistructured interviews to assess main diagnosis at inclusion (K-SADS-PL,[21] ADI-R,[22] Mini International Neuropsychiatric Interview (MINI)[23 24]).
▶ Clinical heteroassessments with specific rating scales (WISC-IV[25] or -V,[26] Positive and Negative Syndrome Scale (PANSS),[27] Scale for the Assessment of Negative Symptoms (SANS)[28] Trail Making Test (TMT) A, TMT B and verbal fluency).
▶ Self-report questionnaires (Cloninger's Temperament and Character Inventory (TCI) 226 and[29 30] Baron Cohen's Autism Quotient (AQ)[31]);
▶ Genetic analysis (including DNA testing for fragile-X syndrome, high-resolution karyotype, DNA CNVs from CGH array, DNA SNPs from exome sequencing).

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 realising 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 centre. 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 (eg, age and 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 prioritisation using validated tools (RVIS and Polyphen2)[32 33] and strategies in accordance with international guidelines.[34]

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

Clinical profile:

► K-SADS-PL for DSM IV-TR/DSM-5 comorbid psychiatric diagnosis of patients and minor siblings.
► MINI assessments for DSM IV-TR/DSM five 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 computerised 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.
► WISC-IV or WISC-V, full version in patient and WISC-IV abridged version in minor siblings; Wechsler Adult Intelligence Scale-III in a short form in adult siblings and parents.[35 36]

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.

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,[37] the pathophysiology of EOS still needs to be elucidated. Importantly, to date, contrary to other neuropsychiatric

pathologies (eg, Alzheimer's disease)[38] only two studies describe the WES genetic approach in patients with EOS.[18 19]

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 and so on).[39]

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 1/20 000)[1 2] 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 (*eg*, because of separation, conflict or death) might be significant obstacles limiting sample size.

ETHICS AND DISSEMINATION

The study protocol was authorised by the French National Agency for Medicines and Health Products Safety (ANSM 2013-A01699-36). All patients, their parents and siblings signed informed consent on enrolment in the study.

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