Missense variants in \textit{ATP1A3} and \textit{FXYD} gene family are associated with childhood-onset schizophrenia

Boris Chaumette\textsuperscript{1} · Vladimir Ferra\textsuperscript{2,3,4} · Amirthagowri Ambalavanan\textsuperscript{5} · Alice Goldenberg\textsuperscript{3,6} · Alexandre Dionne-Laporte\textsuperscript{1} · Dan Spiegelman\textsuperscript{1} · Patrick A. Dion\textsuperscript{1} · Priscille Gerardin\textsuperscript{2,3,4} · Claudine Laurent\textsuperscript{7,8} · David Cohen\textsuperscript{7,9} · Judith Rapoport\textsuperscript{10} · Guy A. Rouleau\textsuperscript{1,5}

Received: 12 September 2017 / Revised: 7 May 2018 / Accepted: 14 May 2018 / Published online: 12 June 2018
© Macmillan Publishers Limited, part of Springer Nature 2018

Abstract
Childhood-onset schizophrenia (COS) is a rare and severe form of schizophrenia defined as onset before age of 13. Here we report on two unrelated cases diagnosed with both COS and alternating hemiplegia of childhood (AHC), and for whom two distinct pathogenic de novo variants were identified in the \textit{ATP1A3} gene. \textit{ATP1A3} encodes the $\alpha$-subunit of a neuron-specific ATP-dependent transmembrane sodium–potassium pump. Using whole exome sequencing data derived from a cohort of 17 unrelated COS cases, we also examined \textit{ATP1A3} and all of its interactors known to be expressed in the brain to establish if variants could be identified. This led to the identification of a third case with a possibly damaging missense mutation in \textit{ATP1A3} and three others cases with predicted pathogenic missense variants in the \textit{FXYD} gene family (\textit{FXYD1}, \textit{FXYD6}, and \textit{FXYD6-FXYD2 readthrough}). \textit{FXYD} genes encode proteins that modulate the ATP-dependant pump function. This report is the first to identify variants in the same pathway for COS. Our COS study illustrates the interest of stratifying a complex condition according to the age of onset for the identification of deleterious variants. Whereas \textit{ATP1A3} is a replicated gene in rare neuropediatric diseases, this gene has previously been linked with COS in only one case report. The association with rare variants in \textit{FXYD} gene family is novel and highlights the interest of exploring these genes in COS as well as in pediatric neurodevelopmental disorders.

Introduction
Schizophrenia is a major mental disorder characterized by a spectrum of symptoms, including delusions, hallucinations, disorganization of speech and behavior, negative symptoms, and cognitive deficits. The age of onset of schizophrenia typically ranges from 15 to 25 years old, but rarely can begin...
before childhood-onset schizophrenia (COS) and has a similar presentation in the Diagnostic and Statistical Manual of Mental Disorders (DSM) compared with poor outcome adult-onset schizophrenia (AOS) [1]. The rate of comorbidity of developmental disorders such as autism spectrum disorder (ASD), motor developmental disorders and learning disabilities are higher than in the later onset forms of schizophrenia. Also, the rate of comorbid medical conditions is increased [2]. The presence of prominent delusions or hallucinations for at least 1 month defines COS and helps to differentiate it from ASD or pervasive developmental disorder (PDD). The prevalence of COS is estimated to be 0.03% [3] compared to 1% for AOS.

Understanding the causes of this rare but severe form of schizophrenia improves our knowledge of the genetic architecture of schizophrenia. COS variants are observed as more penetrant whereas AOS seems to be more driven by genetic and environmental interactions [4]. Stratifying by age of onset has been useful in medicine, in particular for the identification of causal genetic variants. Several publications have shown that some polymorphisms are associated with COS. Identification of rare variants in COS is just starting, including copy number variants [5, 6], truncating variants [7], and de novo mutations [8]. Rare missense variants are more difficult to interpret due to their incomplete penetrance and the limitations of working with a relatively small cohort.

Here we report two COS cases who also have Alternating Hemiplegia of childhood (AHC), a rare disease with a prevalence below 1/100,000. Onset of AHC is typically before the age of 18 months and the clinical presentation is characterized by repeated episodes of hemiplegia that alternately affects one side of the body [9]. Some paroxysmal symptoms are associated, such as seizures, dystonic episodes, visuomotor disorders, dyspnea, dysautonomia signs. Other neurological symptoms include choreoathetosis and ataxia [10]. AHC also causes mild to severe cognitive impairment. Most of the cases are associated with ATP1A3 mutation and very rarely, with a mutation in the ATP1A2 gene. In this current study, two de novo deleterious missense variants were identified in the ATP1A3 gene in two individuals experiencing comorbidities between COS and AHC. This gene has been previously associated with COS in one case with a history of PDD and selective mutism [11]. Very recently, de novo mutations in this gene have been found in ASD [12]. It encodes the catalytic α-subunit of a neuron-specific ATP-dependent transmembrane sodium–potassium pump. Then, we looked for variants in this gene and its interactors in a large COS cohort. One missense mutation was identified in the same exon in the ATP1A3 gene in one affected individual. Missense variants were found in genes that interact with ATP1A3 in four additional COS cases.

### Population and methods

#### Participants

The two cases of COS who also have AHC were identified in the child and adolescent psychiatric unit in the Centre Hospitalier Spécialisé du Rouvray (Sotteville-lès-Rouen, France). This is an inpatient intensive care unit specialized in severe forms of child psychiatric disease. The psychiatric clinical assessment was performed by a specialized expert psychiatrist (VF) using the DSM-V criteria. A specialized geneticist (AG) conducted the general clinical examination including neurological examination. The parents gave their written informed consent for the study. The two probands and their families are Caucasian.

An American COS cohort was recruited by Dr. Rapoport’s group at the National Institute of Mental Health (NIMH) as part of their childhood onset schizophrenia research study. This study was approved by the Institutional Review Board of The National Institute of Mental Health. All participants provided written informed consent from a parent or legal guardian for minors. A total of 361 patients were screened. Seventeen sporadic COS cases (11 males and 6 females) meeting DSM-IIIIR/DSM-IV criteria for schizophrenia with onset of psychosis before age 13 and their unaffected parents were selected for this study. Diagnosis was confirmed with inpatient medication-free observation according previously published recommendations [13]. To address the concern of false positives resulting from inclusion of language disorders, we included only patients with clear positive symptoms (delusions or hallucinations). Medical or neurological disorders were criteria of exclusion. Patients and their available first-degree relatives were interviewed for lifetime and current psychiatric disorders using structured psychiatric interviews and Autism Symptom Questionnaire. The mean age of onset was 9.8 years (range: 6–12 years old). Six of the patients were also diagnosed with ASD. CNV and de novo variants have been previously explored and published in this cohort [5, 8].

#### Genetic study

The molecular analysis of the two French cases was performed by targeted Sanger sequencing in the affected individuals, their parents, and their siblings.

In the American cohort, exome capture of all individuals in the COS trios was performed using SureSelectXT Human All Exon V4 kit (Agilent Technologies Inc., Mississauga, ON, Canada) in two different batches. The first batch consisting of 13 COS trios (39 samples) was captured and sequenced using Illumina HiSeq 2000 at the McGill University and Genome Quebec Innovation Centre (Montreal, QC, Canada). The second batch of four COS trios
(12 samples) was sequenced using the Illumina HiSeq 2000 platform at the Université de Montréal’s Beaulieu-Saucier Pharmacogenomics Centre at the Montreal Heart Institute (Montreal, Canada).

The sequenced reads of all the samples from Illumina HiSeq2000 were aligned to the reference genome (GRCh37/hg19) using Burrow-Wheeler Aligner [14]. The aligned reads were converted to binary format for the convenience of further analysis using SAMtools [15]. Samples had an average coverage of over 90% target covered at a depth of 20 x. The quality of coverage was assessed by the total number of reads mapped to corresponding regions in the reference genome, over the total number of uniquely mapped reads. Next, variant calling was performed using Genome Analysis Tool Kit (GATK) [16]. The variants were called for the sequenced reads available within the coverage region for each of the samples. This process identified single-nucleotide variants and small insertions or deletions at different levels of stringency based on their quality scores.

The identified variants were annotated with ANNOVAR tool [17], including minor allele frequencies from publicly available databases (1000 Genomes project and ExAC database), pathogenicity scores based on Polyphen-2, SIFT, LRT, C-PAP, and MutationTaster, phylogenetic conservation using GERP and PhyloP scores. Segregation analyses and extraction of variants located in genes of interest were performed using an in-house script. Only variants in exonic positions, with a frequency <0.01 in the 1000 Genome project and ExAC database, identified as possibly damaging by at-least three algorithms, and in a phylogenetically-conserved position, were retained.

**Interactome analysis**

In the American cohort, we looked for pathogenic mutations in genetic interactors of ATP1A3. The protein–protein interaction network was identified using the STRING software [18] (http://string-db.org/) with data settings as follow: all active interaction sources, no more than 50 interactions and highest level of confidence (0.9). The pathway was secondarily explored using the curated database Reactome (http://reactome.org/) [19]. Then, all the interactors were retained for further analysis if they are expressed in any part of the brain, based on GTEx database (https://gtexportal.org/home/) [20]. The final list of candidate interactors is given in the Supplementary Table. We also looked for expression of the more interesting interactors across the lifespan using BrainCloud application. BrainCloud allows the query of genome-wide gene expression data in the normal human postmortem dorsolateral prefrontal cortex at different ages [21].

---

**Table 1** Summary of the molecular findings and the clinical presentation of the ATP1A3 mutation carriers

| Case | Mutation in ATP1A3 gene | Pathogenicity | Inheritance | Age of onset for psychiatric symptoms | Sex | Main psychiatric symptoms | Dysmorphic features | Neurodevelopmental delays | Response to treatment | Associated phenotype | Associated phenotype |
|------|-------------------------|---------------|-------------|--------------------------------------|-----|--------------------------|-------------------|------------------------|---------------------|----------------------|----------------------|
| 1    | NM_152296:46:ATP1A3:c.2443G>A (p.Glu815Lys) | Reported in ClinVar (ID:37107) | De novo | 12 years | Male | Visual hallucinations (disorder of lights and shadows) and aggressiveness | Macroglossy, esotropia and short philtrum | Moderate intellectual disability, developmental delays | Poor | Autism spectrum disorder |
| 2    | NM_152296:46:ATP1A3:c.2443G>A (p.Glu815Lys) | Reported in ClinVar (ID:37107) | De novo | 12 years | Male | Visual hallucinations (disorder of lights and shadows) and aggressiveness | Macroglossy, esotropia and short philtrum | Moderate intellectual disability, developmental delays | Poor | Autism spectrum disorder |

**Table 2** Summary of the molecular findings and the clinical presentation of the ATP1A3 mutation carriers

| Case | Mutation in ATP1A3 gene | Pathogenicity | Inheritance | Age of onset for psychiatric symptoms | Sex | Main psychiatric symptoms | Dysmorphic features | Neurodevelopmental delays | Response to treatment | Associated phenotype | Associated phenotype |
|------|-------------------------|---------------|-------------|--------------------------------------|-----|--------------------------|-------------------|------------------------|---------------------|----------------------|----------------------|
| 1    | NM_152296:46:ATP1A3:c.2443G>A (p.Glu815Lys) | Reported in ClinVar (ID:37107) | De novo | 12 years | Male | Visual hallucinations (disorder of lights and shadows) and aggressiveness | Macroglossy, esotropia and short philtrum | Moderate intellectual disability, developmental delays | Poor | Autism spectrum disorder |
| 2    | NM_152296:46:ATP1A3:c.2443G>A (p.Glu815Lys) | Reported in ClinVar (ID:37107) | De novo | 12 years | Male | Visual hallucinations (disorder of lights and shadows) and aggressiveness | Macroglossy, esotropia and short philtrum | Moderate intellectual disability, developmental delays | Poor | Autism spectrum disorder |
Visualizations

To visualize the localization of the predicted pathogenic missense variants, we constructed the 3D picture of the ATP1A3 gene and FXYD gene family using the UCSF ChimeraX software [22] (http://www.rbvi.ucsf.edu/chimera/). Molecular data were obtained from the PHYRE2 Protein Fold Recognition Server [23] (www.sbg.bio.ic.ac.uk/~phyre2/) with the following Uniprot entries: P13637 (ATP1A3), Q9H0Q3 (FXYD6), O00168 (FXYD1), and A0A0A6YYL5 (FXYD6-FXYD2 readthrough). Color markers have been manually placed in ChimeraX.

Results

Our study identified three variants in the ATP1A3 gene in three unrelated individuals with COS (Table 1). Mutations were found in the first two individuals because they also had AHC, which is caused by ATP1A3 mutations in 74% of the patients [24].

In Case 1, we found a de novo mutation in ATP1A3 gene: c.2401 G > A. The patient presented at 3-month-old with seizures and repeated episodes of hemiplegia. Diagnosis of AHC was made at the age of 14 months old. He had severe developmental delays in early childhood and moderate intellectual disability without acquisition of reading and writing skills. He walked at 30 months, spoke his first words at 24 months, and he still has urinary incontinence. He had a failure to thrive, a gait disorder, and global hypotonia. He is the first child of unrelated and unaffected parents and has three siblings who do not carry the mutation, have no neuropsychiatric symptoms and have a normal development. The first psychotic features appeared at the age of 10 when he reported fluctuant symptoms such as visual and auditory hallucinations, delusions of persecution followed by behavioral disorders including psychomotor agitation and aggressiveness (Scale for Assessment of Positive Symptoms [25] (SAPS): patient’s maximal score = 35). He also had depressive symptoms with suicidal ideation and psychomotor slowdown. Hallucinations and delusional ideation seemed to worsen when hemiplegic episodes occurred.

In the unrelated Case 2, we found a de novo missense mutation in ATP1A3: c.2443 G > A in a boy. The diagnosis of AHC was made at the age of 3 months based on nystagmus episodes, major hypotonia, tonic, and myoclonic limb movements and a hemiplegic episode complicated with recurrent seizures. He had a developmental delay with walking acquired at the age of 25 months, and first words around 4 years old. He had impaired social skills compatible with a diagnosis of ASD. The first psychotic symptoms appeared at the age of 12 years with self-reported isolated visual hallucinations described as distortion of lights and shadows, auditory, and tactile hallucinations followed by delusion with persecutory and mystic ideas and bizarre behavior. SAPS scored 40 and he also had negative symptoms (Scale for Assessment of Negative Symptoms (SANS): patient’s maximal score = 35).

No other potentially causative mutations were found for both patients. Neither had obstetrical complications. Their de novo variants have been reported as deleterious in ClinVar (Table 1). Following an approach developed for interpretation of de novo mutation in human disease and especially in autism [26], we estimate that these variants are disease-relevant mutations. The constraint metric for missense variant in ExAC browser [27] is very high (z = 7.38) indicating an intolerance to variation in the ATP1A3 gene.

Response to treatment was evaluated by the May and Dencker scale [28] and the score of four for both the patients indicated that they had a poor response to treatment. In the case 1 the patient did not respond to aripiprazole or antidepressant medication but respond to a combination of risperidone (1.5 mg/day) and lithium 800 mg/day. Antidepressant and lithium were introduced as he presented recurrence of major depressive episodes. In the case 2, he did not respond to risperidone but benefited from treatment with aripiprazole. After a follow-up of 2 years, the pharmacological treatments have not been modified. The tolerance is acceptable and no psychotic relapses have been noticed or reported until now. They also received psychotherapy, cognitive rehabilitation, as well as institutional care including psychomotor-training, physiotherapy, and special needs education.

We looked for mutations in ATP1A3 in our American cohort. A nonsynonymous variant c.2438 T > C was found in an affected male child. This variant has never been reported in any database, it is very conserved across species and all the tested algorithms suggest it was possibly damaging (Table 2). The carrier (NSB1251) was diagnosed with the symptoms of schizophrenia at the age of 10, after an initial diagnosis of ASD. The ethnicity of the trio was Caucasian. None of the parents has a history of psychiatric or neurological diseases. The variant was inherited from the mother. There were no de novo single nucleotide variants identified in the proband in our COS whole exome sequencing study.

The ATP1A3 gene encodes the alpha-3 catalytic subunit of the Na+/K(+)-ATPase transmembrane ion pump, which is exclusively expressed in neurons of various brain regions. The ATP1A3 Na,K-ATPase is heteromeric so we systematically looked for mutations in its known interactors (Supplementary Figure 1 and Supplementary Table). The interactome centered on ATP1A3 identified sixteen genes, essentially the genes coding for Na+/K+-ATPases and their interacting FXYD proteins (Supplementary Figure 2).
Among them, 12 genes are expressed in the brain according to GTEx database (Supplementary Table). Possibly damaging variants in phylogenetically-conserved positions were identified in \textit{ABCA2}, \textit{FXYD1}, \textit{FXYD6}, and \textit{FXYD6-FXYD2} readthrough (Table 2). \textit{FXYD6-FXYD2} readthrough is a conjoined gene that generates transcripts by combining exons from \textit{FXYD6} and \textit{FXYD2}, which are on the same chromosome and in the same orientation. None of the variants were de novo. In total, we found four cases with rare damaging variants in \textit{ATP1A3} and \textit{FXYD} gene family in the American cohort.

We have represented the amino-acid (AA) changes in Fig. 1. The \textit{ATP1A3} variants are very close to each other (less than 15 AA between them) and are part of the transmembrane region of the protein. This region seems critical for the function of the pump. Amino-acid changes in \textit{FXYD} proteins are only found in the N-terminal region. How this \textit{FXYD} region interacts with \textit{ATP1A3} remains unknown. Visualization of the AA changes in \textit{ATP1A3} in other phenotypes has been previously reported using the same tools [11].

As we focused on schizophrenia with an age of onset below 13 years-old, we looked for expression of these genes during childhood. Brain cloud provides data for gene expression in normal postmortem dorsolateral prefrontal cortex during lifespan (Supplementary Figure 3). \textit{ATP1A3}, \textit{FXYD1}, and \textit{FXYD6} were expressed in the brain during childhood, but information is not provided for \textit{FXYD6-FXYD2}. However, this transcript has been experimentally-validated and reported in the human brain in another study [29]. The expression of \textit{ATP1A3} and \textit{FXYD1} are quite stable during the lifespan, whereas the expression of \textit{FXYD6} decreased with age.

### Discussion

We have identified three rare pathogenic variants in the \textit{ATP1A3} gene and three rare possibly damaging variants in \textit{FXYD} gene family. \textit{ATP1A3} is a replicated gene in rare neuropsychiatric diseases and here we strengthen the evidence linking it to COS. The association with \textit{FXYD} gene family is novel. These genes are closely related as they participate to the same heteromeric transmembrane protein complex. Indeed, the transmembrane \textit{Na,K-ATPase} complex is composed of an essential \textit{α}- and \textit{β}-subunit [30], and an auxiliary third subunit belonging to the \textit{FXYD} proteins (sometimes named as the \textit{γ}-subunit) [31]. The \textit{α}-subunit is the catalytic subunit responsible for transport activities of the enzyme. The \textit{FXYD} family has been identified as a modulator subunit of \textit{Na,K-ATPase} by stabilizing the complex, altering its kinetic activity, regulating its affinity for \textit{Na}⁺, \textit{K}⁺, and ATP [32, 33]. The subunits are tissue

---

**Table 2** List of mutations in \textit{ATP1A3} and its interactors in the American cohort annotated with their predicted pathogenicity and their conservative score. Scores in bold are considered as pathogenic.

| Family ID | Chr Position | Gene symbol | Reference allele | Mutant allele | Detailed annotation of the variant | Frequency in the 1000 Genomes project | Frequency in the ExAC database | SIFT Polyphen | LRT Mutation taster | M-CAP PhyloP vertebrate | GERP + score |
|-----------|--------------|-------------|-----------------|---------------|-----------------------------------|----------------------------------------|-------------------------------|-----------------|---------------------|---------------------------|------------|
| NSB1251   | 19 424744411 | ATP1A3      | G               | A             | not reported                       | not reported                          | not reported                | 0.999          | 0.000               | 0.773                     | 9.531       |
| NSB1949   | 9 139906315 | ABCA2       | G               | C             | not reported                       | not reported                          | 0.00001264                  | 0.01355        | 0.999               | 0.000                      | 2.95        |
| NSB1814   | 19 35633635 | FXYD1       | C               | T             | not reported                       | not reported                          | 0.00014                     | 0.007666       | 0.999               | 0.000                      | 4.12        |
| NSB2720   | 11 117693403 | FXYD6-FXYD2 | A               | G             | A1869G                             | A1869G                                 | 0.00004984                  | 0.004175       | 0.999               | 0.000                      | 4.35        |
| NSB1553   | 11 117711076 | FXYD6-FXYD2 | G               | G             | A1869G                             | A1869G                                 | 0.0001911                   | 0.004175       | 0.999               | 0.000                      | 5.44        |

**Table 2** List of mutations in \textit{ATP1A3} and its interactors in the American cohort annotated with their predicted pathogenicity and their conservative score. Scores in bold are considered as pathogenic.

Among them, 12 genes are expressed in the brain according to GTEx database (Supplementary Table). Possibly damaging variants in phylogenetically-conserved positions were identified in \textit{ABCA2}, \textit{FXYD1}, \textit{FXYD6}, and \textit{FXYD6-FXYD2} readthrough (Table 2). \textit{FXYD6-FXYD2} readthrough is a conjoined gene that generates transcripts by combining exons from \textit{FXYD6} and \textit{FXYD2}, which are on the same chromosome and in the same orientation. None of the variants were de novo. In total, we found four cases with rare damaging variants in \textit{ATP1A3} and \textit{FXYD} gene family in the American cohort.

We have represented the amino-acid (AA) changes in Fig. 1. The \textit{ATP1A3} variants are very close to each other (less than 15 AA between them) and are part of the transmembrane region of the protein. This region seems critical for the function of the pump. Amino-acid changes in \textit{FXYD} proteins are only found in the N-terminal region. How this \textit{FXYD} region interacts with \textit{ATP1A3} remains unknown. Visualization of the AA changes in \textit{ATP1A3} in other phenotypes has been previously reported using the same tools [11].

As we focused on schizophrenia with an age of onset below 13 years-old, we looked for expression of these genes during childhood. Brain cloud provides data for gene expression in normal postmortem dorsolateral prefrontal cortex during lifespan (Supplementary Figure 3). \textit{ATP1A3}, \textit{FXYD1}, and \textit{FXYD6} were expressed in the brain during childhood, but information is not provided for \textit{FXYD6-FXYD2}. However, this transcript has been experimentally-validated and reported in the human brain in another study [29]. The expression of \textit{ATP1A3} and \textit{FXYD1} are quite stable during the lifespan, whereas the expression of \textit{FXYD6} decreased with age.

**Table 2** List of mutations in \textit{ATP1A3} and its interactors in the American cohort annotated with their predicted pathogenicity and their conservative score. Scores in bold are considered as pathogenic.

---
speciﬁc [33] and ATP1A3 is selectively expressed in neurons of the central nervous system [34]. FXYD1 encodes the phospholemman protein, a transmembrane phosphoprotein expressed in the cerebellum and the frontal cortex [35]. Phospholemman integrates signals of many different kinases [36] and modulates the neuronal excitability via its effect on the NA,K-ATPase [37]. FXYD6 encodes the phosphohippolin, which plays an important role in neuronal excitability during postnatal development and in adult brain [38]. The heterotrimer ATP1A:ATP1B:FXYD catalyzes the hydrolysis of ATP coupled with the exchange of sodium and potassium ions across the plasma membrane, creating the electrochemical gradient, critical for the neuronal excitability. Interestingly, the expression of ATP1A3, FXYD1, and FXYD6 in the prefrontal cortex is present since birth consistent with the precocious onset of the phenotype.

ATP1A3 has been previously involved in various severe neurological disorders such as rapid-onset dystonia-parkinsonism (RDP), AHC, and CAPOS syndrome (CAPOS = cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss) [39]. Most of the pathogenic ATP1A3 mutations are located in the conserved transmembrane or N-terminus domains [40]. Two rare damaging missense mutations in ATP1A3 have been reported in schizophrenia with neither clinical description nor mention of the age of onset [41]. Overall the frequency of ATP1A3 deleterious variants in AOS seems to be very low by comparison with the frequency we reported in COS. A unique COS case with de novo ATP1A3 has been previously reported in the literature [11]; the proband presented with psychotic symptoms at 6 years of age but also PDD and selective mutism. Interestingly this patient did not have motor phenotypes except decreased muscle tone at 2 months of age for which he received physical therapy. This presentation seems closer to the third case we reported from NIMH cohort, suggesting that ATP1A3 mutations can lead to isolated psychiatric symptoms such as delusions and hallucinations associated with severe behavioral changes.

We screened the literature to identify the cognitive deﬁcits and behavioral problems associated with ATP1A3...
mutations. In AHC, impairment is nearly constant but variable, ranging from mild to moderate with a mean IQ estimation of 62.5 ± 14.0 [42]. The early development ranges from very slow to slight depending on the mutations [43]. However, detailed behavioral phenotypes are not often reported. One study has detailed the behaviour of a girl with AHC and reports deficits in sustained attention, in self-control, in regulation of her emotions and difficulties in inhibition capability [44]. Another case with Attention Deficit Hyperactivity Disorder has been reported [45]. Very recently, de novo mutations in the ATP-binding pathway have been found in ASD [12]. COS is preceded by and comorbid with ASD (or PDD) in 30–50% of cases and a large number of genetic variants are shared by these conditions [46]. Consequently, autistic features reported in ATP1A3 carriers could be considered as an early and prodromal expression of COS.

Interestingly, in RDP, psychiatric conditions (e.g., bipolar disorder) have been reported with a high frequency [47]. Brashear et al. have systematically assessed psychiatric comorbidities in RDP, reporting psychotic symptoms emerging before or at the same time as motor symptom onset [48]. The prevalence of psychotic symptoms was 26% in ATP1A3 mutation carriers with RDP, which is significantly different from the prevalence in RDP-affected non-carrier individuals. Beyond these psychotic features, the carriers suffering from RDP also exhibited depressive symptoms [10] and cognitive impairments specifically in memory and learning, attention, and executive functions [49]. Finally, quantifying protein levels using targeted mass spectrometry showed that ATP1A3 was reduced in auditory cortex gray matter of patients with schizophrenia compared with controls [50]. ATP1A3 was also upregulated by both clozapine and haloperidol in cerebral cortex tissue of antipsychotic-treated monkeys [50]. A heterozygous knock-in mouse model harboring a pathogenic mutation in position 801 (same position as case 1) has been generated and it displayed behavioral abnormalities such as hyperactivity and cognitive deficits [51].

FXYD6 was found to be expressed in glutamatergic synapses [52], one major component and actor in psychosis. Therefore, FXYD6 gene may be highly regulated with a peak of expression around birth in neurons of certain layers from the frontal cortex [53], which represent crucial period and brain region for COS. Ito et al have looked at FXYD6 expression in the post-mortem brain collection of schizophrenia and bipolar disorder called the Stanley brain collection (http://www.stanleyresearch.org/brain/); they found that the expression of FXYD6 in the dorsolateral prefrontal cortex (Brodmann area 46) tended to be decreased compared with healthy subjects [54]. A linkage analysis followed by fine mapping has suggested an association of FXYD6 with AOS [55]. A candidate SNP association study has also identified a SNP and a haplotype associated with AOS in this gene [56]. Two SNPs in this gene have also been associated with schizophrenia in a family-based association study [57]. However, a meta-analysis did not confirm this association concluding that polymorphisms may not have a major influence on susceptibility to schizophrenia [58]. The expression level of FXYD6 in the normal prefrontal cortex decreases during the lifespan and may suggest that variants in this genes are more susceptible to be associated with COS than AOS.

The post-mortem levels of FXYD1 messenger RNA and corresponding protein are decreased in the entorhinal cortex of individuals with schizophrenia compared with controls [59]. FXYD1 has been proposed to regulate the genesis of the neuroepithelium during brain development [60]. The expression of FXYD1 is specifically-regulated in the frontal cortex by the nuclear protein methyl-CpG binding protein 2 (MECP2) [61]. Mutations in MECP2 cause Rett syndrome, a syndromic form of autism in girls, and are associated with an overexpression of FXYD1 in the brain [35]. A case of COS has been reported in a boy carrying a missense mutation in MECP2 [62].

Brain expressed FXYD genes (including FXYD6 and FXYD1) localized in dendrites. The loss of the mRNA localization affects the function of the ATPase in dendrites. Variants in these genes could impact the synaptic functions [63]. It has been proposed that these ATPase regulator genes control the synaptic and perisynaptic membrane potential [52].

Due to the devastating neurologic presentations of ATP1A3 mutations, an international task force has been created to standardize the clinical examination and to provide recommendations [64]. Our data support the idea that there is a purely psychiatric form associated with mutations in ATP1A3. Moreover, there is no previous report of rare mutations in the FXYD gene family in the neurological forms of the disease. It might be worthwhile screening for FXYD6 mutations in AHC, RDP, or CAPOS patients where no ATP1A3 mutation has been identified. Given that we report recurrent missense variants in the same genes in COS, perhaps these genes may be routinely examined in COS.

Response to treatment was poor in our ATP1A3 carriers, as it is frequently the case in COS [65]. However, identification of a molecular target could be helpful in a personalized approach. New therapeutic strategies are currently being explored for ATP1A3 mutation related-pathologies. For example, oral supplementation with adenosine-5′-triphosphate has been reported to improve motor and cognitive skills in one case [66] and could be considered for our patients.

In conclusion, we wish to highlight the interest of studying extreme phenotypes in psychiatric genetics. By
studying COS, which is rare but may result from more penetrant mutations, we are increasing our chances to identify new genes. From a clinical point of view, psychotic symptoms can occur in various medical, neurological, and genetic diseases of children and adolescents. Atypical clinical signs such as early-onset, visual hallucinations, a catatonic syndrome, fluctuation of symptoms, a cognitive regression, or a paradoxical reaction to psychotropic drugs are red flags [67, 68] that should urge psychiatrists to look for an underlying organic condition, including genetic ones.

Acknowledgements We thank the patients and the family members who participate in the study as well as the involved medical teams. We thank Daniel Rochefort and Sylvia Dobrezienicka for the technical support; also Edouard Henrion and Ousmane Diallo for their bioinformatics support. We also thank Dr Maryam Soleimani and Dr Laure Bera for their comments, as well as Dr Elodie Hainque for her advice.

Funding The American cohort was supported by the National institute of Mental health (NIMH). Its genetic assessment was supported by the Canadian Institutes of Health Research (CIHR). Boris Chaumette receives a postdoctoral fellowship from the Healthy Brains for Healthy Lives project (Talent program).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Driver DI, Gogtay N, Rapoport JL. Childhood onset schizophrenia and early onset schizophrenia spectrum disorders. Child Adolesc Psychiatr Clin N Am. 2013;22:539–55.
2. Giannitelli M, Consoli A, Raffin M, Jardri R, Levinson DF, Cohen D, et al. An overview of medical risk factors for childhood psychosis: implications for research and treatment. Schizophr Res. 2017. https://doi.org/10.1016/j.schres.2017.05.011.
3. McKenna K, Gordon CT, Lenane M, Kaysen D, Fahey K, Rapoport JL. Looking for childhood-onset schizophrenia: the first 71 cases screened. J Am Acad Child Adolesc Psychiatry. 1994;33:636–44.
4. Asarnow RF, Forsyth JK. Genetics of childhood-onset schizophrenia. Child Adolesc Psychiatr Clin N Am. 2013;22:675–87.
5. Ahn K, Gotay N, Andersen TM, Anvari AA, Gochman P, Lee Y, et al. High rate of disease-related copy number variations in childhood onset schizophrenia. Mol Psychiatry. 2014;19:568–72.
6. Zhou D, Gochman P, Broadnax DD, Rapoport JL, Ahn K. 15q13.3 duplication in two patients with childhood-onset schizophrenia. Am J Med Genet Part B Neuropsychiatr Genet Pediatrics. 2016;171:777–83.
7. Addington AM, Gauthier J, Piton A, Hamdan FF, Raymond A, Gogtay N, et al. A novel frameshift mutation in UFP13B identified in brothers affected with childhood onset schizophrenia and autism spectrum disorders. Mol Psychiatry. 2011;16:238–9.
8. Ambalavanan A, Girard SL, Ahn K, Zhou S, Dionne-Laporte A, Spiegelman D, et al. De novo variants in sporadic cases of childhood onset schizophrenia. Eur J Hum Genet (EJHG). 2015. https://doi.org/10.1038/ejhg.2015.218.
9. Tenney JR, Schapiro MB. Child neurology: alternating hemiplegia of childhood. Neurology. 2010;74:e57–59.
10. Brashear A, Sweadner KJ, Cook JF, Swoboda KJ, Ozelius L. ATP1A3-related neurologic disorders. In: Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean LJ, et al. (eds). GeneReviews®. Seattle (WA): University of Washington, Seattle; 1993. http://www.ncbi.nlm.nih.gov/books/NBK11115/.
11. Smedegd-Margulies N, Brownstein CA, Vargas S, Tsembulkar SK, Towne MC, Shi J, et al. A novel de novo mutation in ATP1A3 and childhood-onset schizophrenia. Cold Spring Harb Mol Case Stud. 2016;2. https://doi.org/10.1011/mcs.a001008.
12. Takata A, Miyake N, Tsurusaki Y, Fukui R, Miyatake S, Koshimizu E, et al. Integrative analyses of de novo mutations provide deeper biological insights into autism spectrum disorder. Cell Rep. 2018;22:734–47.
13. Gochman P, Miller R, Rapoport JL. Childhood-onset schizophrenia: the challenge of diagnosis. Curr Psychiatry Rep. 2011;13:321–2.
14. Li H, Durbin R. Fast and accurate short read alignment with Burrows-wheeler transform. Bioinformatics. 2009;25:1574–60.
15. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, et al. The sequence alignment/map format and SAMtools. Bioinforma Oxf Engl. 2009:25:2078–9.
16. Van der Auwera GA, Carneiro MO, Hartl C, Poplin R, Del Angel G, Levy-Moonshine A, et al. From FastQ data to high confidence variant calls: the Genome Assembly Toolkit best practices pipeline. Curr Protoc Bioinforma. 2013;43:11.10.1–33.
17. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res. 2010;38:e164–e164.
18. Szkolarczyk D, Franceschini A, Wyder S, Frilund K, Heller D, Huerta-Cepas J, et al. STRINGv10: protein–protein interaction networks, integrated over the tree of life. Nucleic Acids Res. 2015;43:D447–452.
19. Fabregat A, Sidirooulos K, Garapati P, Gillespie M, Hausmann K, Haw R, et al. The reactome pathway knowledgebase. Nucleic Acids Res. 2016;44:D481–487.
20. Melé M, Ferreira PG, Reverter F, DeLuca DS, Monlong J, Sammeth M, et al. Human genomics. Human Transcr Across Tissues Individ Sci. 2015;348:660–5.
21. Colantuoni C, Lipska BK, Ye T, Hyde TM, Tao R, Leek JT, et al. Temporal dynamics and genetic control of transcription in the human prefrontal cortex. Nature. 2011;478:519–23.
22. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, et al. UCSF Chimera—a visualization system for exploratory research and analysis. J Comput Chem. 2004;25:1605–12.
23. Kelley LA, Mezulis S, Yates CM, Hyde TM, Tao R, Leek JT, et al. The reactome pathway knowledgebase. Nucleic Acids Res. 2015;43:D447–452.
29. Fagerberg L, Hallström BM, Oksvold P, Kampf C, Djureinovic D, Odeberg J, et al. Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. Mol Cell Proteom MCP. 2014;13:397–406.

30. Lingrel JB, Kuntzweiler T. Na+,K(+)-ATPase. J Biol Chem. 1994;269:19659–62.

31. Geering K. FXYD proteins: new regulators of Na-K-ATPase. Am J Physiol – Ren Physiol. 2006;290:F241–F250.

32. Garty H, Karlish SJD. Role of FXYD proteins in ion transport. Annu Rev Physiol. 2006;68:431–59.

33. Geering K, Béguin P, Garty H, Karlish S, Füzesi M, Horisberger J-D, et al. FXYD proteins: new tissue- and isoform-specific regulators of Na,K-ATPase. Ann N Y Acad Sci. 2003;986:388–94.

34. Li Z, Langhans SA. Transcriptional regulators of Na,K-ATPase subunits. Front Cell Dev Biol. 2015;3. https://doi.org/10.3389/fcell.2015.00066.

35. Deng V, Matagne V, Banine F, Frerking M, Ohliger P, Budden S, et al. FXYD1 is an MeCP2 target gene overexpressed in the brains of Rett syndrome patients and Mecp2-null mice. Hum Mol Genet. 2007;16:640–50.

36. Mounsey JP, Lu KP, Patel MK, Chen ZH, Horne LT, John JE, et al. Modulation of Xenopus oocyte-expressed phospholemman-induced ion currents by co-expression of protein kinases. Biochim Biophys Acta. 1999;1451:305–18.

37. Crambert G, Fuzesi M, Garty H, Karlish K, Phospholemman (FXYD1) associates with Na,K-ATPase and regulates its transport properties. Proc Natl Acad Sci USA. 2002;99:11476–81.

38. Kadowaki K, Sugimoto K, Yamaguchi F, Song T, Watanabe Y, Singh K, et al. Phosphohippolin expression in the rat central nervous system. Mol Brain Res. 2004;125:105–12.

39. Sweney MT, Newcomb TM, Swoboda KJ. The expanding spectrum of neurological phenotypes in children with ATP1A3 mutations, alternating hemiplegia of childhood, rapid-onset dystonia-parkinsonism, CAPOS and beyond. Pediatr Neurol. 2015;52:56–64.

40. Panagiotakaki E, De Grandis E, Stagnaro M, Heinzen EL, Fons C, Sisodiya S, et al. Clinical profile of patients with ATP1A3 mutations in alternating hemiplegia of childhood – a study of 155 patients. Orphanet J Rare Dis. 2015;10:123.

41. Purcell SM, Moran JL, Ruderfer D, Solovieff N, Roussos P, et al. A polygenic burden of rare disruptive mutations in schizophrenia. Nature. 2014;506:185–90.

42. Sweney MT, Silver K, Gerard-Blanluet M, Renault T, et al. Failure to confirm genetic association of the FXYD6 gene with schizophrenia in a Japanese population. Neurosci Lett. 2008;438:70–75.

43. Choudhury K, McQuillin A, Puri V, Pimm J, Datta S, Thirumalai S, et al. A genetic association study of chromosome 11q22-24 in two different samples implicates the FXYD6 gene, encoding phosphohippolin, in susceptibility to schizophrenia. Am J Hum Genet. 2007;80:664–72.

44. Zhong N, Zhang R, Qiu C, Yan H, Valenzuela RK, Zhang H, et al. A novel replicated association between FXYD6 gene and schizophrenia. Biochem Biophys Res Commun. 2011;405:118–21.

45. Jiao L, Wang B, Niu Y, Ma X, Li J, Shen B, et al. A family-based association study of FXYD6 gene polymorphisms and schizophrenia. Zhonghua Yi Xue Yi Chuan Xue Za Zhi Zhonghua Yixue Xichuanxue Zazhi Chin J Med Genet. 2011;28:539–42.

46. Iwata Y, Yamada K, Iwayama Y, Anitha A, Thanseem I, Toyota T, et al. Failure to confirm genetic association of the FXYD6 gene with schizophrenia: the Japanese population and meta-analysis. Am J Med Genet Part B Neuropsychiatr Genet. 2010;153B:1221–7.

47. Hemby SE, Ginsberg SD, Brunck B, Arnold SE, Trojanowski JQ, Eberwine JH. Gene expression profile for schizophrenia: discrete neuron transcription patterns in the entorhinal cortex. Arch Gen Psychiatry. 2002;59:631–40.

48. Chang JT, Lowery LA, Sive H. Multiple roles for the Na,K-ATPase subunits, Atp1a1 and Fxyd1, during brain ventricle development. Dev Biol. 2012;368:312–22.

49. Sasaki M, Ishii A, Saito Y, Morisada N, Iijima K, Takada S, et al. Genotype-phenotype correlations in alternating hemiplegia of childhood. Neurology. 2014;82:482–90.

50. Muriel V, Garcia-Molina A, Aparicio-Lopez C, Ensenat A, Roig-Rovira T. Neuropsychological deficits in alternating hemiplegia of childhood: a case study. Rev Neurol. 2015;61:25–28.

51. Kawanabe Y, Yamasaki A, Kageyama Y, Hori H, Kato K, et al. Phosphohippolin is a critical component in the development of the human brain. Mol Brain Res. 2004;123:e534–e541.

52. Sasaki M, Ishii A, Saito Y, Morisada N, Iijima K, Takada S, et al. Generation of an animal model of alternating hemiplegia of childhood. Neurology. 2014;82:482–90.

53. Muriel V, Garcia-Molina A, Aparicio-Lopez C, Ensenat A, Roig-Rovira T. Neuropsychological deficits in alternating hemiplegia of childhood: a case study. Rev Neurol. 2015;61:25–28.

54. Hofman TH, Isaksen TJ, Glerup S, Heuck A, Bøttger P, Füchtbauer E-M, et al. Cognitive deficits caused by a disease-mutation in the α3 Na+/K+-ATPase isoform. Sci Rep. 2016;6:https://doi.org/10.1038/srep31972.

55. Frerking M, Ohliger P. New triggers and non-motor findings in a family with rapid-onset dystonia-parkinsonism. Park Relat Disord. 2012;18:737–41.

56. Barbano RL, Hill DF, Snively BM, Light LS, Boggs N, McCall WV, et al. New triggers and non-motor findings in a family with rapid-onset dystonia-parkinsonism. Park Relat Disord. 2012;18:737–41.
65. Kumra S, Oberstar JV, Sikich L, Findling RL, McClellan JM, Vinogradov S, et al. Efficacy and tolerability of second-generation antipsychotics in children and adolescents with schizophrenia. Schizophr Bull. 2008;34:60–71.

66. Ju J, Hirose S, Shi X-Y, Ishii A, Hu L-Y, Zou L-P. Treatment with oral ATP decreases alternating hemiplegia of childhood with de novo ATP1A3 Mutation. Orphanet J Rare Dis. 2016;11:55.

67. Consoli A, Raffin M, Laurent C, Bodeau N, Campion D, Amoura Z, et al. Medical and developmental risk factors of catatonia in children and adolescents: a prospective case-control study. Schizophr Res. 2012;137:151–8.

68. Bonnot O, Klinemann HH, Sedel F, Tordjman S, Cohen D, Walterfang M. Diagnostic and treatment implications of psychosis secondary to treatable metabolic disorders in adults: a systematic review. Orphanet J Rare Dis. 2014;9:65.