Missense variants in *ATP1A3* and *FXYD* gene family are associated with childhood-onset schizophrenia

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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 *ATP1A3* gene. *ATP1A3* encodes the α-subunit of a neuron-specific ATP-dependent transmembrane sodium–potassium pump. Using whole exome sequencing (WES) data

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derived from a cohort of 17 unrelated COS cases, we also examined *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 *ATP1A3* and three others cases with predicted pathogenic missense variants in the *FXYD* gene family (*FXYD1*, *FXYD6* and *FXYD6-FXYD2 readthrough*). *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 *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 *FXYD* gene family is novel and highlights the interest of exploring these genes in COS as well as in pediatric neurodevelopmental disorders.

Keywords

Introduction

Schizophrenia is a major mental disorder characterized by a spectrum of symptoms, including delusions, hallucinations, disorganisation of speech and behaviour, negative symptoms, and cognitive deficits. The age of onset of schizophrenia typically ranges from 15 to 25 years old, but rarely can begin before age 13. This early presentation is referred to as childhood-onset schizophrenia (COS) and has a similar presentation in the DSM compared to 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 one 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 characterised 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]. Alternating hemiplegia of childhood 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

1) 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-IIIR/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].

2) 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 4 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 ×. 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 (1,000 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.

3) 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].

4) Visualisation

To visualize the localisation 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.2401G>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.2443G>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 autism-spectrum disorder (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 & Dencker scale[28] and the score of 4 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 two 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. 2438T>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, twelve genes are expressed in the brain according to GTEx database (Supplementary Table). Possibly damaging variants in phylogenetically-conserved positions were identified in *ABCA2*, *FXYD1*, *FXYD6* and *FXYD6-FXYD2 readthrough* (Table 2). *FXYD6-FXYD2 readthrough* is a conjoined gene that generates transcripts by combining exons from *FXYD6* and *FXYD2*, which are on the same chromosome and in the same orientation. None of the variants were *de novo*. In total, we found 4 cases with rare damaging variants in *ATP1A3* and *FXYD* gene family in the American cohort.

We have represented the amino-acid (AA) changes in Figure 1. The *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 FXYD proteins are only found in the N-terminal region. How this FXYD region interacts with ATP1A3 remains unknown. Visualization of the AA changes in *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). *ATP1A3*, *FXYD1* and *FXYD6* were expressed in the brain during childhood, but information is not provided for *FXYD6-FXYD2*. However, this transcript has been

experimentally-validated and reported in the human brain in another study[29]. The expression of *ATP1A3* and *FXYD1* are quite stable during the lifespan, whereas the expression of *FXYD6* decreased with age.

Discussion

We have identified three rare pathogenic variants in the *ATP1A3* gene and three rare possibly damaging variants in *FXYD* gene family. *ATP1A3* is a replicated gene in rare neuropediatric diseases and here we strengthen the evidence linking it to COS. The association with *FXYD* gene family is novel. These genes are closely related as they participate to the same heteromeric transmembrane protein complex. Indeed, the transmembrane Na,K-ATPase complex is composed of an essential α- and β-subunit[30], and an auxiliary third subunit belonging to the FXYD proteins (sometimes named as the γ-subunit)[31]. The α-subunit is the catalytic subunit responsible for transport activities of the enzyme. The FXYD family has been identified as a modulator subunit of Na,K-ATPase by stabilizing the complex, altering its kinetic activity, regulating its affinity for Na^+, K^+, and ATP[32,33]. The subunits are tissue 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 adult-onset schizophrenia seems to be very low by comparison to 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 behavioural changes. We screened the literature to identify the cognitive deficits and behavioural 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 behavioural 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% to 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 to 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 behavioural 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 gene 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 to 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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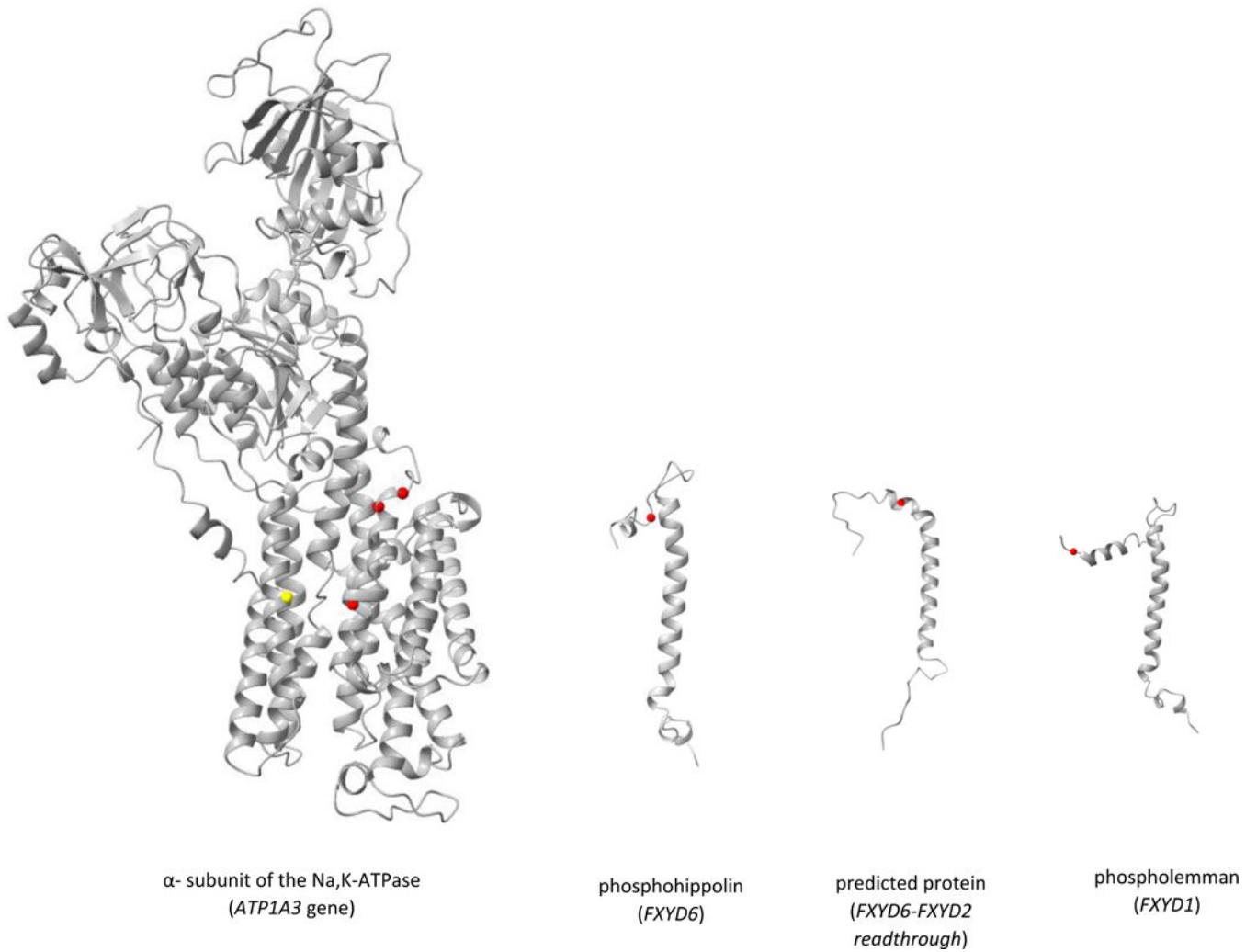
Figure 1.
Model of the mutations observed in our cohort (red) and previously reported in COS[38] (yellow) in *ATP1A3* and its brain-expressed interactors (*FXYD* gene family).

Table 1

summary of the molecular findings and the clinical presentation of the *ATP1A3* mutation carriers

	Case 1	Case 2	NSB1251
Mutation in *ATP1A3* gene	NM_152296.4(ATP1A3):c.2401G>A (p.Asp801Asn)	NM_152296.4(ATP1A3):c.2443G>A (p.Glu815Lys)	NM_152296.4(ATP1A3):c.2438C>T (p.Ala813Val)
Pathogenicity	Reported in ClinVar (ID:37108)	Reported in ClinVar (ID:37107)	Not reported, predicted damaging
Inheritance	De novo	De novo	Inherited from the mother
Age of onset for psychiatric symptoms	10	12	10
Sex	Male	Male	Male
Main psychiatric symptoms	Fluctuant visual and auditory hallucinations, delusions of persecution, psychomotor agitation and aggressiveness	Visual hallucinations (distortion of lights and shadows) followed by auditory and tactile hallucinations, delusion with persecutory and mystic ideas Negative symptoms with major social withdrawal	Positive symptoms: delusions and hallucinations
Dysmorphic features	Short philtrum, large ears with low implantation, gum hypertrophy, exotropia and macrocephaly	Macroglossy, nystagmus, esotropia and short philtrum and cleft palate	?
Neurodevelopmental delays	Moderate intellectual disability, developmental delays, reading and writing skills are not acquired	Walk at 25 months, first word around 4 years old, severe hypotonia	Intellectual disability with verbal intelligence quotient (IQ) of 75 and performance IQ of 57
Response to treatment	Poor	Poor	?
Associated phenotype	Recurrent major depressive disorder	Autism Spectrum Disorder	Autism Spectrum Disorder

Table 2

list of mutations in 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	Reference Allele	Mutant Allele	Gene symbol	Detailed annotation of the variant	Frequency in the 1000 Genome project	Frequency in the ExAC database	SIFT	Poly Phen	LRT	Mutation Taster	M-CAP	PhyloP vertebrate	GERP++ score
NSB1251	19	42474441	G	A	ATP1A3	exon18:c.C2438T:p.A813V	not reported	not reported	**0**	**0.999**	**0.000**	**1.000**	**0.773**	**9.531**	**3.69**
NSB1949	9	139906315	G	C	ABCA2	exon35:c.C5606G:p.A1869G	not reported	0.00002364	**0.01**	**0.955**	0.043	0.998	.	**9.195**	**2.95**
NSB1814	19	35633635	C	T	FXYD1	exon7:c.C268T:p.R90C	0.0014	0.007666	**0**	**1.0**	.	**1.000**	.	**3.554**	**4.12**
NSB2720	11	117693403	A	G	FXYD6-FXYD2	exon7:c.T302C:p.V101A	0.0009	0.0004984	**0**	**0.999**	**0.000**	0.967	**0.143**	**4.289**	**4.35**
NSB1553	11	117711076	C	G	FXYD6	exon6:c.G217C:p.G73R	0.0009	0.001911	**0.04**	0.175	.	**1.000**	**0.133**	**4.818**	**5.44**