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Molecular Case Studies

# A novel de novo mutation in *ATP1A3* and childhood-onset schizophrenia

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**Abstract** We describe a child with onset of command auditory hallucinations and behavioral regression at 6 yr of age in the context of longer standing selective mutism, aggression, and mild motor delays. His genetic evaluation included chromosomal microarray analysis and whole-exome sequencing. Sequencing revealed a previously unreported heterozygous de novo mutation c.385G>A in *ATP1A3*, predicted to result in a p.V129M amino acid change. This gene codes for a neuron-specific isoform of the catalytic α-subunit of the ATP-dependent transmembrane sodium–potassium pump. Heterozygous mutations in this gene have been reported as causing both sporadic and inherited forms of alternating hemiplegia of childhood and rapid-onset dystonia parkinsonism. We discuss the literature on phenotypes associated with known variants in *ATP1A3*, examine past functional studies of the role of ATP1A3 in neuronal function, and describe a novel clinical presentation associated with mutation of this gene.

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## INTRODUCTION

Patients with childhood-onset schizophrenia (COS) meet the same DSM criteria as typical late adolescent–adult onset schizophrenia (SZ) patients but with onset of psychosis before age 13. COS is rare, having a prevalence of ~1 in 40,000 (Gochman et al. 2011). The disease presents with a premorbid phase characterized by impairment in motor, social, and cognitive functioning (Addington and Rapoport 2009; Driver et al. 2013). It progresses to include a characteristic combination of symptoms that can include delusions, hallucinations,

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disorganized speech, grossly disorganized or catatonic behavior, negative symptoms (i.e., diminished emotional expression or avolition), and diminished functioning (Tandon et al. 2013). Although antipsychotics are the mainstay of treatment for COS, response rates are only moderate at best and most patients still suffer from marked functional impairments and lack effective treatment (Kumra 1996).

Efforts to understand the genetic architecture of SZ include genome-wide association studies of large patient cohorts and controls seeking to identify candidate loci in linkage with common variants (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014; Heinzen et al. 2015). Rare genetic variants also play a role in SZ risk; chromosomal microarray and exome sequencing studies have identified excesses of de novo protein-impactful variants in COS cases (Addington and Rapoport 2009; Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014). When these mutations occur in highly conserved or mutation-intolerant genes, the probability that they are disease-relevant mutations is potentially increased (Petrovski et al. 2013; Samocha et al. 2014; Ambalavanan et al. 2016). Subsequently, targeted functional studies of disease-associated mutations need to be undertaken to uncover the physiologic significance of the variants and to start to understand how they give rise to the associated phenotypes, including COS. Studies of COS-causing mutations may provide a window into the etiology of schizophrenia more broadly.

Here we describe a case of COS with a novel heterozygous de novo mutation in the *ATP1A3* gene. This gene codes for the catalytic α-subunit of a neuron-specific ATP-dependent transmembrane sodium–potassium pump. Previously, mutations in this gene have been associated with both sporadic and inherited forms of alternating hemiplegia of childhood (AHC) and rapid-onset dystonia parkinsonism (RDP). This case is noteworthy for the early and severe onset of psychotic symptoms associated with *ATP1A3* mutation without previous or simultaneous onset of motor phenotypes previously linked to the gene.

## RESULTS

### Clinical Presentation and Family History

The proband is a 9-yr-old Caucasian boy with a history of selective mutism and severe aggression who presented with command hallucinations and behavioral worsening meeting full DSM 5 criteria for COS at 6 yr of age. He was born full-term via emergency Cesarean section after maternal preeclampsia and fetal tachycardia. At birth, he weighed 8 lb, 11 oz, was noted to have difficulty breathing, and was admitted to the Neonatal Intensive Care Unit for 24 h. At 2 mo of age, he was diagnosed with decreased muscle tone for which he began receiving early intervention services, including physical therapy. He sat at 10 mo and took his first steps at 13 mo. He began babbling and speaking first words at 5 and 9 mo, respectively. He was toilet trained by age 4. Around 2 yr of age, he showed severe head banging.

At the age of 3, he was diagnosed with selective mutism, pervasive developmental disorder (NOS), and depression. He was described as having mood swings, lack of emotional control, and severe separation anxiety. He had severe self-injurious behaviors. For example, he tried to pull his teeth out and to cut his gingiva out with scissors. For the management of anxiety, he was started on clonazepam, which was then discontinued because of increased aggressive behavior.

At age 6 yr and 2 mo, he reported auditory hallucinations. He was found hitting himself in the head and said he was trying to silence the voices of two small boys that he described as having a “bed in his head.” These voices often said “bad things” and told him to hurt himself and others, and he felt he needed to obey them. He had a delusional conviction that the boys in his head were real. At this time, he also began experiencing diurnal enuresis, although he had toilet trained at age 4. The proband’s history of aggression toward his sister and dog worsened at this time and became highly unpredictable, to the extent that he could not be left alone with them or any other children. His mother noted that he had episodes of stiffness while sleeping. His physical examination at this time was unremarkable. He met diagnostic criteria for DSM 5 schizophrenia with hallucinations and delusions (Criterion A) and decreased level of functioning (Criterion B) persisting for 9 mo (Criterion C) with no major mood episode present during the majority of this time (Criterion D) and no discernible pharmacologic or medical cause explaining his symptoms (Criterion E) (American Psychiatric Association 2013).

There is no family history of birth defects, recurrent miscarriages, stillbirths, infant deaths, or consanguinity. The proband has one full sister who is 1 year younger and healthy.

The proband's father is in his 30s and healthy. He has a maternal half-sister who is healthy. This half-sister has five children—one healthy teenage son, one teenage son with impulsive behavior disorder, one teenage son with bipolar disorder and ADHD but no known psychotic symptoms, one healthy prepubertal son, and one healthy infant daughter. The proband's paternal grandfather's history is unavailable. The proband's paternal grandmother has fibromyalgia.

The proband's mother is in her late 20s and has depression. She has a twin brother with ADHD. That twin has a prepubertal daughter who is healthy. The proband's mother has three maternal half-siblings who are full siblings to one another. One, a female, has epilepsy and developmental delay. Another male has significant anxiety but has two healthy children. There are some distant maternal cousins with autism spectrum disorders (details unknown). The proband's maternal grandfather has a history of addiction. The proband's maternal grandmother has depression.

## Neurological Assessment

Following recognition of psychosis, the patient underwent neurological assessment. His neurologic examination showed intact extraocular movements and pupils that were equally round and reactive. His facial strength was symmetric and normal. His jaw strength was normal. His palate raised symmetrically. His tongue was midline and had normal strength bilaterally. His red reflex was noted bilaterally. He had normal muscle bulk and tone and full strength in the upper and lower extremities bilaterally. His sensory examination was intact to light touch. His deep tendon reflexes were normal and symmetric, as were his finger to nose movements and gait. He was able to walk on his heels and toes and to tandem gait, as well as to hop on either foot and to run 20 ft without difficulty.

He had a clinical brain MRI (magnetic resonance imaging) that was read as within normal limits and an EEG (electroencephalograph) that was read as abnormal because of arrhythmic diffuse slowing. This latter finding may be explained by his medication regimen but is also consistent with excess $\theta$ and $\delta$ activity in the EEG of patients with SZ (Kim et al. 2015).

Following the identification of the de novo *ATP1A3* mutation (below), the proband has been screened for any motor or autonomic symptoms, including episodic symptoms, and has had none other than the episodes of stiffness in sleep noted in his clinical presentation.

## Treatment Outcomes

The proband's auditory hallucinations initially responded to risperidone (with benztropine added to prevent extrapyramidal symptoms), but he was switched to olanzapine in an effort to control his aggression. His selective mutism and enuresis resolved completely. A few months later he developed depressive symptoms that resolved with the addition of fluoxetine. After being stable for 7 mo, he began hearing voices again; thus, haloperidol was added to olanzapine and fluoxetine. Guanfacine and atomoxetine were added to manage his ADHD symptoms, but resulted in increased aggression. Lithium was prescribed to address his mood fluctuations with some initial benefit. At age 9, he began to show echolalia. After 18 mo without auditory hallucinations, and while continuing to take olanzapine, haloperidol, fluoxetine, benztropine, and lithium, he started hearing voices again. This was accompanied by increased aggression and frequent diurnal enuresis and encopresis, similar to when his psychotic symptoms first became evident. Increasing his antipsychotic medication has seemed to reverse this relapse for now.

## Genomic Analyses

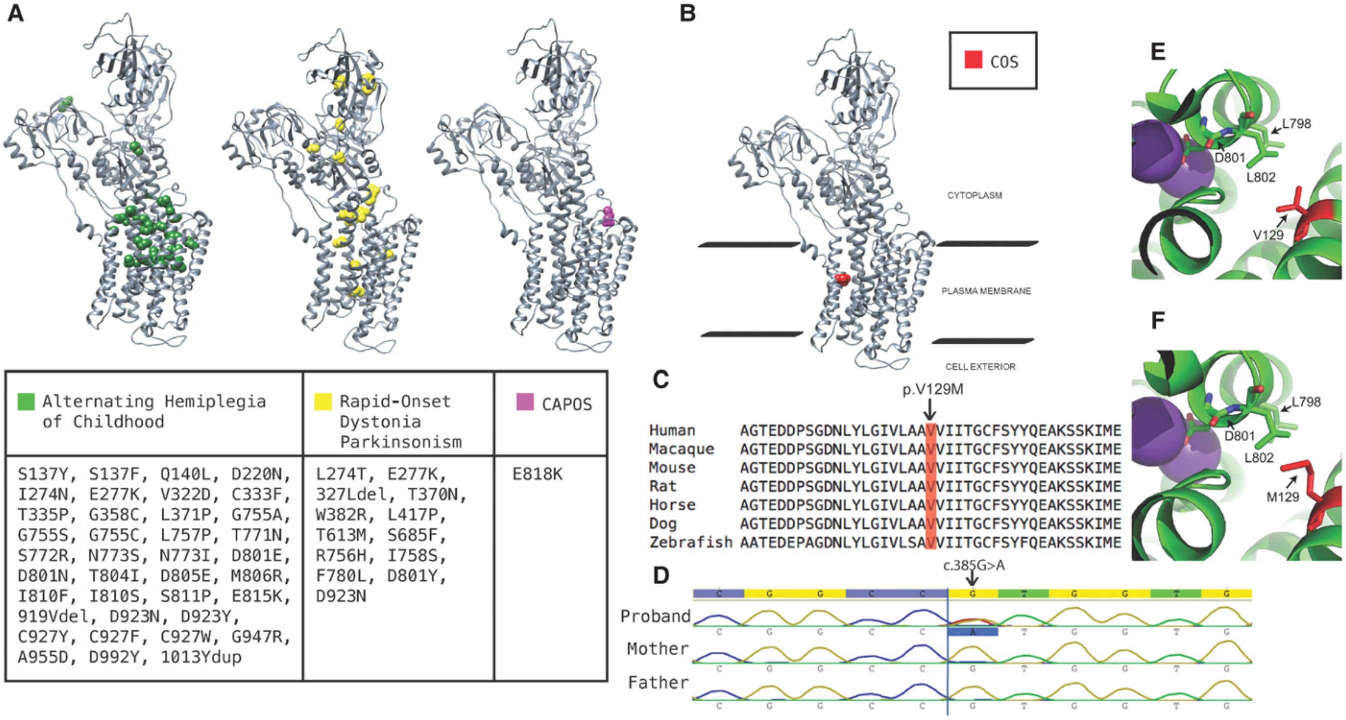
The proband was first assessed using chromosomal microarray, which did not show any copy-number variants. Details about the stringency of this assay are provided in the Methods section. Next, the proband and both parents underwent whole-exome sequencing (WES) from peripheral blood lymphocytes which identified a high-confidence de novo missense change in *ATP1A3* corresponding to NM_152296.4:c.385G>A and p.V129M. Coverage information for this sequencing is provided in Table 1. Sanger sequencing from peripheral blood was performed on the proband, both parents, and the proband's healthy sibling, and the sequencing confirmed the presence of this variant in the proband and its absence in his parents and sister (Fig. 1D). This variant has not been previously described, but other *ATP1A3* variants have been documented in association with a range of autosomal-dominant neurological and psychomotor phenotypes discussed below. Furthermore, this gene was examined using the Exome Aggregation Consortium's browser (Samocha et al. 2014) and the Genic Intolerance database (Petrovski et al. 2013), both of which provide a quantification of the probability of gene-level intolerance to functional variation. Both analyses show that this gene is highly intolerant to variation, increasing the likelihood that mutation in this gene is associated with disease (Table 2). Analysis of the p.V129M variant using the SIFT (Sorting Intolerant from Tolerant) and Polyphen-2 algorithms led to predictions that this change is deleterious by both methods (SIFT score 0, PolyPhen-2 score 0.999) (Kumar et al. 2009; Adzhubei et al. 2010). Additional details of this variant are summarized in Table 2.

Molecular modeling of ATP1A3 p.V129M was performed on several models to examine the potential effect of this mutation on the function of the protein. First, the variant of interest was modeled alongside previously described variants using a predicted structure produced from the relevant amino acid sequence (Fig. 1A,B). Next, the affected residue was examined for conservation across species (Fig. 1C). Finally, the specific amino acid change was modeled relative to the crystal structure of the homologous sodium–potassium pump ATP1B1 in *Squalus acanthias* (Shinoda et al. 2009), which has 92% amino acid sequence homology with human ATP1A3 (see Fig. 1E,F). Homologous positions given here are relative to ATP1A3 numbering, but the relationships described between residues were examined in the ATP1B1 crystal structure. This modeling by homology shows that the mutation of interest may affect the potassium-binding residues in this channel; residue V129 is located in a

**Table 1.** Sequencing coverage information

| Sample | Number of reads (millions) | Mean coverage | Unmapped reads (%) | Target region >10× (%) | Target region >20× (%) | Coverage at *ATP1A3* c.385 (reads) |
|---|---|---|---|---|---|---|
| Proband | 68.4 | 61.9 | 0.16 | 96.30 | 89.80 | 40 |
| Mother | 57.7 | 51.5 | 0.90 | 94.90 | 84.90 | 21 |
| Father | 65.1 | 59.3 | 0.15 | 95.90 | 88.60 | 49 |

Sequencing coverage information for the proband and parents. The target region comprises 44.1 Mb as defined by the EZ Exome 2.0 capture kit used for sequencing. The coverage at the site of the variant of interest is included to give context for the trio genotype obtained from exome sequencing at that site.



| 🟩 Alternating Hemiplegia of Childhood | 🟨 Rapid-Onset Dystonia Parkinsonism | 🟪 CAPOS |
|---|---|---|
| S137Y, S137F, Q140L, D220N, I274N, E277K, V322D, C333F, T335P, G358C, L371P, G755A, G755S, G755C, L757P, T771N, S772R, N773S, N773I, D801E, D801N, T804I, D805E, M806R, I810F, I810S, S811P, E815K, 919Vdel, D923N, D923Y, C927Y, C927F, C927W, G947R, A955D, D992Y, 1013Ydup | L274T, E277K, 327Ldel, T370N, W382R, L417P, T613M, S685F, R756H, I758S, F780L, D801Y, D923N | E818K |

| | |
|---|---|
| Human | AGTEDDPSGDNLYLGIVLAAVVIITGCFSYYQEAKSSKIME |
| Macaque | AGTEDDPSGDNLYLGIVLAAVVIITGCFSYYQEAKSSKIME |
| Mouse | AGTEDDPSGDNLYLGIVLAAVVIITGCFSYYQEAKSSKIME |
| Rat | AGTEDDPSGDNLYLGIVLAAVVIITGCFSYYQEAKSSKIME |
| Horse | AGTEDDPSGDNLYLGIVLAAVVIITGCFSYYQEAKSSKIME |
| Dog | AGTEDDPSGDNLYLGIVLAAVVIITGCFSYYQEAKSSKIME |
| Zebrafish | AATEDEPAGDNLYLGIVLSAVVIITGCFSYFQEAKSSKIME |

**Figure 1.** (*A*) Models of previously published mutations in ATP1A3. List compiled from Termsarasab et al. (2015) and Heinzen et al. (2012). CAPOS, cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss. Mutations modeled using UCSF Chimera package and Phyre2 web portal relative to UniProt P13637 (Pettersen et al. 2004; Kelley et al. 2015). (*B*) V129M mutation. COS, childhood-onset schizophrenia. (*C*) Sequence conservation plot produced using Clustal Omega via EMBL-EBI (European Molecular Biology Laboratory European Bioinformatics Institute) (Goujon et al. 2010; Sievers et al. 2011) (http://www.ebi.ac.uk/Tools/msa/clustalo/). (*D*) Sanger sequencing results plotted using Geneious, version 8.1.4 (Kearse et al. 2012). (*E*,*F*) Molecular modeling of the p.V129M using the homologous protein ATP1B1 in *Squalus acanthias* (PDB code: 2ZXE) with position numberings relative to ATP1A3. The model was visualized using PyMOL (The PyMOL Molecular Graphics System, v.1.8, Schrödinger, LLC).

transmembrane domain and forms hydrophobic interactions with residues L798 and L802 near the potassium-binding residue D801. The additional side-chain bulk from the V129M mutation may thereby push the transmembrane helix containing L798 and L802 toward the sodium–potassium channel and alter channel function.

**Table 2.** Variant table

| Gene | Chr | HGVS DNA | HGVS protein | Variant type | Variant allele fraction | SIFT score | PolyPhen-2 score | Genotype | ExAC MAF | ExAC constraint *z*-score | RVIS Percentile |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *ATP1A3* | 19 | c.385G>A | p.V129M | SNV | 42% of 40 reads | 0 | 0.999 | Het | 0% | 7.38 | 3.37 |

*ATP1A3* reference sequence = NM_152296.4, ENST00000302102. The ExAC constraint *z*-score from Broad Institute's ExAC browser compares the expected frequency of functional variation to the observed frequency, where a large positive *z*-score indicates a gene significantly depleted for variation (Samocha et al. 2014) (http://exac.broadinstitute.org/faq). The RVIS indicates the percentile of variation intolerance, where lower percentiles are more intolerant (Petrovski et al. 2013) (http://genic-intolerance.org).

HGVS, Human Genome Variation Society; ExAC, Exome Aggregation Consortium (http://exac.broadinstitute.org); RVIS, residual variation intolerance score; SNV, single-nucleotide variant; MAF, minor allele frequency.

It should be noted that the Platypus variant calling algorithm used performs local assembly to identify candidate variation, and the authors of this software report that this algorithm is sensitive to deletions up to 1 kb and insertions up to several hundred bases (Rimmer et al. 2014). Thus, for the capture region of the exome performed and within the resolution limits of the chromosomal microarray analysis (CMA) and exome variant calling performed, the patient was deemed to have a normal copy number.

## DISCUSSION

The proband in this report presented with COS with suspected genetic abnormalities. Trio WES identified a single high-confidence de novo variant of strong predicted impact in the *ATP1A3* gene, which codes for isoform 3 of the α-subunit of the $Na^+/K^+$-ATPase complex. Figure 1 shows the location of this patient's mutation in relation to previously observed disease-associated mutations in this protein, as well as known ion-binding sites. Using the ACMG (American College of Medical Genetics and Genomics) guidelines for variant interpretation, this variant meets the criteria for "likely pathogenic," based on the presence of de novo data, multiple lines of computational evidence, population evidence on the prevalence of disease-associated missense variation in this gene, and the proximity of the variant to a well-established functional domain for the protein (Richards et al. 2015). The variant has never been seen in healthy controls, the gene is highly depleted for functional variation compared with a model of expected background variation (ExAC constraint *z*-score 7.38; Samocha et al. 2014) and relative to a common variation in comparable genes (RVIS percentile score 3.37% Petrovski et al. 2013), and missense variation in the gene is previously well-documented to be associated with disease (Brashear et al. 2012). The variant was confirmed to be de novo, and no one else in this family is known to have a psychotic phenotype. Additionally, the variant is predicted to be deleterious by both SIFT and PolyPhen-2 algorithms. As stated, the mutation observed in this patient meets the ACMG criteria for "likely pathogenic," and given the observed association with the patient's phenotype as well as supporting literature discussed below, we investigate the plausibility of a connection between the mutation and the phenotype. However, it should be noted that we do not here present causal evidence to connect this patient's mutation with his phenotype.

The ATP1A3 protein complex plays a key role in establishing the resting membrane voltage in neurons and other electrically active cells by primary active transport of three sodium ions out of the cell and two potassium ions into the cell. There are four genes encoding this α-subunit (*ATP1A1*, *ATP1A2*, *ATP1A3*, *ATP1A4*), which is the major subunit responsible for ATP hydrolysis and for ion binding. The α-3 gene is neuron specific and primarily expressed in central nervous system neurons, notably in GABAergic projection neurons of the basal ganglia (Hieber et al. 1991; Lingrel 1992; Bøttger et al. 2011). Mutations in this subunit of the protein complex have been documented in association with a spectrum of psychological, psychomotor, and neuromuscular abnormalities (Brashear et al. 2007; Ozelius 2012; Rosewich et al. 2014; Sweney et al. 2015). Therefore, it is valuable to review the documented breadth of phenotypic variation associated with mutations found in this protein.

### Motor Phenotypes of *ATP1A3* Mutations

AHC (Heinzen et al. 2012; Ishii et al. 2013) is characterized by paroxysmal eye movements with onset in the first few months of life and paroxysmal focal dystonia and flaccid alternating hemiplegia with onset before 18 mo (Gergont and Kaciński 2014). Symptoms of AHC are often episodic and may be triggered by physical stressors such as extreme change in body temperature, fatigue, and infection, as well as psychological or emotional stressors (Brashear et al. 2007). There is some suggestion that episodes may be alleviated by sleep

(Panagiotakaki et al. 2015). Panagiotakaki et al. (2015) have recently reported that a cumulative total of 132 of 155 observed patients with AHC show likely causal de novo *ATP1A3* mutations (Panagiotakaki et al. 2015).

RDP (Rodacker et al. 2006; Brashear et al. 2007) is diagnosed by bulbar weakness with dysphagia and dysarthria, dystonia, and parkinsonism. Typical onset is during adolescence or adulthood in response to a physiological stressor (Barbano et al. 2012; Rosewich et al. 2014). A variety of triggers have been reported to produce symptom onset, including acute alcohol consumption (Rosewich et al. 2012), extreme temperature change, head trauma, fever, strenuous exercise, and psychological stressors (Goldstein et al. 2009). Brashear et al. (2007) reported that 36 of a group of 49 individuals referred with symptoms of RDP had likely causal mutations in *ATP1A3* (Brashear et al. 2007).

A third clinical disorder recently associated with mutations in *ATP1A3* is CAPOS syndrome. Diagnostic criteria for CAPOS include the constellation described within the name —cerebellar ataxia, areflexia, pes cavus, optic atrophy, and sensorineural hearing loss—as well as abnormal eye movements. Patients were described broadly to show episodic ataxic encephalopathy, commonly triggered by fever (Brashear et al. 2012). CAPOS syndrome has been observed in only 10 patients from three unrelated families to date (Demos et al. 2014). Importantly, these past descriptions all show a causal segregation of mutations in *ATP1A3* with syndromic motor phenotypes. Remarkably, to date, the proband in this study has not experienced motor disturbances resembling any of these previously described phenotypes. Although the proband is older than the typical age of onset for AHC, he is still younger than the typical age of onset for RDP, and he will continue to be monitored for the possible appearance of motor symptoms. The proband's young age and psychosis precludes his ability to provide accurate history needed to determine whether his psychotic symptoms have episodic characteristics, as is seen with motor phenotypes in AHC.

## Psychiatric Phenotypes of Mutations in Gene Family ATP1A

Mutations in *ATP1A3* and in the larger *ATP1A* gene family have also been linked with psychiatric symptoms. Brashear et al. (2012) compared psychiatric histories for a cohort of 26 patients manifesting motor symptoms because of *ATP1A3* mutations and 27 noncarrier control family members. They found that the affected group had approximately two times greater incidence of bipolar disorder and depression than the control group (13 of 26 affected patients also had mood disorder compared with 6 of 27 controls) and a substantial incidence of psychosis where the control group had none (5 of 26 affected patients compared with 0 of 27 controls) (Brashear et al. 2012). This represents a dramatically increased incidence of psychiatric symptoms in association with mutations in *ATP1A3*. Furthermore, Goldstein et al. (2009) found that when comparing 126 subjects with bipolar disorder to their unaffected family members, the presence of single-nucleotide polymorphisms (SNPs) in *ATP1A1*, *ATP1A2*, and *ATP1A3* correlated significantly with disease state (Goldstein et al. 2009).

Mutations in the functionally homologous α-2 isoform (*ATP1A2*) are associated with type 2 familial hemiplegic migraine (FHM2) and benign familial infantile epilepsy (Vanmolkot et al. 2003; Haan et al. 2005; Gardner 2006; Pietrobon 2007). These disorders have motor phenotypes similar to AHC and RDP (Bassi 2004), and FHM2 may additionally be associated with psychotic symptoms in the form of psychotic migraine auras, which may last for days and consist of complex delusions (Barros et al. 2012; LaBianca et al. 2015). Thus, it is plausible to consider that variation in *ATP1A3* may also present with similar psychiatric symptoms.

## Observations from Tissue, and Immortalized and Primary Cell Lines

There is evidence for changes in the level of ATP1A3 protein in postmortem samples of auditory cortex from schizophrenia patients compared with controls (MacDonald et al.

2015). Liquid chromatography–mass spectrometry analysis showed a significant decrease in ATP1A3 protein level, specifically in subjects with a history of auditory hallucinations (MacDonald et al. 2015). This alteration is likely not due to antipsychotic treatment, because chronic treatment with antipsychotics in rhesus monkeys produced an increase in postmortem protein level (MacDonald et al. 2015). *ATP1A3* mRNA levels were also reduced in a microarray study of postmortem samples of prefrontal cortex from schizophrenia patients who committed suicide (Tochigi et al. 2008).

Consistent with findings of decreased mRNA and protein in patient-derived samples, introducing RDP-associated mutant *ATP1A3* into HEK293T cells results in less protein expression compared with wild type (de Carvalho Aguiar et al. 2004). Furthermore, the effect of the potent $Na^+/K^+$ ATPase inhibitor ouabain can be reversed in HEK (human embryonic kidney) cells by transfecting in mutant ATP1A3 protein that is resistant to ouabain. However, if the ouabain-resistant ATP1A3 protein also contains an RDP-associated mutation, it fails to rescue HEK cells from ouabain treatment, indicating that these mutations reduce function (de Carvalho Aguiar et al. 2004). In COS-7 cells, both AHC- and RDP-associated mutations caused a reduction in ATP1A3 enzyme function as measured by an ATPase assay, while only RDP-associated mutations also caused a decrease in protein levels measured on western blot (Heinzen et al. 2012). Finally, slice cultures from heterozygous *Atp1a3* knockout mice show abnormal synaptic behavior, including an increased frequency of mini-iPSC release and a decreased threshold for firing from electrical stimulation, suggesting that reductions in this protein affect neuronal physiology (Ikeda et al. 2013). Similar functional studies of ATP1A3 p.V129M will be necessary to help further establish the pathogenicity of this variant.

## Downstream Affected Pathways and Their Disease Relevance

To understand the potential impact of ATP1A3 p.V129M on this patient, it is useful to consider the potential downstream effects of an altered sodium gradient in affected neurons. At the cellular level, there may be impaired recovery of resting membrane potential after action potential firing, such that prolonged stimulation without recovery could result in a loss of neuronal excitability (Dobretsov 2005). In addition, downstream secondary active transporters such as the sodium–calcium exchanger (NCX) are dependent on the sodium gradient; depletion of this gradient can lead to reversal of NCX (Dobretsov 2005; Khananshvili 2014) as is seen with ouabain-induced toxicity in cardiac cells (Balasubramaniam et al. 2015). Ouabain impairment also causes membrane depolarization, disruption of normal synaptic activity (Azarias et al. 2013), and increased calcium influx in glutamatergic and cholinergic neurons in response to applied neurotransmitter (Song et al. 2013). Transporters for many neurotransmitters are downstream from ATP1A3 function, including glutamate, GABA, glycine, serotonin, and dopamine (Shi et al. 2008; Rose et al. 2009). Therefore, impaired sodium gradients may result in impaired neurotransmitter clearance from the synapse (Camacho and Massieu 2006; Raiteri and Raiteri 2015). Additionally, a number of proteins have been observed to directly interact with ATP1A3, including Src kinase (Li and Xie 2009), a glycine transporter (de Juan-Sanz et al. 2013), and agrin (Hilgenberg et al. 2006). Thus, mutations of *ATP1A3* may produce exacerbated functional changes if these interactions are disrupted.

At the neural circuit level, expression of *ATP1A3* gene product is strongest in GABAergic neurons of basal ganglia and, to some extent, in dopaminergic neurons of the ventral tegmental area (Bøttger et al. 2011), suggesting that these neurons may be particularly vulnerable to functional disturbances because of mutations in the *ATP1A3* gene. Consistent with this distribution, each of the described phenotypes of *ATP1A3* mutations share some features with disturbances of the basal ganglia that also present with a combination of motor and psychiatric abnormalities, including Parkinson's, Huntington's, and progressive supranuclear palsy (Aarsland et al. 2014; Burn et al. 2014; Marras et al. 2014). Furthermore, dysfunction in these circuits fits with existing understanding of the etiology of motor gating abnormalities, schizophrenia, and other sensory gating abnormalities involving GABAergic neurons of basal ganglia and dopaminergic neurons of ventral tegmentum (Davis 1974; Egerton et al. 2013; Heckers and Konradi 2013). Given the evidence for high expression localized to these regions, and their past implication in relevant disease phenotypes, functional defects in this circuit may explain a portion of the phenotype observed in the proband of this study. Further research is needed to show whether cell type–specific functional impairment due to $Na^+/K^+$ ATPase mutation may explain the observed phenotypes in patients.

## METHODS

Upon enrollment in the Manton Center for Orphan Disease Research, a standard assessment of the proband was performed, which documented physical features and recorded medical, developmental, psychiatric, and family history, supplemented by medical records.

The proband underwent CMA of peripheral blood lymphocytes. Briefly, the patient was assessed using an Agilent custom 4 × 180k CGH+SNP chip with a standard Agilent protocol. CNV analysis was performed using Agilent CytoGenomics (v3.0.0.27). The thresholds used for sample quality required a derivative $\log_2$ ratio of <0.2 and a SNP call rate of >0.85, and at least five consecutive oligonucleotide probes were required to support a putative copy-number alteration (Claritas Genomics). It should be noted that CMA is a coarse-grained assay and not intended to identify copy-number alterations at the single-nucleotide or single-exon scale.

WES was provided by Yale University Center for Mendelian Genomics on an Illumina HiSeq 2000 instrument with blood samples pooled six per lane, using 74-bp paired-end sequencing. Libraries (TruSeq DNA v2 Sample Preparation kit) and whole-exome capture (EZ Exome 2.0, Roche) were performed according to manufacturer protocols. FASTQs were filtered, aligned, and variants were called, filtered, and annotated by Codified Genomics. Briefly, reads were aligned to UCSC's hg19 reference genome using BWA (Burrows–Wheeler aligner)-MEM (v0.7.5-a). BAMs were duplicate marked, realigned, and recalibrated with GATK (Genome Analysis Toolkit), v.2.5.2 (McKenna et al. 2010; DePristo et al. 2011; Van der Auwera et al. 2013) and Picard Tools (v.1.38; http://picard.sourceforge.net). Variants were identified in the proband and parents using pooled variant calling with Platypus using default parameters (Rimmer et al. 2014); the resulting variants were processed to obtain de novo variants as described previously (Bainbridge et al. 2013). Analysis showed that sequencing in the proband achieved an average of 61.9-fold coverage; for the 44.1-Mb target region in the exome capture kit used, 96.3% of sites were covered at least 10-fold in the proband, and 89.8% of sites were covered at least 20-fold in the proband. Coverage information for the trio is provided in Table 1. Sanger confirmation of the candidate variant was performed at Boston Children's Hospital Manton Center Gene Discovery Core. Sanger sequencing data were viewed using Geneious (v8.1.4) (Kearse et al. 2012). Protein models were constructed using the Phyre2 web portal (Kelley et al. 2015) and viewed using the UCSF Chimera package (Pettersen et al. 2004). The variant identified in the patient was also modeled using PyMOL (The PyMOL Molecular Graphics System, v.1.8, Schrödinger, LLC). Multiple sequence alignment was performed using Clustal Omega via EMBL-EBI (Goujon et al. 2010; Sievers et al. 2011).

## ADDITIONAL INFORMATION

### Data Deposition and Access

National Human Genome Research Institute/National Heart, Lung, and Blood Institute (NHGRI/NHLBI) Centers for Mendelian Genomics facilitate data sharing via public release of causal variants and candidate genes and submission of exome and genome sequence data to dbGaP (http://www.ncbi.nlm.nih.gov/gap; accession number phs000744.v4.p2) and ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/; SCV000267646).

## Ethics Statement

DNA for genetic testing and the medical records of the patient and his biological parents were collected by The Manton Center for Orphan Disease Research, Gene Discovery Core under written informed consent governed by the Institutional Review Board of Boston Children's Hospital.

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## Author Contributions

N.S.M. analyzed the data, researched and interpreted the literature relevant to mutation, and wrote the manuscript. C.A.B. directed the genetic investigation, including sequencing, and data interpretation, found the *ATP1A3* mutation, and helped edit the manuscript. S.V. reviewed the clinical history and revised the manuscript. S.K.T. reviewed and revised the manuscript and made the figures and helped review the clinical history. M.C.T. worked with and consented the patient and family and helped gather patient data. J.S. helped with modeling the variant. E.G.-C. performed Sanger confirmation of the variant of interest. K.X.L. helped research and interpret the literature relevant to the mutation. K.B. helped perform the sequencing. R.J.K. helped interpret the data and literature relevant to the mutation and helped review and revise the manuscript. T.W.Y. helped analyze and interpret the data. A.T. and G.T.B. contributed to the clinical understanding of the case. A.H.B. contributed to the interpretation of the genetic data. M.-J.H. contributed to the genetic analysis. P.B.A. aided in the interpretation of the genetic and clinical data. J.G.-H. oversaw the project, treated the patient, recognized the clinical phenotype, referred the subject for sequencing, led the clinical interpretation of the data and literature, and helped edited the manuscript.

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### Competing Interest Statement

In the past 3 years, J.G.-H. has received grant support from the Tommy Fuss Fund, the Al Rashed Family, and GlaxoSmithKline. He holds equity in Neuro'motion, a company that develops emotional regulation training technology. In previous years, he has served as a consultant to Abbott Laboratories, Pfizer Inc., Johnson & Johnson (Janssen, McNeil Consumer Health), Novartis, Parke-Davis, GlaxoSmithKline, AstraZeneca, and Seaside Therapeutics; has been a speaker for Abbott Laboratories, Pfizer Inc., Novartis, Bristol-Meyers Squibb; and has received grant support from Abbott Laboratories, Pfizer Inc., Johnson & Johnson (Janssen, McNeil Consumer Health), Akzo-Nobel/Organon, and the NIMH. R.J.K. is currently a consultant for Ironwood Pharmaceuticals and has consulted for En Vivo Pharmaceuticals in the past. She was formerly an employee of Pfizer for 12 years and of Selventa for 1 year.

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