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Consolidating the Role of Mutated *ATP2B2* in Neurodevelopmental and Cerebellar Pathologies

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Keywords: *ATP2B2* | cerebellar atrophy | developmental delay | intellectual disability | movement disorder | neurodevelopmental disorder

ABSTRACT

Plasma membrane calcium ATPases (PMCAs) encoded by *ATP2B* genes have been implicated in Mendelian diseases with ataxia, dystonia, and intellectual disability. Work to date has shown that *ATP2B2* (encoding PMCA2) is required for synaptic function and Purkinje-cell integrity in the cerebellum. A recent case series has linked *ATP2B2* to a novel entity, characterized by neurodevelopmental and movement phenotypes, in only seven individuals. We called for collaboration to collect five unpublished families affected by the new rare *ATP2B2*-related condition. Exome-/genome sequencing-identified genotypes included four likely pathogenic/pathogenic heterozygous de novo missense variants and one dominantly inherited end-truncating frameshift allele. The six affected individuals shared features with the described patients including developmental delay, cognitive disturbances, epilepsy, autistic traits, and motor disorders. Striking cerebellar atrophy was observed in one affected individual. In association with hearing loss and movement abnormalities, we report a recurrent p.(Glu457Lys) substitution, previously documented in a neurologically impaired *ATP2B2* mouse mutant. Our study further delineates the mutational spectrum and presentation of a human syndrome caused by *ATP2B2* variants, confirming the importance of PMCA2 in neurotypical and cerebellar development.

1 | Introduction

Plasma membrane calcium (Ca^{2+}) ATPase (PMCA) is a high-affinity calmodulin-dependent protein that ejects cytoplasmic Ca^{2+} to the extracellular space [1]. Intracellular Ca^{2+} concentrations are regulated within a tight range via an orchestrated system of channels, exchangers, and pumps including PMCAs [1]. PMCA binds an intracellular Ca^{2+} ion, transporting it through the plasma membrane and releasing it into the external compartment after a series of conformational changes. Four PMCA isoforms are known, each encoded by a separate gene (*ATP2B1-4*) [1].

Variants in three of the four PMCA-encoding genes have been linked to monogenic disorders in OMIM: heterozygous variants in *ATP2B1* underlie "intellectual developmental disorder, autosomal dominant 66" (MIM:619910), and hemizygous *ATP2B3* variants cause "spinocerebellar ataxia, X-linked 1" (MIM:302500); further, heterozygous variants in *ATP2B2* are reported to be implicated in "deafness, autosomal dominant 82" (MIM:619804), supported by the publication of independent hearing loss-affected families carrying loss-of-function (LoF) *ATP2B2* alterations [2]. Only recently, a new allelic disorder associated with *ATP2B2* has begun to be delineated based on our pilot study in which we identified a set of seven patients with neurodevelopmental-disease features and rare de novo variants in this gene [3]. A single individual with *ATP2B2*-related neurodevelopmental disorder was described in 2018 [4]. However, no additional cases with this distinct *ATP2B2*-related condition have been reported.

Herein, we provide additional evidence for a causal role of missense and C-terminally located LoF variants in *ATP2B2* in neurodevelopmental syndromes with movement abnormalities and cerebellar involvement. Via multisite collaboration, we collected six previously undescribed *ATP2B2*-mutated individuals from five unrelated families with heterogenous developmental, cognitive, epileptic, and motor phenotype presentations (Table 1).

2 | Methods

Case recruitment and genetic analysis were performed as previously reported [3]. Extended methods are provided in the Supporting Information. The study received ethical approval by Technical University Munich (#5360/12S). For patients who were sequenced within routine diagnostic settings, institutional review board approval was not required. Legal representatives provided consent to share clinical information and molecular results.

3 | Results

3.1 | *ATP2B2* Genotypes

Four unique *ATP2B2* de novo missense variants were identified (Table 1; Figure 1): NM_001001331.4: c.1369G > A, p.(Glu457Lys) (Family 1); c.1340G > A, p.(Gly447Asp) (Family 2); c.2750T > C, p.(Met917Thr) (Family 3); and c.673G > A, p.(Asp225Asn) (Family 4). The first variant was listed in ClinVar in two separate entries as a de novo event in independent individuals (ClinVar-ID: 421722). Additionally, an unreported dominantly inherited LoF variant, NM_001001331.4: c.3266_3267del, p.(Leu1089GlnfsTer60), was ascertained (Family 5; Table 1; Figure 1). The missense variants were located within transmembrane domains and the N-terminal cytosolic loop domain of the encoded protein. One of these variants, p.(Glu457Lys), was identical to a missense variant reported in a rodent model of PMCA2-related disease (designated as p.(Glu412Lys) in the alternative transcript NM_001683.5) [5]. Moreover, the missense variants affected highly conserved residues in protein regions that were depleted for rare nonsynonymous variation (MetaDome) [6]. The LoF variant was positioned in the penultimate exon in close proximity to two previously described frameshift alleles causing *ATP2B2*-associated neurodevelopmental disorder. For additional variant details, see Table 1 and Figure 1.

3.2 | Associated Conditions

Patient I from Family 1 was an 8-year-old girl from Germany. Motor and cognitive developmental delay became evident in the first 2 years of life. She was able to walk unaided at the age of 20 months, and she presented with profound speech impairment. Muscular hypotonia was associated with feeding difficulties and she was always dystrophic. Neuropsychological evaluation at 6 years of age established a mild form of intellectual disability with a moderately reduced IQ of 60 and a behavioral disorder with autistic-type symptoms. Motor delay evolved into incoordination with falls, and neurological assessment at the age of 4 years documented the presence of ataxia with gait impairment. She showed neural hearing loss, which was formally diagnosed by age 5.5 years. The patient has received speech therapy and she had hearing aids since age 6. Bilateral myopia (−5 dpt) was also present. She had no seizures and a brain MRI was not performed.

Patient II from Family 2 was a 3-year-old boy from the United States. Global developmental delay was noted, and he was diagnosed with intellectual disability and severe speech abnormalities. He remained non-verbal but understood simple commands. Myoclonic and generalized seizures began at 1.5 years of age and were only partially controlled with antiepileptic medication. Multiple follow-up examinations revealed a dominant movement disorder with low muscle tone, unsteadiness of postures and gait, tremor, and head titubation. He was found to have signs of cerebellar dysfunction including truncal and appendicular hypotonia, ataxia of the trunk and limbs, and intermittent horizontal nystagmus. The course of the disease was progressive over 2.5 years. Serial MRI was performed (9 months to 2.5 years). At 9 months of age, imaging showed severe diffuse cerebellar atrophy, particularly in the vermis and hemispheres, with the presence of an abnormal cross-like pontine signal, indicative of brainstem atrophy ("hot cross bun" sign). By 2 years of age, the cerebellar atrophy had mildly progressed, along with a new elongated focus of signal abnormality in the right frontal centrum semiovale, potentially related to gliosis. The most recent MRI at 2.5 years revealed no significant interval change, with persistent severe cerebellar and brainstem atrophy, the same elongated

TABLE 1 | Overview of clinical details for patients with heterozygous likely pathogenic/pathogenic *ATP2B2* variants.

Family/patient	1/I	2/II	3/III	4/IV	5/V	5/VI	Vicario et al. [4]	Poggio et al. [3]	Summary
ATP2B2 variant	NM_001001331.4: c.1369G > A, NP_001001331.1: p.(Glu457Lys)[a]	NM_001001331.4: c.1340G > A, NP_001001331.1: p.(Gly447Asp)	NM_001001331.4: c.2750T > C, NP_001001331.1: p.(Met917Thr)	NM_001001331.4: c.673G > A c. NP_001001331.1: p.Asp225Asn	NM_001001331.4: c.3266_3267del, NP_001001331.1: p.(Leu1089GlnfsTer60)	NM_001001331.4: c.3266_3267del, NP_001001331.1: p.(Leu1089GlnfsTer60)			
Variant inheritance	De novo	De novo	De novo	De novo	Paternally inherited	Unknown			
REVEL score	0.941	0.939	0.917	0.886	NA	NA			
CADD score	33	33	27	29.6	NA	NA			
gnomAD v4.1.0/ other controls	Not found	Not found	Not found	Not found	Not found	Not found			
Gender	Female	Male	Female	Female	Male	Male	Male	Female: 3/7; male: 4/7	Female: 6/14; male: 8/14
Current age	8 years	3 years	6 years 6 months	6 years 7 months	6 years 9 months	45 years	27 years	3–43 years	3–45 years
Anthropometric measures	Normal	No abnormalities reported	Normal	No abnormalities reported	No abnormalities reported	NA			
Developmental delay	Yes—motor and cognitive delay	Yes	Yes—motor and cognitive delay	Yes—motor and cognitive delay	Yes—motor and cognitive delay	Yes—motor and cognitive delay	Yes	6/7	13/14
Hypotonia	Yes—feeding problems and dystrophy	Yes—appendicular and axial hypotonia	No	Yes	Yes	NA	No	2/7	6/13
Intellectual impairment	Yes	Yes	Yes	Yes	Yes	Yes	Yes	6/7	13/14
Speech impairment	Yes	Yes—no speech	Yes	Yes	Yes	Yes	Yes	6/7	13/14
Dysmorphia	No	Yes—upslanting palpebral fissures, deeply set eyes; bulbous nasal tip; retrognathia	No	No	Yes—retrognathia and unilateral incomplete palmar crease	NA	NA	2/7	4/12

(Continues)

TABLE 1 | (Continued)

Family/patient	1/I	2/II	3/III	4/IV	5/V	5/VI	Vicario et al. [4]	Poggio et al. [3]	Summary
Behavioral deficits	Yes—autistic traits and stereotypies	No	Yes—autistic traits	Yes—autistic and ADHD traits	No	No	NA	5/7	8/13
Hearing abnormalities	Yes—hearing impairment, diagnosed age 5.5 years, hearing aids	No	No	No	No	NA	No	2/7	3/13
Visual abnormalities	Yes—myopia (minus 5 dpt)	Yes—intermittent horizontal nystagmus with lateral gaze	No	No	No	NA	NA	4/7	6/12
Ataxia	Yes	Yes	No	Yes	No	NA	Yes	5/7	9/13
Other movement disorders	No	Yes—tremor	No	No	No	NA	No	3/7	4/13
Seizures	No	Yes	No	No	Yes	Yes	No	6/7	9/14
Brain MRI abnormality	Not performed	Severe progressive cerebellar volume loss; abnormal cross-like pontine signal, similar to the “hot cross bun” sign	Not performed	Not performed	Normal at 2 months	Normal	Global cerebellar atrophy	2/7	4/11
Other features	No	Yes—eczema and constipation	Yes—severe constipation (with fecaloma)	Yes—severe constipation and eczema	Yes—growth delay	No			4/6

Abbreviations: CADD, combined annotation dependent depletion; MRI, magnetic resonance imaging; NA, not available; REVEL, rare exome variant ensemble learner.
[a]Corresponds to c.1234G > A, p.(Glu412Lys) on alternative transcript NM_001683.5.

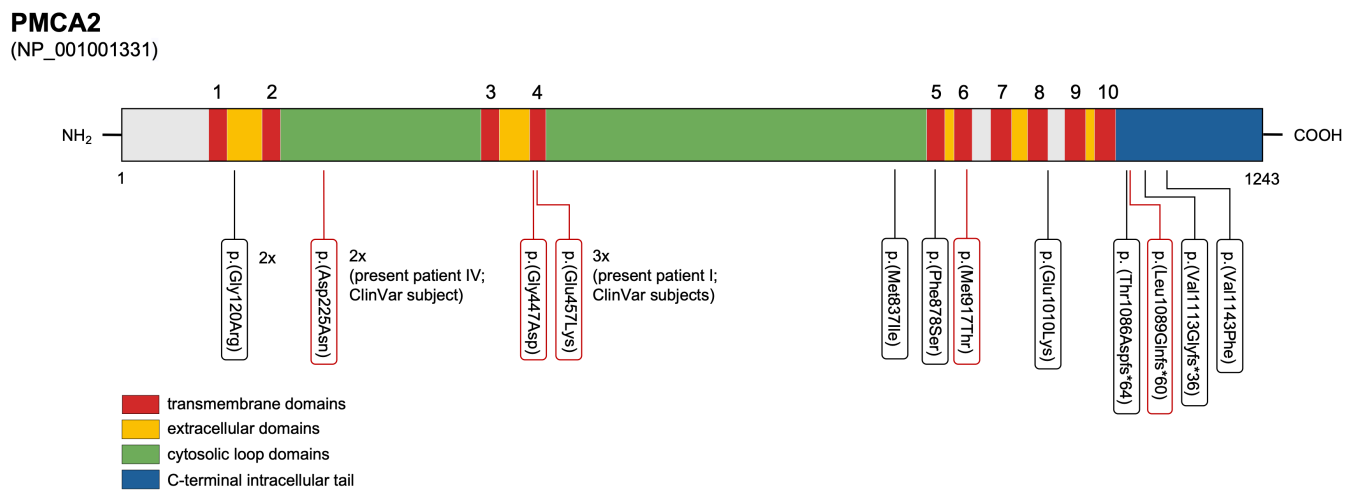

FIGURE 1 | Genetic variation in *ATP2B2*-associated neurodevelopmental disorders Schematic illustration of the *ATP2B2*-encoded protein PMCA2 (GenBank: NP_001001331) showcasing 10 transmembrane domains (red, numbered), two cytosolic loop domains (green), five extracellular domains (yellow), and a C-terminal intracellular tail that includes the calmodulin-binding regulatory domain (blue). The variants identified herein are indicated in red, while the previously reported variants [3, 4] are shown in black. [Colour figure can be viewed at wileyonlinelibrary.com]

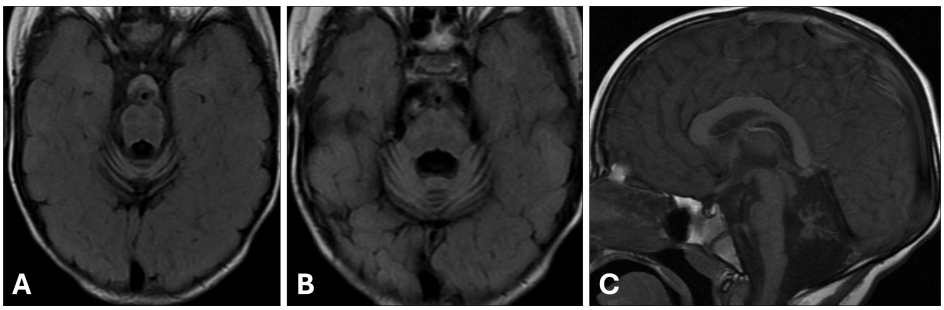

FIGURE 2 | Magnetic resonance imaging showing cerebellar atrophy in Patient II carrying the de novo missense variant p.(Gly447Asp) in *ATP2B2*. Axial (A and B) and sagittal (C) brain MRI scans of Patient II at the age of 2.5 years. The scans demonstrate severe cerebellar atrophy (A–C) with inferior vermian hypoplasia (C). Associated volume loss of the brainstem with pontine signal abnormalities resembling the "hot cross bun sign" was also evident (A and B).

white matter abnormality, and no new acute abnormalities (Figure 2). Additional physical findings in the patient included upslanting palpebral fissures, deeply set eyes, a bulbous nasal tip, retrognathia, chronic constipation, and eczema.

Patient III from Family 3 was a 6.5-year-old female from France. The early postnatal period was unremarkable, but medical attention was sought around the age of 12 months because of a delay in developmental milestones. Neurocognitive testing revealed signs of motor and speech delays with deficits in different domains including gross motor function, perception, and verbal comprehension. By age 3 years, she was diagnosed with intellectual disability. She was only capable of using short sentences. Additionally, major impairments in social skills and behavior became apparent. She was reported to be nonmodulating under social circumstances and a suspected diagnosis of autism spectrum disorder was given. She could not perform complex daily tasks and currently needs assistance due to cognitive and behavioral issues. Her condition was complicated by chronic constipation. She never had seizures and there were no overt movement disorders. A brain MRI was not performed.

Patient IV from Family 4 was a 6-year-old girl from Australia who was referred to a pediatrician at the age of 3 years because of global developmental delay. She was born via elective cesarean section at term with developmental dysplasia of the hips diagnosed in the newborn period that was managed with hip bracing. She had an adenotonsillectomy for obstructive sleep apnea, grommets inserted for conductive hearing loss, and eczema managed with topical steroids and emollients. After the age of 3 years, she developed difficulties with constipation that needed ongoing management with laxatives. The patient walked at 22 months of age, but she had an unsteady gait that resulted in frequent falls. Neuropsychology evaluation was performed at the age of 6 years that confirmed a mild intellectual disability with an IQ of 66 and a behavioral disorder with ADHD symptoms and autistic symptoms. She was noted to have significant difficulties with both of her receptive and expressive language in the context of normal hearing. Difficulties were observed in her expressive language with word finding, vocabulary, syntax, and pragmatic language. She required verbal instructions to be repeated and clarified. Her clinical examination at 6 years of age was notable for dysmetria and dysdiadochokinesis. A brain MRI was not performed.

Patient V, a 6-year-old boy, and his affected 45-year-old father (Patient VI) from Family 5 of French descent were under medical surveillance due to epileptic symptoms that began in early childhood. The first clonic seizure in Patient V was noted 9 days after birth, followed by multiple seizures up to the age of 4 years. A diagnosis of benign familial neonatal-infantile convulsions was made and the child was treated with carbamazepine. Hypotonia and developmental delay with major language impairment were also noted. At the age of 5 years, an endocrinology follow-up was performed because of growth delay with short stature, but no growth hormone deficiency was found. He needed specialized education. He presented some dysmorphic features with mild retrognathia and an incomplete unilateral palmar crease. Brain MRI was normal. His father Patient VI was treated for epilepsy until the age of 7, and he received special education for intellectual disability and speech impairment. His brain MRI was unremarkable.

4 | Discussion

In this study, we delineate the genetic and phenotypical profile of six additional patients with rare variants in *ATP2B2*, presenting with complex neurological manifestations that overlap with other Ca^{2+} defect-related syndromes [1].

Similar to the genotypic spectrum that we reported previously for the *ATP2B2*-linked condition [3], we identified both missense and LoF alterations. All missense substitutions were absent from population databases, affected invariant amino-acid positions in functional domains, and were categorized as deleterious by prediction models (Table 1). In in vitro assays, the published disease-related *ATP2B2* missense mutations exhibited deleterious consequences including loss- and gain-of-function effects [3]. Despite the absence of functional data for the missense variants we have acquired here, the observed mutational recurrence at an invariant Glu residue in transmembrane domain 4 is intriguing. Although no further detailed data were obtainable for two human subjects with the p.(Glu457Lys) variant listed in ClinVar, disturbed Ca^{2+} extrusion from murine cells consistent with a functional LoF effect is a remarkable finding in a mouse model bearing an identical amino-acid substitution [5]. Based on these data and classification guidelines [7], we label the variant p.(Glu457Lys) in our present Patient I as "pathogenic." The remaining three missense variants detected in our Patients II–IV qualified as "likely pathogenic" alleles according to ACMG [7]. The "likely pathogenic" frameshift variant in relatives from Family 5 mapped between two recently reported neurodevelopmental disease-causing LoF variants in the protein C terminus, predicted to result in abnormally truncated PMCA2 with altered pump activity due to impaired calmodulin-dependent autoregulation [3, 8, 9].

Our results shed light on the variable developmental and neurological outcomes in additional *ATP2B2*-mutated patients (Table 1). The disorder appears to cover a spectrum of clinical presentations, beginning with infantile milestone delay, followed by varying degrees of intellectual, behavioral, motor, and sensory impairments as well as seizures. Although data are still limited, our present collection of five families may be representative of different *ATP2B2*-related disease subtypes: (1) developmental delay coupled with ataxia with or without cerebellar atrophy or hearing loss (Family 1, Family 2, Family 4); (2) unspecific neurodevelopmental signs with autism (Family 3); and (3) epilepsy-predominant manifestations (Family 5).

The present study encourages us to enhance awareness of this new *ATP2B2*-related disorder, which is likely to be underdiagnosed.

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Conflicts of Interest

J.F.'s spouse is Principal of Friedman Bioventure, which holds a variety of publicly traded and private biotechnology interests. The other authors delcare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Peer Review

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1111/cge.14622.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.