

De Novo *ATP1A1* Variants in an Early-Onset Complex Neurodevelopmental Syndrome

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Abstract

ATP1A1 encodes the $\alpha 1$ subunit of the sodium-potassium ATPase, an electrogenic cation pump highly expressed in the nervous system. Pathogenic variants in other subunits of the same ATPase, encoded by *ATP1A2* or *ATP1A3*, are associated with syndromes such as hemiplegic migraine, dystonia, or cerebellar ataxia. Worldwide, only 16 families have been reported carrying pathogenic *ATP1A1* variants to date. Associated phenotypes are axonal neuropathies, spastic paraplegia, and hypomagnesemia with seizures and intellectual disability. By whole exome or genome sequencing, we identified 5 heterozygous *ATP1A1* variants, c.674A>G;p.Gln225Arg, c.1003G>T;p.Gly335Cys, c.1526G>A;p.Gly509Asp, c.2152G>A;p.Gly718Ser, and c.2768T>A;p.Phe923Tyr, in 5 unrelated children with intellectual disability, spasticity, and peripheral, motor predominant neuropathy. Additional features were sensory loss, sleep disturbances, and seizures. All variants occurred de novo and are absent from control populations (MAF GnomAD = 0). Affecting conserved amino acid residues and constrained regions, all variants have high pathogenicity *in silico* prediction scores. In HEK cells transfected with ouabain-insensitive *ATP1A1* constructs, cell viability was significantly decreased in mutants after 72h treatment with the ATPase inhibitor ouabain, demonstrating loss of ATPase function. Replicating the haploinsufficiency mechanism of disease with a gene-specific assay provides pathogenicity information and increases certainty in variant interpretation. This study further expands the genotype-phenotype spectrum of *ATP1A1*.

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Table 1 Patient Overview and Phenotype Characterization

ID, sex, age at examination	1, M, 2y	2, M, 9y	3, M, 15y	4, M, 14y	5, M, 18y
Country of origin	The Netherlands	Poland	Italy	USA	USA
Age at onset	neonatal	early infancy	infancy	early childhood	infancy
Symptoms at onset	developmental delay, obstipation	developmental delay	developmental delay	developmental delay, autism	developmental delay
Intellectual disability	yes	yes	yes	yes	yes
Speech development	limited to a few words	nonverbal	limited to ~100 words	borderline to normal	delayed, hypophonic
Epileptic seizures	no, but EEG abnormal	no	no	no	yes
Hyperactivity	NA*	yes	yes	yes	NA*
Motor milestones	delayed	delayed	delayed	normal	delayed
Ambulatory capacities	ambulant	uses a wheelchair, never been ambulant	ambulant	ambulant	Uses a wheelchair, motor regression since the age of 7y
Fine motor skills	MD	impaired	impaired	normal	impaired
Foot deformities	yes, pes equinus	yes, valgus feet (Figure 1B/C)	yes, flat feet	no	yes, pes planovalgus
Self-mutilation	no	yes	no	no	no
Scoliosis	no	yes	no	no	no
Muscle atrophies	no	diffuse in arms and legs	no	no	no
LL distal weakness (MRC)	NA*	yes (2)	yes (4)	no (5)	no (5)
UL distal weakness (MRC)	NA*	yes (4)	no (5)	no (5)	yes (4)
LL tendon reflexes	brisk	brisk	normal	normal	brisk
UL tendon reflexes	normal	normal	normal	normal	brisk
Spasticity	increased muscle tone	increased muscle tone	no	no	increased muscle tone, foot clonus, positive Babinski sign
Sensory deficits	NA*	yes	no	no	no
Brain MRI	hypomyelination, hydrocephalus e vacuo	hypomyelination	low cerebellar tonsils	normal	demyelination
EMG/NCS	ND	ND	ND	ND	borderline low sensory nerve action potentials (median and sural nerves), normal motor NCS and EMG
Serum magnesium levels	normal	normal	normal	normal	normal
Serum potassium levels	MD	MD	normal	normal	normal

Continued

Table 1 Patient Overview and Phenotype Characterization (continued)

ID, sex, age at examination	1, M, 2y	2, M, 9y	3, M, 15y	4, M, 14y	5, M, 18y
Additional information	sleep disturbances	sleep disturbances, episodes of “stiff limbs”, hypotonia, failure to thrive, laryngomalacia, gastrointestinal reflux	NA	NA	history of prematurity (29 wk), gastrointestinal reflux, asthma
ATP1A1 variant	p.Gln225Arg	p.Gly335Cys	p.Gly509Asp	p.Gly718Ser	p.Phe923Tyr
De novo status (PM6)	confirmed	confirmed	confirmed	confirmed	confirmed
Functional relevance by in vitro assay (fig. 2C PS3)	shown	shown	shown	shown	shown
In-silico predictions (PP3)	deleterious	deleterious	deleterious	deleterious	deleterious
Absent from controls (GnomAD; PM2)	MAF = 0	MAF = 0	MAF = 0	MAF = 0	MAF = 0
Critical domain/region (fig. 2A PM1)	—	transmembrane	—	metal ion binding	transmembrane
ACMG interpretation of pathogenicity	pathogenic	pathogenic	pathogenic	pathogenic	pathogenic

Demographic background, disease onset, leading symptoms, clinical examinations, and additional findings are shown for all 5 patients; all carrying de novo variants in *ATP1A1*. Abbreviations: ACMG = American College of Molecular Genetics; EMG = electromyography; F = female; LL = lower limb; M = male; MD = missing data; MRC = Medical Research Council; MRI = magnet resonance imaging; NA = not applicable; NCS = nerve conduction studies; PM = moderate pathogenicity criterion; PP = supporting pathogenicity criterion; PS = strong pathogenicity criterion; UL = upper limb; y = years; * cannot be assessed at the age of 2 y.

ATP1A1 encodes the $\alpha 1$ unit of a sodium-potassium ATPase being highly expressed in the nervous system. With an autosomal-dominant inheritance, associated phenotypes comprise Charcot-Marie-Tooth disease (CMT)^{1,2}, hereditary spastic paraplegia (HSP)³, and intellectual disability⁴, hypomagnesemia, hypokalemia, and seizures.⁵ Heterozygous missense mutations lead to a reduced ATPase function, indicating haploinsufficiency.¹

We aimed at showing the pathogenicity of 5 newly identified heterozygous missense variants and broadening the known genotype-phenotype spectrum^{1-3,5} of *ATP1A1*.

Methods

All patients or legal representatives gave written informed consent. Clinical examinations were performed by experienced child neurologists. Using site-specific protocols, *ATP1A1* variants were identified by whole exome or genome sequencing. In both children and parents, variants were validated by Sanger sequencing. Cosegregation analyses confirmed that all 5 variants occurred de novo.

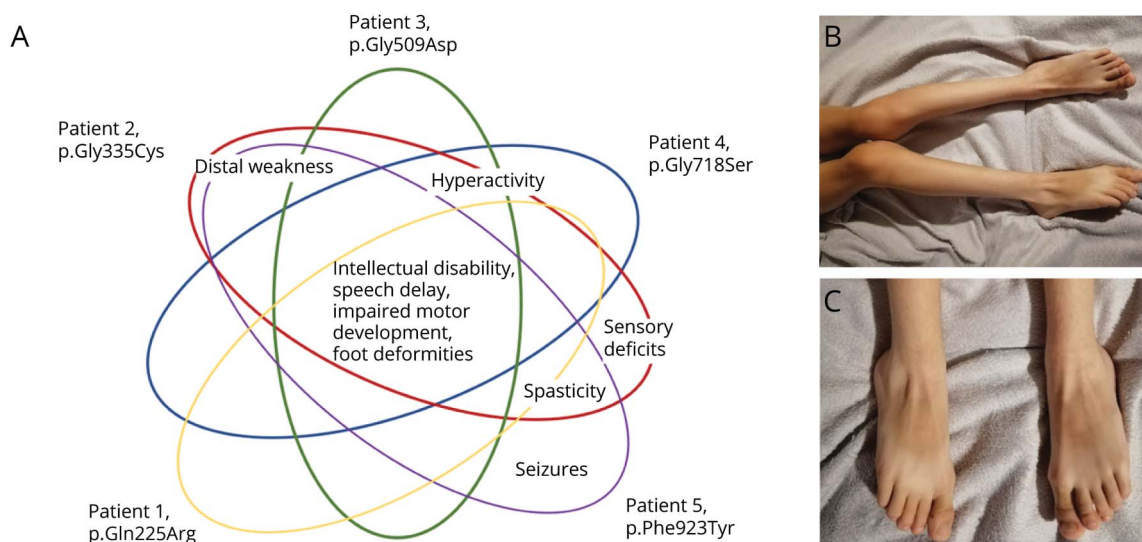
To assess variant pathogenicity, HEK cells were transfected with ouabain-insensitive *ATP1A1* plasmids encoding either the wildtype (wt) or mutants in replicates of 8. As a negative control, we used wt-*ATP1A1* plasmids that are not insensitive to ouabain. 24 hours after transfection, cells were treated with the ATPase inhibitor ouabain at a concentration of 0.5 μ M. After 72 hours, cell survival was measured by luciferase (CellTiter-Glo) assay. For normalization, we calculated the viability ratio of treated and untreated cells, each transfected with the same plasmid. For statistical analyses, we used a 1-way ANOVA to compare all mutants with wt, wt-ouabain, and untransfected cells, using the Tukey post-test for α -error correction.

Results

Phenotype Description

All 5 children were male and of Caucasian origin. First symptoms were observed in early childhood or even neonatally (Table 1). At the time of examination, the patients were 2–18 years old. A variable degree of intellectual disability was present in all patients (Figure 1A). Speech development ranged from absent to borderline normal. Motor milestones were delayed in 4 of 5 children. At current age, spasticity and distal muscle weakness were the leading motor symptoms (3/4) and foot deformities occurred in all examined patients (4/4). Deep tendon reflexes were brisk in 3 of 4 cases. Additional sensory deficits were reported in one patient (patient 2), who had a tendency to self-mutilation and distal muscle atrophies (Figure 1B, C) as well. This and one other patient (patient 5) were using a wheelchair by the time of examination. One patient (patient 5) had epileptic seizures. Brain MRIs had shown signs of hypomyelination or demyelination in 3 of 5 patients, and low

Figure 1 Clinical Phenotypes Associated With Novel *ATP1A1* Variants



(A) Graphic depiction of distinct and overlapping phenotypes in 5 patients. (B) Diffuse muscle atrophies, increased muscle tone, and pes valgus in patient 2. (C) Pes planovalgus in patient 2, aggravated by neuropathy.

cerebellar tonsils were reported in one patient (patient 3). Serum magnesium and potassium levels were normal in all examined patients. Additional findings were sleep disturbances and gastrointestinal reflux in 2 patients.

Molecular Genetics

We identified 5 de novo missense variants in *ATP1A1* (ENST00000295598.10; NM_000701.8; Figure 2A) that are all absent from control populations (GnomAD): c.674A>G; p.Gln225Arg, c.1003G>T; p.Gly335Cys, c.1526G>A; p.Gly509Asp, c.2152G>A; p.Gly718Ser, and c.2768T>A; p.Phe923Tyr (Table 1). All variants affect conserved (GERP scores: 4.82–5.33) and constrained amino acid positions (Figure 2B). The variants p.Gly335Cys and p.Phe923Tyr are located within transmembrane domains, whereas p.Gln225Arg, p.Gly509Asp, and p.Gly718Ser lie in intracellular loops, the latter affecting the metal binding site (Figure 2A). *In silico* pathogenicity predictions (CADD scores: 23.9–29.9, median: 29.0) were deleterious for all exchanges.

Functional Assessment

Compared with the ouabain-insensitive wt, cell viability was significantly reduced for all variants (Figure 2C). In the presence of the pathogenic mutant, the otherwise resistant plasmid led to cell death when treated with the unselective ATPase inhibitor ouabain, demonstrating the expected loss of function. To show that cell death was not an effect of transfection, we normalized each treated mutant to untreated cells transfected with the same plasmid.

Discussion

In this work, we describe a complex but consistent phenotype (Figure 1A) not adhering to distinct disease patterns such as

neuropathy, spastic paraplegia, or intellectual disability that have all been described in association with pathogenic *ATP1A1* variants before,^{1–5} (eTable 1, links.lww.com/WNL/B742) but combining those and other clinical features in a broader spectrum. In a linear protein model (Figure 2A), the variant positions do not correlate with the distinct subphenotypes. Why a certain variant causes one specific clinical syndrome and what determines disease severity is therefore not clear today. Although it is often challenging to determine pathogenicity of single variants in large proteins, the availability of a specific bioassay allowed us to show haploinsufficiency of each variant. All herein reported missense variants occurred de novo, and other genetic causes more likely to be causative were ruled out by whole exome or genome sequencing.

We conclude that the *ATP1A1*-associated disease spectrum is broader than previously thought. Our results show each variant's pathogenicity and expand the genotype–phenotype spectrum.

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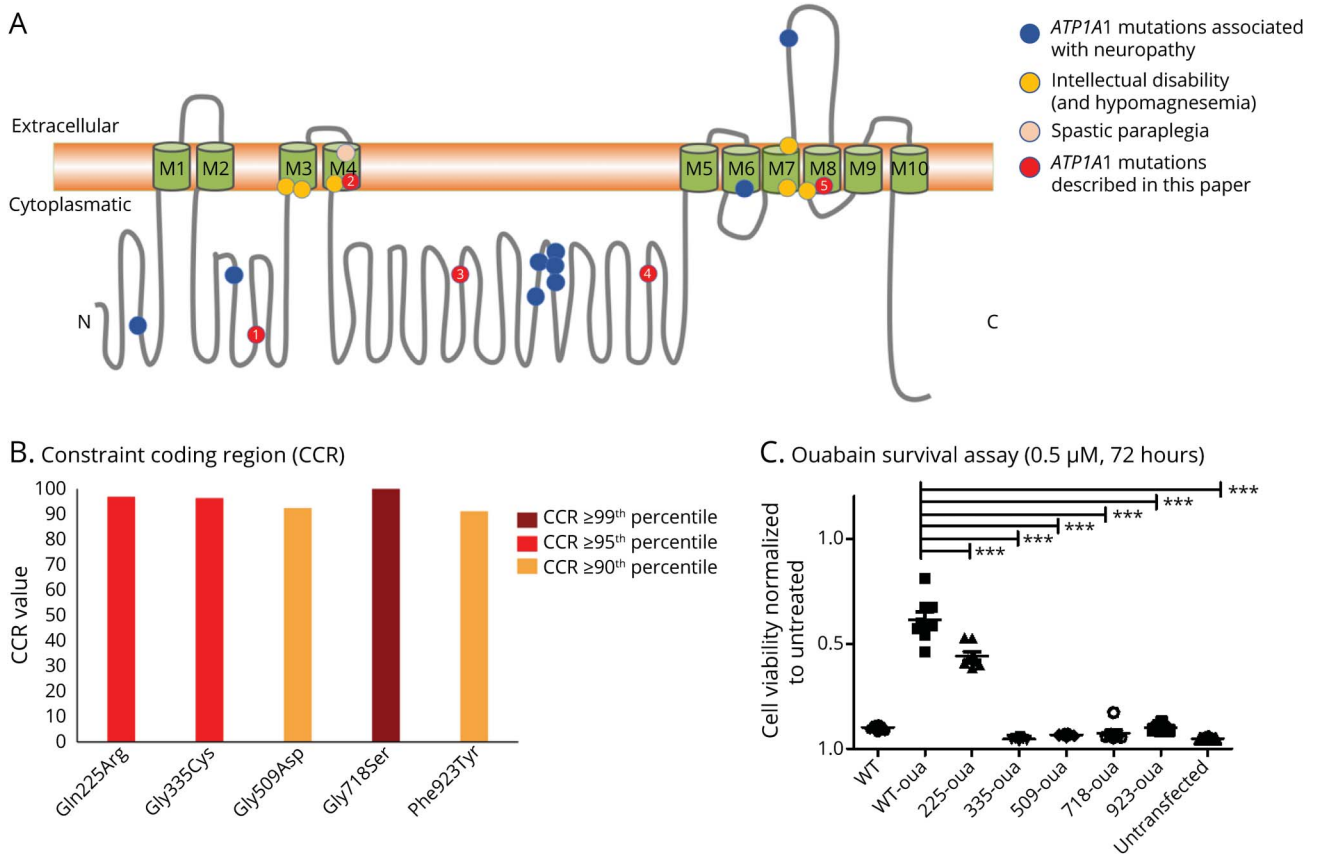
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Disclosure

A. Malhotra and R. J. Taft are full-time employees at Illumina, Inc. The remaining authors report no disclosures relevant to the manuscript. Go to Neurology.org/N for full disclosures.

Figure 2 Functional Characteristics of Novel *ATP1A1* Variants



(A) Schematic depiction of the *ATP1A1* protein showing the localization of previously reported and novel variants, sorted by phenotypes. Blue: *ATP1A1* mutations associated with neuropathy: Leu48Arg, Ser207Phe, Ile592Thr, Ala597Thr, Pro600Ala, Pro600Thr, Asp601Phe, Asp811Ala, Gly877Ser. Yellow: *ATP1A1* mutations associated with intellectual disability (and renal hypomagnesemia): Leu302Arg, Gly303Arg, Pro333Arg, Met859Arg, Gly864Arg, Asp933Asn. Rose: *ATP1A1* mutation associated with spastic paraplegia: Leu337Pro. Red: Novel *ATP1A1* mutations described in this article: Gln225Arg (patient 1), Gly335Cys (patient 2), Gly509Asp (patient 3), Gly718Ser (patient 4), Phe923Tyr (patient 5). (B) *ATP1A1* variants are mapped to highly constrained coding regions (CCRs) of the gene.⁶ High constraint means that the disruption of this region would most likely cause haploinsufficiency. (C) Luminescence-based ouabain survival assay in HEK cells transfected with ouabain-insensitive *ATP1A1* plasmids for all 5 mutants and wildtype (oua-wt), as well as wt-*ATP1A1* (not ouabain-insensitive) in comparison with untransfected cells. After ouabain (0.5μM) treatment for 72h, cell viability is shown as a ratio with untreated cells (mean) transfected with the same plasmid. N, 8 Statistics: One-way ANOVA, α -error correction: Tukey posttest.

Publication History

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Appendix (continued)

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Appendix (continued)

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