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## De novo variants in *ATP2B1* lead to neurodevelopmental delay

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### Summary

Calcium (Ca<sup>2+</sup>) is a universal second messenger involved in synaptogenesis and cell survival; consequently, its regulation is important for neurons. ATPase plasma membrane Ca<sup>2+</sup> transporting 1 (ATP2B1) belongs to the family of ATP-driven calmodulin-dependent Ca<sup>2+</sup> pumps that participate in the regulation of intracellular free Ca<sup>2+</sup>. Here, we clinically describe a cohort of 12 unrelated individuals with variants in *ATP2B1* and an overlapping phenotype of mild to moderate global development delay. Additional common symptoms include autism, seizures, and distal limb abnormalities. Nine probands harbor missense variants, seven of which were in specific functional domains, and three individuals have nonsense variants. 3D structural protein modeling suggested that the variants have a destabilizing effect on the protein. We performed Ca<sup>2+</sup> imaging after introducing all nine missense variants in transfected HEK293 cells and showed that all variants lead to a significant decrease in Ca<sup>2+</sup> export capacity compared with the wild-type construct, thus proving their pathogenicity. Furthermore, we observed for the same variant set an incorrect intracellular localization of ATP2B1. The genetic findings and the overlapping phenotype of the probands as well as the functional analyses imply that *de novo* variants in *ATP2B1* lead to a monogenic form of neurodevelopmental disorder.

Global developmental delay (DD), often leading to intellectual disability (ID), has a high prevalence of about 2% and is among the most frequent indications for genetic testing.<sup>1</sup> Despite its frequency and importance, a genetic cause can only be found in 27% to 42% of routine diagnostic cases.<sup>2,3</sup> One reason for this inadequate clarification rate is the extreme genetic heterogeneity of DD. According to the SysNDD<sup>4</sup> database, pathogenic variants in 1,497 (gene statistics from July 29, 2021) different genes are an established cause of a neurodevelopmental disorder. Furthermore, several genes are under investigation to identify their association with DD/ID.

Calcium (Ca<sup>2+</sup>) is a second messenger that is involved in the regulation of signal transduction, gene expression, cell metabolism, and cell survival. Ca<sup>2+</sup> homeostasis is especially important for neurons, as it controls the release of neurotransmitters and regulates neurite outgrowth, cell membrane depolarization, and depression of synaptic transmission, which are important processes for learning and memory consolidation (for a review, see Brini et al.<sup>5</sup>). The dysregulation of Ca<sup>2+</sup> homeostasis by pathogenic variants in Ca<sup>2+</sup> channel genes, such as *CACNA1A* (MIM: 183086), is a common cause for several forms of ataxia. Moreover, other genes whose protein products control

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**Table 1. Clinical spectrum of the *ATP2B1*-related disorder**

Individual	Variant	Inheritance	Sex	Age <sup>a</sup>	DD	ID	Behavior	Seizures	Other
1	c.716A>G (p.Asp239Gly)	<i>de novo</i>	F	6 years	yes	moderate	normal	yes	secundum atrial septal defect, toe clindactyly, facial dysmorphism, hypotonia
2	c.791C>T (p.Thr264Ile)	<i>de novo</i>	M	8 years	yes	mild	ASD	no	transposition of large vessels, low set ears
3	c.1274C>A (p.Thr425Lys)	<i>de novo</i>	M	9 years	yes	mild	N/A	pathological EEG	cerebral cavernous malformation, sparse hair
4	c.1376A>G (p.His459Arg)	<i>de novo</i>	M	17 years	yes	mild	ASD	yes	marfanoid habitus, arachnodactyly, scoliosis, hypermobile thumb
5	c.2288G>C (p.Arg763Pro)	<i>de novo</i>	M	21 years	yes	mild	ASD	no	facial dysmorphism
6	c.2365C>T (p.Arg789Cys)	<i>de novo</i>	M	3 years	yes	unclassified	sleeping difficulties	yes	facial dysmorphism, hypotonia
7	c.2470G>A (p.Glu824Lys)	<i>de novo</i>	F	22 years	yes	moderate	compulsive behavior	yes	N/A
8	c.2570A>G (p.Gln857Arg)	<i>de novo</i>	F	3 years	yes	unclassified	normal	no	brachycephaly, facial dysmorphism, clinodactyly of the 5th finger
9	c.2972G>A (p.Arg991Gln)	unknown	M	51 years	yes	mild	ASD	no	marfanoid habitus, aortic root dilation, pectus carinatum, scoliosis, arachnodactyly, facial dysmorphism
10	c.2632C>T (p.Gln878*)	<i>de novo</i>	M	6 years	yes	mild	hyperactivity	no	facial dysmorphism
11	c.458G>A (p.Tip153*)	unknown	F	6 years	yes	moderate	N/A	no	short stature
12	c.1789C>T (p.Arg597*)	unknown	M	5 years	yes	unclassified	ASD	infantile spasms	short stature, pectus excavatum, plagiocephaly

Abbreviations: ID, intellectual disability; DD, development delay; ASD, autism spectrum disorder; N/A, not available; M, male; F, female. Variant descriptions based on GenBank: NM\_001001323.2.

<sup>a</sup>Age of last examination.

intracellular levels of free Ca<sup>2+</sup>, such as *CACNA1B* (MIM: 618497),<sup>6</sup> *CAMK4*,<sup>7</sup> *ANXA11*,<sup>8</sup> and *MICU2*,<sup>9</sup> have been reported to be associated with DD/ID.

ATPase plasma membrane Ca<sup>2+</sup> transporting 1 (*ATP2B1*, formerly known as plasma membrane Ca<sup>2+</sup> pump isoform 1; *PMCA1*; MIM: 108731) encodes an ATP-driven calmodulin-dependent Ca<sup>2+</sup> pump that maintains the homeostasis of intracellular Ca<sup>2+</sup> by removing it from the cytosol. Moreover, Ca<sup>2+</sup> pumps are considered to have a crucial role on neuronal function (for a review, see Cali et al.<sup>10</sup>) and certain variants in isoforms of *ATP2B1* have been reported to be pathogenic. Cali and colleagues<sup>11</sup> reported

one individual with DD, generalized hypotonia, cerebellar ataxia, and a hemizygote missense variant in *ATP2B3* (MIM: 302500). In another case report, Li and colleagues<sup>12</sup> describe four members of Chinese family with progressive spastic paraplegia and a heterozygous missense variant in *ATP2B4*.

In this study, we report 12 individuals with an overlapping phenotype of mild to moderate DD/ID, each harboring a different variant in *ATP2B1*. Table 1 presents an outline of the clinical symptoms of each individual. Additionally, a comprehensive clinical description of each individual is in Table S1 and in the supplemental



**Figure 1. Photos of individuals with *ATP2B1* variants**

No shared dysmorphic features in four individuals (for individual numbering, see Table 1).

by six individuals (individuals 1, 5, 6, 8, 9, and 10), there was no apparent shared facial gestalt nor specific malformations (Figure 1, Table S1). Additionally, four individuals had anomalies of digits (individuals 1, 4, 8, and 9).

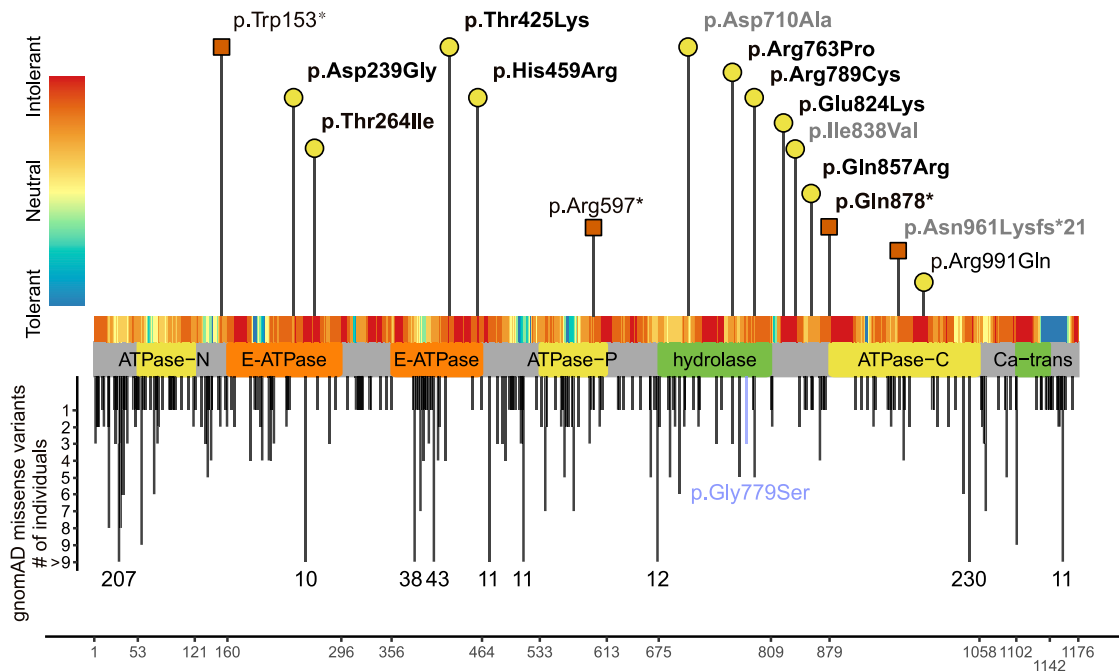
According to data from gnomAD,<sup>13</sup> *ATP2B1* contains a significantly reduced number of truncating and missense variants, indicating a selective constraint on both types of variants in a population without severe, early-onset phenotypes such as DD/ID (probability of being loss-of-function intolerant [pLI] score = 1.00; Z score missense = 5.29). In the present cohort, we identified 12 variants in *ATP2B1*, nine missense and three nonsense, that we consider of relevance. Nine (eight missense and one nonsense) were detected *de novo*,

**notes.** Written consents for genetic testing and its publication, as well as the publication of photographs, were obtained from all subjects studied or their legal representatives after counseling and information about risks and benefits in accordance with national ethical standards and laws as confirmed by the responsible local committees. This study was approved by the ethics committee of the University of Leipzig (approval code: 402/16-ek).

All individuals showed clear signs of DD. These included speech development delay in all individuals with a median age of first words of 26 months (spectrum of 12 to 36 months) and motor development delay in most individuals (median age at first walking 20 months; 16 to 36 months). The DD/ID was of variable degree; borderline to mild: 7/12, 58%; moderate: 3/12, 25%; moderate to severe: 2/12, 17%. Furthermore, five individuals (5/11, 45%, no information on one proband) were diagnosed with an autism spectrum disorder (ASD) and three additional individuals showed other behavior abnormalities. Dissimilar forms of seizures were reported in six individuals (6/12, 50%). Noteworthy, abnormal brain MRI findings were reported in three individuals without overlapping aspects. Individuals 1 and 6 had hypotonia. Other neurological symptoms, such as ataxia, were not reported. Individuals 11 and 12 had short stature, while all other individuals had normal growth development. Of note, two individuals, 4 and 9, exhibited a marfanoid habitus. Although minor facial dysmorphisms have been reported

while for the remaining three individuals, parents were unavailable for segregation. All variants were absent from gnomAD, except the *de novo* variant from individual 6 (c.2365C>T [p.Arg789Cys]), which was detected in gnomAD once. The three truncating variants affect neither the last exon, the last 55 bp of the penultimate exon, nor the first 100 bp of *ATP2B1*; thus, nonsense-mediated mRNA decay is likely.<sup>14</sup> The missense variants seem to be pathogenic *in silico* (conserved and predicted pathogenic by multiple tools) and have a combined annotation-dependent depletion (CADD)<sup>15</sup> score between 25.6 and 32 (mean 28.5) and a rare exome variant ensemble learner (REVEL)<sup>16</sup> score between 0.85 and 0.98 (mean 0.92, overview in Table S1). Additionally, seven out of nine missense variants of the cohort are located in established domains of *ATP2B1* (Figure 2), which are critical for the protein's function. The E1-E2 ATPase as well as the cation transporter ATPase domains, which are affected by six variants, are involved in Ca<sup>2+</sup> binding, phosphoenzyme formation, and Ca<sup>2+</sup> release.<sup>17</sup> The haloacid dehalogenase-like hydrolase domain is affected by two variants and generates the energy for Ca<sup>2+</sup> transport via hydrolysis of ATP to ADP. Two missense variants do not affect an established domain but are located in areas that are intolerant to missense variation, as proposed by MetaDome.<sup>18</sup> Hence, an altered protein function of these seems plausible.

To better understand how missense variants affect *ATP2B1* function, we investigated the effect of the variants



**Figure 2. Variants in *ATP2B1***

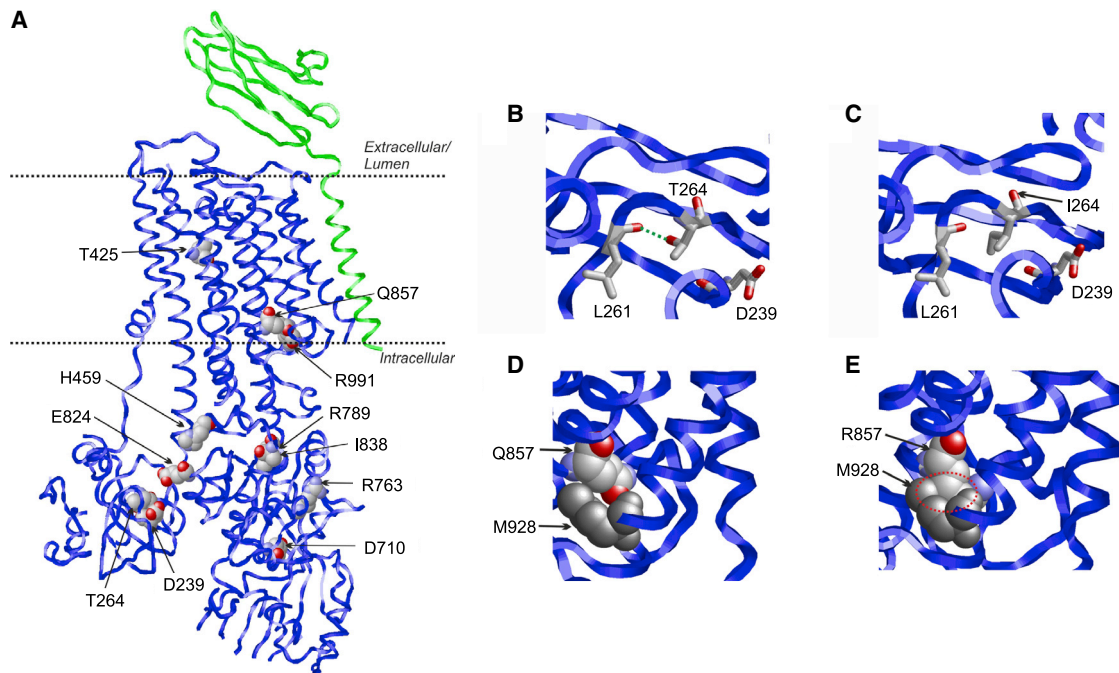
Location of missense and loss-of-function variant in *ATP2B1* with respect to the domain structure of *ATP2B1* (GenBank: NM\_001001323.2). The x axis represents the corresponding amino acid position of *ATP2B1*. Variants reported in this study are labeled with the corresponding p-code and are indicated by a yellow circle (missense) or a red square (loss of function). Confirmed *de novo* variants are indicated in bold. Deciphering Developmental Disorders study variants with a lacking detailed phenotypic description are indicated in gray (see also Table S1 and supplemental methods). Missense variants in gnomAD with allele count are shown below the protein scheme. The gnomAD variant p.Gly779Ser, which was used as negative control for the  $\text{Ca}^{2+}$  imaging experiments, is blue color-coded. The tolerance landscape (MetaDome<sup>18</sup>) is shown color-coded above the protein scheme. Abbreviations: ATPase-N, cation transporter/ATPase, N terminus; ATPase-P, cation transport ATPase (P-type); ATPase-C, cation transporter/ATPase, C terminus; E-ATPase, E1-E2 ATPase; hydrolase, haloacid dehalogenase-like hydrolase; Ca-trans, plasma membrane calcium transporter ATPase C-terminal.

by an *in silico* structural protein modeling. The availability of an experimental structure for *ATP2B1*<sup>19</sup> allows us to map the sequence positions of the variants in the 3D structure (Figure 3A). Three missense variants affect conserved amino acid (AA) residues in the transmembrane domain (Thr425, Gln857, and Arg991) that constitutes the  $\text{Ca}^{2+}$  exit pathway to the extracellular space. The remaining variants affect AA residues in the cytosolic protein part that harbors the active site. The observation that damaging variants are distributed over the structure is in line with the fact that *ATP2B1* is a complex molecular machine that requires structural integrity of all domains for proper function.

Most of the residues affected by the identified variants form tight interactions in the protein structure and are predicted to affect protein stability by a variety of structural mechanisms: for example, p.Thr264Ile results in a loss of a stabilizing hydrogen bond (Figures 3B and 3C). The p.Gln857Arg exchange causes protein destabilization by a steric clash between the Arg857 and Met928 sidechain in the altered protein (Figures 3D and 3E). The p.Arg991Gln exchange most likely causes the loss of a stabilizing interaction between the positively charged arginine and the negatively charged phospholipids. The p.Thr425Lys exchange in the transmembrane domain gen-

erates a positive charge close to the  $\text{Ca}^{2+}$  exit pathway, which most likely interferes with the transport of the positively charged  $\text{Ca}^{2+}$  ions. The p.Asp710Ala exchange disrupts a salt-bridge between the Asp710 sidechain and a conserved lysine (Lys476) of the active site.

As the *in silico* predictions suggest that the variants would affect the function of *ATP2B1*, we tested this assumption experimentally. Therefore, we generated plasmids expressing *ATP2B1* that harbor the variants of interest. Although the variants in our cohort affect several isoforms of *ATP2B1*, we decided to investigate the isoform (*ATP2B1a*) that is mainly detected in the human brain<sup>20</sup> (GenBank: NM\_001001323.2; Ensembl: ENST00000359142.7; compare also GTEx for expression data). As expected, we observed a membranous fluorescence after transfection of the *ATP2B1*-yellow fluorescent protein (YFP) wild-type expression plasmid in HEK293 cells (Figure 4A). More important, we also observed a significantly altered cellular localization of *ATP2B1*-YFP harboring the variants p.Asp239Gly, p.Thr264Ile, and p.Arg991Gln (Figures 4A and 4B). Of the remaining six missense variants, the variants p.Thr425Lys, p.Arg763Pro, p.Glu824Lys, and p.Gln857Arg also showed a shift of membranous to cytoplasmic localization of *ATP2B1*. However, this effect was not statistically significant, as



### Figure 3. Structural effects in ATP2B1

(A) Structure of ATP2B1 (PDB: 6A69<sup>19</sup>) indicating the sites of genetic variants. The ATP2B1 structure is shown as blue ribbon and the neuroplastin subunit in green. Sites of genetic variants are shown in space-filled presentation (atom-type coloring) and labeled. The location of the membrane is indicated by two dotted lines.

(B) Enlargement of the ATP2B1 structure showing the effect of the p.T264Ile and p.Gln857Arg variants. (B) T264 forms a hydrogen bond (green dotted line) to L261.

(C) In the p.Thr264Ile variant, this hydrogen bond cannot be formed by the nonpolar isoleucine sidechain.

(D) Q857 is in close spatial proximity to M928 (shown in gray).

(E) The bulkier R857 sidechain of the variant causes steric clashes (red dotted circle) with M928 leading to a destabilization of ATP2B1.

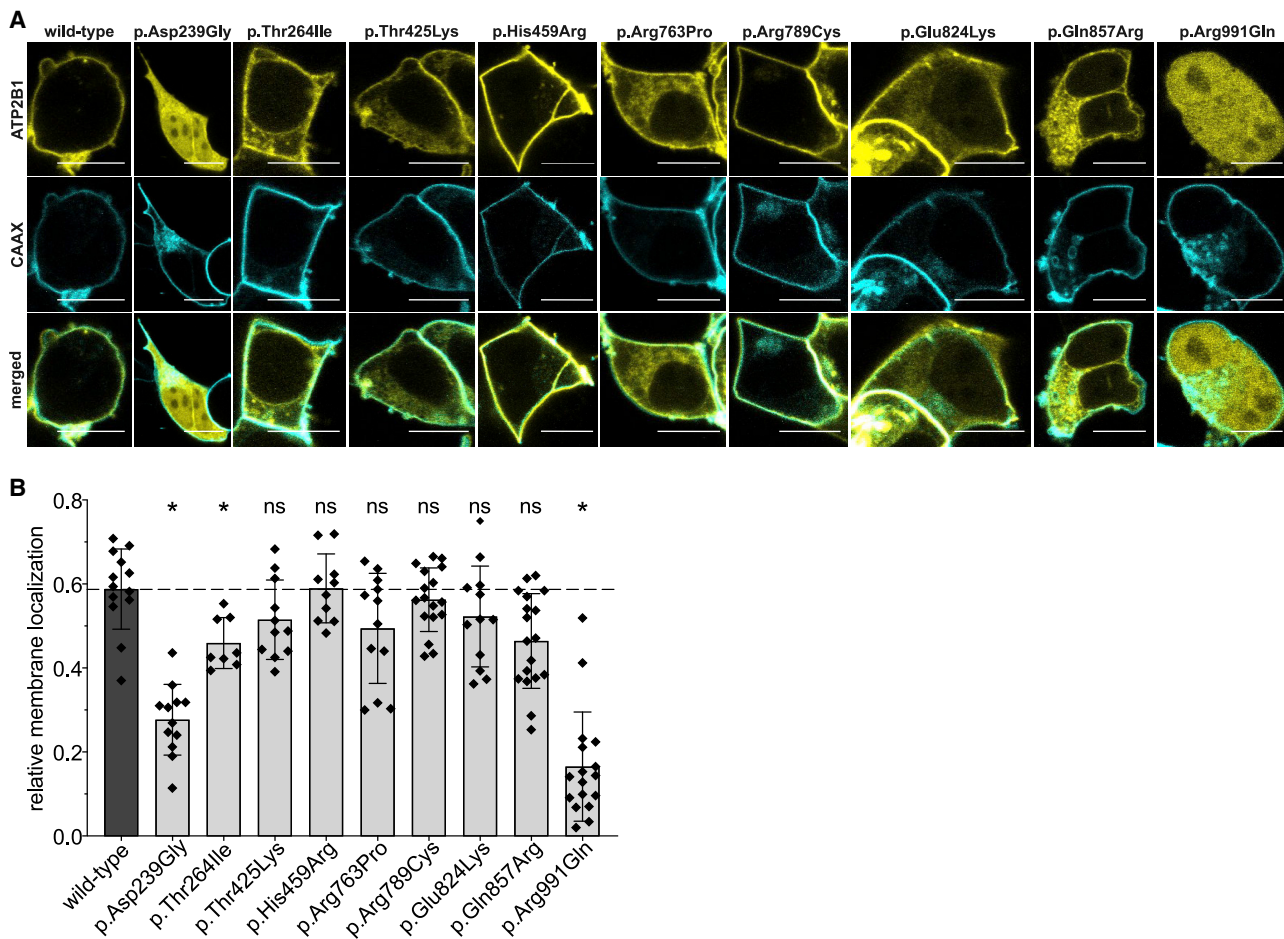
this was observable only in a proportion of analyzed cells (Figure S1). The variants p.His459Arg and p.Arg789Cys did not show a change and were comparable with the wild type.

Next, we investigated whether the identified variants in *ATP2B1* affect the  $\text{Ca}^{2+}$  transport function of ATP2B1. Therefore, we performed  $[\text{Ca}^{2+}]_i$  imaging in HEK293 cells after transfection of the corresponding expression plasmids. We chose this method<sup>21</sup> because it allows a continuous measurement with a perfusion system to infuse the cells with different solutions. This makes it possible for us to investigate the cells in an approximate physiological environment and change of cellular  $\text{Ca}^{2+}$  gradients. The method allows even higher sensitivity because transfected cells can be separated from non-transfected ones by single-handedly picking the cells with a YFP signal, expressed through the plasmid. The result of this experiment is shown in Figure 5A. To investigate the ATP2B1 activity, we determined the relative  $\text{Ca}^{2+}$  extrusion rate. Therefore, after initial perfusion with EGTA,  $\text{Ca}^{2+}$  store depletion, and  $\text{Ca}^{2+}$  loading, we analyzed  $[\text{Ca}^{2+}]_i$  decline after perfusion with EGTA, which is represented by the time constant tau (see also Figure S2). Hence, a lower tau value indicates a higher ATP2B1 activity. Accordingly, we could detect a significantly reduced tau value after transfection of the wild-type ATP2B1 plasmid compared to untransfected

HEK293 cells (Figure 5B). As an additional negative control, we analyzed a presumably non-pathogenic variant (p.Gly779Ser) that was detected three times in gnomAD. As expected, the  $[\text{Ca}^{2+}]_i$  imaging experiments revealed no statistically different  $\text{Ca}^{2+}$  extrusion rate of this variant compared to the wild type. The investigation of the identified variants in the present cohort revealed a significantly increased tau value after  $\text{Ca}^{2+}$  loading in all nine variants compared to the wild type (Figure 5B).

Interestingly, by ranking the variants on the basis of reduction of  $\text{Ca}^{2+}$  transport and on changes in cellular localization, we observe a significant correlation between the influence of a *ATP2B1* variant on membrane localization and the tau value ( $p < 0.001$ ;  $r^2 = 0.768$ ; Figure S3A). Of note, we also found a correlation between the REVEL score and the tau value ( $p < 0.02$ ;  $r^2 = 0.5$ ; Figure S3B) on the basis of the rank. Although speculative, these observations indicate that the missense variants identified in this study reduce  $\text{Ca}^{2+}$  transport by disturbing the cellular trafficking and/or by inducing the degradation of abnormal ATP2B1 protein. A similar mechanism has also been reported for the creatine transporter *SLC6A8*.<sup>22</sup>

At this point, it should be noted that we identified another individual with DD and a *de novo* variant in *ATP2B1* (c.1793T>C [p.Ile598Thr]; see supplemental note, "individual 16"). We have not included this



**Figure 4. Subcellular localization of transiently overexpressed *ATP2B1* variants**

(A) Representative confocal laser scanning microscopy images of transfected HEK293 cells expressing YFP-fused *ATP2B1* and a CAAX-box-modified cyan fluorescent protein. Scale bars: 10  $\mu$ m.

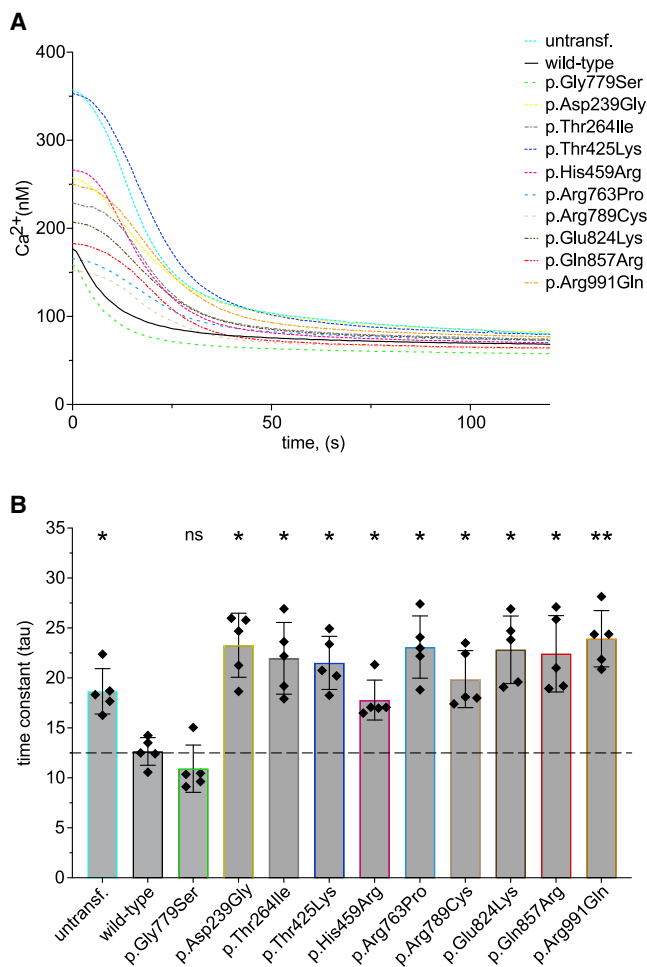
(B) Quantification of relative membrane localization (for details, see supplemental methods). Data presented as mean and standard deviation from 8 to 17 independent analyzed cells. Data are shown as mean  $\pm$  standard deviation represented by error bars. The results of a one-way ANOVA with the Games-Howell post-hoc test (each compared to wild type) is indicated as: \* $p < 0.05$ ; ns:  $p > 0.05$ .

individual into the clinical description of the present cohort, as the membrane localization and the tau value were indistinguishable from the wild type. It is possible that the variant causes a functional effect not measured by our assays. However, because the clinical relevance of this variant remains uncertain, we have excluded this individual from the phenotypic analysis.

The results of the present study imply that *de novo* missense variants in *ATP2B1* cause a loss-of-function mechanism on the basis of the following observations. (1) The *in silico* structural modeling supports a reduced *ATP2B1* activity resulting from changes of protein structure or stability. (2) The  $\text{Ca}^{2+}$  transport is significantly decreased by the missense variants. (3) The correlation between intracellular localization and tau value suggests the degradation of abnormal *ATP2B1*. (4) *ATP2B1* exhibits a pLI score = 1.00, which supports a potential haploinsufficiency of this gene. (5) Three of the probands carry nonsense variants. This notion is further supported by the reports of seven independent individuals that harbor

gross deletions, which affect *ATP2B1* (summarized by Klein et al.<sup>23</sup> and James et al.<sup>24</sup>). More important, these individuals exhibited an overlapping phenotype (DD, digit anomalies, facial dysmorphism, and growth deficiency) with the probands reported in the present study. We cannot exclude a dominant negative effect by the missense variants, even though this mechanism is unlikely. Instead of acting as monomers or homodimers, *ATP2B1*–4 form heteromeric complexes with neuroplastin or basigin.<sup>25</sup> In accordance, none of the missense variants affect the interacting region of *ATP2B1* and neuroplastin (Figure 3A).

Taken together, the overlapping phenotype of the probands and the experimental evidence strongly support that *ATP2B1* is a DD/ID-associated gene. Furthermore, the role of *ATP2B1* in neurodevelopment is strengthened by its expression pattern. While *ATP2B1* is expressed at the early stages during development, the three other isoforms of the ATPase plasma membrane  $\text{Ca}^{2+}$  transporter family (*ATP2B2*–4) are expressed at later stages.<sup>26</sup> The



**Figure 5. De novo missense variants in *ATP2B1* affect  $\text{Ca}^{2+}$  transport**

(A) Fluorometric  $[\text{Ca}^{2+}]_i$  analysis (for details, see [supplemental methods](#)) in untransfected (untransf.) HEK293 cells and cells expressing wild-type or mutated *ATP2B1*. Data are shown as mean  $[\text{Ca}^{2+}]_i$  from five independent experiments after loading of the  $[\text{Ca}^{2+}]_i$  indicator dye fura-2/AM and the final addition of EGTA (for a representative complete sequence of the experiment, see [Figure S2](#)).

(B) In order to investigate the  $\text{Ca}^{2+}$  transport of transfected HEK293, time-dependent  $[\text{Ca}^{2+}]_i$  decline was analyzed after final addition of EGTA that is represented by the time constant  $\tau$ . Data presented as mean and standard deviation from five independent experiments. As negative control served the likely non-pathogenic variant (3 times in gnomAD) p.Gly779Ser. The dashed line indicates the median  $\tau$  value of *ATP2B1* wild type. Data are shown as mean  $\pm$  standard deviation represented by error bars. The results of a one-way ANOVA with the Games-Howell post-hoc test (each compared to wild type) is indicated as: \*\*  $p < 0.005$ ; \*  $p < 0.05$ ; ns:  $p > 0.05$ .

important role of *ATP2B1* for neurological development is further corroborated by the fact that it can compensate for the absence of those other isoforms to a certain extent.<sup>27</sup> *ATP2B1* encodes different isoforms, which differ in calmodulin affinity and tissue expression, by alternative splicing (for a review, see Krebs<sup>28</sup>). Kenyon and colleagues found that *Atp2b1b* is nearly ubiquitously expressed in most tissues, while *Aatp2ba* is specifically expressed in

the central nervous system. In rat brains, they found out that *Atp2b1a* is detected only in neurons, where it concentrates in the membrane of the soma, dendrites, and spines.<sup>20</sup> It thus seems logical to consider *ATP2B1* as a candidate gene for an early neurodevelopmental disorder as diagnosed in the probands of this study. *Atp2b1-4* accumulate during the maturation of hippocampal neurons, which indicates the need of an increased  $\text{Ca}^{2+}$ -exclusion capacity.<sup>26</sup> Interestingly, *Atp2b1-4* are transcriptionally regulated by  $\text{Ca}^{2+}$  itself.<sup>29</sup> Likewise, studies with rats demonstrated the expression of *ATP2B1b* in the developing brain is later replaced by *ATP2B1a*, therefore further underlining its role in neurodevelopment.<sup>30</sup>

Thus, as *ATP2B1* has a significant impact on  $\text{Ca}^{2+}$  homeostasis in the central nervous system<sup>31</sup> during development on the basis of its expression pattern, it strongly supports that variants that affect *ATP2B1*'s function will impact neurons and therefore an individual's neurodevelopment.

We also considered whether variants that affect a certain domain lead to a variable phenotype. However, we could observe neither a particular domain-specific correlation with the symptoms of the individuals nor a correlation between the severity of the symptoms and the impact of each variant on *ATP2B1* activity. This may be due to the relatively small size of the herein reported cohort. Therefore, further studies with more individuals harboring a pathogenic variant in *ATP2B1* may clarify a potential correlation.

In conclusion, the overlapping phenotype of 12 probands, the genetic findings, the *in silico* data, the structural modeling, the role of *ATP2B1* in the central nervous system, and the pathogenicity of the variants prompt us to add *ATP2B1* loss-of-function variants as a monogenetic cause of DD/ID.

#### Data and code availability

The data of this study are available from the corresponding author with reasonable request. There was no code used for this study.

#### Supplemental information

Supplemental information can be found online at <https://doi.org/10.1016/j.ajhg.2022.03.009>.

#### Author contributions

M.J.R., R.J., M.W., and H.O. contributed to the concept and design and coordinated the study. Data curation and genetic analysis were performed by D.K., D.M., N.H., A.T., M.M., V.N., R.P., A.S., and T.B. Clinical investigations were performed by G.M., D.B., S.M., F.M., B.V., D.M., U.H., S.N., N.R., E.B., M. Sinnema, A.M., and I.D. The experiments were performed by M.J.R., N.U., and H.O. under the support of E.G. and M. Schaefer. H.S. performed the structural analysis and molecular modeling. M.J.R. and H.O. contributed to the acquisition, interpretation, statistical analysis, and visualization of the results. The original draft was written by M.J.R., R.J., and H.O., and all authors critically revised the



manuscript and ensured the final approval of the manuscript for publication.

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## Declaration of interests

R.P. is an employee of GeneDx, Inc. All other authors declare no competing interests.

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## Web resources

DECIPHER, <https://www.deciphergenomics.org/>  
ENSEMBL, <https://www.ensembl.org/>  
GeneMatcher, <https://genematcher.org/>  
GenBank, <https://www.ncbi.nlm.nih.gov/genbank/>  
gnomAD, <https://gnomAD.broadinstitute.org/>  
MetaDome, <https://stuart.radboudumc.nl/metadome>  
MutationTaster, <https://www.mutationtaster.org/>  
OMIM, <http://www.omim.org/>  
STRING database, <https://www.string-db.org/>  
SysNDD, <https://www.sysid.dbmr.unibe.ch/>  
The Genotype-Tissue Expression (GTEx) Project, <https://gtexportal.org/home/>  
The National Center for Biotechnology Information (NCBI), <https://www.ncbi.nlm.nih.gov/>  
UCSC Cell Browser (human cerebral cortex), <http://hgw1.soe.ucsc.edu/>  
UniProt database, <https://www.uniprot.org/>  
Variant Effect Predictor (VEP) from ENSEMBL, <https://www.ensembl.org/Tools/VEP>  
WebAutoCasC, <https://autocasc.uni-leipzig.de/>

## References

- McRae, J.F., Clayton, S., Fitzgerald, T.W., Kaplanis, J., Prigmore, E., Rajan, D., Sifrim, A., Aitken, S., Akawi, N., Alvi, M., et al. (2017). Prevalence and architecture of de novo mutations in developmental disorders. *Nature* *542*, 433–438.
- Gilissen, C., Hehir-Kwa, J.Y., Thung, D.T., van de Vorst, M., van Bon, B.W., Willemsen, M.H., Kuint, M., Janssen, I.M., Hoischen, A., Schenck, A., et al. (2014). Genome sequencing identifies major causes of severe intellectual disability. *Nature* *511*, 344–347.
- Aspromonte, M.C., Bellini, M., Gasparini, A., Carraro, M., Bettella, E., Polli, R., Cesca, F., Bigoni, S., Boni, S., Carlet, O., et al. (2019). Characterization of intellectual disability and autism comorbidity through gene panel sequencing. *Hum. Mutat.* *40*, 1346–1363.
- Kochinke, K., Zweier, C., Nijhof, B., Fenckova, M., Cizek, P., Honti, F., Keerthikumar, S., Oortveld, M.A.W., Kleefstra, T., Kramer, J.M., et al. (2016). Systematic Phenomics Analysis Deconvolutes Genes Mutated in Intellectual Disability into Biologically Coherent Modules. *Am. J. Hum. Genet.* *98*, 149–164.
- Brini, M., Cali, T., Ottolini, D., and Carafoli, E. (2014). Neuronal calcium signaling: function and dysfunction. *Cell. Mol. Life Sci.* *71*, 2787–2814.
- Gorman, K.M., Meyer, E., Grozeva, D., Spinelli, E., McTague, A., Sanchis-Juan, A., Carss, K.J., Bryant, E., Reich, A., Schneider, A.L., et al. (2019). Bi-allelic Loss-of-Function CACNA1B Mutations in Progressive Epilepsy-Dyskinesia. *Am. J. Hum. Genet.* *104*, 948–956.
- Zech, M., Lam, D.D., Weber, S., Berutti, R., Poláková, K., Havránková, P., Fečíková, A., Strom, T.M., Růžicka, E., Jech, R., and Winkelmann, J. (2018). A unique de novo gain-of-function variant in *CAMK4* associated with intellectual disability and hyperkinetic movement disorder. *Cold Spring Harb. Mol. Case Stud.* *4*, a003293.
- Nahm, M., Lim, S.M., Kim, Y.-E., Park, J., Noh, M.-Y., Lee, S., Roh, J.E., Hwang, S.-M., Park, C.-K., Kim, Y.H., et al. (2020). *ANXA11* mutations in ALS cause dysregulation of calcium homeostasis and stress granule dynamics. *Sci. Transl. Med.* *12*, eaax3993.
- Shamseldin, H.E., Alasmari, A., Salih, M.A., Samman, M.M., Mian, S.A., Alshidi, T., Ibrahim, N., Hashem, M., Faqeih, E., Al-Mohanna, F., and Alkuraya, F.S. (2017). A null mutation in *MICU2* causes abnormal mitochondrial calcium homeostasis and a severe neurodevelopmental disorder. *Brain* *140*, 2806–2813.
- Cali, T., Brini, M., and Carafoli, E. (2018). The PMCA pumps in genetically determined neuronal pathologies. *Neurosci. Lett.* *663*, 2–11.
- Cali, T., Lopreiato, R., Shimony, J., Vineyard, M., Frizzarin, M., Zanni, G., Zanotti, G., Brini, M., Shinawi, M., and Carafoli, E. (2015). A Novel Mutation in Isoform 3 of the Plasma Membrane Ca<sup>2+</sup> Pump Impairs Cellular Ca<sup>2+</sup> Homeostasis in a Patient with Cerebellar Ataxia and Laminin Subunit 1 $\alpha$  Mutations. *J. Biol. Chem.* *290*, 16132–16141.
- Li, M., Ho, P.W.-L., Pang, S.Y.-Y., Tse, Z.H.-M., Kung, M.H.-W., Sham, P.-C., and Ho, S.-L. (2014). PMCA4 (ATP2B4) mutation in familial spastic paraplegia. *PLoS ONE* *9*, e104790.
- Karczewski, K.J., Francioli, L.C., Tiao, G., Cummings, B.B., Alfoldi, J., Wang, Q., Collins, R.L., Laricchia, K.M., Ganna, A., Birnbaum, D.P., et al. (2020). The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature* *581*, 434–443.
- Lindeboom, R.G.H., Supek, F., and Lehner, B. (2016). The rules and impact of nonsense-mediated mRNA decay in human cancers. *Nat. Genet.* *48*, 1112–1118.
- Rentzsch, P., Schubach, M., Shendure, J., and Kircher, M. (2021). CADD-Splice-improving genome-wide variant effect

- prediction using deep learning-derived splice scores. *Genome Med.* *13*, 31.
16. Ioannidis, N.M., Rothstein, J.H., Pejaver, V., Middha, S., McDonnell, S.K., Baheti, S., Musolf, A., Li, Q., Holzinger, E., Karyadi, D., et al. (2016). REVEL: An Ensemble Method for Predicting the Pathogenicity of Rare Missense Variants. *Am. J. Hum. Genet.* *99*, 877–885.
  17. Mangialavori, I.C., Ferreira-Gomes, M.S., Saffioti, N.A., González-Lebrero, R.M., Rossi, R.C., and Rossi, J.P.F.C. (2013). Conformational changes produced by ATP binding to the plasma membrane calcium pump. *J. Biol. Chem.* *288*, 31030–31041.
  18. Wiel, L., Baakman, C., Gilissen, D., Veltman, J.A., Vriend, G., and Gilissen, C. (2019). MetaDome: Pathogenicity analysis of genetic variants through aggregation of homologous human protein domains. *Hum. Mutat.* *40*, 1030–1038.
  19. Gong, D., Chi, X., Ren, K., Huang, G., Zhou, G., Yan, N., Lei, J., and Zhou, Q. (2018). Structure of the human plasma membrane Ca<sup>2+</sup>-ATPase 1 in complex with its obligatory subunit neuroplastin. *Nat. Commun.* *9*, 3623.
  20. Kenyon, K.A., Bushong, E.A., Mauer, A.S., Strehler, E.E., Weinberg, R.J., and Burette, A.C. (2010). Cellular and subcellular localization of the neuron-specific plasma membrane calcium ATPase PMCA1a in the rat brain. *J. Comp. Neurol.* *518*, 3169–3183.
  21. Lenz, J.C., Reusch, H.P., Albrecht, N., Schultz, G., and Schaefer, M. (2002). Ca<sup>2+</sup>-controlled competitive diacylglycerol binding of protein kinase C isoenzymes in living cells. *J. Cell Biol.* *159*, 291–302.
  22. Salazar, M.D., Zelt, N.B., Saldivar, R., Kuntz, C.P., Chen, S., Penn, W.D., Bonneau, R., Koehler Leman, J., and Schleich, J.P. (2020). Classification of the Molecular Defects Associated with Pathogenic Variants of the *SLC6A8* Creatine Transporter. *Biochemistry* *59*, 1367–1377.
  23. Klein, O.D., Cotter, P.D., Schmidt, A.M., Bick, D.P., Tidymann, W.E., Albertson, D.G., Pinkel, D., and Rauen, K.A. (2005). Interstitial deletion of chromosome 12q: genotype-phenotype correlation of two patients utilizing array comparative genomic hybridization. *Am. J. Med. Genet. A.* *138*, 349–354.
  24. James, P.A., Oei, P., Ng, D., Kannu, P., and Aftimos, S. (2005). Another case of interstitial del(12) involving the proposed cardio-facio-cutaneous candidate region. *Am. J. Med. Genet. A.* *136*, 12–16.
  25. Schmidt, N., Kollwe, A., Constantin, C.E., Henrich, S., Ritzau-Jost, A., Bildl, W., Saalbach, A., Hallermann, S., Kulik, A., Fakler, B., and Schulte, U. (2017). Neuroplastin and Basigin Are Essential Auxiliary Subunits of Plasma Membrane Ca<sup>2+</sup>-ATPases and Key Regulators of Ca<sup>2+</sup> Clearance. *Neuron* *96*, 827–838.e9.
  26. Kip, S.N., Gray, N.W., Burette, A., Canbay, A., Weinberg, R.J., and Strehler, E.E. (2006). Changes in the expression of plasma membrane calcium extrusion systems during the maturation of hippocampal neurons. *Hippocampus* *16*, 20–34.
  27. Okunade, G.W., Miller, M.L., Pyne, G.J., Sutliff, R.L., O'Connor, K.T., Neumann, J.C., Andringa, A., Miller, D.A., Prasad, V., Doetschman, T., et al. (2004). Targeted ablation of plasma membrane Ca<sup>2+</sup>-ATPase (PMCA) 1 and 4 indicates a major housekeeping function for PMCA1 and a critical role in hyperactivated sperm motility and male fertility for PMCA4. *J. Biol. Chem.* *279*, 33742–33750.
  28. Krebs, J. (2015). The plethora of PMCA isoforms: Alternative splicing and differential expression. *Biochim. Biophys. Acta* *1853*, 2018–2024.
  29. Guerini, D., García-Martin, E., Gerber, A., Volbracht, C., Leist, M., Merino, C.G., and Carafoli, E. (1999). The expression of plasma membrane Ca<sup>2+</sup> pump isoforms in cerebellar granule neurons is modulated by Ca<sup>2+</sup>. *J. Biol. Chem.* *274*, 1667–1676.
  30. Brandt, P., Neve, R.L., Kammesheidt, A., Rhoads, R.E., and Vanaman, T.C. (1992). Analysis of the tissue-specific distribution of mRNAs encoding the plasma membrane calcium-pumping ATPases and characterization of an alternately spliced form of PMCA4 at the cDNA and genomic levels. *J. Biol. Chem.* *267*, 4376–4385.
  31. Brini, M. (2009). Plasma membrane Ca(2+)-ATPase: from a housekeeping function to a versatile signaling role. *Pflugers Arch.* *457*, 657–664.