Pathogenic heterozygous TRPM7 variants and hypomagnesemia with developmental delay

Running head: Characterizing novel TRPM7 variants

Willem Bosman¹, Kameryn M. Butler², Caitlin A. Chang³, Mythily Ganapathi⁴, Edwin Guzman⁵, Femke Latta¹, Wendy K. Chung⁶, Felix Claverie-Martin⁷, Jessica M. Davis², Joost G. J. Hoenderop¹, Jeroen H. F. de Baaij¹

¹Department of Medical BioSciences, Radboudumc, Nijmegen, the Netherlands
²Greenwood Genetic Center, Greenwood, SC, USA
³Department of Medical Genetics, BC Women and Children’s Hospital, Vancouver, British Columbia, Canada
⁴Department of Pathology and Cell Biology, Columbia University Irving Medical Center, New York, NY, USA
⁵Department of Pediatrics, Columbia University Irving Medical Center, New York, NY, USA
⁶Department of Pediatrics, Boston Children’s Hospital and Harvard Medical School, Boston, MA, USA
⁷Unidad de Investigación, RenalTube Group, Hospital Universitario Nuestra Señora de Candelaria, Santa Cruz de Tenerife, Spain

Correspondence to:
Jeroen H. F. de Baaij; E-mail: jeroen.debaaij@radboudumc.nl
Background.

Heterozygous variants in TRPM7, encoding an essential and ubiquitously expressed cation channel, may cause hypomagnesemia, but current evidence is insufficient to draw definite conclusions and it is unclear whether any other phenotypes can occur.

Methods.

Individuals with unexplained hypomagnesemia underwent whole exome sequencing which identified TRPM7 variants. Pathogenicity of the identified variants was assessed by combining phenotypic, functional and in silico analyses.

Results.

We report three new heterozygous missense variants in TRPM7 (p.Met1000Thr, p.Gly1046Arg, p.Leu1081Arg) in individuals with hypomagnesemia. Strikingly, autism spectrum disorder and developmental delay, mainly affecting speech and motor skills, was observed in all three individuals, while two out of three also presented with seizures. The three variants are predicted to be severely damaging by in silico prediction tools and
structural modeling. Furthermore, these variants result in a clear loss-of-function of TRPM7-mediated magnesium uptake \textit{in vitro}, while not affecting TRPM7 expression or insertion into the plasma membrane.

**Conclusions.**

This study provides additional evidence for the association between heterozygous \textit{TRPM7} variants and hypomagnesemia and adds developmental delay to the phenotypic spectrum of \textit{TRPM7}-related disorders. Considering the \textit{TRPM7} gene is relatively tolerant to loss-of-function variants, future research should aim to unravel by which mechanisms specific heterozygous \textit{TRPM7} variants can cause disease.
KEY LEARNING POINTS

What was known:

- Two studies have reported heterozygous variants in TRPM7 in three families with hypomagnesemia.
- Considering the low number of cases, current evidence is insufficient to conclude that TRPM7 variants can indeed be causative for hypomagnesemia.
- Beyond hypomagnesemia, the exact nature of other phenotypes associated with potentially pathogenic TRPM7 variants is incompletely described.

This study adds:

- With the addition of three independent cases, we confirm that heterozygous TRPM7 variants can be disease-causing and lead to hypomagnesemia.
- In addition to hypomagnesemia, TRPM7 variants may also be associated with global developmental delay and autism spectrum disorder.

Potential impact:

- TRPM7 can be added to gene panels in the diagnosis of individuals with unsolved hypomagnesemia, developmental delay and/or autism spectrum disorder.

Keywords: autism spectrum disorder, developmental delay, genetics, hypomagnesemia, TRPM7
INTRODUCTION

Magnesium (Mg$^{2+}$) is implicated in many processes in health and disease and its homeostasis has to be maintained tightly by intestinal absorption, storage in bone and soft tissues and renal reabsorption (1). Transient receptor potential melastatin type 7 (TRPM7) is a divalent cation channel responsible for the influx of ions such as Mg$^{2+}$, calcium (Ca$^{2+}$) and zinc (2). Although the channel is ubiquitously expressed, within the kidney TRPM7 expression is significantly higher in the distal convoluted tubule (DCT), the only segment of the nephron where transcellular Mg$^{2+}$ reabsorption occurs (3-5). Recently, two independent groups identified heterozygous variants in the TRPM7 gene in three families with hypomagnesemia (serum Mg$^{2+}$ <0.7 mmol/L) (6, 7). In addition, these studies report secondary hypocalcemia, seizures and hemiplegic migraine attacks as potential consequences of the TRPM7 variants. These phenotypes are reminiscent of hypomagnesemia with secondary hypocalcemia (HSH, MIM #602014) caused by bi-allelic variants in TRPM6 (8, 9). The hypocalcemia can be explained by inadequately low parathyroid hormone (PTH) levels, caused by a hypomagnesemia-induced reduction in PTH production and secretion (10). Sporadically, individuals with HSH also present with neurological symptoms such as intellectual disability and psychomotor delays, both observed in 3 out of 88 reported cases (11). Since TRPM6 and TRPM7 are thought to function as heterotetramers to facilitate Mg$^{2+}$ (re)absorption in colon and DCT and thereby maintain sufficient Mg$^{2+}$ levels in the body (12), it is not surprising that TRPM7 variants could cause a similar phenotype. However, TRPM7 is more broadly expressed and has various TRPM6-independent functions, suggesting that phenotypes associated with TRPM7 variants could be more extensive.

Considering the low number of reported cases, the current evidence is insufficient to unequivocally conclude that heterozygous TRPM7 variants cause hypomagnesemia and it is unclear which other phenotypes can occur. Therefore, the aim of this study is to characterize additional TRPM7 variants in order to provide additional evidence for their pathogenicity and
enhance understanding of the associated phenotypic spectrum. This led to the identification of three novel variants that were tested for effects on protein expression, structure and function.

MATERIALS AND METHODS

Study participants
Individuals were included when a novel TRPM7 variant that was suspected but unconfirmed to be causative for their phenotypes was identified. GeneMatcher was employed to find and connect multiple cases (13). Variant identification was part of routine genetic diagnostics without the need for additional procedures. Written informed consent was obtained for the genetic analysis and the publication of anonymized data.

Genetic analysis
Variants were identified by whole exome sequencing (WES) at the respective institutes: Columbia University Irving Medical Center, NY, USA by reanalysis of WES data in 2023. The clinical WES was previously done at GeneDx (Stamford, USA) in 2013 and was non-diagnostic (case 1); Greenwood Genetic Center, SC, USA using the SureSelectXT Clinical Research Exome kit (Agilent) and the NovaSeq 6000 System (Illumina) with 150 bp paired-end reads (case 2); University of British Columbia, Canada in collaboration with GeneDx for sequencing in 2022 (case 3). Reads were aligned to human genome build GRCh37/hg19. Only canonical splice site or non-synonymous coding variants with ≥5 reads, variation in ≥20% of reads and minor allele frequencies <0.1% were included. Allele frequencies of the newly identified TRPM7 variants were checked in the GnomAD v2.1.1 (14) and GME Variome (15) databases in September 2023. For in silico prediction of pathogenicity, REVEL (16) and BayesDel v1 (17) scores were used with cut-off values of ≥0.644 and ≥0.13 based on the analysis by Pejavar et al. (18).
DNA constructs

The pTracer-CMV2 construct encoding mouse TRPM7 (NM_021450.2; 97% sequence similarity with human TRPM7) with a carboxy-terminal HA-tag was a kind gift from Dr. David E. Clapham. The variants were introduced using the Q5 site-directed mutagenesis kit (New England BioLabs; E0554) following manufacturer’s instructions.

Cell culture and transfection

Human Embryonic Kidney 293 (HEK293) cells were maintained in Dulbecco’s Modified Eagle Medium (Gibco, 42430-035) supplemented with 10% (v/v) fetal bovine serum, 1 mmol/L sodium pyruvate and 0.1 mmol/L non-essential amino acids (Westburg, CA NEAA-B) at 37°C, 5% (v/v) CO₂. Lipofectamine 2000 (Invitrogen, 11668-019) was used for transfection at a 1:2 DNA to Lipofectamine ratio according to manufacturer’s instructions.

²⁵Mg²⁺ uptake assay

HEK293 cells were seeded in 12-well plates (three wells per condition) coated with poly-L-lysine (Sigma-Aldrich, P2636). 6 hours later, cells were transfected with empty vector, wild-type (WT) or variant Trpm7. Two days after transfection, cells were washed once with uptake buffer (125 mmol/L NaCl, 5 mmol/L KCl, 0.5 mmol/L CaCl₂, 0.5 mmol/L Na₂HPO₄, 0.5 mmol/L Na₂SO₄, 15 mmol/L HEPES, set to pH 7.5 with NaOH) without Mg²⁺ and then incubated for 15 minutes in uptake buffer containing 1 mmol/L ²⁵MgO (CortecNet, M-Mg25). Cells were then washed three times with ice-cold PBS, lysed in HNO₃ and analyzed with inductively coupled plasma mass spectrometry.

Cell surface biotinylation

HEK293 cells were seeded in poly-L-lysine-coated 6-well plates (two wells per condition) and transfected with empty vector, WT or mutant Trpm7. Two days later, cells were transferred to a cold room (4°C) and washed twice with PBS-CM (PBS with 0.5 mmol/L CaCl₂ and 1 mmol/L MgCl₂, pH 8.0). PBS-CM containing 0.5 mg/ml Sulfo-NHS-LC-LC-Biotin (Thermo
Scientific, 21338) was then added to the cells for 30 minutes. For cells transfected with WT, a negative control without biotin was included to confirm specificity of the assay. After washing twice in PBS-CM with 0.1% BSA and twice in PBS, cells were lysed in lysis buffer (1% (v/v) Triton X-100, 50 mmol/L Tris-HCl pH 7.5, 1 mmol/L EDTA, 1 mmol/L EGTA, 10 mmol/L sodium glycerophosphate, 50 mmol/L NaF, 10 mmol/L sodium pyrophosphate, 270 mmol/L sucrose, 150 mmol/L NaCl) containing freshly-added protease and phosphatase inhibitors (1 mmol/L phenylmethylsulfonyl fluoride, 5 µg/ml leupeptin hemisulfate, 1 µg/ml pepstatin A, 1 µg/ml aprotonin, 1 mmol/L sodium orthovanadate). Equal amounts of proteins were added to 20 µl NeutrAvidin Agarose Resin (Thermo Scientific, 29201) and input samples were prepared to check expression. Samples were incubated with the Resin overnight while rotating. After washing three times with lysis buffer, samples were eluted with 40 µl 2x Laemmli/dithiothreitol buffer at 37°C for 30 minutes.

**SDS-PAGE and Western blotting**

Biotinylation samples were loaded on 8% (v/v) SDS-PAGE gels and transferred to polyvinylidene difluoride membranes. After blocking with 5% (w/v) milk in TBS-Tween, membranes were incubated overnight with primary antibody targeting HA (Cell signaling technology, 2367) diluted 1:5,000 or β-actin (Sigma-Aldrich; A5441) diluted 1:10,000 in 1% (w/v) milk. Blots were then incubated for 1 hour with peroxidase-conjugated secondary antibody (Jackson ImmunoResearch, 515-035-003) diluted 1:10,000 and visualized with PICO chemiluminescent substrate (ThermoFisher, 34580).

**In silico modelling**

The following reference sequences were used for alignment of the TRPM7 amino acid sequence of different species: NP_060142.3 (*Homo sapiens*), NP_446157.2 (*Rattus norvegicus*), NP_067425.2 (*Mus musculus*), NP_001384973.1 (*Gallus gallus*), NP_001025232.1 (*Danio rerio*). The variants were modelled in the structure of closed apo state mouse TRPM7 reconstituted in lipid nanodiscs solved by cryogenic electron
microscopy (Protein Data Bank, 8SI2) (19). Considering the heterozygosity, the variants should affect TRPM7 function even if not all subunits of the tetramer are affected, so they were modelled in only one subunit. Analysis and visualization were performed in YASARA (20).

Statistical analysis
Welch's ANOVA with Dunnett's T3 multiple comparisons test was used to compare $^{25}$Mg$^{2+}$ uptake capacity and cell surface expression between WT and variants. A P-value of <0.05 was considered statistically significant.

RESULTS
Hypomagnesemia and developmental delay in individuals with novel TRPM7 variants
To provide an overview of phenotypes associated with heterozygous TRPM7 variants, characteristics of 3 individuals with newly identified variants are summarized together with the 5 cases from previous studies (Table 1) (6, 7). The 3 novel cases are described in more detail below.

Case 1 is a 14-year-old girl with global developmental delay, seizures, autism spectrum disorder (ASD) and attention deficit hyperactivity disorder (ADHD). Her developmental delay is characterized by speech impairment (first words at 32 months, echolalic speech), motor skill disorders (dyspraxia) and borderline normal intellectual functioning. She also has torticollis and severe myopia and shows rapid weight gain. Brain MRI showed no abnormalities (data not shown). Serum analysis revealed hypomagnesemia (0.37 mmol/L) but normal Ca$^{2+}$ (2.36 mmol/L) and PTH levels (3.8 pmol/L). A de novo missense variant was identified in TRPM7 (NM_017672.6:c.2999T>C, p.Met1000Thr) after WES trio analysis, which was the most likely candidate out of 12 identified variants (Table S1).
Case 2 is a 2-year-old boy with global developmental delay (absent speech and motor skill disorder), seizures, ASD and eczema. In addition, he was recently diagnosed with obstructive sleep apnea and enamel hypoplasia. Brain MRI did not show abnormalities (data not shown). Out of the candidate variants identified after WES trio analysis (Table S2), only a de novo variant (c.3136G>C, p.Gly1046Arg) in TRPM7 was not inherited from the healthy parents. Subsequently, serum Mg$^{2+}$ was measured at 0.58 mmol/L. He is treated with Topamax and Onfi for the seizures and magnesium supplements were started resulting in some improvements in sleep and attention, but Mg$^{2+}$ levels remained low (0.45-0.62 mmol/L).

Case 3 is a 4-year-old boy with ASD and developmental delay, characterized by speech delay (first words at 18 months) and motor skill disorders (dyspraxia, axial hypotonia, wide-based gait with toe-walking). There are some dysmorphic features in the face and hands (upslanting palpebral fissure, bulbous nasal tip, low-set ears, persistent fetal pads). MRI was unremarkable apart from an incidental suspicion of hemangioma at the T3 vertebral body (data not shown) and he does not have a history of seizures. He also presented with hypomagnesemia (0.47-0.50 mmol/L) but normal serum Ca$^{2+}$ (2.47 mmol/L) and PTH (6.4 pmol/L). The p.Leu1081Arg (c.3242T>G) variant was identified in TRPM7 after singleton WES analysis. Targeted Sanger sequencing in the parents confirmed a de novo origin. In addition, a maternally inherited variant was found in the mitochondrial gene encoding the cytochrome c oxidase I (MT-CO1) subunit of Complex IV of the electron transport chain (m.7023G>A; YP_003024028.1:p.Val374Met). This variant was considered not to be the disease cause based on its presence in the healthy mother and a very low blood heteroplasmy level (2%).

None of the variants are present in the GnomAD and GME Variome databases, while all three are predicted to be pathogenic by the in silico tools (Table 2).
p.Met1000Thr, p.Gly1046Arg and p.Leu1081Arg severely affect TRPM7 function while membrane expression is maintained

To obtain functional evidence for the pathogenicity of the newly identified variants, we performed an uptake assay using the stable isotope $^{25}\text{Mg}^{2+}$. Compared to cells transfected with WT TRPM7, p.Met1000Thr (18.7%, P=0.025), p.Gly1046Arg (18.4%, P=0.025) and p.Leu1081Arg (21.1%, P=0.027) demonstrated severely reduced uptake capacity (Figure 1A). These levels were comparable with cells transfected with the empty vector (23.9% compared to WT), indicating that all three variants result in a complete loss-of-function. We also examined whether variant TRPM7 proteins are present at the plasma membrane by cell surface biotinylation. Overall protein expression was comparable to WT (Figure S1A) and all variants were present at the cell surface. The relative membrane expression of all three variants was not significantly different compared to WT (149%, 108% and 89% for p.Met1000Thr, p.Gly1046Arg and p.Leu1081Arg respectively) (Figure 1B and C). To assess potential dominant-negative effects, we co-expressed WT TRPM7 with either empty vector or the variants and measured $^{25}\text{Mg}^{2+}$ uptake. Total TRPM7 expression was ~2-fold higher in the presence of the variants compared to the empty vector, indicating WT TRPM7 expression was similar in all conditions (Figure S1B and C). When the p.Gly1046Arg variant was co-expressed, uptake capacity was significantly reduced compared to WT alone (58.3%, P=0.049) (Figure 1D). Although co-transfection with the p.Met1000Thr and p.Leu1081Arg variants also seemed to affect $\text{Mg}^{2+}$ uptake, no significant reductions in $\text{Mg}^{2+}$ uptake by WT TRPM7 were observed (68.3 and 73.0%, P=0.11 and 0.36 respectively).

To support our functional findings, we assessed conservation of the affected residues in different species and modeled the amino acid substitutions in the Cryo-EM structure of the TRPM7 tetramer (Figure 2A and B). All three residues are highly conserved among vertebrates. The Gly1046 residue is located in the pore-forming domain, where the small glycine residue allows passage of cations through the pore. In the p.Gly1046Arg variant, the pore is blocked by the introduction of the large arginine side chain (Figure 2C). The Leu1081
residue is part of the S6 transmembrane helix, which partially lines the pore. Residues 1078-1083 form a hydrophobic region of this helix that is present right below the pore, in close proximity to Gly1046 (Figure 2C). In the p.Leu1081Arg variant a large hydrophilic side chain is introduced at the center of this hydrophobic region. Lastly, the Met1000 residue is at the start of the S5 transmembrane helix, where the S5 helix bends in relation to the S4-S5 linker (Figure 2B and D). The S5 helix is involved in the channel’s transition from a closed to an open conformation by moving relative to the S4-S5 linker and away from the pore center (19). The interaction of the methionine side chain at this bend with other residues is expected to be lost when replaced by the smaller threonine residue, similar to what was observed for p.Met1000Val (6).

**DISCUSSION**

In this study, we provide evidence that heterozygous pathogenic TRPM7 variants are causative for hypomagnesemia and can also be associated with developmental delay and ASD. This is based on the identification of three de novo variants (p.Met1000Thr, p.Gly1046Arg and p.Leu1081Arg) in individuals with a similar combination of low serum Mg²⁺ levels and developmental delay with ASD. Two of the three variants affect the same residues, Met1000 and Gly1046, as two recently reported missense variants (6, 7). In addition, all three variants severely affect the Mg²⁺ uptake capacity of TRPM7 in HEK293 cells and are predicted to be damaging by various in silico methods. This work demonstrates that heterozygous TRPM7 variants at specific positions can be disease-causing and that the resulting phenotypes may be broader than previously described.

Our results combined with the cases from earlier work have now led to the identification of 6 heterozygous TRPM7 variants causative for hypomagnesemia, 5 missense variants and 1 splice variant. Our data indicate that individuals with TRPM7 missense variants can also present with global developmental delay, especially affecting motor skills and speech.
Although global developmental delay was not reported in the two previous studies, Lei et al. do report difficulties with ordered movement and slurred speech in a child with variant p.Met1000Val, while case F2-II.1 described by Vargas-Poussou et al. (p.Gly1046Asp) was too young at the time of examination to draw conclusions concerning developmental delay (6, 7). Indeed, clinical follow-up of this child revealed delays in maturation and speech. This same study also described a family that carries a splice variant (c.3+1G>C), which leads to loss of expression of the affected allele as it causes defective splicing and a premature stop codon in exon 2. Developmental delay seems to be absent and ages of diagnosis were much higher in this family, indicative of a less severe phenotype compared to the missense variants. However, with only one splice variant described so far, it is too early to conclude whether a less severe phenotype is a specific characteristic of such a variant or if it is simply part of the variable phenotypic spectrum of TRPM7-related disorders.

Since TRPM7 can form functional heteromeric channels with TRPM6, it is not surprising that the phenotypes of individuals with TRPM7 variants show similarities to HSH. In both disorders, hypomagnesemia is consistently observed as well as seizures in the majority of cases. Considering both TRPM proteins are co-expressed highly in the colon and DCT, it is likely that in both cases the underlying mechanism of the hypomagnesemia is intestinal malabsorption and renal wasting caused by decreased functioning of TRPM6/TRPM7 heterotetramers. Indeed, a combined reduction in intestinal and renal (re)absorption of Mg²⁺ has been observed in both disorders (7, 8). Furthermore, in line with the cases from this study, HSH is sometimes also associated with neurodevelopmental disorders, especially when hypomagnesemia is detected and treated late (10, 21, 22). However, there also seem to be some distinct differences between the disorders. Based on the small cohort described so far, neurodevelopmental delay seems to be more prevalent in TRPM7-related disorders compared to HSH, also indicated by the presence of ASD in all three cases described here. Clinical follow-up of the patient carrying variant p.Gly1046Asp has shown ASD as well. A broader and more severe phenotype could be a result of the more ubiquitous expression and
general function of TRPM7 compared to TRPM6 (23, 24). Indeed, variants in TRPM7 have been associated with increased susceptibility to a wide range of diseases, including neurodegenerative disorders (25), colorectal cancer (26), breast cancer (27) and thrombocytopenia (28). In line with the widespread role of TRPM7 and the heterozygous state of TRPM7 variants described thus far, global deletion of TRPM7 is not compatible with life in cell and animal models (24, 29). Considering this broad and essential function of TRPM7, identification of a larger cohort of individuals with pathogenic TRPM7 variants is required to obtain a more accurate and complete understanding of the associated phenotypes. It is therefore essential that TRPM7 variants are considered in the diagnosis of individuals with unsolved hypomagnesemia with or without developmental delay and ASD.

It is important to note that GnomAD reports a relatively high number of predicted loss-of-function variants in TRPM7 in the healthy population, resulting in a probability of being loss-of-function intolerant (pLI) of 0. If haploinsufficiency is assumed as disease mechanism, these loss-of-function variants would be expected to be disease-causing. This indicates that only specific variants are pathogenic, which is supported by the fact that two of the three novel variants (p.Met1000Thr and p.Gly1046Arg) affect the same residue as previously reported cases. Our data suggest an equally important role for the Leu1081 residue. Indeed, similar to Met1000 and Gly1046, there are no missense variants reported at position Leu1081 in GnomAD or GME Variome. Structurally, the importance of these residues can be understood, as they are predicted to cause significant alterations either directly in the pore region (p.Gly1046Arg, p.Gly1046Asp and p.Leu1081Arg) or in the gating machinery (p.Met1000Thr and p.Met1000Val). In addition, there are other examples of autosomal dominant disorders caused by specific variants in genes that are otherwise tolerant to loss-of-function. Heterozygous variants in the calcium-sensing receptor gene CASR (pLI=0.05) can cause hypocalciuric hypercalcemia (30) while autosomal-dominant tubulointerstitial kidney disease can be caused by variants in UMOD (pLI=0), MUC1 (pLI=0.02) or REN (pLI=0) (31). These observations might be explained by toxic accumulation of mutant protein
such as in the case of MUC1, in which all pathogenic variants are frameshift variants that result in the same aberrant protein (32), although our data do not point towards accumulation of TRPM7 variants. Alternatively, variants could have specific effects on protein-protein interactions. In addition to TRPM6, TRPM7 interacts with Cyclin and CBS-domain Divalent Metal Cation Transport Mediator proteins (CNNMs), Phosphatases of Regenerating Liver proteins (PRLs) and ADP Ribosylation Factor Like GTPase 15 (ARL15), all of which are implicated in Mg\(^{2+}\) homeostasis (33, 34). Lastly, the most likely explanation for variants affecting the pore region is a dominant-negative effect. The finding that TRPM7-mediated \(^{25}\)Mg\(^{2+}\) uptake is reduced when the p.Gly1046Arg variant is co-expressed indeed indicates that this variant interferes with WT TRPM7 functioning. However, although trends were observed, these findings could not be replicated for p.Met1000Thr, p.Leu1081Arg or p.Gly1046Asp (7). This could be explained by less severe effects or the number of subunits of the tetramer that has to be affected. Of note, the aforementioned disease mechanisms do not explain the pathogenicity of the c.3+1G>C variant, as this variant results in loss of TRPM7 expression rather than expression of a non-functional or toxic protein. It is possible that the disease mechanism of the splice variant differs, considering the less severe phenotype of the family carrying this variant. Evidently, although a dominant-negative effect seems likely for the p.Gly1046Arg variant, understanding why only certain variants in TRPM7 are disease-causing while other loss-of-function variants are not is still an important question for future research.

In conclusion, we have identified three novel pathogenic TRPM7 missense variants in individuals with hypomagnesemia and developmental delay. These variants affect essential residues in the TRPM7 protein and completely abolish Mg\(^{2+}\) uptake capacity. By screening the TRPM7 gene in individuals with hypomagnesemia, developmental delay and/or ASD the diagnosis of these disorders can be improved and the phenotypic spectrum of TRPM7-related disorders can be expanded.
ACKNOWLEDGEMENTS
We would like to thank Dr. Hanka Venselaar for the visualization of the variants in the 3D TRPM7 structure.

CONFLICT OF INTEREST STATEMENT
None declared

AUTHORS’ CONTRIBUTIONS
JGJH and JHFdB conceptualized the study. KMB, CAC, MG, EG, WKC, FCM and JMD identified and interpreted genetic variants and acquired clinical data. WB and FL conducted experiments and analyzed data. WB, JGJH and JHFdB wrote the manuscript. All authors corrected and approved the manuscript.

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DATA AVAILABILITY STATEMENT
The variants described in this study have been submitted to ClinVar (p.Met1000Thr: SCV004175909; p.Gly1046Arg: SCV003799049; p.Leu1081Arg: SCV004175910). All
relevant data is present within the manuscript. Additional data can be requested from the corresponding author.

REFERENCES


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ADHD: attention deficit hyperactivity disorder; CKD: chronic kidney disease; N, no; NA, not assessed; OSA: obstructive sleep apnea; Y, yes.
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Figure 1. Effects of Met1000Thr, Gly1046Arg and Leu1081Arg on Mg$^{2+}$ uptake and cell membrane expression. (A) $^{25}$Mg$^{2+}$ uptake after 15 minutes in cells transfected with mock, WT or variant TRPM7. (B) Quantification of cell surface biotinylation assay for HEK293 cells transfected with WT or variant TRPM7. (C) Representative Western blot of cell surface biotinylation assay. (D) $^{25}$Mg$^{2+}$ uptake after 15 minutes in cells co-transfected with WT TRPM7 with either mock or variant. Data points represent 3 independent experiments. Data are presented as mean ± SEM. *P<0.05; **P<0.01.
Figure 2. Visualization of Met1000Thr, Gly1046Arg and Leu1081Arg in the TRPM7 protein structure. (A) Alignment of the TRPM7 amino acid sequence of different species for the affected residues and surrounding region. Dr, Danio rerio; Gg, Gallus gallus; Hs, Homo sapiens; Mm, mus musculus; Rn, Rattus norvegicus. (B) Position of the variants in the complete apo state TRPM7 tetramer structure. One subunit (gray) is shown as ribbon diagram, while the other three are shown as Cryo-EM density maps. (C) Zoom-in of the Gly1046 vicinity, showing the Gly1046Arg substitution blocking the pore and the Leu1081Arg substitution affecting the S6 helix just below the pore-forming region. (D) Zoom-in of the Met1000 vicinity, showing the position of this residue in the bend between the S4-S5 linker and the S5 helix. WT residues are shown in gray, variant residues in red. Unaffected subunits of the tetramer are shown in light green, dark green and blue.