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ORIGINAL ARTICLE

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Missense variants in the TRPMr7 α-kinase domain are associated with recurrent pediatric acute liver failure

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Abstract

Background: Pediatric acute liver failure (PALF) is a rare and life-threatening condition. In up to 50% of PALF cases, the underlying etiology remains unknown during routine clinical testing. This lack of knowledge complicates clinical management and liver transplantation decisions. Recently, whole-exome sequencing has identified genetic disorders in a large number of cases without specific laboratory biomarkers or metabolic fingerprints.

Abbreviations: ALF, acute liver failure; CADD, Combined Annotation Dependent Depletion; LT, liver transplantation; PALF, pediatric acute liver failure; PALFES, Pediatric Acute Liver Failure Exome Sequencing; TMT, tandem mass tag; TRPM7, transient receptor potential cation channel subfamily M member 7; WES, whole-exome sequencing.

Holger Prokisch and Georg F. Vogel contributed equally to this work.

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Methods: We describe how further analysis of whole-exome sequencing data combined with proteomic analyses in 5 previously unsolved PALF patients, where no pathogenic variants in genes previously associated with acute liver failure were identified, revealed rare biallelic variants in transient receptor potential cation channel subfamily M member 7 (*TRPM7*).

Results: We establishe TRPM7 as a novel disease gene for PALF. Yet, the cation channel kinase TRPM7 has not been associated with any Mendelian disorder. No homozygous loss-of-function variants were found in in-house exomes or publicly available databases. Rare biallelic *TRPM7*-variants were significantly enriched in the PALF cohort compared with a pediatric control cohort. Viral infections preceded the majority of PALF episodes. Recurrent PALF episodes characterized the disease course with rapid progression, leading to early death in 3 cases. Proteomic analyses of patient fibroblasts unveiled significantly reduced TRPM7 protein levels, indicative of functional impairment. Severely reduced Mg^{2+} levels in one individual with a mutation in the channel domain suggests a potential interaction between disturbed Mg^{2+} homeostasis and PALF. The consistent presence of mutations in the TRPM7 protein-kinase-domain across all patients suggests its specific relevance in PALF.

Conclusions: Our data extend the genetic spectrum of recurrent PALF and prompt consideration of TRPM7 in children with unexplained liver failure.

Keywords: pediatric acute liver failure, TRPM7, whole-exome sequencing

INTRODUCTION

Pediatric acute liver failure (PALF) is a rare and life-threatening condition predominantly affecting children during their first years of life.[1,2] It is characterized by high morbidity and mortality rates.[3] Effective management of PALF requires the expertise of specialized pediatric centers with an interdisciplinary team. The spectrum of underlying etiologies primarily comprises intoxication, metabolic disorders, and viral infections.[3,4] A timely diagnosis is of paramount importance, not only for the initiation of optimal treatment but also to allow prediction of the disease course. In the setting of recurrent episodes of acute liver failure (ALF), a molecular diagnosis can help to prevent additional PALF episodes and optimize care.

However, in both American and European cohorts, in approximately half of PALF cases the underlying etiology remains unknown after routine diagnostics. This uncertainty significantly complicates clinical management, hinders the implementation of disease-specific therapies, and poses challenges in decisions related to liver transplantation (LT).[1,5–7]

In recent years, whole-exome sequencing (WES) has been established as a tool to search for genetic causes in undetermined PALF cases. Genetic disorders have been identified in a large number of cases, particularly in those without specific laboratory biomarkers or metabolic fingerprints. WES revealed a higher impact of genetic disorders on PALF than previously thought.[8,9] For example, in the international PALFES study, of 250 indeterminate PALF cases, WES identified a genetic basis in ~40% of cases.[2]

We describe 5 children from 3 unrelated families with PALF who were found by WES to carry biallelic variants in transient receptor potential cation channel subfamily M member 7 (*TRPM7*). Functional studies in patient fibroblasts showed significantly reduced TRPM7 protein levels, suggesting that deleterious variants in *TRPM7* are associated with PALF.

METHODS

Patient recruitment

All 5 pediatric patients were extracted from the Pediatric Acute Liver Failure Exome Sequencing (PALFES) study. PALFES is an international, multicenter observational study designed to investigate the genetic causes of indeterminate PALF.[2] The study includes both prospective and retrospective cases, enrolling 260

patients aged 0–18 years from 19 countries between 2011 and 2022. Patients were included if no cause of PALF was identified after routine clinical workup, with both initial and recurrent presentations considered. WES was performed in all PALF cases to identify genetic etiologies, with a special focus on 243 genes previously reported to be associated with ALF. If no diagnosis was found within these genes, the entire exome was evaluated. Prior to study participation, informed consent was obtained from the patients and their parents in accordance with the local regulations. The study was conducted in accordance with both the Declaration of Helsinki[10] and Istanbul and approved by the ethics committees of the participating centers where biological samples were collected. Written consent was given in writing by all subjects must be included within the methods section of the manuscript.

WES and variant prioritization

WES was performed at the Institute of Human Genetics, Klinikum rechts der Isar, Technische Universität München (TUM, Munich, Germany). An in-house pipeline at TUM was used for the analysis of the WES data.[11] WES was performed on blood-extracted genomic DNA as previously published.[12] Sequencing reads were aligned to the human genome assembly GRCh37/hg19 (UCSC Genome Browser) using the Burrows-Wheeler Aligner (v.0.7.5a).[13] Single nucleotide variants, as well as small insertions and deletions, were detected using the Genome Analysis Toolkit.[14] Copy number variants were detected using ExomeDepth.[15] Mitochondrial DNA variants were detected from exonic data as previously outlined.[16]

Cell culture

Fibroblasts from family 1 (individuals 2 and 3) were collected postmortem, while fibroblasts from individual 5 were collected during the patient's lifetime. Primary patient-derived fibroblast cell lines were cultured in high glucose DMEM supplemented with supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin, and 200 μM uridine at 37 °C and 5% CO_2.

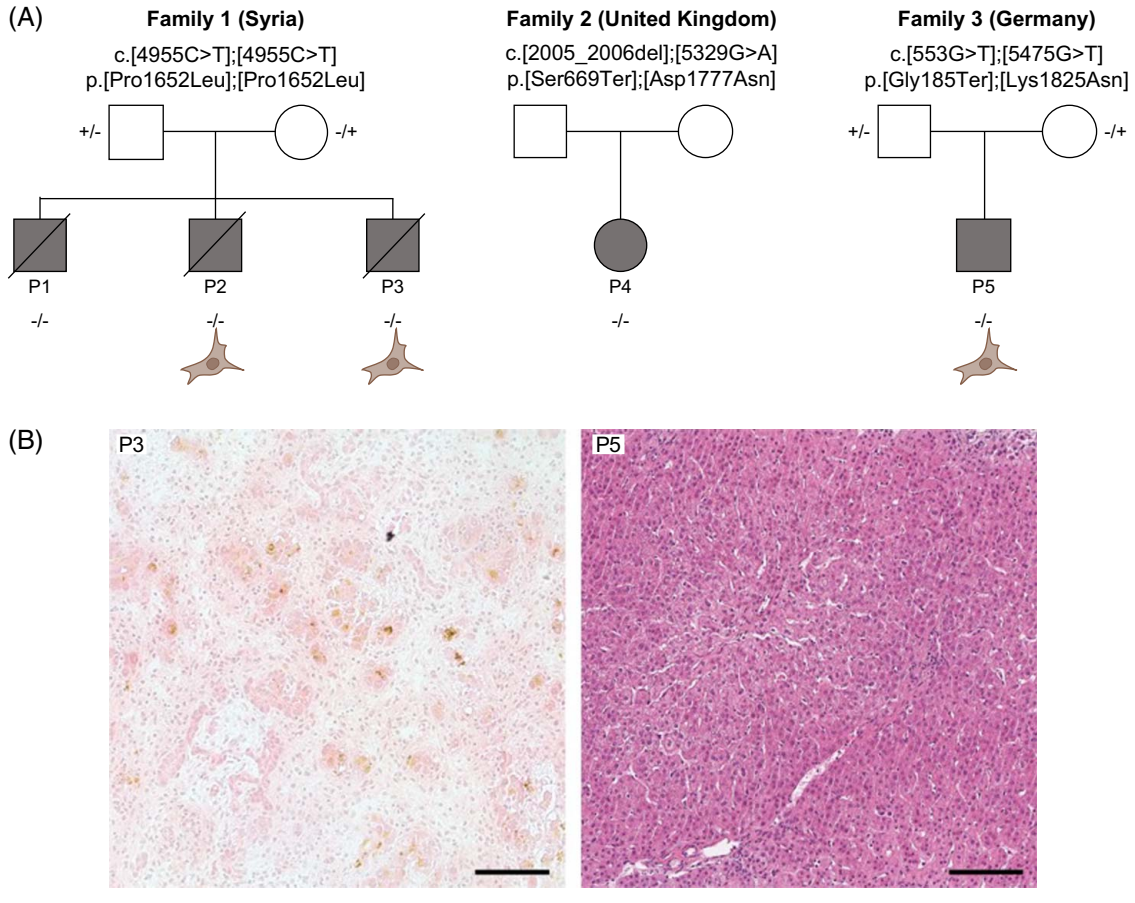


FIGURE 1 Family pedigrees and liver histopathology. (A) Pedigrees of 3 families identified with identified recessive inherited mutations in transient receptor potential cation channel subfamily M member 7 (*TRPM7*). (B) Hematoxylin-eosin staining of liver biopsies from individuals 3, 4, and 5 (I3, I4, and I5), showing micronodular cirrhosis, canalicular, and hepatocellular cholestasis in I3; parenchymal damage, bridging collapse, and severe cholestasis in I4; low hepatic damage and chronic cholestasis with portal and septal fibrosis in I5. The scale bar is 500 μm.

RNA sequencing

RNA was isolated from patients' fibroblasts whole cell lysates using standard procedures.[17] RNA integrity number was assessed using Agilent 2100 BioAnalyze. Strand-specific, polyA-enriched RNA sequencing was performed according to previously published protocols, and reads were aligned to the GRCh37/hg19 reference using the spliced transcripts alignment to a reference aligner.[17] RNA sequencing data analysis was carried out using DROP,[18] an integrative pipeline that integrates quality control and expression outlier calling with Outlier in RNA-Seq Finder.[19]

Tandem mass tag–labeled quantitative proteomics

Quantitative tandem mass tag (TMT) proteomics analysis was performed at the BayBioMS Core Facility of the Technical University of Munich (Freising, Germany) on patient fibroblasts. Fibroblast cell pellets containing 0.5 million cells each were lysed under denaturing conditions in a buffer containing urea and quantified using the BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA). Protein extract (15 µg) was further reduced and alkylated, and the tryptic digestion was performed using Trypsin Gold (Promega, Madison, WI). Digests were acidified and desalted, and TMT-labeling was performed according to Zecha et al[20] using the TMT 11-plex labeling reagent (Thermo Scientific, Waltham, MA). Each TMT batch consisted of 9 patient samples and 2 reference samples. The TMT 11-plex peptide mixture was fractionated using trimodal mixed-mode chromatography, according to Yu et al.[21] LC-MS measurements were performed on a Fusion Lumos Tribrid mass spectrometer (Thermo Scientific) in the data-dependent acquisition mode and multinotch MS3 mode. Peptide identification was performed using MaxQuant (version 1.6.3.4),[22] resulting in the identification of protein groups. Protein group intensities were then normalized to account for variability within and between TMT batches. Protein expression outlier identification was performed by comparing normalized protein intensities of the 3 case samples to those of 18 controls of the same batch using the Wilcoxon test approach. All statistical analyses were performed using R version 4.0.4.

RESULTS

As part of the PALFES study, we performed WES in 3 unrelated families from Syria, Ireland, and Germany but did not identify any pathogenic or likely pathogenic variants in genes previously associated with ALF.[2] Instead, we identified biallelic variants in *TRPM7* within these families, establishing *TRPM7* as a novel disease gene for PALF (Figure 1).

Individual 1 is the eldest of the 3 affected brothers in family 1 (including individuals 2 and 3, Figure 1). Two of the 3 presented with recurrent episodes of ALF starting at 6 and 7 months of age. These episodes were always associated with viral infections of the upper respiratory tract (Table 1, Figure 2). Treatment during ALF episodes was symptomatic. For individual 3, a high-urgency LT was opted. All 3 children passed away due to ALF at the ages of 37, 42, and 9 months without receiving LT.

Individual 4 from family 2 presented with episodes of ALF at the age of 9 and 15 months, along with encephalopathy during intercurrent viral illness that required intubation and ventilation (Table 1, Figure 2). Both episodes settled on conservative treatment. At the age of 19 months, she presented again with ALF and encephalopathy following a 2-day history of vomiting illness. The patient subsequently underwent a liver transplantation. At the most recent follow-up, the patient was 17 years old and in good health with standard immune suppression (tacrolimus, mycophenolate, and prednisolone).

Individual 5 from family 3 presented with ALF episodes at the age of 35 and 40 months (Table 1, Figure 2). An influenza A virus infection was detected during the first episode. After almost complete recovery from the first episode of ALF, the second episode, preceded by respiratory adenovirus infection, was noted 5 months later (Supplemental Table S1, http://links.lww.com/HC9/B152). Management was symptomatic for the first episode and included plasmapheresis, as well as N-acetylcysteine and magnesium substitution during the second episode. Both episodes were resolved. The patient was diagnosed with a urachal cyst that required surgical removal. During the latest follow-up examination at the age of 4 years, the patient was in good health. His AST/ALT levels were slightly above the normal limit, and synthetic liver function was normal. He is currently treated with ursodeoxycholic acid and receives vitamin K and vitamin D supplementation, as well as potassium and magnesium.

A concise summary of the clinical findings is given in Table 1.

In summary, the first episodes of ALF were recorded at a median age of 9 months (IQR: 28) with a median of 2 (IQR: 2) episodes of ALF per individual. Time to International normalized ratio recovery after episodes of ALF was a median of 12 days (range 4–28 d, IQR: 5.5) (Supplemental Table S2, http://links.lww.com/HC9/B153). Liver enzyme tests revealed elevated levels of ALT and AST (ALT: median 1.070 U/L, IQR 6.081; AST: median 3.465 U/L, IQR 20.049), lactate dehydrogenase (median 5.309.5 U/L, IQR 10.833 U/L), total and direct bilirubin (median 103.36 µmol/L, IQR: 120.66; direct bilirubin: median 56.01 µmol/L, IQR 117.3), GGT

TABLE 1 Clinical characteristics of the patient cohort.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Sex	Male	Male	Male	Female	Male
Alive	No	No	No	Yes	Yes
Age last follow-up (mo)	37	42	9	212	42
Pregnancy	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable
Anthropometry at birth	Unremarkable	Unremarkable	Unremarkable	Unremarkable	Unremarkable
TRPM7 variant 1	c.4955C > T; p.Pro1652Leu	c.4955C > T; p.Pro1652Leu	c.4955C > T; p.Pro1652Leu	c.[2005_2006del]; p.[Ser699Ter]	c.[553G > T]; p[Gly185Ter]
TRPM7 variant 2	c.4955C > T; p.Pro1652Leu	c.4955C > T; p.Pro1652Leu	c.4955C > T; p.Pro1652Leu	c. [5329G > A]; p.[Asp1777Asn]	c.[5475G > T]; p.[Lys1825Asn]
Hepatic symptoms					
Age at first symptoms (mo)	36	6	7	9	35
ALF episodes	1	4	2	3	2
HE	Yes	Yes	Yes	Yes	Yes
trigger of ALF	Viral infection	Viral infection	Viral infection	NA	Viral infection
TB (μmol/L)	37.57	94.52	467.16	NA	112.2
DB (μmol/L)	30.43	24.65	340.34	NA	81.6
ASAT (IU/L)	25,318	21,000	178	951	3465
ALAT (IU/L)	7435	6800	100	719	1070
GGT (IU/L)	n.a.	154	45	NA	1730
LDH (IU/L)	14,600	10,167	413	NA	452
INR	> 6	> 6	> 6	> 6	1.57
Albumin (g/L)	NA	NA	26359	NA	25.9
Ammonia (μmol/L)	144	366	165	NA	67
Mg (mmol/L)	Normal	Normal	Normal	Normal	0.3
Liver transplantation	No	No	Listed	Yes	No
age at LT (mo)	NA	NA	NA	68	NA
Clinical course	Deceased during ALF	Deceased during ALF	Deceased during ALF	Unremarkable after LT	Recovered from ALF
Other organ manifestations	No	No	No	No	No

Abbreviations: ALF, acute liver failure; DB, direct bilirubin; INR, international normalized ratio; LDH, lactate dehydrogenase; LT, liver transplantation; NA, not available; TB, total bilirubin.

(median 154 U/L, IQR: 842.5). International normalized ratio was > 6 in individuals 1–4 and 1.57 in individual 5.

All patients presented with HE. Ammonia levels were elevated in all patients, with a median of 150.0 μmol/L (IQR: 21). Serum magnesium levels were within the normal range for individuals 1–4 but decreased in individual 5, who showed chronic hypomagnesemia even under oral substitution (0.3 mmol/L, expected 0.7–0.95 mmol/L).

Evaluation for inborn errors of metabolism, hepatotropic infections (except for 2 episodes of ALF) or intoxication was negative in all cases (Supplemental Table S2, http://links.lww.com/HC9/B153). Viral infections of the upper respiratory tract were identified as triggers for all but 2 ALF episodes (10/12, 2 NA) (Supplemental Table S1, http://links.lww.com/HC9/B152).

Treatment was primarily symptomatic. Plasmapheresis as well as administration of N-acetylcysteine and magnesium were reported for individual 5. Three of the 5 patients died due to ALF (individuals 1–3, family 1). Individual 4 underwent LT during the second ALF episode at the age of 19 months. Individual 5 is alive with his native liver.

Liver biopsy and histopathological evaluation were performed in all except individual 2, and histology revealed varying degrees of parenchymal damage or hepatocyte steatosis with no features common to all patients (Table 2, Figure 1). Of note, no CD8-positive T-cell infiltrates were found.

Pregnancy and birth were unremarkable in all patients. No overt extrahepatic manifestations were reported in individuals 1–4. In individual 5, growth retardation and a ventricle septum defect were reported

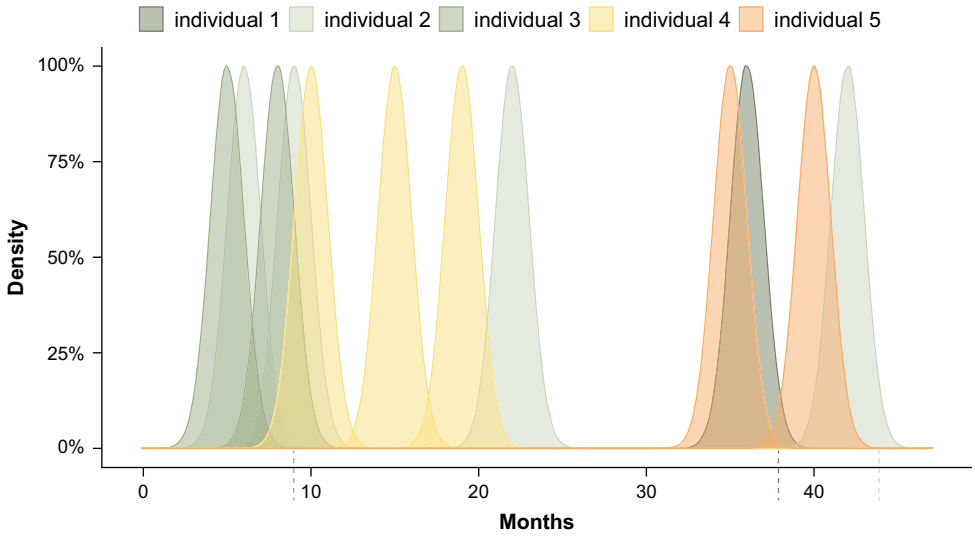


FIGURE 2 The occurrence of acute liver failure episodes for each patient depicted as density plot. The occurrence of acute liver failure episodes is indicated by curves. The age of death of individuals is indicated by dashed lines. Individuals 1, 2, and 3 are from family 1 (green).

(Table 1). Dysmorphism or inborn mental retardation was not reported in any of the 5 individuals during the follow-up period.

As no variants explaining the ALF phenotype were identified in any of the 5 affected individuals in the PALFES study, a genome-wide search for rare potential biallelic variants (allele frequency < 0.001) with predicted deleterious effects (Combined Annotation Dependent Depletion [CADD] score > 20) was conducted in all 3 families. In all 5 affected individuals, potential biallelic variants were identified in *TRPM7*, a gene so far not associated with a Mendelian disorder (Figure 1A). In family 1, only 1 homozygous missense variant (c.[4955C > T]; p.[Pro1652Leu]) was identified in all 3 affected individuals (Figure 3). The 3 affected siblings did not share any other rare biallelic variants with a CADD score of ≥ 20. Remarkably, this homozygous *TRPM7* missense variant appears to be a founder mutation situated within a broader genomic region (≥ 20 Mb), where the affected individuals share an identical haplotype. The search for rare potential biallelic variants (allele frequency < 0.001) with predicted deleterious effects (CADD score > 20) in the affected individuals from families 2 and 3 also only revealed compound heterozygous variants in *TRPM7* and no other rare biallelic variants. Each of them presents a distinctive combination of a missense and a loss-of-function variant (Figure 3).

Next, to determine whether rare biallelic variants in *TRPM7* are frequent in the population, all 30,000 in-house WES cases were searched by applying the same filters as for the unsolved ALF cases. Only 1 additional case with rare potential biallelic variants and a CADD score of > 20 in *TRPM7* was discovered in our database of 30,000 WES cases. Notably, this particular case has already been resolved with another genetic diagnosis, as the patient carried a homozygous variant in the GBE1 gene. Thus, we found a significant enrichment of rare biallelic *TRPM7* variants in 150 unsolved PALF cases compared to the WES data from individuals without ALF ($p \leq 0.0001$, Fisher exact test).

To elucidate the consequences of the *TRPM7* variants, functional analyses of primary patient-derived fibroblasts were performed. These analyses included fibroblasts from individuals 2 and 3 with the homozygous *TRPM7* c.[4955C > T] p.[Pro1652Leu] variant. Additionally, fibroblasts from individual 5, who carries compound heterozygous variants in *TRPM7* (c.[553G > T];[5475G > T], p.[Gly185Ter];[Lys1825Asn]), were analyzed. Proteomic analyses showed a significant and specific decrease in TRPM7 protein levels in fibroblasts from all 3 affected individuals compared to 18 control fibroblasts (Figure 4). The reduced TRPM7 protein level supports the deleterious character of the rare biallelic variants identified in *TRPM7*. Notably, there were differences in TRPM7 protein levels between family 1 and family 3. Yet all 3 children in family 1 died due to ALF within the first 4 years of life, while individual 5 was in good condition at the age of 4.

DISCUSSION

In summary, we describe 3 unrelated families with PALF carrying biallelic variants in *TRPM7* and provide convincing evidence that deleterious variants in *TRPM7* are associated with PALF. We identified a homozygous *TRPM7* variant in all 3 affected PALF family members and biallelic variants in 2 further families with PALF. Rare biallelic potentially damaging variants were significantly enriched in PALF cases versus controls and all affected individuals share the phenotype of mostly

TABLE 2 Histopathologic characteristics of the patient cohort

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Liver histology	Microvesicular steatosis, centro-lobular necrosis, portal fields, and bile ducts regular, no signs of cholestasis, no signs of inflammation	NA	Micronodular cirrhosis, canalicular and hepatocellular cholestasis, lack of steatosis	Severe parenchymal damage, bridging collapse, and severe cholestasis. Steatosis maximal in regenerative nodules, few multinucleate giant cells	Low hepatic damage, low bilirubinostasis, low chronic cholestasis signs, signs of clearance reaction and regeneration, portal/septal fibrosis, signs of ER activation, lack of steatosis.

Abbreviations: ER, endoplasmic reticulum; NA, not available.

recurrent ALF episodes. Finally, proteomics analyses further supported the deleterious effects of the identified variants in *TRPM7*.

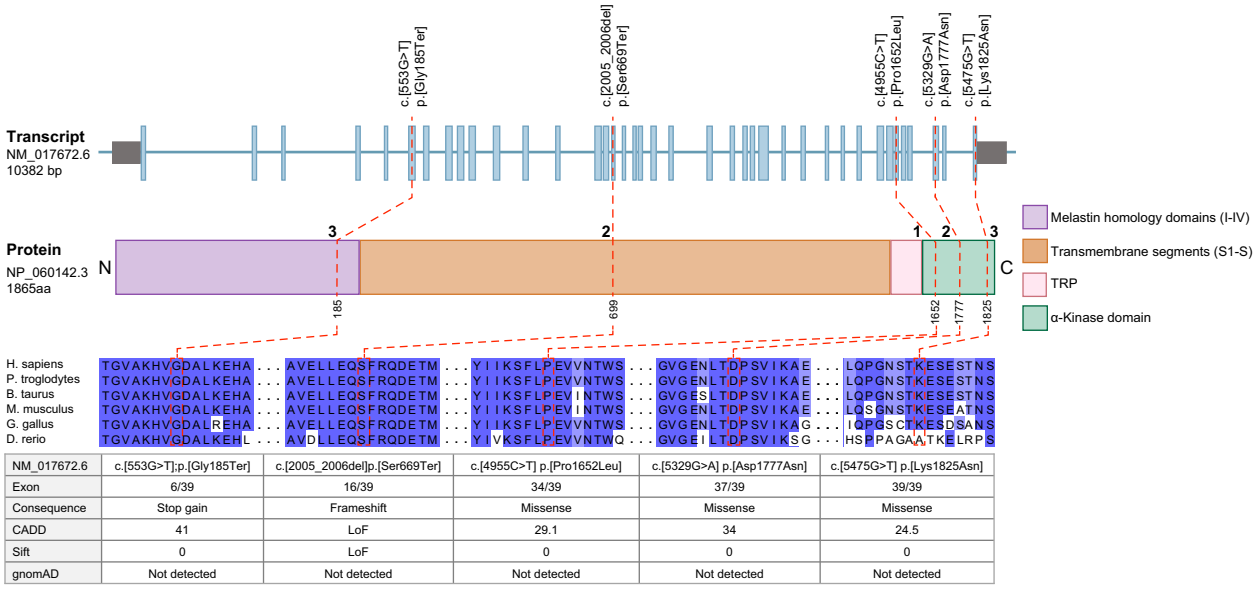
The *TRPM7* gene, located on chromosome 15, is 129.64 kb in size and consists of 39 exons that encode a protein of 1865 amino acids (Figure 3). TRPM7 is a divalent cation channel that is highly selective for Mg^{2+}, Zn^{2+}, and Ca^{2+}. It is involved in the maintenance of cellular and organismal bivalent cation homeostasis and is regulated by intracellular Mg^{2+} nucleotide levels, PIP2, and mechanical cues.[23–25] The transient receptor potential melastatin family is a group of cell membrane cation channels consisting of 8 members, including the transient receptor potential cation channel subfamily M member 7 (TRPM7) [MIM:605692].[26,27] It is a plasma membrane protein with a dual function consisting of a transient receptor potential ion channel fused to a protein kinase domain[28,29] (Figure 5). In order to form a channel pore, TRPM7 is presumed to form a tetrameric complex consisting of 4 TRPM7 monomers, which have a high permeability to bivalent cations.[32,33] In contrast to the relatively tissue-specific expression of the other 7 members of the transient receptor potential melastatin subfamily, the TRPM7 channel kinase is ubiquitously expressed.[34,35]

The importance of TRPM7 in developmental processes and cell viability has been demonstrated in several studies. The germline deletion of *TRPM7* in mice resulted in embryonic lethality, with death occurring prior to embryonic day 7.5.[25,36,37] The essential character of *TRPM7* in humans is supported by the absence of homozygous loss-of-function variants in public databases. So far, *TRPM7* has not been linked to Mendelian diseases in the OMIM database.[38]

In agreement with the predicted essential function of TRPM7, all patients carry at least 1 missense variant. All missense variants identified in our study are localized within the C-terminal α-kinase domain of the TRPM7 protein. All other variants are loss-of-function variants. In all PALF cases, this results in the sole expression of TRPM7 proteins with a missense variant in the α-kinase domain but otherwise functional channel domains, indicating a regulatory deficiency of TRPM7 function (Figure 3).

In 2020, rare heterozygous missense variants affecting the transmembrane region of *TRPM7* were found in a population of unexplained stillbirth. It was hypothesized that the variants could affect ion channel function and potentially cause lethal arrhythmias in utero.[39] In another study, heterozygous missense variants in the transmembrane segment have been suggested to result in impaired ion channel function and thus may cause recurrent hemoplegic migraine attacks accompanied by intractable hypomagnesemia.[40]

In 2022, biallelic variants in *TRPM6*, which has a high level of homology with *TRPM7*, were identified to be causative for hypomagnesemia and secondary



NM_017672.6	c.[553G>T];p.[Gly185Ter]	c.[2005_2006del]p.[Ser669Ter]	c.[4955C>T] p.[Pro1652Leu]	c.[5329G>A] p.[Asp1777Asn]	c.[5475G>T] p.[Lys1825Asn]
Exon	6/39	16/39	34/39	37/39	39/39
Consequence	Stop gain	Frameshift	Missense	Missense	Missense
CADD	41	LoF	29.1	34	24.5
Sift	0	LoF	0	0	0
gnomAD	Not detected	Not detected	Not detected	Not detected	Not detected

FIGURE 3 Transient receptor potential cation channel subfamily M member 7 (*TRPM7*) variants and gene structure. Genomic organization of *TRPM7* depicting the localization and phylogenetic conservation of amino acid residues affected by mutations identified in the 3 families. Positions of mutations are highlighted in red. All altered sites demonstrate high evolutionary conservation across diverse species, underscoring their functional significance. The variants were absent in the gnomAD database, as well as in over 30,000 in-house exomes. Based on in silico prediction tools, all 5 distinct variants had a CADD score above 24, supporting their deleterious effect. Regions are not drawn to scale. Abbreviations: CADD, Combined Annotation Dependent Depletion; TRP, transient receptor potential.

hypocalcemia.[41,42] Vargas-Poussou et al[43] presented *TRPM7* as a candidate gene for autosomal dominant hypomagnesemia and secondary hypocalcemia. Interestingly, Mg^{2+} levels were normal in all of our patients except for individual 5, who had severe hypomagnesemia during ALF episodes and showed chronic hypomagnesemia even under oral substitution. Therefore, we consider it unlikely that a generalized disruption of Mg^{2+} homeostasis is causative for ALF but suggest that a regulatory dysfunction of TRPM7 in the liver is responsible for PALF, consistent with the role of TRPM7 in intestinal magnesium reabsorption.[25,44]

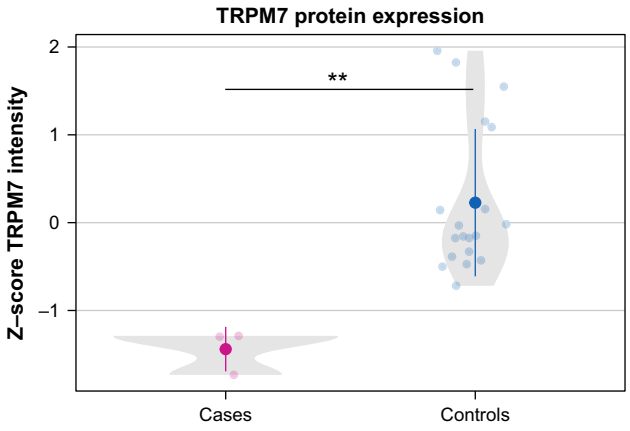


FIGURE 4 The rare biallelic variants in TRPM7 result in significantly reduced TRPM7 protein level in fibroblasts. TRPM7 protein z-score distribution in 3 cases and 18 control cell lines. Cases include individuals 2 and 3 of family 1 and individual 5 of family 3. Controls are pediatric fibroblasts from patients with no rare variants in TRPM7. Abbreviation: TRPM7, transient receptor potential cation channel subfamily M member 7.

Surprisingly, in a recent publication, a biallelic frameshift variant in the transmembrane segment of *TRPM7* was reported in 2 siblings with Hallermann-Streiff syndrome [MIM:234100] in a highly consanguineous Pakistani family, characterized by craniofacial dysmorphism.[45,46] The frameshift variant was predicted to cause complete loss of TRPM7 protein, but this was not validated. The absence of ALF in the 2 children aged 7 and 10 years could be by chance or suggest a gain of function of the identified missense variants in the *TRPM7* kinase domain in individuals with PALF. The gain of function in the ubiquitously expressed *TRPM7* may provide an explanation for the liver-specific phenotype. Further studies are needed to understand the pathomechanism leading to ALF. Fibroblast cell lines are likely not the best model system, nor is the embryonic lethal TRPM7 knockout mouse model, suggesting modeling of the identified variants or taking advantage of hepatic model systems.

Benign viral infections preceded 83%, 2 of 10 potentially hepatotropic, of the PALF episodes in this cohort. It seems that hepatic homeostasis of the 5 patients reported is easily affected by viral infections. This might implicate a potential role of TRPM7 in cellular stress responses, such as the unfolded protein response. This has been demonstrated in neurons with TRPM7 overexpression.[47] Similarly, patients with Wolcott-Rallison syndrome, which affects the unfolded

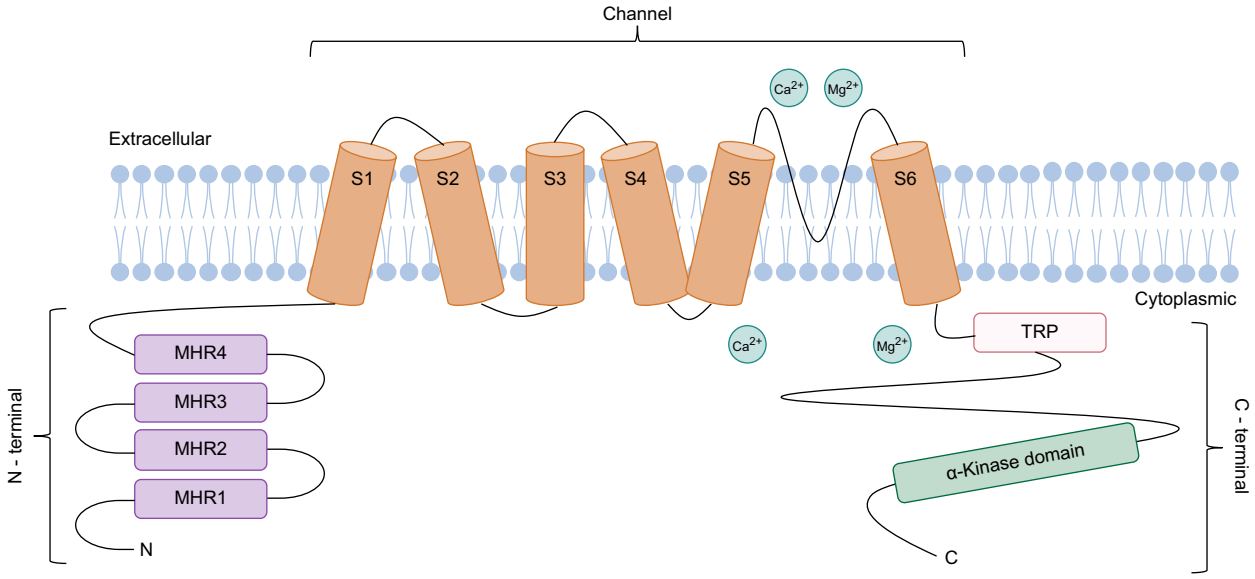


FIGURE 5 TRPM7 protein structure. The protein comprises 4 melastatin homologous regions in the N-terminal domain and 6 transmembrane segments with a pore region located between segments 5 and 6. The C-terminal domain consists of the transient receptor potential region, followed by the connecting loop of the coiled-coil domain and the serine/threonine protein kinase domain, which is able to phosphorylate substrates and regulate the activity of downstream target proteins.[30,31] Abbreviations: MHR, melastatin homologous regions; TRP, transient receptor potential.

protein response, are prone to PALF during episodes of viral infection.[48]

In conclusion, we provided statistical evidence and functional data supporting TRPM7 as a cause of PALF, thereby expanding the clinical and mutational spectrum of the disease. We reported pathogenic variants in TRPM7 in 3 unrelated families. Further families and cases are needed to better understand the genotype-phenotype correlation and underlying pathomechanism. Based on the clinical presentation and course of the 5 reported individuals, we can deduce that timely and specialized care for PALF is of paramount importance. While PALF episodes may resolve in these children, the presence of hepatic encephalopathy requires consideration of LT. This is further supported by the absence of neurocognitive impairment in patients with *TRPM7* variants in our cohort, including individual 4 aged 17 years with LT at the age of 19 months.

AUTHOR CONTRIBUTIONS

Lea D. Schlieben, Johannes A. Mayr, Holger Prokisch, and Georg F. Vogel were responsible for the conception and design of this study. Lea D. Schlieben interpreted the genetic and proteomic results and was responsible for the statistical analysis and data visualization. Holger Prokisch and Georg F. Vogel supervised the study.

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CONFLICTS OF INTEREST

Max Diesner is employed by Immundiagnostik AG. Vassiliki Konstantopoulou consults, advises, and received grants from Chiesi. He consults and advises Amicus. He is on the speakers' bureau for Sanofi and Takeda. He received grants from Nutricia Metabolics and Biomarin. The remaining authors have no conflicts to report.

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