SUPPLEMENTAL MATERIAL

TRPM7 mutations cause autosomal dominant hypomagnesemia with secondary hypocalcemia type 2 (HSH2)

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TABLE OF CONTENTS

Full Methods

Patient examinations

Electrolyte measurements in blood and 24 hours urine of the patients were performed according to standard procedures. Mg^{2+} loading tests were performed in family 1. During the test, fasting patients and controls were seated and were allowed to stand to void. They were maintained on their usual diet with a normal NaCl intake the days before the test. Urine was collected at 30-minute intervals, and venous blood was drawn through an indwelling catheter at the midpoint of each period. After two 30-minute control periods, four additional 30-minute urine collections were obtained during continuous $MgCl_2$ infusion (10 mmol in 5% (w/v) dextrose over two hours) for measurement of urinary creatinine and Mg, and serum creatinine and Mg concentrations.

Informed consent was obtained in accordance with Institutional Review Board guidelines. Informed written consent for the genetic analysis of Family 2 was obtained from the patients' parents. The Ethics Committee of Nuestra Señora de Candelaria University Hospital (Santa Cruz de Tenerife, Spain) approved this study

Whole exome sequencing

Genomic DNA of subjects was isolated from whole blood according to standard protocols. WES for proband of Family 1 was performed at BGI-Europe (Copenhagen, Denmark), employing an Illumina HiSeq2000TM machine (Illumina, San Diego, CA, USA). For these samples, 'read alignment' using BWA and 'variant calling' with GATK were performed at BGI. For all patients, variants were annotated with an in-house developed annotation and prioritization pipeline.[22] Variants in genes associated with hypomagnesemia were selected and analyzed, but no known hypomagnesemia-causing mutations were found.

Supplementary table 1 provides an overview of the rare variants detected by whole exome sequencing in individual F1-II.2 after excluding variants in introns and UTRs, synonymous variants, variants in dbSNP with frequency >1%, variants in our inhouse database with frequency >1% and variants in poorly conserved regions (phyloP <2).

WES analysis for patient and parents of Family 2 was performed by Macrogen Inc. (Seoul, South Korea) using an Illumina NovaSeq 6000 platform (Seoul, South Korea) with a read length of 100bp PE. A standard analysis of the raw data was performed by Macrogen for each individual. We analyzed variants in genes encoding Mg^{2+} transporters and genes associated with hypomagnesemia. No other mutation suspected of causing hypomagnesemia was found. A summary of rare variants detected in patient F2-II.1 by WES analysis is shown in Supplementary Table 2. The following variants were excluded: variants in introns and UTRs, synonymous variants, variants in databases with frequency >1%, variants with quality <100 and variants with a low probability to be damaging, according to Polyphen score (<0,80).

PCR and Sanger sequencing

Genomic DNA of subjects was isolated from whole blood according to standard protocols. For Sanger sequencing, primers were designed on TRPM7 (Fw: GGTGTCGCCTCTAACTTGCT, Rv: TGAAAGACTCGGCTTCTGCT), and used for polymerase chain reaction (PCR). mRNA isolation of HEK293 cells was performed using TRIzol reagent (Invitrogen) according to manufacturer's protocol. mRNA isolation of blood was performed using the PAXgene Blood RNA kit (Qiagen), according to the manufacturer's protocol. Isolated mRNA was treated with DNase (Promega) and subsequently cDNA was synthesized using Moloney-Murine Leukemia Virus (M-MLV) reverse transcriptase (Invitrogen). TRPM7 splicing was determined using PCR primers designed on exons 1 and 2 and intron 1 (Figure 2A and Supplementary Table 3). Amplified sequences from both genomic DNA and cDNA were separated on agarose gel by electrophoresis and directly Sanger sequenced according to standard methods. NM_017672.6 was used as a reference sequence.

To identify the sequences of the alternatively spliced TRPM7, a 5' RACE PCR was performed according to the manufacturer's protocol (Invitrogen). In short, a gene-specific antisense oligonucleotide (GSP1) primer is used for first strand cDNA synthesis. Subsequently the first strand product is purified from unincorporated dNTPs and GSP1, and a homopolymeric tail is added to the 3' end of the cDNA by terminal deoxynucleotidyl transferase (TdT). The tailed

cDNA is then amplified by PCR using a mixture of three primers: a nested gene-specific primer (GSP2), which anneals 3' to GSP1; and a combination of a complementary homopolymer-containing anchor primer and corresponding adapter primer, to amplify the homopolymeric tail. The amplified sequences were cloned in the pGEM-T Easy Vector (Promega) according to the manufacturer's protocol and subsequently Sanger sequenced.

Cell culture

Primary fibroblast cultures were established from a skin biopsy as described previously.[23] Subsequently, fibroblasts were cultured in Medium 199 containing EBSS, L-glutamine and 2.2 g/L $NaHCO_3$ (Lonza, Walkersville, MD, USA) at 37˚C in a humidity-controlled incubator with 5% (v/v) CO_2. HEK293 cells were grown in Dulbecco's modified eagle's medium (DMEM, Bio Whittaker-Europe, Verviers, Belgium) containing 10% (v/v) fetal bovine serum (Biowest, Nuaillé, France), 2 mmol/L L-glutamine and 10 µg/mL non-essential amino acids, at 37°C in a humidity-controlled incubator with 5% (v/v) CO_2. The cells were transiently transfected with the respective DNA constructs using Lipofectamin 2000 (Invitrogen, Breda, The Netherlands) at 1:2 DNA:Lipofectamin ratio for 48 hours unless otherwise stated.

Cells were fixed at different time points with 0.5% crystal violet (santa cruz sc-207460) (in 20% methanol (VWR 20903.368), 80% distilled water) for 15 minutes at room temperature, after which they were washed with distilled water. After overnight drying, methanol (100%) was added and absorbance was measured at 570nm with a spectrophotometer.

Constructs

The pTracer-mTRPM7 construct with amino-terminal HA tag was a kind gift from Dr. David E. Clapham. The p.Gly1046Asp mutation was introduced using the Q5 site-directed mutagenesis kit (NEB, Leiden, Netherlands) using primer F: 5'-ATGATTTTTGATGAAGTTTATGCATATG-3' and R: 5'-CCAGTATGGATGAAAAACTATATC-3'.

Electrophysiology

HEK293 cells were transiently transfected with HA-mTRPM7 wildtype and HA-mTRPM7 p.Gly1046Asp pTracer plasmids, either alone or as co-transfection in a 1:1 ratio. Additionally, cells were transiently co-transfected with HA-hTRPM6 pCINeo IRES GFP in combination with either HA-mTRPM7 wildtype or HA-mTRPM7 p.Gly1046Asp pTracer plasmids (1:1 ratio), empty pcDNA3 as mock control. Transfected cells were identified by green fluorescence, and non-transfected (green fluorescent-negative) cells from the same batch were used as controls. Electrophysiological recordings were undertaken in whole-cell configuration using an EPC-10 USB patch-clamp amplifier controlled by the Patchmaster software (HEKA, Lambrecht, Germany). Cells were kept in an extracellular bath solution (150 mM NaCl, 1 mM $CaCl_2$, 10 mM Hepes/NaOH, pH 7.4). Pipettes were pulled from thin-walled borosilicate glass (Harvard Apparatus, March-Hugstetten, Germany) and had resistance between 1 and 3 MΩ when filled with the pipette solution (150 mM NaCl, 10 mM Na_2-EDTA, 10 mM Hepes/NaOH, pH 7.2). The capacitance and access resistance were monitored continuously. A linear voltage ramp from −100 to +100 mV (in 450 ms) was applied every 2 seconds from a holding potential of 0 mV. The time course of current development was determined by extracting the current amplitudes at +80 and -80 mV. Current densities were obtained by normalizing current amplitudes to the respective cell membrane capacitances, with a bar graph showing the channel activity at 200 seconds after opening the cell membrane. All experiments were performed at room temperature. Analysis and display of patch clamp data was performed using Igor Pro software (WaveMetrics, Lake Oswego, USA) and Graphpad Prism (San Diego, California, USA), using one-way ANOVA with Sidak's posthoc test for statistical testing.

$^{25}Mg^{2+}$ uptake experiments

HEK293 cells were seeded in poly-L-lysin coated 12-well plates and transfected with mock or TRPM7 constructs. After 48 hours, cells were 30 minutes pre-incubated with 30 µmol/L NS8593 or 0.4 % (v/v) DMSO. Transfected HEK293 cells were washed in basic uptake buffer (in mmol/L: 125 NaCl, 5 KCl, 0.5 CaCl2, 0.5 Na2HPO4, 0.5 Na2SO4, 15 HEPES/NaOH pH 7.5) and subsequently placed in basic uptake buffer with 1 mmol/L $^{25}Mg^{2+}$ for 0 or 15 minutes

with or without 30 µmol/L NS8593 or 0.4 % DMSO. The buffer was removed, cells were washed 3 times with PBS, lysed in HNO_3 and subjected to inductively coupled plasma mass spectrometry (ICP-MS) analysis as described previously.[24] Naturally occurring $^{25}Mg^{2+}$ content of the cells was determined at time point 0 and was subtracted from the other time points when calculating net fluxes.

Immunoblotting

Protein lysates were denatured in Laemmli sample buffer (250 mM Tris-HCl pH 6.8, 10% (w/v) SDS, 30% (v/v) glycerol and 0.02% (w/vo) bromophenol blue). Samples were subjected to 8% (w/v) SDS/PAGE, transferred to polyvinylidene fluoride membranes that were blocked for 30 min with 5% (w/v) nonfat dry milk in Tris-buffered saline, 0.1% (v/v) Tween 20 (TBS-T) and immunoblotted overnight at 4 °C using anti-HA (Roche, high affinity 3F10, 1:5,000). The blots were then washed with TBS-T and incubated with secondary peroxidase-labeled goat anti-mouse IgG (1:10,000; Jackson Immunoresearch) for 1 h at room temperature. After subsequent washes, they were visualized with an enhanced chemiluminescence reagent using the Bio-Rad ChemiDoc XRS imaging system.

Cell surface biotinylation

HEK293 cells were transfected with wild type and mutant TRPM7 constructs. Cell surface proteins were biotinylated as described previously.[24] Protein lysates were subjected to SDS-PAGE and immunoblots were incubated using mouse anti-HA (1:5,000; Cell Signaling, Danvers, MA, USA).

Statistics

All results are depicted as mean ± standard deviation (S.D.) or standard error of the mean (S.E.M.). Statistical analyses were conducted by one-way or two-way ANOVA, or a T-test, depending on the experimental set-up. Tukey's post-test was used to identify significantly different groups. $P < 0.05$ was considered statistically significant.

Supplementary Table 1. Variants found in hypomagnesemia genes of individual F1-II.2

Chr	Position	Ref.	Var.	Gene Name	Effect	AA Ref.	AA Mut.	SNP ID	Freq.	ClinVar Interpretation
chr1	16371067	G	T	CLCNKB	EXON_REGION	R	L	rs2015352	0,55	Benign
chr1	16375354	C	T	CLCNKB	UTR	-	-	rs9442227	0,68	Not reported
chr1	16376191	C	T	CLCNKB	EXON_REGION	A	V	rs7367494	0,92	Benign
chr1	16380196	T	C	CLCNKB	EXON_REGION	M	T	rs5253	0,88	Benign
chr1	16380243	A	G	CLCNKB	EXON_REGION	K	E	rs2275166	0,72	Benign
chr1	43200526	T	C	CLDN19	UTR	-	-	rs912075	0,59	Benign
chr1	55464743	T	C	BSND	UTR	-	-	rs2500340	0,66	Benign
chr1	55464790	C	G	BSND	UTR	-	-	rs2500341	0,66	Benign
chr1	55474325	A	C	BSND	UTR	-	-	rs6682884	0,43	Benign
chr3	122003832	G	C	CASR	EXON_REGION	E	Q	rs1801726	0,92	Benign
chr3	122004098	A	T	CASR	UTR	-	-	rs4677948	0,92	Benign
chr4	110914427	A	T	EGF	EXON_REGION	E	V	rs4698803	0,86	Benign
chr7	55229255	G	A	EGFR	EXON_REGION	R	K	rs2227983	0,23	Benign
chr10	72643671	C	T	PCBD1	UTR	-	-	rs9712	0,75	Benign
chr10	104836940	C	T	CNNM2	UTR	-	-	rs2296568	0,12	Benign
chr12	5022041	G	A	KCNA1	UTR	-	-	rs4766310	0,48	Benign

Other variants associated with hypomagnesemia detected by whole exome sequencing in patient F1-II.2. Deep intronic variants, synonymous variants and variants with quality <100 were not included. Chr: chromosome, Ref: reference, Var: variant, AA: amino acid, Mut: mutation, Freq: frequency. The genes included in the analysis were: *CLDN16, CLDN19, CaSR, CLCNKB, SLC12A3, BSND, KCNJ10, FXYD2, HNF1B, PCBD1, SARS2, TRPM6, EGF, EGFR, CNNM2, KCNA1 FAM111A, SLC41A1, ATP1A1* and *MAGT1*.

Supplementary Table 2. Rare genetic variants of individual F1-II.2

Chr.	Position	Ref.	Var.	Gene name	Component	AA Ref.	AA Mut.	Allele freq.
chr1	36075012	G	C	PSMB2	SA_SITE			NP
chr1	109814994	A	G	CELSR2	EXON_REGION	K	R	$1.77x10^{-5}$
chr1	150443574	T	A	RPRD2	EXON_REGION	I	N	$4.85x10^{-5}$
chr1	165175260	C	T	LMX1A	EXON_REGION	G	S	$3.41x10^{-5}$
chr1	182555526	T	C	RNASEL	EXON_REGION	K	R	NP
chr1	204951032	G	A	NFASC	EXON_REGION	R	H	$1.44x10^{-5}$
chr1	228539078	A	G	OBSCN	EXON_REGION	E	G	NP
chr1	235966304	C	G	LYST	EXON_REGION	D	H	$6.38x10^{-5}$
chr2	9000857	G	C	MBOAT2	EXON_REGION	S	C	$4.20x10^{-6}$
chr2	71895939	C	T	DYSF	EXON_REGION	P	L	$8.00x10^{-6}$
chr2	152421631	T	C	NEB	EXON_REGION	K	R	$1.10x10^{-4}$
chr2	190530947	G	A	ASNSD1	EXON_REGION	R	Q	$2.48x10^{-5}$
chr2	227898171	C	T	COL4A4	EXON_REGION	G	S	$8.56x10^{-5}$
chr3	50326736	C	T	IFRD2	EXON_REGION	A	T	$1.06x10^{-4}$
chr3	50327861	T	G	IFRD2	EXON_REGION	E	A	NP
chr3	52559025	G	A	NT5DC2	EXON_REGION	S	L	$1.30x10^{-4}$
chr3	63981362	G	A	ATXN7	EXON_REGION	G	R	$4.01x10^{-6}$
chr3	100469375	C	A	ABI3BP	EXON_REGION	W	C	NP
chr4	1807488	G	A	FGFR3	EXON_REGION	V	M	$1.76x10^{-4}$
chr4	184585057	T	C	TRAPPC11	EXON_REGION	C	R	$1.19x10^{-5}$
chr5	75871587	G	A	IQGAP2	EXON_REGION	A	T	$4.23x10^{-5}$
chr6	7889033	C	T	TXNDC5	EXON_REGION	V	M	$1.13x10^{-4}$
chr6	36693650	C	T	RAB44	EXON_REGION	R	W	$2.01x10^{-5}$
chr6	51909793	T	A	PKHD1	EXON_REGION	M	L	$7.96x10^{-6}$
chr6	64416000	A	G	PHF3	EXON_REGION	N	S	$1.99x10^{-5}$
chr6	117859876	G	A	DCBLD1	EXON_REGION	W	*	$3.98x10^{-6}$
chr7	151093051	C	A	WDR86	EXON_REGION	Q	H	NP
chr8	24333949	C	T	ADAM7	EXON_REGION	R	C	$1.71x10^{-4}$
chr8	74334861	C	A	STAU2	EXON_REGION	G	V	$4.49x10^{-5}$
chr8	106811144	G	A	ZFPM2	EXON_REGION	R	Q	$4.82x10^{-5}$
chr9	73254127	T	A	TRPM3	SA_SITE			$3.20x10^{-5}$
chr9	100234679	C	T	TDRD7	EXON_REGION	L	F	$2.13x10^{-5}$
chr9	136223836	A	G	SURF2	EXON_REGION	E	G	NP
chr11	57466661	TCC		ZDHHC5	EXON_REGION	S		NP
chr11	132306003	G	A	OPCML	EXON_REGION	T	I	NP
chr12	72092874	A	G	TMEM19	EXON_REGION	T	A	$5.30x10^{-5}$
chr14	78221423	C	T	SNW1	EXON_REGION	E	K	$3.54x10^{-5}$
chr15	**50978727**	**C**	**G**	**TRPM7**	**SD_SITE_CANONICAL**			**NP**
chr15	86076851	G	A	AKAP13	EXON_REGION	C	Y	$3.90x10^{-5}$
chr16	57741550	A	T	CCDC135	EXON_REGION	N	I	$3.99x10^{-6}$

chr17	3854559	A	G	ATP2A3	EXON_REGION	I	T	$1.25x10^{-4}$
chr17	30648174	T	C	RHBDL3	EXON_REGION	F	L	NP
chr17	41223246	G	A	BRCA1	EXON_REGION	P	L	NP
chr18	8253288	G	A	PTPRM	EXON_REGION	R	Q	$7.31x10^{-5}$
chr18	52937094	G	A	TCF4	EXON_REGION	T	M	$3.99x10^{-6}$
chr19	8434121	A	G	ANGPTL4	EXON_REGION	Q	R	$4.04x10^{-6}$
chr19	10670133	C	T	KRI1	EXON_REGION	E	K	$6.36x10^{-5}$
chr19	31768897	G	A	TSHZ3	EXON_REGION	P	L	$1.59x10^{-5}$
chr19	36149535	C	T	COX6B1	EXON_REGION	P	S	$1.13x10^{-4}$
chr19	39860678	C	T	SAMD4B	EXON_REGION	P	S	NP
chr19	40698183	C	T	MAP3K10	EXON_REGION	A	V	NP
chr19	47258718	C	G	FKRP	EXON_REGION	T	S	$1.08x10^{-4}$
chr20	42972148	A	G	R3HDML	EXON_REGION	Q	R	$4.19x10^{-6}$

Overview of the rare variants detected by whole exome sequencing in individual F1-II.2 after excluding variants in introns and UTRs, synonymous variants, variants in dbSNP with frequency >1%, variants in our in-house database with frequency >1% and variants in poorly conserved regions (phyloP <2). TRPM7 mutation indicated in bold. Chr: chromosome, Ref: reference, Var: variant, AA: amino acid, Mut: mutation, Freq: population frequency with respect to gnomAD (Karczewski et al 2020). NP: Not present.

Supplementary Table 3. Variants found in hypomagnesemia genes of individual F2-II.1

Chr	Position	Ref.	Var.	Gene Name	Effect	AA Ref.	AA Mut.	SNP ID	Freq.	ClinVar Interpretation
chr1	16366234	T	G	*CLCNKB*	upstream_gene	-	-	rs1815526	0,90	Not reported
chr1	16374994	C	T	*CLCNKB*	splice_region	-	-	rs1889788	0,74	Benign
chr1	16375063	C	G	*CLCNKB*	missense	A	G	rs1889789	0,83	Benign
chr1	16375354	C	T	*CLCNKB*	5_prime_UTR	-	-	rs9442227	0,73	Not reported
chr1	16376191	C	T	*CLCNKB*	missense	A	V	rs7367494	0,83	Benign
chr1	16378000	A	G	*CLCNKB*	missense	I	V	rs6650119	0,10	Benign
chr1	16378725	A	T	*CLCNKB*	missense	T	S	rs12140311	0,10	Benign
chr1	16380196	T	C	*CLCNKB*	missense	M	T	rs5253	0,84	Benign
chr1	16380243	A	G	*CLCNKB*	missense	K	E	rs2275166	0,70	Benign
chr1	16383448	A	C	*CLCNKB*	3_prime_UTR	-	-	rs10803415	0,90	Not reported
chr1	16383581	G	T	*CLCNKB*	3_prime_UTR	-	-	rs1057844	0,53	Not reported
chr1	16383605	A	G	*CLCNKB*	3_prime_UTR	-	-	rs1057845	0,59	Not reported
chr1	16383668	T	G	*CLCNKB*	3_prime_UTR	-	-	rs6660218	1,00	Not reported
chr1	16383742	C	G	*CLCNKB*	3_prime_UTR	-	-	rs1057857	0,65	Not reported
chr1	16383914	C	T	*CLCNKB*	downstream_gene	-	-	rs10927896	0,31	Not reported
chr1	43201534	G	A	*CLDN19*	missense	R	C	rs4660658	0,19	Benign
chr1	55464743	T	C	*BSND*	5_prime_UTR	-	-	rs2500340	0,59	Benign
chr1	55464790	C	G	*BSND*	5_prime_UTR	-	-	rs2500341	0,60	Benign
chr3	122003832	G	C	*CASR*	missense	E	Q	rs1801726	0,92	Benign
chr3	122004098	A	T	*CASR*	3_prime_UTR	-	-	rs4677948	0,92	Benign
chr3	190106071	AG	A	*CLDN16*	frameshift	-	-	rs368234054	0,12	Benign
chr3	190106074	G	C	*CLDN16*	missense	A	P	rs3214506	0,12	Benign
chr4	110901198	G	A	*EGF*	missense	M	I	rs2237051	0,62	Benign
chr4	110914427	A	T	*EGF*	missense	E	V	rs4698803	0,92	Benign
chr7	55086780	A	C	*EGFR*	5_prime_UTR	-	-	rs712830	0,93	Likely-Benign
chr9	77376647	T	C	*TRPM6*	missense	K	E	rs2274924	0,29	Benign
chr9	77502160	G	A	*TRPM6*	missense	T	I	rs1333342	0,40	Not reported
chr12	5022041	G	A	*KCNA1*	3_prime_UTR	-	-	rs4766310	0,48	Benign
chr16	56904587	C	G	*SLC12A3*	missense	A	G	rs1529927	0,99	Benign
chr16	56906338	G	A	*SLC12A3*	missense	E	K	rs146834675	$3{,}99 \times 10^{-4}$	Likely-Benign
chr16	56917953	T	C	*SLC12A3*	splice_region	-	-	rs2304483	0,57	Benign
chr16	56947522	A	G	*SLC12A3*	3_prime_UTR	-	-	rs5805	0,59	Benign
chr17	36047275	C	T	*HNF1B*	3_prime_UTR	-	-	rs1800929	0,78	Benign
chr19	39408360	A	G	*SARS2*	splice_region	-	-	rs7508411	0,83	Benign
chr19	39421388	A	G	*SARS2*	5_prime_UTR	-	-	rs730078	0,84	Benign

Other variants associated with hypomagnesemia detected by whole exome sequencing in patient F2-II.1. Deep intronic variants, synonymous variants and variants with quality <100 were not included. Chr: chromosome, Ref: reference, Var: variant, AA: amino acid, Mut: mutation, Freq: frequency. The genes included in the analysis were: *CLDN16, CLDN19, CaSR, CLCNKB, SLC12A3, BSND, KCNJ10, FXYD2, HNF1B, PCBD1, SARS2, TRPM6, EGF, EGFR, CNNM2, KCNA1 FAM111A, SLC41A1, ATP1A1* and *MAGT1*.

Supplementary Table 4. Rare genetic variants of individual F2-II.1

Chr.	Position	Ref.	Var.	Gene Name	Effect	AA Ref.	AA Mut.	Freq.
chr1	10703237	C	T	CASZ1	missense_variant	D	N	$1,07x10^{-5}$
chr1	204378823	C	G	PPP1R15B	missense_variant	A	P	$8,49x10^{-5}$
chr2	20205777	G	C	MATN3	missense_variant	A	G	NP
chr2	61258814	T	A	PEX13	missense_variant	F	Y	$3,18x10^{-5}$
chr2	74687357	G	A	WBP1	missense_variant	S	N	NP
chr2	110325533	A	C	SEPT10	missense_variant	I	M	$8,14x10^{-6}$
chr2	185803250	A	G	ZNF804A	missense_variant	K	E	$1,59x10^{-5}$
chr2	209195297	A	G	PIKFYVE	missense_variant	H	R	NP
chr3	43389333	C	T	SNRK	missense_variant	R	W	$1,2x10^{-5}$
chr5	75964596	A	T	IQGAP2	missense_variant	N	Y	NP
chr5	115824656	C	T	SEMA6A	missense_variant	A	T	$1,51x10^{-4}$
chr5	162868260	G	C	CCNG1	missense_variant	W	C	$4,59x10^{-5}$
chr5	177649545	A	C	PHYKPL	missense_variant	D	E	NP
chr6	26056107	T	C	HIST1H1C	missense_variant	K	E	$1,19x10^{-5}$
chr6	26409806	G	A	BTN3A1	missense_variant	G	E	$3,98x10^{-6}$
chr6	132966494	C	T	TAAR1	missense_variant	A	T	$1,46x10^{-4}$
chr6	170713671	G	T	FAM120B	missense_variant	R	M	$3,98x10^{-6}$
chr7	4947145	A	C	MMD2	missense_variant	I	S	NP
chr7	138732453	T	C	ZC3HAV1	missense_variant	S	G	$3,98x10^{-6}$
chr9	95048101	T	C	IARS	missense_variant	Y	C	$3,98x10^{-6}$
chr9	113563101	G	A	MUSK	missense_variant	V	M	$2,42x10^{-5}$
chr9	131193497	C	G	CERCAM	missense_variant	P	R	$3,98x10^{-6}$
chr9	139848372	G	A	LCN12	missense_variant	R	H	$3,27x10^{-5}$
chr10	98115022	G	T	OPALIN	missense_variant	P	Q	NP
chr11	1471010	C	T	BRSK2	missense_variant	R	W	$3,23x10^{-5}$
chr15	40846173	C	G	C15orf57	missense_variant	Q	H	$3,26x10^{-5}$
chr15	**50891345**	**C**	**T**	**TRPM7**	**missense_variant**	**G**	**D**	NP
chr16	20360451	C	T	UMOD	missense_variant	G	S	$1,59x10^{-5}$
chr17	27896036	G	C	TP53I13	missense&splice_reg._var.	V	L	$2,53x10^{-5}$
chr17	38133186	G	A	GSDMA	missense_variant	G	R	$1,42x10^{-5}$
chr17	41891721	G	A	MPP3	missense_variant	R	W	$4,23x10^{-6}$
chr17	72952028	A	T	HID1	missense_variant	S	T	$1,19x10^{-5}$
chr19	2422141	C	T	TMPRSS9	missense_variant	T	M	$3,39x10^{-5}$
chr19	16874700	T	G	NWD1	missense_variant	L	W	NP
chr19	44039540	T	A	ZNF575	missense_variant	Y	N	NP
chr20	31609529	C	T	BPIFB2	missense&splice_reg._var.	A	V	$1,13x10^{-4}$
chr21	47817319	T	G	PCNT	missense_variant	C	G	NP
chr21	47817321	T	G	PCNT	missense_variant	C	W	NP
chrX	147022144	C	T	FMR1	missense_variant	A	V	NP
chrX	153069484	C	T	PDZD4	missense_variant	R	H	$4,46x10^{-5}$

Rare variants detected by whole exome sequencing in individual F2-II.1 after excluding variants in introns and UTRs, synonymous variants, variants in databases with frequency >1%, variants with quality <100 and variants with a low probability to be damaging (score <0,80 in

Polyphen). TRPM7 mutation indicated in bold. Chr: chromosome, Ref: reference, Var: variant, AA: amino acid, Mut: mutation, Freq: population frequency with respect to gnomAD (Karczewski et al 2020). NP: Not present.

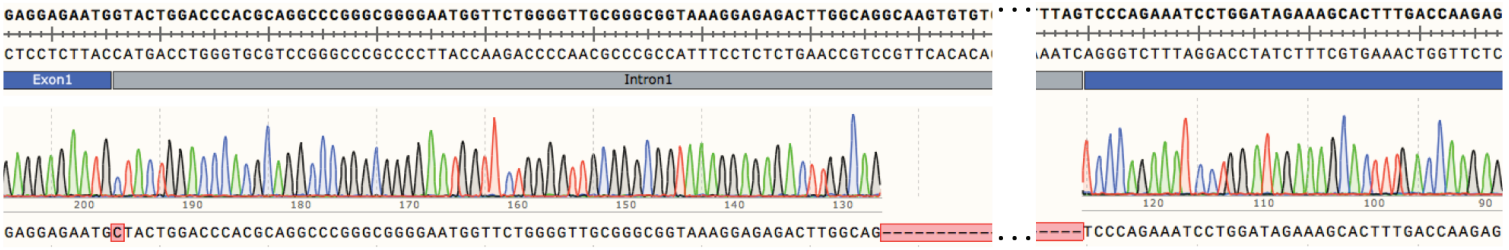
Supplementary Table 5. Primers used for PCR and sequencing

Primer	Sequence
TRPM7-Fw	5’ - CGCCGGAGCTGAGTTAGTTCTG – 3’
TRPM7-RvA	5’ - CTTTTTGCACCCTTCTAGCCCTC – 3’
TRPM7-RvB	5’ - CACATTCCCTCTTGGTCAAAGTGC – 3’
TRPM7-GSP1	5’ – TTTAAGCAGAAGTTGC – 3’
TRPM7-GSP2	5’ – AGAATGACTTCAGGTTTGGTGTC – 3’
TRPM7-GSP amplification	5’ – ATATGATAGCCTCACATACTTAGC – 3’
TRPM7-G1046Df	5' - TGTAGATAAAGATGCCCATGACTTGC - 3'
TRPM7-G1046Dr	5' - TGTTTCTTCTGGAGTTCACTAACGCC - 3'

Supplementary Figure 1. Overview of the defective spliced transcript of TRPM7

Sanger sequencing of the alternatively spliced TRPM7 containing regions of intron 1 and resulting in a preliminary stop codon.

Supplementary Figure 1



atgctactggacccacgcaggcccgggcggggaatggttctggggttgcgggcggtaaaggag

Met	Leu	Leu	Asp	Pro	Arg	Arg	Pro	Gly	Arg	Gly	Met	Val	Leu	Gly	Leu	Arg	Ala	Val	Lys	Glu

Ex 1	Intron 1

agacttggcagtcccagaaatcctggatag

Arg	Leu	Gly	Ser	Pro	Arg	Asn	Pro	Gly	*

Intron 1	Exon 2