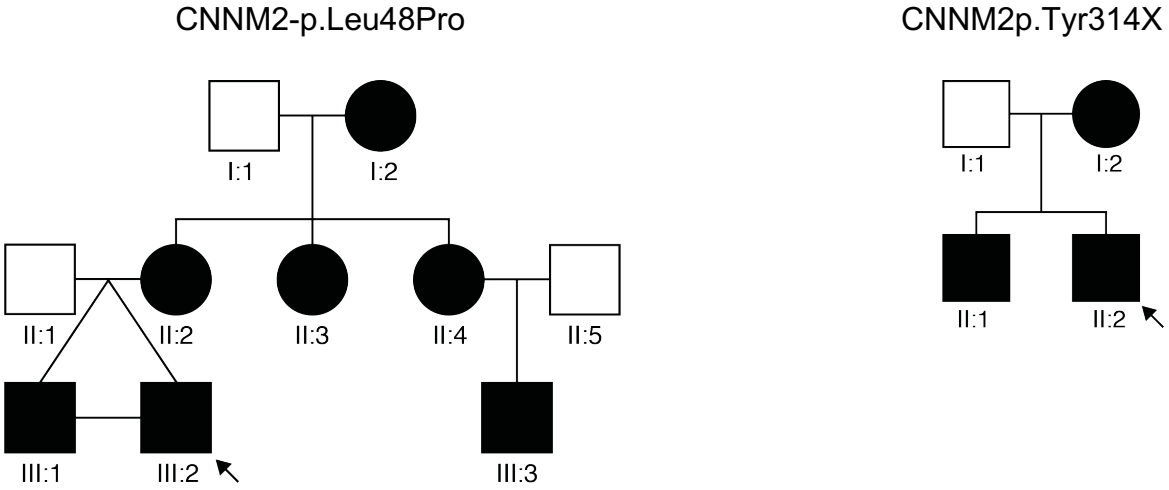
SUPPLEMENTARY MATERIALS

Phenotypic and genetic spectrum of patients with heterozygous mutations in Cyclin M2 (CNNM2)

Gijs A.C. Franken[1], Dominik Müller[2], Cyril Mignot [3], Boris Keren[4], Jonathan Lévy[5], Anne-Claude Tabet[5], David Germanaud[6], María-Isabel Tejada [7,8], Hester Y. Kroes[9], Rutger A.J. Nievelstein[10], Elise Brimble[11], Maria Ruzhnikov[11], Felix Claverie-Martin[12], Maria Szczepańska[13], Martin Ćuk[14], Femke Latta[1], Martin Konrad[15], Luis A. Martínez-Cruz[16], René J.M. Bindels Bindels[1], Joost G.J. Hoenderop [1], Karl-Peter Schlingmann*[15], Jeroen H.F. de Baaij*[1]

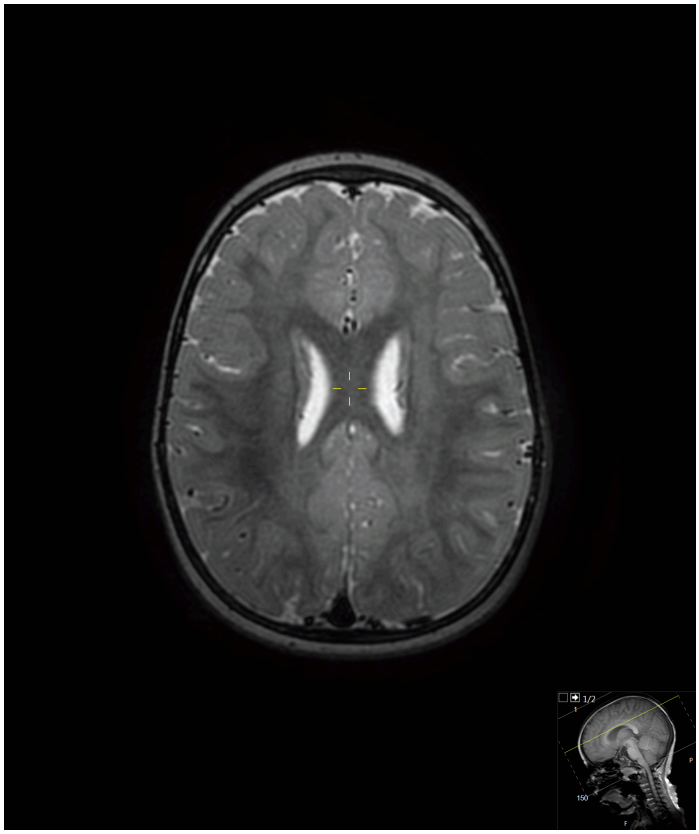
SUPPLEMENTARY FIGURES

Supplementary figure 1



The inheritance pattern of patients carrying CNNM2-p.Leu48Pro (left) and p.Tyr314* (right) mutations depicted in pedigrees. Arrows indicate proband patients.

Supplementary figure 2



Magnetic resonance imaging studies of patient carrying the CNNM2-p.Arg797* mutation.

Supplementary table 1

Phenotypic overview of patients with novel non-pathogenic CNNM2 mutations

Proband	10	11
Genetic Findings		
DNA	NM_017649.4:c.1016G>A	NM_017649.4:c.1094C>T
Protein	p.Gly339Asp	p.Ser365Phe
Genetic diagnosis	Next-Generation-Sequencing panel for hypomagesemia	Exome sequencing
General Parameters		
Gender	F	M
Inherited	De novo	De novo
Ethnicity	Caucasian	Caucasian
Age of manifestation	10 years	Birth
Body mass index (percentile)	19.3 (P50-P85)	13.83 (<P3)
eGFR (mL/min)	136	N/A
Neurological manifestations		
Seizures	N	Y
Brain Malformations	N/A	Y
Intellectual disability	Y	Y
Speech/Communication defects	N/A	Delayed
ASD	N/A	N
Motor skill defects	N/A	Delayed
Electrolyte levels		
Serum Na^+ (mmol/L)	140	142
Serum K^+ (mmol/L)	3.7	3.5
Serum Mg^{2+} (mmol/L)	0.49	0.7
Serum Ca^{2+} (mmol/L)	2.42	1.25 (ionized)
Urinary Mg^{2+} (%FE)	11.2	N/A
Mg^{2+} supplementation	Oral	N/A
Serum Mg^{2+} after supplementation (mmol/L)	N/A	N/A

Y = yes, N = no, N/A = unknown. FE = fractional excretion in %. ASD = autism spectrum disorder.

Supplementary table 2

Phenotypic overview of clinically affected family members of the index patient carrying the CNNM2-p.Leu48Pro variant, described in supplementary figure 1.

Patient	**III:1**	**III:3**	**II:2**	**II:4**	**II:5**	**I:2**
Gender	M	M	F	F	F	F
Seizures	N	N	N/A	N/A	N/A	N/A
Motor skills defects	N/A	N/A	Y	N/A	N/A	N/A
Speech/Communication	Dyslexia and dyslalia	Dyslalia	Dyslexia	N/A	N/A	N/A
Serum Mg^{2+} (mmol/L)	0.53	0.66	0.58-0.66	0.58-0.66	0.58-0.66	0.58-0.66
Urinary Mg^{2+} (mmol/L)	13.2	8.4	N/A	N/A	N/A	N/A
Serum Mg^{2+} after suppl (mmol/L)	0.49-0.66	0.58-0.62	N/A	N/A	N/A	N/A

Y = yes, N = no, N/A = unknown. Suppl = supplementation.
Full correction by Mg^{2+} supplementation was defined as increasing serum Mg^{2+} levels to normal range (0.70 – 1.05 mmol/L).

Supplementary table 3

Primers used for mutagenesis. For the mutants p.Tyr314X and p.Arg797X reverse primers were used to build in a HA-tag right before the pre-mature stop codon.

Primer mutagenesis	Sequence (5'-3')
mCNNM2-Leu48Pro Fwd	CAGCTGCGGGCCGGCCGCTGCCGCTGCTACTG
mCNNM2-Leu48Pro Rev	CAGTAGCAGCGGCAGCGGCCGGCCCGCAGCTG
mCNNM2-Tyr314X Fwd	CGGCTAGCGCCACCATGATTGG
mCNNM2-314-HAX Rev	CCGCTCGAGCTATGCGTAGTCTGGCACGTCGTATGGGTAGTTGCCCTGCCTGCG
mCNNM2-Leu321del Fwd	CTGTGCTCGCTGCTGGGCAACGTACTGGTC
mCNNM2-Leu321del Rev	GACCAGTACGTTGCCCAGCAGCGAGCACAG
mCNNM2-Val324Met Fwd	CTGCTGCTGGGCAACATGCTGGTCAACACCACG
mCNNM2-Val3244Met Rev	CGTGGTGTTGACCAGCATGTTGCCCAGCAGCAG
mCNNM2-Ser365Phe Fwd	CCCAAGCCATCTGCTTCCGACACGGCCTGGC
mCNNM2-Ser365Phe Rev	GCCAGGCCGTGTCGGAAGCAGATGGCTTGGG
mCNNM2-Leu418Pro Fwd	GAAAAACTGCTGGAGATGCCCCGGGTTACTGACCC
mCNNM2-Leu418Pro Rev	GGGTCAGTAACCCGGGGCATCTCCAGCAGTTTTTC
mCNNM2-Ser795Leu Fwd	CTACATCCCTGACTACTTAGTACGAGCCCTCTC
mCNNM2-Ser795Leu Rev	GAGAGGGCTCGTACTAAGTAGTCAGGGATGTAG
mCNNM2-Arg797X Fwd	CGGCTAGCGCCACCATGATTGG
mCNNM2-Arg797-HAX Rev	CCGCTCGAGCTATGCGTAGTCTGGCACGTCGTATGGGTATACTGAGTAGTCAGGG

Supplementary table 4

Primers for building in FLAG-tag at N-terminus.

FLAG-mCNNM2 Fwd	CGGCTAGCGCCACCATGGACTACAAGGATGACGATGACAAGATTG GCTGTGGCGCTTG
mCNNM2-HA Rev	GTGGCGCGCCGCCATCTTCCAAG

Supplementary table 5

Components of the $^{25}Mg^{2+}$-uptake buffer

Components	Concentration (mM)
Sodium chloride	120
Potassium chloride	5
Calcium chloride	0.5
Sodium diphosphate	0.5
Sodium disulfate	0.5
HEPES/NaOH pH 7.5	15

Supplementary table 6

Components of cell lysis buffer for protein extraction

Components	Concentration
Tris-HCl pH 7.5	50 mM
EGTA	1 mM
EDTA	1 mM
Triton-X100	1% (v/v)
Sodium glycerophosphate	10 mM
Sodium orthovanadate	1 mM
Sodium Fluroide	50 mM
Sodium pyrophosphate	10 mM
Sucrose	270 mM
Sodium chloride	150 mM
Peptatin	1 µg/mL
Phenylmethlysulfonyl fluoride	1 mM
Leupeptin	5 µg/mL
Aproptin	5 µg/mL

Supplementary table 7

In silico prediction of identified CNNM2 mutations using the Combined Annotation Dependent Depletion tool GRCh37 v1.6 (https://cadd.gs.washington.edu) to obtain PolyPhen-2, SIFT, and CADD scores. PolyPhen-2 scores ≥0.4, SIFT-scores ≤0.05, and CADD-scores ≥15 were considered as deleterious.

Variant	PolyPhen-2			SIFT			CADD	
	Score	Effect		Score	Effect		Score	Effect
p.Leu48Pro	0.043	Benign		0.26	Tolerated		21.1	Moderate: Deleterious
p.Val324Met	0.953	Probably damaging		0.00	Deleterious		28.2	Deleterious
p.Gly339Asp	0.905	Probably damaging		0.01	Deleterious		28.3	Deleterious
p.Ser365Phe	0.791	Possibly damaging		0.00	Deleterious		28.4	Deleterious
p.Leu418Pro	0.999	Probably damaging		0.00	Deleterious		28.5	Deleterious
p.Ser795Leu	0.998	Probably damaging		0.00	Deleterious		29.9	Deleterious