

# Novel mutations in *TRPM6* gene associated with primary hypomagnesemia with secondary hypocalcemia. Case report

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**Background.** Primary hypomagnesemia with secondary hypocalcemia (HSH) is a rare genetic disorder. Dysfunctional transient receptor potential melastatin 6 causes impaired intestinal absorption of magnesium, leading to low serum levels accompanied by hypocalcemia. Typical signs at initial manifestation are generalized seizures, tetany, and/or muscle spasms.

**Case report.** We present a 5 w/o female manifesting tonic-clonic seizures. Laboratory tests detected severe hypomagnesemia and hypocalcemia. The molecular genetic analysis revealed two novel mutations within the *TRPM6* gene c.3308dupC (p.Pro1104Thrfs\*28) (p.P1104Tfs\*28) and c.3958C>T (p.Gln1302\*) (p.Q1302\*) and the patient was successfully treated with Mg supplementation.

**Conclusion.** Ion disbalance should be taken into account in the differential diagnosis of infantile seizures. Accurate diagnosis of HSH together with appropriate treatment are crucial to prevent irreversible neurological outcomes.

**Key words:** hypomagnesemia, hypocalcemia, transient receptor potential melastatin 6, infantile seizures

Received: January 23, 2021; Revised: April 13, 2021; Accepted: April 22, 2021; Available online: May 18, 2021

<https://doi.org/10.5507/bp.2021.027>

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## INTRODUCTION

Magnesium (Mg) is the fourth most abundant mineral in the human body. This divalent cation plays roles in many biological pathways. It acts as a cofactor for more than 300 enzymes, regulating a number of fundamental functions such as muscle contraction, neuromuscular conduction, glycemic control, myocardial contraction, and blood pressure<sup>1,2</sup>. Moreover, Mg is essential in energy production, synthesis of nuclear materials, active transmembrane transport for other ions, and bone development<sup>2</sup>. Approximately 99% of total body Mg is stored in bone or within cells<sup>3</sup>. Extracellular Mg comprises only 1% of the total body magnesium content. Mg homeostasis in humans is tightly regulated and depends primarily on the balance between intestinal uptake and renal excretion. This mechanism is provided by a combination of para- and transcellular pathways. Genetic screening of families suffering from primary hypomagnesemia with secondary hypocalcemia has led to identification of the *TRPM6* gene (OMIM acc. No. ID 607009, gene ID 140803), an essential player for systemic Mg regulation<sup>4,5</sup>. This gene encodes transient receptor potential melastatin 6 (TRPM6), which

serves as a constitutively active cation channel highly permeable to Mg.

Herein, we report the case of 5-week-old girl presenting with refractory seizures and severe hypomagnesemia and who was successfully treated with Mg supplementation.

## CASE REPORT

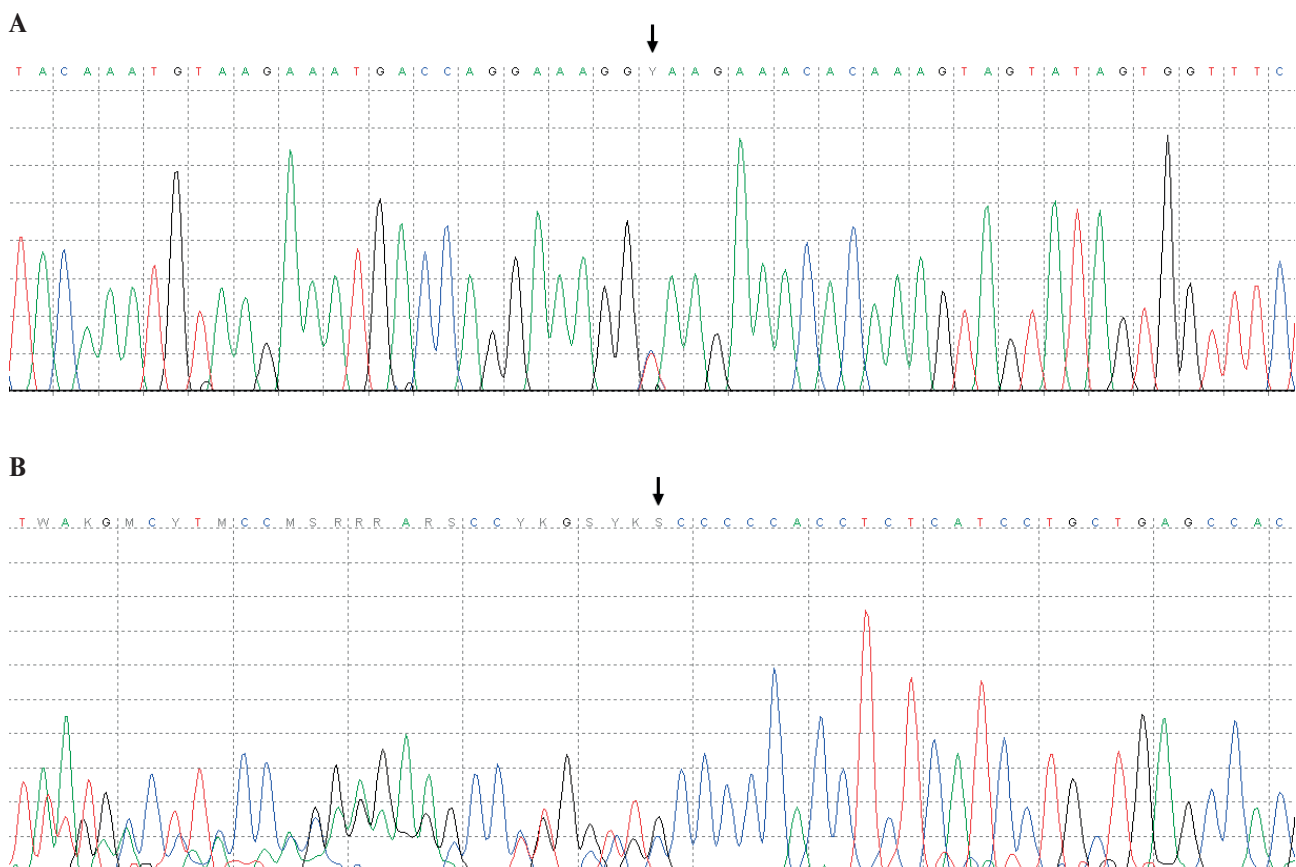
The girl was born at gestational age 40 weeks to nonconsanguineous parents with no adverse perinatal events. The family history was unremarkable. Her birth weight was 2,900 g (-0.83 SD) and birth length 49 cm (-0.48 SD). She began to be breastfed in maternity hospital. After physiological weight loss during the first 7 days of life she began to thrive. Vitamin D was supplemented to prevent rickets. At the age of 5 weeks she was admitted to the Department of Pediatrics because of sudden development of tonic-clonic seizures. According to the mother, the child had shown no sign of infection during recent days. She also precluded any possibility of intoxication. Bedside blood glucose test revealed normal glycemia. We administered phenobarbital intravenously as an empiri-

cal and non-causal treatment of acute convulsions, but the effect was insignificant. An electroencephalographic examination was performed immediately and showed generalized slowing of the background activity without any epileptiform abnormalities.

Blood tests detected severe hypomagnesemia (0.22 mmol/L; reference range 0.7-1.0 mmol/L) and hypocalcemia (1.8 mmol/L; reference range 2.2-2.7 mmol/L) at admission. Blood count was normal, as were Na, K, Cl, P, serum creatinine, blood urea nitrogen, liver transaminases, alkaline phosphatase, serum osmolality, total protein and albumin, glycemia, and parameters of acid-base balance. Fractional excretion of magnesium (FEMg) was 0.9% (reference range 3-5%). Urinary calcium/creatinine was 0.35 mol/mol (reference range <2 mol/mol) (ref.<sup>6</sup>). Intravenous replacement therapy (magnesium sulfate + calcium gluconate) was successfully initiated to stop the seizures. Calcium infusion therapy was discontinued when normal calcium levels were achieved. After normalization of Mg, plasma level of parathormone (PTH) was 6.2 pmol/L (reference range 1.6-6 pmol/L) and that of serum 25-hydroxyvitamin D was 61.1 nmol/L (reference range 50-200 nmol/L). On the third day, we switched intravenous magnesium replacement to oral magnesium administration uneventfully (magnesium lactate, 60 mg/kg/day). At the age of 1 year and 6 months, the patient showed age-appropriate physical development. To date,

there have been no adverse effects of magnesium administration.

We performed molecular genetic analysis on the proband after obtaining written informed consent from her parents. Due to strong clinical suspicion of hypomagnesemia type 1 (intestinal), we performed whole gene sequencing of the *TRPM6* gene (all coding regions and flanking regions). Genomic DNA was extracted from peripheral blood samples using the magnetic bead method (MagPurix, Zinexts Life Science, New Taipei City, Taiwan). Sequencing was done by the next-generation sequencing method using MiSeq Illumina equipment (Illumina, San Diego, CA, USA) while following the manufacturer's instructions. Oligo sequences are available upon request from the corresponding author. Data were evaluated using IGV 2.3 (Broad Institute) software. Two heterozygous mutations were detected in *TRPM6*: (NM\_017662.4 (TRPM6\_v001): c.3308dupC (p.Pro1104Thrfs\*28) (p.P1104Tfs\*28) and c.3958C>T (p.Gln1302\*) (p.Q1302\*). Both variants were further verified using PCR with subsequent Sanger sequencing. Amplification was performed using two primer sets (5'-ATGACCTACCACGAGAAGCC-3' and 5'-GCTCTCTTTCTCCTGCCTCA-3' for *TRPM6* c.3308dupC, 5'-CATGGAGATCGCTGGAGAGA-3' and 5'-CTTTGAGTGTGCTTGCCTGT-3' for *TRPM6* c.3958C>T) (Integrated DNA Technologies, Coralville,



**Fig. 1.** Sanger sequencing results. Molecular analysis presenting *TRPM6* variant c.3958C>T inherited from mother (A) and variant c.3308dupC from father (B).

IA, USA) with the following cycling conditions: 94 °C for 3 min, 35 cycles of 94 °C for 45 s, 58 °C for 1 min, 72 °C for 1 min, and 72 °C for 10 min. Purified amplicons were sequenced on an ABI 3130XL device (Applied Biosystems, Foster City, CA, USA).

Data were analyzed using Mutation Surveyor software version 4.0.9 (SoftGenetics, State College, PA, USA). In analyzing the parents' DNA, the c.3958C>T mutation was detected in the mother and the c.3308dupC was found to be inherited from the father (Fig. 1). These two novel variants, leading into premature stop codons, have been documented for the first time and were classified as pathogenic according to ACMG criteria.

## DISCUSSION

Primary hypomagnesemia with secondary hypocalcemia (HSH, OMIM 602014), also termed hypomagnesemia 1 (HOMG 1) or intestinal hypomagnesemia with secondary hypocalcemia, is a rare autosomal recessive disease (the population frequency remains unknown) that was first described by Paunier and colleagues in 1968 (ref.<sup>7</sup>).

Mutations in the *TRPM6* gene cause loss of TRPM6 function (ref.<sup>4</sup>). This member of the transient receptor potential (TRP) family serves as a constitutively active cation channel transporting  $Mg^{2+}$  and  $Ca^{2+}$  ions. It has a restricted expression pattern along the apical membranes of small intestine and distal convoluted tubule<sup>8</sup>. Impaired intestinal reabsorption of Mg together with renal Mg wasting results in extreme hypomagnesemia<sup>9</sup>. The secondary hypocalcemia is caused by inhibition of the parathyroid hormone synthesis and release from parathyroid gland in the presence of profound hypomagnesemia<sup>10</sup>. In our case, signs of hypoparathyroidism were not detected in the laboratory because we examined the plasma level of PTH just after normalization of Mg.

During intrauterine development Mg is supplied by free exchange through the placenta. After birth, Mg level is progressively depleted until hypomagnesemia becomes clinically apparent within a few weeks. In the presence of hypomagnesemia, the kidneys attempt to preserve magnesium by lowering fractional excretion Mg below 0.5-1% (reference range 3-5%) (ref.<sup>11</sup>). The most common manifestations of HSH are neurological symptoms that include seizures, tetany, tremors, and restlessness<sup>12</sup>. Our patient manifested seizures in early infancy at 5 weeks of age, which is similar to previously reported cases<sup>9,13-18</sup>. If misdiagnosed, HSH can lead to failure to thrive, mental retardation, and even death<sup>15</sup>.

HSH is treated by parenteral magnesium followed by long-term therapy administering oral magnesium salt. It has been reported that 18-87 mg/kg/day (0.7-3.5 mmol/kg/day) of elemental magnesium keep the patients free of symptoms<sup>17</sup>.

Genetic testing of the *TRPM6* gene in our patient revealed new frame-shift mutations that had never previously been reported.

## CONCLUSION

HSH is a treatable condition necessitating early and accurate diagnosis together with appropriate treatment to prevent irreversible neurological outcomes or even death.

**Acknowledgments:** This work was supported by the Ministry of Health of the Czech Republic - conceptual development of research organization (FNBr, 65269705).

**Author contributions:** JP, JS: designed the study and wrote the manuscript; KS, JP, SA, MU: participated in collecting the laboratory and clinical data; JAH, SC, PV: performed mutational analysis; OS, PJ: revised and edited the draft. All authors read and approved the final manuscript.

**Conflict of interest statement:** None declared.

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