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Original article

New TRPM6 mutation and management of hypomagnesaemia with secondary hypocalcaemia

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Abstract

Background: TRPM6 gene mutation has been reported to cause hypomagnesemia with secondary hypocalcemia (HSH). However, the genotype–phenotype correlation for TRPM6 gene mutations has not been clarified.

Objective: To elucidate the factors underlying the severe neurological complications in HSH and evaluate the potential association between the location of TRPM6 gene mutations and clinical data of HSH.

Methods: A Japanese patient diagnosed with HSH at 10 weeks of age exhibited neurological damage and failed to thrive. Magnesium supplements were therefore started at 12 weeks of age. Mutational analysis of the TRPM6 gene was performed using a direct sequencing method to determine the position and type of mutation. Using the data of 29 HSH patients reported in the literature, linear regression analysis was also performed to examine the association between TRPM6 gene mutation location and HSH onset age, initial serum magnesium and calcium concentrations, and dose of oral magnesium.

Results: A novel stop-codon homozygous mutation [c.4190 G > A] W1397X was identified in exon 26 of the patient's TRPM6 gene. No statistical correlation was found between the location of mutations in the TRPM6 gene and the clinical data for 4 clinical indicators of HSH.

Conclusions: We identified the first Japanese HSH patient with a novel nonsense mutation in the TRPM6 gene. Regression analysis of mutation locations in the protein-coding region of TRPM6 and the reported clinical data for 4 clinical indicators of HSH in 30 HSH patients did not detect a genotype–phenotype correlation.

Keywords: TRPM6; HSH; Genotype–phenotype correlation; Magnesium; Mental retardation; Failure to thrive

1. Introduction

Hypomagnesemia with secondary hypocalcemia (HSH, OMIM #602014) is a rare autosomal recessive disorder that is characterized by the development of neurological symptoms, including tetany, muscle spasms, and seizures, in early infancy due to low serum magnesium [1]. The low serum magnesium levels

Abbreviations: HSH, hypomagnesemia with secondary hypocalcemia; TRPM6, transient receptor potential channel melastatin 6

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associated with HSH result from defective intestinal magnesium absorption and increased renal magnesium clearance, which lead to secondary hypocalcemia due to insufficient secretion and resistance to parathyroid hormone (PTH) [2,3]. To help prevent the negative outcomes of PTH deficiency, which include severe neurological damage and even death, HSH patients require life-long oral magnesium supplementation [4,5]. In 2002, an association was identified between HSH and mutations in the gene encoding the transient receptor potential channel melastatin 6 (TRPM6), which is involved in transepithelial magnesium transport and belongs to the transient receptor potential (TRP) family of cation channels [6,7]. To date, at least 38 *TPRM6* mutations have been reported and include stop codon, frame-shift, and splice-site mutations, and exon deletions [4]. Although an apparent association exists between the development of HSH and *TRPM6* abnormality, no definitive genotype–phenotype correlation has been established between TRPM6 gene mutations and disease severity [5].

2. Patient

The patient was an infant female born as the first child of consanguineous parents of Japanese origin. The patient's family pedigree is presented in Fig. 1A. The patient was delivered without problems and had a birth weight of 2795 g. At 72 days of age, the patient had a generalized tonic seizure with apnea, and was then admitted to a local hospital. Blood examination showed hypocalcemia (1.98 mmol/l; reference value: 2.2–2.7 mmol/l); however, the symptoms improved without any specific treatment after 1 week.

At 81 days of age, the patient was referred to our hospital because of recurrent seizure. On admission, the patient presented with seizure, muscle hypotonia, and severe irritability. Although blood examination showed hypocalcemia (1.55 mmol/l), the level of parathyroid hormone (PTH) was normal (17 pg/ml, reference value: 12–92 pg/ml). Blood urea nitrogen, creatinine, electrolytes, blood glucose and urinalysis were within normal ranges. Electroencephalogram and magnetic resonance imaging of the brain did not show any specific findings. Midazolam (0.2 mg/kg/h) for seizure and calcium gluconate (calcium; 35.2 mg/kg/day) for hypocalcemia were administered. After admission to our hospital, hypomagnesemia was detected (0.10 mmol/l; reference value: 0.75–1.25 mmol/l). Fractional magnesium excretion ($FEMg^{+2}$) was 2.7% (reference value: 1.0–8.0%), indicating that the renal absorption of magnesium was impaired. For this reason, magnesium was intravenously administered (4.37–5.47 mg/kg/day) at 84 days of age. All symptoms disappeared in accordance with normalization of the serum magnesium level. Creatinine clearance and urinary electrolytes, including calcium excretion, were within normal ranges. Renal ultrasonography was normal. Based on these findings, the patient was clinically diagnosed with HSH.

At 93 days of age, the patient was orally administered magnesium (26.73 mg/kg/day) four times daily in place of intravenous magnesium treatment. After 2 days of oral magnesium, the patient had mild diarrhea and irritability due to hypomagnesemia (0.49 mmol/l). The patient's irritability was reduced by increasing the dose of oral magnesium to 53.46 mg/kg/day, and the mild diarrhea also improved following the administration of oral magnesium with probiotics six times daily. The

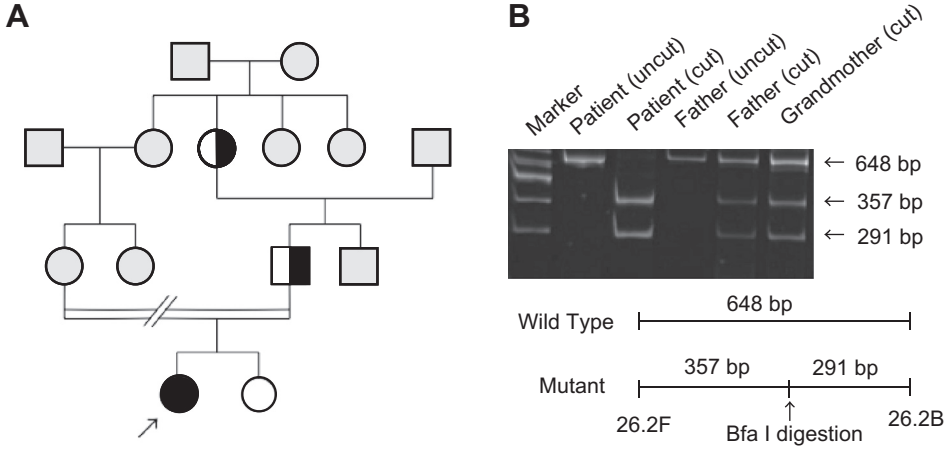

Fig. 1. Patient's family pedigree and genetic analysis of the TRPM6 gene. (A) Patient's family tree. The parents and sister of the proband did not have symptoms of HSH. Filled symbols, study patient; open symbols, wild-type haplotype; semi-filled symbols, heterozygous mutation carriers; grey symbols, unknown genotype; female; square, male; double slash, divorce. (B) Identification of the W1397X mutation in the TRPM6 gene. PCR-RFLP analysis was performed to identify mutations in the patient's TRPM6 gene using SDS–PAGE separated on an 0.8% gel. The patient has a homozygous W1397X mutation in the TRPM6 gene, and the patient's father and grandfather are heterozygous for this mutation. The restriction enzyme *Bfa*I was used in the analysis.