DOI: 10.4274/jcrpe.galenos.2021.2020.0192

Original article

Long-Term Clinical Follow-up of Patients with Familial Hypomagnesemia with Secondary Hypocalcemia

Bayramoğlu E et al. Patients with Hypomagnesemia due to Novel TRPM6 Mutations

Elvan Bayramoğlu, Melikşah Keskin, Zehra Aycan, Şenay Savaş-Erdeve ve Semra Çetinkaya
Clinic of Pediatric Endocrinology, University of Health Sciences, Dr. Sami Ulus Obstetrics and Gynecology and Pediatrics Training and Research Hospital, Ankara-TURKEY

What is already known?
HSH is a rare autosomal recessive disease which is characterized by selective magnesium malabsorption related to a mutation on transient receptor potential melastatin 6 *(TRPM6*)' gene. Affected cases are usually diagnosed when seizures occur due to severe hypocalcemia and hypomagnesemia during infancy. Early diagnosis and treatment play a crucial role in the prevention of sudden deaths which rarely occur due to irreversible neurological deficits and arrhythmias

What this study adds?
In this study, we present long-term follow-up data and treatment responses of our 6 cases, in which three novel TRPM6 mutations were identified. We have discussed controversial clinical findings of TRPM6 mutations such as short stature and testicular hypofunction. In addition, we have reviewed the genetic and clinical features of all Turkish patients previously reported in literature.

Abstract
Objective: Familial hypomagnesemia with secondary hypocalcemia (HSH) is an autosomal recessive disease caused by mutation on transient receptor potential melastatin 6 *(TRPM6*) gene and characterized by selective magnesium malabsorption. Affected cases are usually diagnosed at infancy with seizures due to hypocalcemia and hypomagnesemia. Irreversible neurological deficits and arrhythmias can be observed without appropriate treatment. We aimed to evaluate the long-term follow-up of six patients with genetically confirmed HSH.
Methods: A total of six patients with HSH, two of whom were siblings, were included in the study. Age at diagnosis, clinical, laboratory and follow-up data on admission were recorded. All the 39 exons of TRPM6 gene and flanking exon-intron junctions from genomic DNA were amplified and sequenced in all cases**.**
Results: The median follow-up duration was 12.1 years (minimum 7.6, maximum 21.7 years). All cases were diagnosed in infancy. Four different mutations, three of which were not previously identified, were detected in *TRPM6* gene. The treatment compliance was good and there was no severe complication in the long-term follow-up of cases. We observed mental retardation, specific learning difficulty and attention deficit / hyperactive disorder as comorbidities.
Conclusion: We identified four different *TRPM6* mutations and three of these mutations were newly identified. The long-term prognosis of HSH is good with an early diagnosis and good treatment compliance in our case series. The given long-term follow-up data, prognosis and recently identified mutations in HSH cases will contribute to the increase of knowledge about this rare disease and to prevent negative outcomes.
Keywords: Hypomagnesemia, Hypocalsemia, TRPM6 mutation

Elvan BAYRAMOĞLU, University of Health Sciences, Dr. Sami Ulus Obstetrics and Gynecology and Pediatrics Training and Research Hospital, Pediatric Endocrinology Clinic, Altındağ/Ankara, 06020, Turkey
+90 03123056513
elvanbayramoglu@gmail.com
0000-0002-6732-8823
14.08.2020
03.02.2021

Introduction

Familial hypomagnesemia with secondary hypocalcemia (HSH) is a rare autosomal recessively inherited disease which is related to transient receptor potential melastatin 6 **(***TRPM6)* gene mutation, seen in early infancy period; also characterized by hypocalcemia secondary to hypomagnesemia and neuromuscular excitability symptoms like generalized seizures, muscle cramps, agitation (1). *TRPM6* functions as a cation channel with high permeability to Mg^{+2} and its activity is regulated by intracellular Mg^{+2} levels (2). It is expressed on intestine and distal convoluted tubules. The mutations cause hypomagnesemia by intestinal and renal magnesium wasting. Hypocalcemia in this disease is secondary to parathyroidhormone (PTH) resistance and decreased PTH release because of hypomagnesemia (3,4). Magnesium plays essential roles in the normal cell physiology throughout the body. It is difficult to distinguish the clinical manifestations of HSH from other causes of hypocalcemia such as hypoparathyroidism. If hypomagnesemia cannot be detected and treated soon, fatal convulsions, irreversible neurodevelopmental deficits and life-threating arrhythmias may develop. Any genotype-phenotype correlations between mutations on *TRPM6* and the severity of HSH have not been identified.
In this study, clinical features and long-term follow-up data of six patients with HSH who had *TRPM6* mutation were presented.

Patients and Methods:
The clinical files of six HSH patients who were members of five different families and were followed-up in our clinic since 1998 were evaluated retrospectively. Primary hypomagnesemia is diagnosed with low serum Mg^{+2} levels with a requirement of high dose Mg^{+2} treatment. Exclusion criteria were secondary hypomagnesemia such as being an infant of diabetic mother, intestinal malabsorption, clinical conditions such as short bowel syndrome and exposure to various drugs (e.g. proton pump inhibitors, antibiotics, diuretics, chemo-therapeutic agents). The levels of serum electrolytes, serum creatinine, alkaline phosphatase, PTH, 25 (OH) vitamin D and urinary Mg^{+2}, Ca, P, creatinine were evaluated in all cases.
The ultra-filtrated fraction of serum Mg^{+2} was calculated as UFMg=0.7xSMg. Renal Mg^{+2} handling was assessed by calculating fractional Mg^{+2} excretion (normal range 3 to 5% for normomagnesemic individuals) with FeMg=(UMgxSCr)/(UFMgxUCr) x100 where Fe is fractional excretion, SMg is serum Mg^{+2}, UMg is urinary Mg^{+2}, SCr is serum creatinine and UCr is urine creatinine. Hypercalciuria was defined as urine Ca/creatinine ratio (UCa/UCr) higher than 0.21mg/mg. Renal ultrasound was performed to rule out nephrocalcinosis. Clinical and laboratory findings of the patients under Mg^{+2} treatment were evaluated regularly at the outpatient clinic visits. Diarrhea as the mean side effect of high oral magnesium administration was defined as three or more loose or watery bowel movements per day. Diagnoses of all cases were confirmed by genetic analysis. Neurodevelopmental status was assessed by Revised Wechsler Intelligence Scale for children (WISC-R), which is evaluated by the Department of Child and Adolescent Psychiatry.
The clinical and laboratory findings (serum Ca, serum Mg^{+}, alkaline phosphatase, PTH, 25 (OH) vitamin D and urinary Mg^{+2}, Ca, P, creatinine) of the parents were also evaluated.
All participants and their parents received oral and written information concerning the study before providing signed consent. All procedures performed in this study were in accordance with the ethical standards. This study was approved by the local ethical committee (Sultangazi Haseki Training and Research Hospital, no: 2020–58).

Mutational analysis:
Extraction of DNA from leukocytes was performed using standard protocols. *TRPM6* mutational screening was performed by SSCP analysis. For that purpose, an overlapping set of PCR primers based on the sequence of the human *TRPM6* gene (genomic contig GenBank accession number AL354795) was used to amplify the complete coding sequence and the intron/exon boundaries from genomic DNA (primer sequences available upon request). Amplified products were separated on polyacrylamide gels by electrophoresis (Multiphor II; Pharmacia Biotech®). Subsequently, exons with conformational variants were directly sequenced from both strands (Applied Biosystems 310 Genetic Analyzer).

Results:
Five male and one female cases diagnosed with primary hypomagnesemia were included in the study. All patients presented with afebrile convulsions during the early infancy (1 to 9 months old on admission). The consanguineous marriage was present in three families, which one of had two affected siblings. The clinical and laboratory findings of the subjects at the time of diagnosis and follow-up are presented in Table 1. The follow-up period of the subjects ranged from 7.5 to 21.6 years. All cases were diagnosed with hypomagnesemia on admission except one case (F4). This patient admitted with a seizure at 3.5 months of age and the diagnose was considered as hypoparathyroidism because the serum magnesium was not assesed. Anticonvulsant treatment (phenytoin, phenobarbital) was started due to persistence of seizures despite calcitriol treatment. The hypomagnesemia was diagnosed at 7 months of age with the observation of hypomagnesemia and recurrent resistant seizures despite calcitriol and anticonvulsant therapy.
On admission the range of serum Mg^{+2} levels were 0.13-0.23 mmol/L, serum Ca levels were 1.42-1.92 mmol/L, PTH levels were 3.8-9.1 pg/mL. Under Mg^{+2} treatment, serum Ca and PTH levels were observed within normal levels during follow-up of all patients.
Oral Mg^{+2} treatment doses were adjusted to sustain normocalcemia according to the tolerances of the cases. Dose was not increased in patients with diarrhea. Treatment doses were between minimum 12.5 and maximum 31.5 mg / kg / day. In two cases (F3, F4), serum Mg^{+2} concentrations were maintained at the reference intervals with oral treatment and were subnormal in four cases (0.51-0.59 mmol/l). However, no symptoms were observed. Fractional Mg^{+2} excretion was between 1.2% and 5.9%. Hypercalciuria and nephrocalcinosis were not detected in any case.
None of the patients had short stature at the last follow-up. Height standard deviation (SD) scores ranged from -0,93 to 2,36 SD. In one case who was diagnosed at 9 months of age (F1.1) mild-to-moderate mental retardation was observed. One case had mild-to-moderate mental retardation (F1.1) and his brother had specific learning difficulty (F1.2). In another case (F5) attention deficit and attention deficit / hyperactivity disorder was diagnosed. Neurodevelopmental status of all patients except F1.1 and F1.2 were consistent with their age.

Mutation Analysis
Four different mutations were detected in *TRPM6* gene in six cases out of five families. (Table 1) A homozygous missense mutation which has been identified previously in a Turkish family on the Exon 23 was detected in F1.1 and F1.2. [c.3158A>G, (p.Tyr1053Cys)]. Both of the parents were heterozygous carriers of this mutation. Two patients with non-consanguineous families (F2 and F4) had the same splice-site mutation which was not previously described. (IVS7 splice-site, c.841 (+1)G>A). This mutation affected an essential splice site. The parents of these patients were heterozygous for the same mutation. The missense mutation [c.1751A> G, (p.His584Arg)] on the Exon 16 was detected in F3. This variant was reported in the ExAC database (exac.broadinstitute.org) as a rare variant at a frequency of 29/120000 and classified as a variant of unknown significance. However, this variant was predicted to be disease causing by in silico analysis such as Mutation Taster. Furthermore, the parents of these patients were heterozygous for the same variant. In F5, nonsense mutation on the exon 25 [c.3514C> T, (p.Arg1172)], previously reported in the ExAC database (exac.broadinstitute.org) as a rare variant of 1/120000 frequency, was detected. However, this mutation causes stop codon formation and the parents were heterozygous for this mutation. Therefore, this mutation, which was not previously reported in any patient, was accepted as a pathogenic variant (Figure 1).

The clinical and laboratory findings (serum Ca, serum Mg^{+}, alkaline phosphatase, PTH, 25 (OH) vitamin D and urinary Mg^{+2}, Ca, P, creatinine) of the parents, who had heterozygous mutations, were evaluated. The clinical and laboratory findings were unremarkable.

Discussion

In this study we have given the long-term follow-up results of the six genetically confirmed HSH patients. Three novel mutations on *TRPM6* gene were identified in addition to one known pathogenic mutation. The first presentation of all cases was afebrile seizures in the first year of life. Mild mental retardation, specific learning difficulty and attention deficit / hyperactive disorder were found as comorbidities. In the long-term follow-up, we found that growth was normal with magnesium supplementation and maintenance of normal serum calcium levels.

Magnesium (Mg^{+2}) is a cofactor for many enzymes and transporters, including phosphatases and phosphokinases. It is required for energy storage and use, and plays an important role in the synthesis of nucleic acids and proteins (5). Therefore, insufficient cellular Mg^{+2} concentrations affect many systems. Magnesium is strictly regulated by intestinal absorption and renal excretion and/or reabsorption. Intestinal absorption of Mg^{+2} occurs in the jejunum and the ileum. Most of the renal-filtered Mg^{+2} is absorbed by passive paracellular transport from the proximal tubule and the thick ascending loop of Henle. In the distal convoluted tubule, the fine tuning of Mg^{+2} equilibrium is made by active transcellular transport (6).

Hypomagnesemia in children may develop secondary to clinical conditions such as intestinal malabsorption, short bowel syndrome; being diabetic mother infant; and various drugs (proton pump inhibitors, antibiotics, diuretics, chemotherapeutic agents) and also may develop as a result of primary familial hypomagnesemia disorders (7). HSH is usually characterized by clinical findings such as restlessness, tremor, muscle cramps, tetany, perioral cyanosis and generalized convulsions in neonates or early infancy period. Magnesium levels are normal at delivery due to free passage of Mg^{+2} from placenta. It progressively decreases within weeks / months and clinical findings begin (8). The age at presentation varies between 2 weeks and 9 months, and in 96% of the patients, generalized seizures have been reported as presenting symptom which is similar to all of our cases (9, 10).

Hypocalcemia and hypoparathyroidism may cause misdiagnosis of primary hypoparathyroidism if the serum magnesium level was not assessed (11), as in our F4 case. Improper or delayed diagnosis and treatment may cause recurrent fatal convulsions and irreversible neurological damage (9). The mechanism by which hypomagnesemia causes neurological damage is not known; defect of voltage dependent magnesium passage on N-methyl-D aspartate receptor is thought to trigger convulsions (12). Abnormal development and neural tube defects have been reported in *TRPM6* knock-out mice. In addition, mental retardation, paranoid delusions and death due to recurrent seizures were reported in cases with delayed diagnosis (8, 9, 13). Mental retardation was diagnosed in one of our cases (F1.1), who was diagnosed relatively late, at 9 months of age. On the other hand, his brother had specific learning difficulty eventhough (F1.2) he had a early diagnose at 1 month of age and treated properly. Interestingly, Lainez et al. (14) have also described a case with mental retardation and the same genetic mutation decribed in these siblings of our study. Lastly, in one patient who was diagnosed at 5 months of age, we observed attention deficit / hyperactivite disorder (F5), and another patient with recurrent seizures had normal neurological development (F4).

Short stature has been rarely reported in HSH patients and the underlying mechanism is not completely explained (9, 12, 15). Short stature may be the result of late diagnosis and non-compliance with the treatment, but it is also seen in cases diagnosed in early infancy and treated appropriately. So, it has been suggested that short stature may be a clinical feature of the disease (15). There was no short stature in any of our patients.

TRPM6 has been shown to be expressed in testicles, but the effect of mutations on male fertility is unknown (16). In two 21-years-old male patients, puberty was consistent with tanner stage 5 and the sperm number, motility and morphology were normal in the spermiogram. In our 15-years-old male, puberty was consistent with stage 5, but a spermiogram could not be performed. None of the patients had a child.

The standard treatment in HSH is high dose Mg^{+2}. On diagnosis, intravenous or intramuscular administration can be preferred and maintenance therapy is high dose oral Mg^{+2}. A significant variation of mean oral magnesium dose (0,41-3,9 mmol/kg) has been reported between patients and centers (9,17). In the literature, it has been shown that serum Mg levels do not reach normal values other than 3 cases under high dose Mg treatment in patients with HSH (9, 14). In line with the litherature in our study, oral Mg doses ranged between 0.51-1.28 mmol / kg (12.5-31.5 mg / kg). Normal serum Mg levels near the lower limit of the reference intervals were obtained in only two patients under Mg treatment.

Physiological fractional renal Mg excretion is 3-5%, it falls below 0.5-1% in order to maintain serum Mg levels in the presence of hypomagnesemia (18, 19). In the current study fractional renal Mg excretion was measured over 5% in two patients whose serum Mg levels were normal but close to the lower limit of the reference intervals. In patients with subnormal course of serum Mg levels, renal Mg excretion was over 1% (1.2-2.1%). Increased renal Mg excretion has a clear role in the pathogenesis of the disease and prevents to reach physiological serum Mg values despite adequate treatment. In other words, the treatment should not provide normomagnesemia, but should provide normocalcemia, and if serum Ca is normal, Mg doses should not be increased.

It has been shown that the mutations previously identified in patients with HSH are not localized in a specific region and distributed all over the *TRPM6* protein (3, 9, 14). Up to date, 11 different mutations have been identified in 17 Turkish patients (Table 2) (9, 14,15,20-22). The most common *TRPM6* mutations in Turkish patients were c.5775A>G (in five cases from three non-consanguineous families), c.469G>T (in three cases from three family with), c.3158A>G (in three cases from two non-consanguineous families). In our study, missense mutation (F1.1 and 1.2) was found on the 23^{th} exon which was previously described in a Turkish case by Lainez et al. In addition, we demonstrated three novel mutations. The first mutation was c.841(+1)G>A on the IVS7 splice site was identified in two unrelated patients (F2 and F4). The second novel mutation was c.3514C>T (p.Arg1172) on the 25^{th} exon. It was reported as a rare variant in the database, however it causes a stop codon formation and both parents were heterozygous carriers for the same mutation, therefore this mutation is accepted as pathogenic. The missense variant found in F3 was c.1751A>G (p.His584Arg) and both parents were heterozygous carriers for the same mutation. This variant was reported in ClinVar database as a variant of unknown significance. It may be

pathogenic when evaluated together with the clinical features and segregation analysis of the patient, however functional analysis is required.

In a study, in which 28 HSH cases were evaluated regarding genotype-phenotype relationship, normal serum Mg levels were obtained by magnesium treatment in two cases with a deletion on the exon 32 and 33 (9). Since mutations on the exon 32 and 33 only affect a small portion of the *TRPM6* protein, it has been suggested that the channel function may be partially protected. However, it was reported with functional analysis that all mutations resulted in complete loss of function in *TRPM6* ion channel and no genotype-phenotype correlation was reported (9). In another study, the age of admission, serum Mg and Ca levels and oral Mg doses have been compared in 30 cases previously described in the literature. No relation has been found between genotype and these clinical and laboratory parameters (10). In our study, clinical and laboratory findings of all patients with different mutations were similar. In addition, in one patient, normal serum Mg levels were obtained with Mg treatment, while Mg levels were subnormal in another patient with the same mutation.

Magnesium treatment and close follow-up are essential to prevent clinical symptoms and to obtain normal Ca metabolism in HSH patients. In our case series, we observed that with an early diagnosis, appropriate treatment and a good treatment compliance the long-term (about 15 years) prognosis was good and no serious complications developed in HSH patients, similar to the study of Astor et al. which reported long-term (about 40 years) follow-up data (16).

Study Limitation

The main limitation of our study was the small number of patients. Additionally, functional analysis could not be performed although we identified new pathogenic mutations.

Conclusion

Evaluation of our cases with HSH revealed a homogenous clinical picture at manifestation with onset in early infancy with generalized seizures, however revealed heterogenous molecular findings including four different *TRPM6* mutations that three of them novel. Early diagnosis, appropriate treatment and good treatment compliance are crutial for the prognosis. Enlightment of the genetic etiology of autosomal recessive disorders like HSH is important, which can reveal mutations especially in populations where consanginous marriages are prevalent. Molecular studies in the cases with HSH and their families will contribute to increase our knowledge about the magnesium homeostasis. Determination of the genetic mutation is also useful to know the prognosis and associated comorbidities.

Acknowledgement

We thank Professor, Karl Peter Schlingmann, University Children's Hospital, Department of General Pediatrics in Münster/Germany and Naz Güleray, University of Health Sciences, Dr. Sami Ulus Obstetrics and Gynecology and Pediatrics Training and Research Hospital, Department of Medical Genetic in Ankara/ Turkey for genetic analyses and sequencing data.

Conflict interest

There is no conflict interest in this paper

Funding

There are no funding sources in this paper.

References:

1. Schlingmann KP, Weber S, Peters M, Niemann Nejsum L, Vitzthum H, Klingel K, Kratz M, Haddad E, Ristoff E, Dinour D, Syrrou M, Nielsen S, Sassen M, Waldegger S, Seyberth HW, Konrad M. Hypomagnesemia with secondary hypocalcemia is caused by mutations in TRPM6, a new member of the TRPM gene family. Nat Genet 2002; 31: 166-170.

2. Topala CN, Groenestege WT, Thébault S, van den Berg D, Nilius B, Hoenderop JG, Bindels RJ. Molecular determinants of permeation through the cation channel TRPM6. Cell Calcium 2007; 41: 513-523

3. Walder RY, Landau D, Meyer P, Shalev H, Tsolia M, Borochowitz Z, Boettger MB, Beck GE, Englehardt RK, Carmi R, Sheffield VC. Mutation of TRPM6 causes familial hypomagnesemia with secondary hypocalcemia. Nat Genet 2002; 31: 171-174.

4. Voets T, Nilius B, Hoefs S, van der Kemp AW, Droogmans G, Bindels RJ, Hoenderop JG. TRPM6 forms the Mg2+ influx channel involved in intestinal and renal Mg2+ absorption. J Biol Chem 2004; 279: 19-25.

5. de Baaij JH, Hoenderop JG, Bindels RJ. (2015) Magnesium in man: implications for health and disease. Physiol Rev 2015; 95: 1-46.

6. Dai LJ, Ritchie G, Kerstan D, Kang HS, Cole DE, Quamme GA. Magnesium transport in the renal distal convoluted tubule. Physiol Rev 2001; 81: 51-84.

7. Sutton RA, Domrongkitchaiporn S. Abnormal renal magnesium handling. Miner Electrolyte Metab 1993; 19: 232-240.

8. Zhao Z, Pei Y, Huang X, Liu Y, Yang W, Sun J, Si N, Xing X, Li M, Wang O, Jiang Y, Zhang X, Xia W. Novel TRPM6 mutations in familial hypomagnesemia with secondary hypocalcemia. Am J Nephrol 2013; 37: 541-548.

9. Schlingmann KP, Sassen MC, Weber S, Pechmann U, Kusch K, Pelken L, Lotan D, Syrrou M, Prebble JJ, Cole DE, Metzger DL, Rahman S, Tajima T, Shu SG, Waldegger S, Seyberth HW, Konrad M. Novel TRPM6 mutations in 21 families with primary hypomagnesemia and secondary hypocalcemia. J Am Soc Nephrol. 2005; 16: 3061-3069.

10. Katayama K, Povalko N, Yatsuga S, Nishioka J, Kakuma T, Matsuishi T, Koga Y. New TRPM6 mutation and management of hypomagnesaemia with secondary hypocalcaemia. Brain Dev 2015; 37: 292-298.

11. Jin-no Y, Kamiya Y, Okada M, Hirako M, Takada N, Kawaguchi M. Primary hypomagnesemia caused by isolated magnesium malabsorption: atypical case in adult. Intern Med 1999; 38: 261-265.

12. Hartnett KA, Stout AK, Rajdev S, Rosenberg PA, Reynolds IJ, Aizenman E. NMDA receptor-mediated neurotoxicity: a paradoxical requirement for extracellular Mg2+ in Na+/Ca2+-free solutions in rat cortical neurons in vitro. J Neurochem 1997; 68: 1836-1845.

13. Walder RY, Yang B, Stokes JB, Kirby PA, Cao X, Shi P, Searby CC, Husted RF, Sheffield VC. Mice defective in Trpm6 show embryonic mortality and neural tube defects. Hum Mol Genet 2009; 18: 4367-4375.

14. Lainez S, Schlingmann KP, van der Wijst J, Dworniczak B, van Zeeland F, Konrad M, Bindels RJ, Hoenderop JG. New TRPM6 missense mutations linked to hypomagnesemia with secondary hypocalcemia. Eur J Hum Genet 2014; 22: 497-504.

15. Guran T, Akcay T, Bereket A, Atay Z, Turan S, Haisch L, Konrad M, Schlingmann KP. Clinical and molecular characterization of Turkish patients with familial hypomag nesaemia: novel mutations in TRPM6 and CLDN16 genes. Nephrol Dial Transplant 2012; 27: 667-673.

16. Astor MC, Løvås K, Wolff AS, Nedrebø B, Bratland E, Steen-Johnsen J, Husebye ES. Hypomagnesemia and functional hypoparathyroidism due to novel mutations in the Mg channel TRPM6. Endocr Connect 2015; 4: 215-222.

17. Shalev H, Phillip M, Galil A, Carmi R, Landau D. Clinical presentation and outcome in primary familial hypomagnesaemia. Arch Dis Child 1998; 78: 127-130.

18. Dimke H, Hoenderop JG, Bindels RJ. Molecular basis of epithelial Ca2+ and Mg2+ transport: insights from the TRP channel family. J Physiol 2011; 589: 1535-1542.

19. Agus ZS. Hypomagnesemia. J Am Soc Nephrol 1999; 10: 1616-1622. Review.

20. Altıncık A, Schlingmann KP, Tosun MS. A Novel Homozygous Mutation in the Transient Receptor Potential Melastatin 6 Gene: A Case Report. J Clin Res Pediatr Endocrinol 2016; 8: 101-104.

21. Apa H, Kayserili E, Agin H, Hizarcioglu M, Gulez P, Berdeli A. A case of hypomagnesemia with secondary hypocalcemia caused by Trpm6 gene mutation. Indian J Pediatr 2008; 75:632–634.

22. Özlü SG, Kasapkara CS, Ceylaner S, Erat Nergız M, Alan B, Yılmaz S, Citak Kurt AN. Mild hypotonia and recurrent seizures in an 8-month-old boy: Answers. Pediatr Nephrol 2010; 34: 1729-1731.

Table 1. Laboratory, genetic and follow-up features of cases with familial hypomagnesemia with secondary hypocalcemia

	F1.1	**F1.2**	**F2**	**F3**	**F4**	**F5**
Age on admission (months)	9	1	1.5	3	3.5	5
Age on diagnosis (months)	9	1	1,5	3	7	5
Gender	Male	Male	Male	Female	Male	Male
Symptoms on admission	Seizures	Seizures	Seizures	Seizures	Seizures	Seizures
Serum Ca on admission (2.1-2.55 mmol/L)	1.68	1.82	1.92	1.62	1.42	1.55
PTH on admission (pg/mL) (n: 9-67)	4.9	3.8	5.5	6.5	9.1	8.7
Serum Mg on admission (0.66-1.07 mmol/L)	0.23	0.16	0.21	0.18	0.13	0.17
Age at last control (years)	22	15.3	8.4	7.8	9.4	22
Height SDS at last control	-0,93	-0,92	0,8	2,32	-0,22	-0,43
Follow-up period (years)	21.2	15.2	8.3	7.6	8.9	21.7
Magnesium doses at last control (mg/kg/day)	12.5	13.6	13.5	31.5	24	13
Neurodevelopmental status	Mild-Moderate MR	SLD	Normal	Normal	Normal	ASHD
Serum Mg at final control (0.66-1.07 mmol/L)	0.62	0.59	0.51	0.71	0.72	0.59
Serum Ca at final control (2.1-2.55 mmol/L)	2.25	2.52	2.37	2.52	2.42	2.52
FeMg(%) (n:%3-5)	2.1	1.9	1.2	5.1	5.9	1.8
Urinary Ca/Cre	0.1	0.06	0.19	0.04	0.06	0.036
Nefrocalsinosis	No	No	No	No	No	No

Parental consanguinity	Yes	Yes	Yes	Yes	No	No
Affected gene	TRPM6	TRPM6	TRPM6	TRPM6	TRPM6	TRPM6
Mutation	c.3158A>G (p.Tyr1053Cy)	c.3158A>G (p.Tyr1053Cys)	*c.841(+1)G>A	*c.1751A>G (p.His584Arg)	*c.841(+1)G>A	*c.3514C>T (p.Arg1172*)
Localization	Exon 23	Exon 23	IVS7 donorsplice site	Exon 16	IVS7 donorsplice site	Exon 25
Mutation/Mother	c.3158A>G (p.Tyr1053Cy) (het)	c.3158A>G (p.Tyr1053Cy) (het)	*c.841(+1)G>A (het)	*c.1751A>G (p.His584Arg) (het)	*c.841(+1)G>A (het)	*c.3514C>T (p.Arg1172*) (het)
Mutation/Father	c.3158A>G (p.Tyr1053Cy) (het)	c.3158A>G (p.Tyr1053Cy) (het)	*c.841(+1)G>A (het)	*c.1751A>G (p.His584Arg) (het)	*c.841(+1)G>A (het)	*c.3514C>T (p.Arg1172*) (het)

*no association with the disease has been reported before. Ca: calcium, Mg: magnesium, PTH: Parathormone, FeMg: fractional magnesium excretions MR: mental retardation, SLD: specific learning difficulty, ADHD: attention deficit/hyperactive disorder

Turkish Patients (references)	Gender	Age at diagnosis	Symptoms at manifestation	İnitial serum Mg^{+2} (mmol)	İnitial serum Ca^{+2} (mmol)	Oral/İMM g^{+2} (mmol/kg/d)	Mg^{+2} under therapy	Fe Mg (%)	Additional finding	Mutation
P1 [9]	F	2 mo	Seizures	0.21	1.63	1.03 (o)	0.59	2.6	-	c.1769G>G
P2 [9]	M	6 yr	Seizures	ND	1.29	0.62 (o)	0.57	2.8	MR	c.2667+G>A
P3 [9] [a]	M	3 mo	Seizures	0.09	1.6	0.54 (o)	0.33	ND	-	c.5775A>G
P4 [9] [a]	M	4 mo	Asymptomatic	0.16	1.75	0.94 (o)	0.53	ND	-	c.5775A>G
P5 [9]	M	4 mo	Seizures	0.1	1.45	2.0 (o)	0.50	3.7	-	c.469G>T
P6 [9]	F	3 wk	Seizures	0.2	1.72	0.93 (o)	0.52	ND	-	c.2667+G>A
P7 [14]	F	infancy	Seizures	0.05	1.78	0.97 (o)	0.50	ND	MR	c.3158A>G
P8 [14]	F	8 mo	Seizures	0.2	1.6	0.5 (o)	0.53	ND	-	c.469G>T + 5261G>A
P9 [21]	F	2 mo	Seizures	<0.24	1.5	1.6 (o)	0.57	3.9	-	c.3447delT>p.F1149fs
P10 [15] [b]	M	3 mo	Seizures	0.16	1.8	0.2 (o)/ 0.8 (im)	0.38	0.1	-	c.3556C>T
P11 [15] [b]	F	3 mo	Seizures	0.08	1.0	0.4 (o)/ 0.9 (im)	0.45	0.1	Short stature	c.3556C>T
P12 [15]	M	1 mo	Seizures	0.2	2.4	0.9 (im)	0.41	0.8	-	c.5775A>G
P13 [15]	M	1yr	Seizures	0.14	2.6	0.8 (o)	0.75	2.7	-	c.1444-1 G>T
P14 [15] [c]	M	1 mo	Seizures	0.5	1.7	0.6 (o)/3.7 (im)	0.58	2.3	Short stature	c.5775A>G
P15 [15] [c]	F	3 mo	Seizures	0.5	1.7	0.5 (o)/ 7 (im)	0.66	1.9	-	c.5775A>G
P16 [22]	M	1 mo	Seizures	0.16	1.42	0.7 (o)	0.69	ND	-	469G>T +
P17 [23]	M	8 mo	Seizures +hypotonia	0.19	1.67	1.72 (o)	ND	0.18	-	3178A > T

Table 2: Clinical data and results of the TRPM6 mutational analyses of Turkish patient with familial hypomagnesemia with secondary hypocalcemia

a, b, c: siblings, F;female, M; male , o; oral, im; intramusculer, ND; not defined, MR: mental retardation

Figure 1. Pedigree and mutational analysis of the patients with novel TRPM6 variant.