CEN Case Reports (2019) 8:42–47
https://doi.org/10.1007/s13730-018-0362-x

CASE REPORT

Exome sequencing identifies a novel frameshift variant causing hypomagnesemia with secondary hypocalcemia

M. Kamran Azim[1,3] · Aisha Mehnaz[2] · Javeria Z. Ahmed[2] · Ghulam Mujtaba[1]

Received: 6 February 2018 / Accepted: 20 August 2018 / Published online: 24 August 2018
© Japanese Society of Nephrology 2018

Abstract

Hypomagnesemia with secondary hypocalcemia is a rare autosomal-recessive disorder characterized by intense hypomagnesemia associated with hypocalcemia (HSH). Mutations in the *TRPM6* gene, encoding the epithelial Mg^{2+} channel TRPM6, have been proven to be the molecular cause of this disease. This study identified causal mutations in a 2-month-old male patient of hypomagnesemia from a consanguineous marriage. Biochemical analyses indicated the diagnosis of HSH due to primary gastrointestinal loss of magnesium. Whole exome sequencing of the trio (i.e. proband and both parents) was carried out with mean coverage of > 150×. ANNOVAR was used to annotate functional consequences of genetic variation from exome sequencing data. After variant filtering and annotation, a number of single nucleotide variants (SNVs) and 2 bp deletion at exon26:c.4402_4403delCT in *TRPM6* gene were identified. This deletion which resulted in a novel frameshift mutation in exon 26 of this gene was confirmed by Sanger sequencing. With these investigations in hand, the patient was managed with magnesium sulphate. The patient remained asymptomatic and was developmentally and neurologically normal till his last follow up.

Keywords TRPM6 gene · Hypomagnesimia · TRPM7 gene · Magnesium metabolism

Introduction

The second most abundant cellular cation after potassium is magnesium. Physiologic serum concentration of magnesium (Mg^{2+}) in a healthy individual is around 0.75–0.95 mmol/l (1.8–2.3 mg/dl) [1]. Mg^{2+} has a crucial role in numerous cellular functions and enzymes, including ion channels, metabolic cycles, and signalling pathways. Mg^{2+} homeostasis is kept by renal and intestinal (re)absorption. Predominantly kidney plays important role for Mg^{2+} homeostasis. In the glomeruli, approximately one-third of Mg^{2+} is ultra-filterable. In the kidney, 80–99% Mg^{2+} reabsorption is generally accomplished by paracellular pathway in the proximal tubule and the thick ascending limb of the loop of Henle. The 10–15% reabsorption takes place in the distal convoluted tubule (DCT) by active transcellular pathway and ultimately Mg^{2+} excretion is finalized [2].

Low serum Mg^{2+} concentration has been reported in different genetic disorders including isolated dominant hypomagnesaemia with hypocalciuria, isolated recessive hypomagnesemia with normocalciuria, familial hypomagnesemia with hypercalciuria/nephrocalcinosis, hypomagnesaemia with secondary hypocalcaemia, autosomal dominant hypoparathyreoidism, Gitelman syndrome and sometimes classic Bartter syndrome [3]. Molecular genetic analyses of patients suffering from Mg^{2+} metabolic disorder revealed disruptive mutations in genes including *FXYD2, CNNM2, CLDN16 (PCLN1), TRPM6, CASR, SLC12A3, KCNJ1, HNF1B, KCNJ10, CLDN19, SLC12A1, KCNA1* and *CLC-NKB* [5]. Therefore molecular genetic studies revealed involvement of these genes in Mg^{2+} (re)absorption and retention [4].

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13730-018-0362-x) contains supplementary material, which is available to authorized users.

✉ M. Kamran Azim
Kamran.azim@jinnah.edu; mkamranazim@yahoo.co.uk

[1] International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan

[2] Department of Paediatrics, Dow Medical College, Dow University of Health Sciences, Karachi, Pakistan

[3] Department of Biosciences, Mohammad Ali Jinnah University, Karachi, Pakistan

Primary hypomagnesemia with secondary hypocalcemia (HSH) is a rare autosomal recessive disease characterized by deficiency of Mg^{2+} [5]. The onset is usually seen in early infancy with generalized convulsion, seizures and sometimes headed by muscles cramp and agitation [4]. Prolong and intense hypomagnesemia causes hypoparathyroidism which eventually leads to hypocalcemia in patients [6]. Late diagnosis may lead patients to permanent neurological injury or even sudden death as a result of arrhythmias. The phenotypes can be overcome by augmenting the patient's diet with Mg^{2+}.

Molecular genetic analysis of HSH provided evidences for involvement of *TRPM6* and *TRPM7* genes in Mg^{2+} transport in the DCT [5]. The *TRPM6* gene mutations have been reported as underlying genetic defect in patients with HSH [7]. TRPM6 and TRPM7 proteins are close members of the TRP superfamily of ion channels with ion channel and protein kinase activities and have been shown to play important roles in magnesium homeostasis. TRPM6 and TRPM7 are highly permeable to magnesium [7]. In contrast to TRPM7, the expression pattern of TRPM6 seems to be more confined, with expression mainly along the gastrointestinal tract as well as in kidney predominantly in the distal convoluted tubule (DCT) [8]. The Mg^{2+}-enriched diet could not compensate for either embryonic lethality or hypomagnesemia caused by TRPM6 deficiency [9]. Dietary Mg^{2+} restriction promoted a compensatory increase in Mg^{2+} absorption and reabsorption by enhancing TRPM6 expression in intestine and kidney [10].

Here we present the molecular pathology of a patient of HSH by whole exome sequencing and Sanger sequencing which resulted in the identification of a novel frameshift mutation in the *TRPM6* gene.

Case study

A 2-month-old male infant, 7th product of consanguineous marriage, home delivered via spontaneous vaginal delivery at term, partially vaccinated, was referred from Sukkur, Pakistan to Civil hospital, Karachi, Pakistan with complain of uncontrollable generalized tonic clonic fits off and on since 1 month of age. Fits were not associated with fever, altered level of consciousness, any focal deficits or any other associated complains. The patient was developmentally appropriate for age. Past history was significant for inappropriate startle response starting at the 15th day of life. He had a significant family history of early sibling death at the age of 2 months with history of similar kind of convulsions.

On examination, the infant weighed 4 kg. He was vitally stable with BP below 90th centile and all anthropometric measurements lying below 10th centile for age. He had no other significant neurological or physical findings except for brisk reflexes bilaterally in both upper and lower limbs.

On investigating the patient, the serum calcium and magnesium levels were markedly low [serum calcium = 4.5 mg/dl (normal range 9–11 mg/dl) and magnesium = 0.4 (normal range 1.8–3.6 mg/dl)]. Rest of the markers including urea, creatinine and electrolytes (including phosphate), complete blood count, random blood sugar, serum alkaline phosphatase, serum albumin, blood gases and ECG were within the normal range. The patient was investigated further and serum para-thyroid hormone (PTH) level was also turned out to be normal (32 pg/ml; normal range 10–65 pg/ml). 24 h urinary electrolytes were also within normal range. Ultrasound KUB showed no element of nephrocalcinosis. Calcium creatinine ratio was calculated that was 0.16 (normal) and fractional excretion of magnesium was 0.8% (< 4% = renal conservation and > 4% = renal wasting). Therefore, on the basis of history and clinical investigations, a strong clinical diagnosis of hereditary hypomagnesemia due to primary gastrointestinal loss of magnesium (i.e. familial hypomagnesemia with secondary hypocalcemia) was made.

With these investigations in hand, the patient was managed with I/V calcium gluconate initially at 1 ml/kg/dose early 6 hourly that was later increased to 2 ml/kg/dose and I/V magnesium sulphate initially at 25 mg/kg/dose 6 hourly that was later increased up to 50 mg/kg/dose 6 hourly. Repeated serum calcium and magnesium levels showed improvement and fits of the patient were eventually controlled. The patient was discharged on oral calcium and magnesium supplements. On discharge, his serum calcium and magnesium levels were 9.2 and 1.6 mg/dl, respectively. Consistent with the observation reported by Lainez et al. [4], serum Mg^{2+} levels never normalised for this child even with aggressive management.

The patient was followed regularly till 5 months of age. He was admitted once again at 3.5 months of age with fits as they had discontinued calcium and magnesium supplements for 2 weeks duration. Otherwise the patient remained asymptomatic and was developmentally and neurologically normal till his last follow up.

Whole exome sequencing Blood samples of the HSH patient and his parents were obtained in Civil hospital, Karachi, Pakistan after obtaining informed consent. The genomic DNA was extracted from the blood by CTAB method [11]. The purified genomic DNA samples of HSH patient and his parents were subjected to exome sequencing (trio exome sequencing). The whole exome sequencing was performed using SureSelect Human All Exon V6 (Agilent Inc., USA) and the Complete Genomics sequencing platform (http://www.completegenomics.com/). Bioinformatics analysis of exome sequence data was carried out by GATK (https://software.broadinstitute.org/gatk/), Samtools [12], Annovar

and wAnnovar [13] and Phenolyzer [14] (Fig. 1; Table 1). Sequence data were aligned to the human reference genome (NCBI37/hg19). PCR amplification followed by Sanger sequencing of exon 26 of *TRPM6* gene was carried out.

Bioinformatic analysis of exome sequencing data (i.e. proband and parents) compiled lists of heterozygous, homozygous and de novo SNVs and indels (Supplementary Tables 1 and 2). We found 531 heterozygous and 177 homozygous variants after trio-based filtering. Analysis of VCF files by wANNOVAR identified 10,343 of missense, nonsense and splicing variants; and 385 indels. Filtering of these variants (with MAF > 0.01) in 1K genome project database resulted in 1796 SNVs and 230 indel variants. Further filtering of variants in genomAD exome database (http://gnomad.broadinstitute.org/) (with MAF > 0.01) resulted in 1365 SNVs and 203 indels. Next, based on disease model, wANNOVAR compiled two lists of 206 and 13 candidate genes attributable to SNVs and indels respectively (Fig. 2). TRPM6 gene was present in both the lists. When VCF files of proband and parents were compared and analysed in the light of Variant Effect Predictor (VEP) scores and VarElect tool (http://varelect.genecards.org), we could trim down the list of candidate genes to 11 (Table 2). Subsequent evaluation of the mutations in these genes by pointed out *TRPM6* as the causal gene.

Several known synonymous SNVs were found in *TRPM6* gene (eg. dbSNP id rs11144089). These SNVs had low impact on structure-function of TRPM6 as predicted by VEP (https://www.ensembl.org/vep). A novel 2 bp deletion (exon26:c.4402_4403delCT) was identified in proband which resulted in frame shift mutation in exon 26 of *TRPM6* gene. This deletion in *TRPM6* gene which was not found in 'Genome Aggregation Database' (genomAD data set) was confirmed by Sanger sequencing (Fig. 3). The genomAD

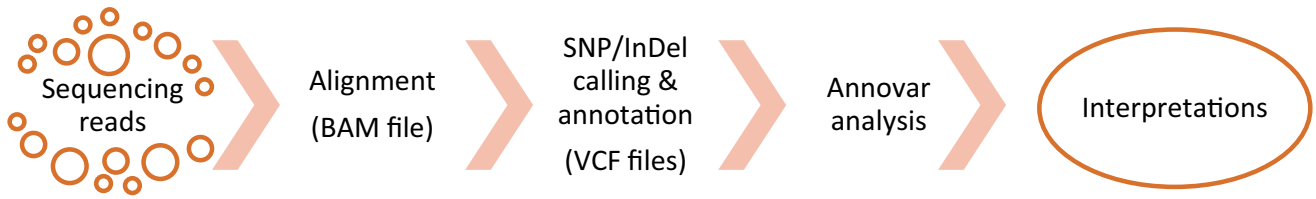

Fig. 1 Flowchart of bioinformatics analysis of exome sequence data

Table 1 Statistics of exome sequencing data

	Proband	Mother	Father
Total base mapped (Gb)	17.48	18.6	19.08
No. of bases in target regions	59,168,140	59,061,609	59,168,140
No. of bases covered on target regions	58,743,040	58,624,755	58,695,292
Coverage of target regions (%)	99.28	99.26	99.20
Average sequencing depth on target regions	165.19	176.4	174.41

Table 2 List of candidate genes in the light of analysis carried out by wANNOVAR, VEP and VarElect

Genes	Mutation type	dbSNP id (if known)		Minor allele frequency	Impact predicted by VEP	Related diseases
PLCE1	Frame shift	Novel	p.L781fs	–	High	Nephrotic syndrome, type 3
TBX4	Frame shift	Novel	p.V483fs	–	High	Ischiocoxopodopatellar syndrome
NPAT	Frame shift	Novel	p.T113fs	–	High	Ataxia-telangiectasia
UROS	Frame shift	Novel	p.I176fs	–	High	Congenital erythropoietic porphyria
SCAF4	Frame shift	Novel	p.K1137fs	–	High	Wells syndrome
TRPM6	Frame shift	Novel	p.L1435fs	–	High	Hypomagnesemia
NT5M	Frame shift	Novel	p.T186fs	–	High	Adiaspiromycosis
EGF	Frame shift	rs79786166	p.P1135fs	0.06	High	Hypomagnesemia
SLC6A2	SNV	rs1805068	p.L319=	0.01	Low	Orthostatic intolerance
SLC26A1	SNV	rs144218313	p.Val445Ile	0.0018	Moderate	Nephrolithiasis
SLC44A4	SNV	rs116706632	p.Pro397Ser	0.0027	Moderate	Deafness, autosomal dominant 72

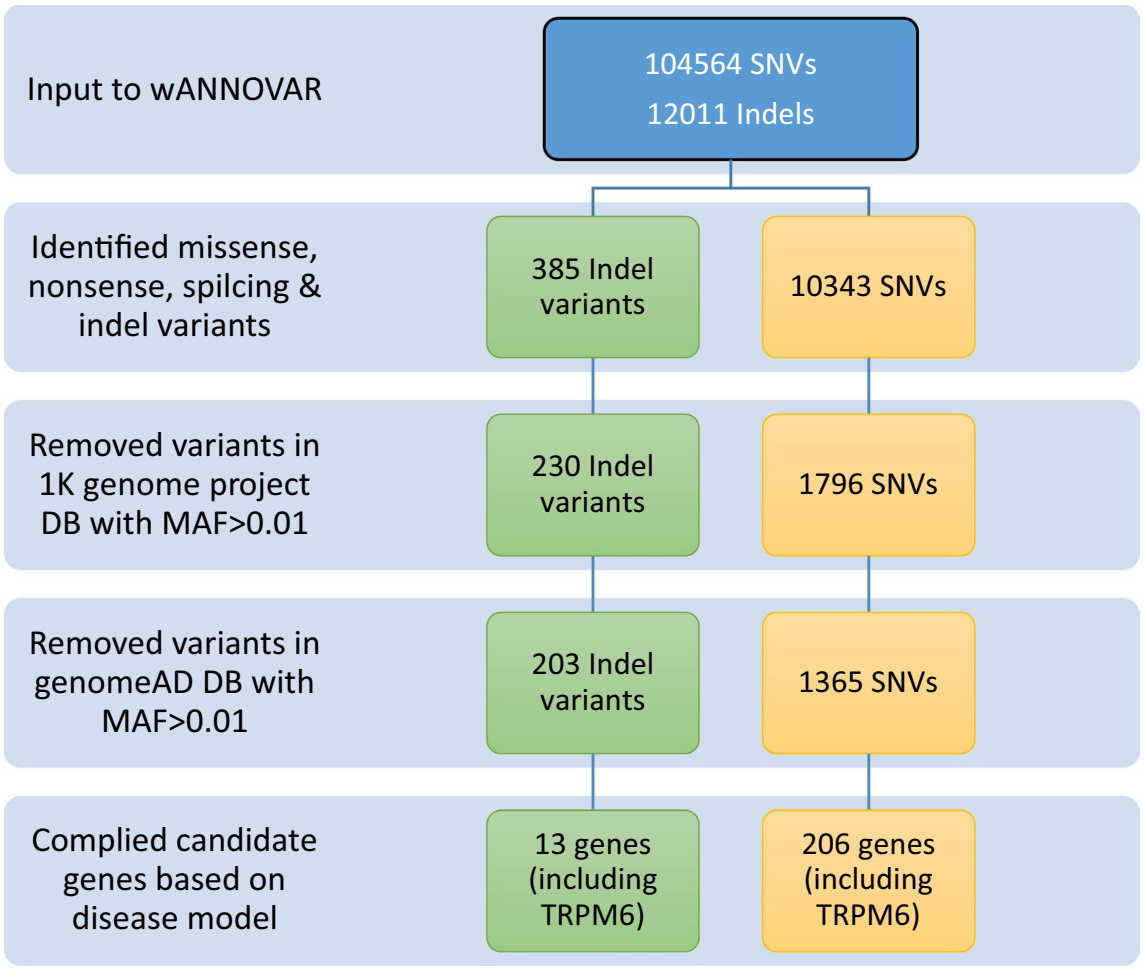

Fig. 2 Identification of genes responsible in patient of hereditary hypomagnesemia with secondary hypocalcemia by wANNOVAR pipeline. The input data included all SNVs and indels in patient generated by exome sequencing

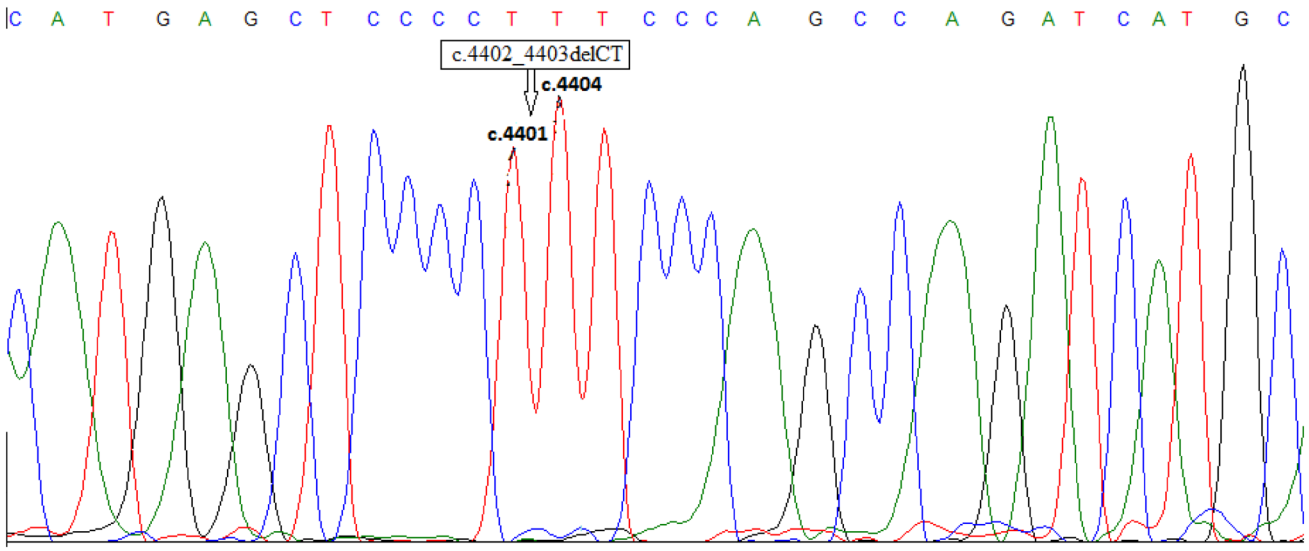

Fig. 3 Electrophorogram generated by Sanger sequencing shown here confirmed the deletion of two bp at exon26:c.4402_4403delCT (HGSV nomenclature) in the coding strand of genomic DNA of TRPM6 gene of patient

Table 3 Data related to frame shift mutations in TRPM6 gene of 15 HSH patients analysed in the present study

Cases	Gender	Origin	Zygosity	Nucleotide mutation(s)	Exon	Consequence(s) of mutation	References
1	M	Israel	Comp-hetero	(1280delA), (3779-91del)	11 and 26	H427fsX429, E1260fsX1283	[22]
2,3	F	Albania	Homo	2207delG	17	R736fsX737	
4	F	India	Homo	del Ex21 + 23	22 and 23	Frame shift	
5	F	France	Hetero	5017-18delT	30	L1673fsX1675	
6	F	Rumania	Homo	Del2831_2832insG	21	I944fsX959	
7,8	F, M	Pakistan	Homo	Del Ex25-Ex27	25–27	Frame shift	
9,10,11	F, F, M	UK	Hetero	668delA	6	D223fsX263	
12	F	Japan	Comp-hetero	Del1796_1797	16	P599fsX609	
13	F	Greece	Homo	Del Ex 21	21	Frame shift	
14		China	Comp-hetero	c.1196delC		Frame shift	[24]
15	M	Pakistan	Homo	c.4303_4304delAG	26	L1435fs	Present

dataset contains 123,136 exome sequences and 15,496 whole genome sequences from unrelated individuals sequenced as part of several disease-specific and population genetic studies (including 1000 genome project data set). The trio analysis showed that the frame shift mutation was heterozygous in parents and homozygous in the proband. The high impact (according to VEP) frame shift mutation was found to be in the carboxy terminal cytoplasmic domain in between TRP box and kinase domain. Therefore, we predicted that this mutation would result in disruption of TRPM6 and TRPM7 complex formation.

Discussion

Whole exome sequencing has significantly assisted the identification of mutations causing genetic disorders [15]. This technology has the potential to implicate novel gene variations. The purpose of this study was to search for causal mutations in a patient diagnosed with HSH from a consanguineous marriage. Trio whole exome sequencing and Sanger sequencing identified a homozygous novel frame shift variant in *TRPM6* gene in the proband. Exome sequencing of family pedigrees (trio analysis) offers a powerful method for the characterization of transmitted alleles and/or de novo mutations that may confer predisposition to disease. The "trio" analysis leverages the family relationship to accurate variant calling and identify high-confidence de novo mutations. Trio analysis classifies the inheritance patterns of genetic mutations to identify SNVs and indels.

Among several candidate genes with SNVs and indels, we identified a novel 2 bp deletion in proband which resulted in frame shift mutation in *TRPM6* gene of this HSH patient. Trio exome sequence analysis and the reported clinical data supported the notion that the 2 bp deletion would be responsible for this rare autosomal recessive disorder. Several frame shift mutations in *TRPM6* gene have been characterized in hypomagnesemia patients (Table 3). Schlingmann et al. [16] reported 23 different mutations in TRPM6 gene among a cohort of 21 families with 28 affected individuals. Nine of these were frame shift mutations in exons 6, 11, 16, 17, 21, 22, 23 and 25–27. It is interesting that two patients of Pakistani ancestry with consanguinity in this cohort had homozygous deletion of exons 25–27. Likewise a patient from India (with consanguinity) had deletion of exons 22–23. These deletions would lead to frame shifts and consequently a preterm stop codon. Zhao et al. [17] reported a novel heterozygous frame shift mutation (c.1196delC) in two Chinese sisters with HSH.

Mutations in an array of genes have been identified in hypomagnesemia patients. A mutation in the EGF gene encoding pro-EGF was reported, which was responsible for a rare form of renal magnesium (Mg^{2+}) wasting in isolated recessive hypomagnesemia [18]. We identified a known frame shift mutation (dbSNP id rs79786166) in the present study. Mutations in KCNJ10 gene, which codes for Kir4.1, and mutations in FYXD2 gene, which codes for the γ-subunit of Na^{+}-K^{+}-ATPase have been linked with hypomagnesemia [19]. Mutations in the transcription factor hepatocyte nuclear factor (HNF1B) gene, which regulates the transcription of FXYD2 caused a similar phenotype [20]. The CLDN16 gene has also been associated with renal hypomagnesemia. It has been proposed that claudin-16 (encoded by CLDN16 gene) is involved in controlling magnesium and calcium permeability of the paracellular pathway in the cTAL [6, 21]. Several known intronic mutations in HNF1B and CLDN16 genes were found in our patient. Moreover, mutations in the DCT-expressed cyclin M2 (CNNM2) gene have been linked with hypomagnesemia [22, 23].

Known moderate and low-impact SNVs were found in genes encoding Solute Carrier Family proteins (i.e. SLC6A2, SLC26A1, SLC44A4) (Table 2). These variants appear to be benign, but we could not completely exclude

the possibilities of their contribution to hypomagnesemia in the patient.

In conclusion, among several candidate genes, present trio exome sequencing study identified a novel homozygous frame shift mutation in TRPM6 gene of HSH patient. However, it should be noted that exome sequencing does not cover large genomic rearrangement such as copy number variations (CNVs).

Compliance with ethical standards

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the Independent Ethics Committee, ICCBS, University of Karachi, Karachi, Pakistan (IEC Approval Number ICCBS/IEC-010-SS-2016).

Conflict of interest The authors have declared that no conflict of interest exists.

References

1. Konrad M, Weber S. Recent advances in molecular genetics of hereditary magnesium-losing disorders. J Am Soc Nephrol. 2003;14:249–60.
2. Sanders GT, Hujjgen HJ, Sanders R. Magnesium in disease: a review with special emphasis on the serum ionized magnesium. Clin Chem Lab Med. 1999;37:1011–33.
3. Cole DEC, Quamme GA. Inherited disorders of renal magnesium handling. J Am Soc Nephrol. 2000;11:1937–47.
4. Lainez S, Schlingmann KP, van der Wijst J, Dworniczak B, van Zeeland F, Konrad v M, Bindels RJ, Hoenderop JG. New *TRPM6* missense mutations linked to hypomagnesemia with secondary hypocalcemia. Eur J Hum Genet. 2014;22(4):497–504.
5. Chubanov V, Schlingmann KP, Waring J, Heinzinger J, Kaske S, Waldegger S, Schnitzler MM, Gudermann T. Hypomagnesemia with secondary hypocalcemia due to a missense mutation in the putative pore-forming region of TRPM6. Proc Natl Acad Sci USA. 2007;101(9):2894–99.
6. De Baaij JH, Hoenderop JG, Bindels RJ. Magnesium in man: implications for health and disease. Physiol Rev. 2015;95:1–46.
7. Chubanov V, Waldegger S, Schnitzler MM, Vitzthum H, Sassen MC, Seyberth HW, Konrad M, Gudermann T. Disruption of TRPM6/TRPM7 complex formation by a mutation in the TRPM6 gene causes hypomagnesemia with secondary hypocalcemia. 2004; 101:2894–99.
8. Schmitz C, Dorovkov MV, Zhao X, Bennett JD, Ryazanov AG, Perraud A-L. The channel kinases TRPM6 and TRPM7 are functionally nonredundant. J Biol Chem. 2005;280:37763–71.
9. Schlingmann KP, Gudermann T. A critical role of TRPM channel-kinase for human magnesium transport. J Physiol. 2005;566:301–8.
10. Schuchardt JP, Hahn A. Intestinal absorption and factors influencing bioavailability of magnesium-an update. Curr Nutr Food Sci. 2017;13(4):260–78.
11. Zhang YJ, Zhang S, Liu XZ, Wen HA, Wang M. A simple method of genomic DNA extraction suitable for analysis of bulk fungal strains. Lett Appl Microbiol. 2010; https://doi.org/10.1111/j.1472-765X.2010.02867.x.
12. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R. 1000 Genome Project Data Processing Subgroup. The sequence alignment/map format and SAMtools. Bioinformatics. 2009;25(16):2078–79.
13. Yang H, Wang K. Genomic variant annotation and prioritization with ANNOVAR and wANNOVAR. Nat Protoc. 2015;10:1556–66.
14. Yang H, Robinson PN, Wang K. Phenolyzer: phenotype-based prioritization of candidate genes for human diseases. Nat Methods. 2015;12:841–3.
15. Retterer K, Juusola J, Cho MT, Vitazka P, Millan F, Gibellini F, Vertino-Bell A, Smaoui N, Neidich J, Monaghan KG, McKnight D, Bai R, Suchy S, Friedman B, Tahiliani J, Pineda-Alvarez D, Richard G, Tracy Brandt T, Haverfield E, Chung WK, Bale S. Clinical application of whole-exome sequencing across clinical indications. Genet Med. 2016; 18:696–704.
16. 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;16:3061–69.
17. 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;37:541–48.
18. Thebault S, Alexander RT, Groenestege WMT, Hoenderop JG, Bindels RJ. EGF increases TRPM6 activity and surface expression. J Am Soc Nephrol. 2009;20:78–85.
19. Li H, Sun S, Chen J, Xu G, Wang H, Qian Q. Genetics of magnesium disorders. Kidney Dis (Basel). 2017;3(3):85–97.
20. van der Made CI, Hoorn EJ, de la Faille R, Karaaslan H, Knoers NV, Hoenderop JG, Vargas Poussou R, de Baaij JH. Hypomagnesemia as first clinical manifestation of ADTKD-HNF1B: a case series and literature review. Am J Nephrol. 2015;42(1):85–90.
21. Hou J. Claudins and mineral metabolism. Curr Opin Nephrol Hypertens. 2016;25(4):308–13.
22. Viering DHHM, de Baaij JHF, Walsh SB, Kleta R, Bockenhauer D. Genetic causes of hypomagnesemia, a clinical overview. Pediatr Nephrol. 2017;32(7):1123–35.
23. de Baaij JH. The art of magnesium transport. Magnes Res. 2015;28(3):85–91.
24. Lü Q, Zhang Y, Song C, An Z, Wei S, Huang J, Huang L, Tang L, Tong N. A novel SLC12A3 gene homozygous mutation of Gitelman syndrome in an Asian pedigree and literature review. J Endocrinol Invest. 2016;39(3):333–340