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Magnesium-permeable *TRPM6* polymorphisms in patients with meningomyelocele

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Abstract

Background: To evaluate whether there is an association between single nucleotide polymorphisms in magnesium-permeable *TRPM6* ion channel and development of meningomyelocele (MMC). Therefore, we examined a total of 150 children with MMC, along with age- and gender-matched controls. DNA collected from whole blood was analyzed for the presence of two polymorphisms, rs2274924 (A > G; K1579E; Leu1579Glu) and rs3750425 (G > A; Val1393Ile), in *TRPM6*. Serum Mg²⁺ and calcium levels were also examined.

Results: A statistically significant difference in the distribution of rs2274924 genotypes ($p = 0.049$) was observed between the groups. Decreases in the AA genotype, and increases in the AG heterozygous genotype were also detected in the study group. The distribution of polymorphisms in the rs3750425 genotype and alleles was not statistically different between groups. Serum Mg²⁺ levels were lower in the GG genotype of rs3750425 compared with the GA and AA genotypes ($p = 0.003$).

Conclusions: A statistically significant difference in rs3750425 genotypes was observed between the patients with MMC and the controls, which corresponded to lower serum Mg²⁺ concentrations in these patients. Taken together, these results suggest that genetic variations in the Mg²⁺-permeable *TRPM6* ion channel may play a role in the etiopathogenesis of MMC during embryonic development.

Keywords: Neural tube defect, Spina bifida cystica, Etiopathogenesis, Genetic, Embryogenesis, Development, Human, Children

Background

Neural tube defects (NTDs) occur during embryonic period when the neural tube fails to close normally and neural tissue exposed to the intrauterine environment. It affects about 0.1–0.4 % worldwide, 0.3 % in Turkey (Mandiracioğlu et al. 2004; van Rooij et al. 2003) and 2.6/1000 in East Anatolia region of Turkey in live births (Saraç et al. 2007). Meningomyelocele (MMC) is a nonlethal but severely disabling form of NTD (Shuttleff and Graaf 2001). Etiology of MMC remains poorly understood, most likely driven by a combination of genetic and

environmental factors (Miao et al. 2016; Au et al. 2010). Genetic factors may contribute up to 70 % of NTD prevalence based on epidemiological evidences to date (Leck 1974; Copp and Greene 2010; Bassuk and Kibar 2009). Maternal health problems, including inadequate nutrition have also been shown to affect neural tube closure through a variety of mechanisms (Copp et al. 2013).

Magnesium (Mg²⁺) is the second most abundant cation in cell and is essential for all stages of life, from the early embryo to adult. Regulation of cellular as well as whole body Mg²⁺ homeostasis is critical for human health. Although the concentration of Mg²⁺ in the extracellular environment can vary significantly, the total intracellular Mg²⁺ concentration is actively maintained within a relatively narrow range (14–20 mM) via tight (Komiya and

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Runnels 2015; Romani 2011). This responsibility largely achieves to Mg^{2+} transporters and ion channels. Transient receptor potential cation channel, subfamily M, member 6 (*TRPM6*; 602014), located on chromosome 9q21.13, plays a central role in the regulation of Mg^{2+} homeostasis via absorption and reabsorption of Mg^{2+} from the small intestines and kidneys, respectively (Song et al. 2009; Hruby et al. 2013; Groenestege et al. 2006; Schlingmann et al. 2005).

Homozygous *TRPM6* knockout embryos died by E12.5 and exhibited neural tube closure defects (Walder et al. 2002). The expression pattern of *TRPM6* during embryogenesis demonstrated a significant increase at E10, suggesting *TRPM6* is also temporally required for early development. Heterozygous *TRPM6* knockout mice exhibited mild hypomagnesemia (Walder et al. 2002; Woudenberg-Vrenken et al. 2011). Mutations in *TRPM6* occur in the rare autosomal-recessive disease hypomagnesemia with secondary hypocalcemia (HSH) in human (Schlingmann et al. 2002). As indicated in many studies, hypomagnesemia in null mice is sufficient to induce NTDs, as well as developmental disorders of the bones (Walder et al. 2009; Chubanov and Gudermann 2014). Proper Mg^{2+} levels are also of particular importance to pregnant women, with deficiencies associated with premature birth and intrauterine growth retardation, along with increases in both mortality and morbidity during the embryonic period. Embryotoxic and teratogenic effects of Mg^{2+} have also been reported in animal models. Lower serum Mg^{2+} levels are strongly associated with skeletal malformations, osteogenesis imperfecta, and growth retardation of the embryo, as well as dysregulation of fetal temperature and sudden infant death (Hurley et al. 1976; Günther et al. 1981).

The fundamental principles underlying *TRPM6* ion channel operation have been described in detail. *TRPM7*, an ion channel permeable to both Ca^{+} and Mg^{2+} , functions via the formation of heterodimers with *TRPM6* (Günther et al. 1981). Single nucleotide polymorphisms (SNPs) of *TRPM6* have been associated with diabetes mellitus and serum magnesium level in human (Song et al. 2009; Hruby et al. 2013; Walder et al. 2009). Given these associations, we sought to investigate the role of rs2274924 (A > G; K1579E; Leu1579Glu) ve Rs3750425 (G > A Val1393Ile) polymorphisms in *TRPM6* comparing with the frequency of specific genotypes and alleles in patients with MMC and healthy controls and the potential correlation between these SNP variants and serum Mg^{2+} and calcium concentrations to identify potential functional outcomes associated with disease genotypes.

Methods

This case–control study was conducted after obtaining approval from the institutional ethics committee (protocol number 31.01.2013/02/12). Oral and written consent were obtained from all families prior to inclusion in this study. A total of 150 patients diagnosed with MMC admitted to the outpatient clinics of neurosurgery, pediatric surgery, and pediatrics were enrolled in this study. An additional 150 patients admitted to the pediatric surgery outpatient clinic with no evidence of abnormal sacral spine examination findings were used as a control group. The demographic characteristics and clinical history of each patient were investigated in detail. Evidence of consanguineous marriage between parents or earlier generations was also noted. Due to the possibility of teratogenic effects, maternal use of any drug during the periconceptual period was recorded. Serum calcium and Mg^{2+} levels were assessed using a colorimetric assay.

Isolation of DNA

DNA isolation was performed using the Promega Wizard® Genomic DNA Isolation Kit according to the manufacturer's instructions. The DNA concentration was measured using a MaestroNano® (MaestroNanodrop; Maestrogen, USA), and diluted to 1–10 ng.

Genotyping using TaqMan® probes

SNPs of rs2274924 (A > G; K1579E; Leu1579Glu) and rs3750425 (G > A; Val1393Ile) in *TRPM6* were analyzed using TaqMan® probes in combination with an ABI 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). The genomic sequence of rs2274924 was assessed using the probe AGTCCTTGAGTATTCTTCTTTTCT[C/T]TGACAGTCTCCTGTCTTTGGTTAGC (Assay ID: C_16183233_20). Polymorphism at this nucleotide confers an AAG to GAG substitution, resulting in a leucine to glutamic acid substitution at residue 1579. For genotyping, the kit used FAM and VIC fluorescent dyes for the A and G alleles, respectively, as forward and reverse primers. The genomic sequence of rs3750425 was assessed using the probe TCCACTGATGCCAGTCAGAGACAA[C/T]TGGGGTCTGCCAGTCAGATGAACA (Assay ID: C_25943493_20). Polymorphism at this nucleotide confers an ATT to GAG substitution, resulting in a valine to isoleucine substitution at residue 1393. For genotyping, the kit used FAM and VIC fluorescent dyes for the A and G alleles, respectively, as forward and reverse primers. All polymerase chain reaction (PCR) reaction mixtures were prepared on ice.

From each sample, we combined 2.5 μ L DNA with 7.5 μ L PCR reaction mixture for a final volume of 10 μ L.

Plates were then covered with optical film, centrifuged, and analyzed using a 7500 Fast Real-Time PCR System for 40 cycles. The device software was used to identify homozygous mutant, heterozygous, and homozygous normal genotypes for each allele.

Statistical analysis

Data were analyzed using the IBM Statistical Package for the Social Sciences v. 21 (SPSS Inc., Chicago, IL, USA). Relationships between parameters were assessed using Pearson's correlation analysis. Distributions of genotype and allele frequency were analyzed using a Chi square test. The Mann–Whitney *U* test was used to evaluate differences between groups. Data were expressed as the mean \pm standard deviation. A *p* value <0.05 was considered statistically significant.

Results

The study group ($n = 150$) consisted of 60 female (40 %) and 90 (60 %) male patients. The mean age of the study group was 3.1 ± 3.22 years (range 1 month–18 years). The mean age of the mothers was 28.15 ± 4.71 years (range 19–44 years). A detailed list of demographic, clinical, and laboratory findings are presented in Table 1.

No significant differences in genotype or allele distributions of *TRPM6* rs3750425 polymorphisms were evident between the groups (Table 2). In contrast, a statistically significant difference was observed between the study and control groups in terms of the distribution of the rs2274924 genotype ($p = 0.049$). Prevalence of the AA genotype was markedly decreased in patients with MMC, while the heterozygous AG genotype was increased. However, no significant difference in the distribution of rs2274924 alleles was observed between the groups (Table 2).

Within the study group, we observed a statistically significant difference in serum Mg^{2+} levels associated with

rs3750425 genotypes but not for rs2274924 genotype. Serum Mg^{2+} levels were significantly lower in patients harboring the rs3750425 GG genotype relative to those possessing either the GA or AA genotypes ($p = 0.003$; Table 3). No differences were observed in relation to rs2274924 genotypes.

Discussion

Our statistical finding focused on two coding SNPs in *TRPM6* in NTD patients. *TRPM6* polymorphism may contribute to different ways the NTD development, such as regulating osteogenesis during embryonic development, effecting the maternal serum magnesium level in pregnant women and changing in the expression level under the influence of estrogen hormone during pregnancy.

A limited number of studies have investigated the potential links between *TRPM6* variations and human disease. In our study population, approximately 31.5 % are heterozygous for the rare *G* allele of rs2274924 and 22 % heterozygous for the allele *A* of rs3750425. The HapMap studies were shown that population frequencies are 0.07–0.21 for the rs3750425 *A* allele (1393 Ile) and 0.15–0.36 for the rs2274924 *G* allele (1584Glu) in four ethnic groups (Walder et al. 2009). Song et al. (2009) provide suggestive evidence that two common non-synonymous *TRPM6* coding region variants, Ile1393Val (rs3750425) and Lys1584Glu (rs2274924) polymorphisms, might confer susceptibility to type 2 diabetes in women with low magnesium intake. However, a follow-up study of Romero et al. (2010), Shuen et al. (2009) failed to duplicate these results for rs3750425 and rs2274924. A significant association was observed between common variants of *TRPM6* and hypomagnesemia (Hruby et al. 2013; Meyer et al. 2010), with a specific T allele of rs11144134 in *TRPM6* associated with higher femoral neck and lumbar spine BMD (Meyer et al. 2010). Magnesium-deficiency has been linked to osteoporosis and low BMD in observational and animal studies (Alexander et al. 2008). Disorders in osteogenesis responsible from inhibition of bone matrix mineralization (Semczuk and Semczuk-Sikora 2001; Durlach 2004; Takaya et al. 2006). The transcripts of canonical *TRPM6* channel were also revealed in human MG-63, SaOS and U2 OS osteoblast cell lines (Abed et al. 2009). These observations show that *TRPM6* may important the control of bone homeostasis during embryonic and adulthood. Polymorphic amino acids for two polymorphisms investigated in present study are located between the coiled region and kinase near the C-terminal (Schlingmann et al. 2004). Although these amino acid changes in coiled region of *TRPM6* protein are improbable responsible for direct regulation of trans-membrane structures and kinase activity, they may alter

Table 1 Demographic, clinical, and laboratory findings of each group

	Study group	Control group
Age (years)	3.1 ± 3.22	9.7 ± 2.21
Female/male	60/90	75/75
History of another baby with MMC in the family	18 (11.9 %)	–
Consanguinity between parents	41 (27.3 %)	15 (10 %)
History of another baby with a congenital anomaly in the family	32 (21.3 %)	10 (6.6 %)
Drug use during pregnancy	25 (16.6 %)	15 (10 %)
Calcium (mg/dL)	9.09 ± 1.24	10.0 ± 1.25
Mg^{2+} (mg/dL)	1.98 ± 0.62	2.3 ± 0.39

MMC Meningomyelocele

Table 2 Frequencies of the genotypes and alleles of rs3750425 and rs2274924 polymorphisms in the transient receptor potential cation channel, subfamily M, member 6 (TRPM6) gene

	Genotypes			p value	Odds ratio (95 %)
	GG	GA	AA		
rs3750425 Polymorphism groups					
Study group	94	53	3	0.14	1.45 (0.88–2.39)
Control group	103	40	7		
Alleles					
	G	A			
Study group	241	59	0.68	1.11 (0.74–1.67)	
Control group	246	54			
Genotypes					
	AA	AG	GG		
rs2274924 Polymorphism groups					
Study group	71	66	13	0.049	1.84 (1.13–3)
Control group	91	46	13		
Alleles					
	A	G			
Study group	214	86	0.22	1.27 (0.88–1.83)	
Control group	228	72			

GG wild type, GA heterozygous, AA polymorphic allele, AA wild type, AG heterozygous, GG polymorphic allele

Table 3 Mean serum Mg²⁺ and Ca⁺ levels in patients with meningomyelocele (MMC) according to their genotype

Polymorphism	Calcium	p value	Mg ²⁺	p value	
rs3750425	GG	9.2 ± 1.20	0.48	1.51 ± 0.31	0.003
	GA	9.0 ± 1.21		2.0 ± 0.34	
	AA	9.5 ± 1.18		2.1 ± 0.38	
rs2274924	AA	9.35 ± 1.17	0.33	1.80 ± 0.41	0.37
	AG	9.02 ± 1.15		1.82 ± 0.48	
	GG	9.30 ± 1.2		1.81 ± 0.42	

protein conformation and thus decrease TRPM6 channel activity.

Mg²⁺ deficiency causes or contributes to some abnormalities during embryonic development in human and rats. The decreased maternal Mg²⁺ intake because of inadequate nutrition and embryonic genetic defects might be responsible from Mg²⁺ deficiency. A maternal Mg²⁺ intake below 378 mg/day has been associated with a twofold to threefold higher risk for the neural tube defect, spina bifida in offspring, suggesting a pivotal role for Mg²⁺ not just in growth but in morphogenesis as well (Groenen et al. 2004). A decrease in basal [Mg²⁺]_i from cord blood platelets is associated with small for gestational age (SGA)

babies in humans (Venu et al. 2008). Oral Mg²⁺ supplementation given before the 25th week of gestation is also associated with a lower frequency of preterm births, a lower frequency of low birth weight, and fewer SGA infants compared with placebo (Makrides and Crowther 2001). Pups of pregnant females fed a Mg²⁺ deficient diet demonstrated an increased incidence of resorptions, gross malformation, growth retardation, abnormal fat metabolism, insulin resistance and diabetes (Hurley et al. 1976; Venu et al. 2008). Despite these apparent links between maternal serum Mg²⁺ levels and some embryonic and fetal anomalies, no correlation was found between maternal or neonatal serum Mg²⁺ levels and MMC (Golalipour and Mansourian 2010). These observations are broadly consistent with the results presented here, with no significantly different neonatal serum Mg²⁺ or Ca⁺ levels evident between the groups, although lower serum Mg²⁺ levels were detected in patients with GG genotypes in rs3750425 compared with either the GA or AA variants. As Mg²⁺ homeostasis in the embryo is critically dependent on the maternal blood supply, a lack of genetic and biochemical analysis of maternal serum samples to assess whether Mg²⁺ homeostasis was altered during prenatal development was the main limitation of our study.

Recent studies have continued to add to the growing number of Mg²⁺ transporters and ion channels

involved in Mg^{2+} homeostasis during embryogenesis. The expression pattern of TRPM6 during embryogenesis demonstrated a significant increase at E10, suggesting TRPM6 is also temporally required for early development. Heterozygous TRPM6 knockout mice exhibited mild hypomagnesemia (Walder et al. 2002; Woudenberg-Vrenken et al. 2011). Interestingly, in one of the studies dams fed with a high Mg^{2+} diet slightly suppressed the embryonic lethality caused by knockout of TRPM6, suggesting that embryonic lethality may in part be due to Mg^{2+} deficiency. However, in the second study by Woudenberg-Vreken et al. (2011) a high Mg^{2+} diet failed to rescue the lethality caused by homozygous deletion of TRPM6. Rare mutations or polymorphic variants in TRPM6, including amino acid substitutions that prevent its heterooligomerization with TRPM7 or changing channel activity, may cause the imbalance in intracellular magnesium level and likely to contribute the abnormal bone development during embryogenesis.

Recent studies have shown that estrogen directly increases the transcription of TRPM6, as well as its activity (Cao et al. 2009); this effect is of particular importance during pregnancy, due to the marked increase in maternal estrogen levels. As 90 % of maternal estradiol consists of fetal precursors, these effects take on added significance (Buster et al. 1976). Given the strong evidence that estrogen influences TRPM6 synthesis during pregnancy, this gene is likely to play an important role in the regulation of Mg^{2+} homeostasis in both the mother and fetus. Genetic variations in TRPM6 may therefore affect intracellular Mg^{2+} levels during embryonic development, with significant implications for fetal development.

Several limitations in present study must be completed with further consideration. First, our study had relatively small sample size, however, consistency with laboratory findings makes our results acceptable. Second, population's ethnic diversity may be a concern but impossible to clarify our findings, because our populations are racially homogeneous, all participant lived in Elazig region. Third, although serum magnesium may be a good indicator for heavy magnesium deficiency, we didn't performed the measures of extracellular or intracellular magnesium status and the patch clamp experiment to display the effect on channel activity of studying polymorphic variant in TRPM6 gene.

Clinical data show that sufficient magnesium intake can partially compensate for the severe magnesium deficiency caused by genetic defect in the TRPM6 gene (Altıncık et al. 2016). High magnesium concentrations in intestinal tracts may overcome the genetic defects of magnesium absorption and independently increase magnesium absorption via the other pathways. Inadequating of dietary magnesium intake, the function of TRPM6 in

active intestinal magnesium absorption and renal reabsorption happen very important. Based on these observations, we strongly recommend checking serum Mg^{2+} values of babies born with MMC. Further studies performed on a large number of patients will be necessary to confirm these findings, as well as clarify the etiology of MMC, particularly in the context of Mg^{2+} homeostasis.

Authors' contributions

MS drafted and wrote the manuscript. EÖ carried out the genetic studies, participated in the sequence alignment and performed the statistical analysis. UB provided related articles and helped the writing of manuscript. TT participated in the design of the study and transferred blood samples to laboratory. AT participated in the genetic studies and sequence alignment. MA conceived of the study, and participated in its design and coordination and helped to draft the manuscript. AO cared of the patients and collected blood samples from the patients. ET gave care to the patients and redacted the English. FSE performed surgery to the patients. AK supervised the study throughout from planning to writing of the paper. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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References

- Abed E, Labelle D, Martineau C, Loghin A, Moreau R (2009) Expression of transient receptor potential (TRP) channels in human and murine osteoblast-like cells. *Mol Membr Biol* 26(3):146–158
- Alexander RT, Hoenderop JG, Bindels RJ (2008) Molecular determinants of magnesium homeostasis: insights from human disease. *J Am Soc Nephrol* 19(8):1451–1458
- Altıncık A, Schlingmann KP, Tosun MS (2016) A novel homozygous mutation in the transient receptor potential melastatin 6 gene: a case report. *J Clin Res Pediatr Endocrinol* 8(1):101–104
- Au KS, Ashley-Koch A, Northrup H (2010) Epidemiologic and genetic aspects of spina bifida and other neural tube defects. *Dev Disabil Res Rev* 16(1):6–15
- Bassuk AG, Kibar Z (2009) Genetic basis of neural tube defects. *Semin Pediatr Neurol* 16(3):101–110
- Buster JE, Sakakini J Jr, Killam AP, Scragg WH (1976) Serum unconjugated estradiol levels in the third trimester and their relationship to gestational age. *Am J Obstet Gynecol* 125(5):672–676
- Cao G, van der Wijst J, van der Kemp A, van Zeeland F, Bindels RJ, Hoenderop JG (2009) Regulation of the epithelial Mg^{2+} channel TRPM6 by estrogen and the associated repressor protein of estrogen receptor activity (REA). *J Biol Chem* 284(22):14788–14795
- Chubanov V, Gudermann T (2014) TRPM6. *Handb Exp Pharmacol* 222:503–520
- Copp AJ, Greene ND (2010) Genetics and development of neural tube defects. *J Pathol* 220(2):217–230
- Copp AJ, Stanier P, Greene ND (2013) Neural tube defects: recent advances, unsolved questions, and controversies. *Lancet Neurol* 12(8):799–810

- Durlach J (2004) New data on the importance of gestational Mg deficiency. *J Am Coll Nutr* 23(6):694–700
- Golalipour MJ, Mansourian AR (2010) Maternal and neonatal serum Mg²⁺ concentrations in neural tube defects pregnancies in Gorgan (North of Iran)—a case control study. *J Clin Diagn Res* 4:2817–2821
- Groenen PM, van Rooij IA, Peer PG, Ocké MC, Zielhuis GA, Steegers-Theunissen RP (2004) Low maternal dietary intakes of iron, magnesium, and niacin are associated with spina bifida in the offspring. *J Nutr* 134(6):1516–1522
- Groenestege WM, Hoenderop JG, van den Heuvel L, Knoers N, Bindels RJ (2006) The epithelial Mg²⁺ channel transient receptor potential melastatin 6 is regulated by dietary Mg²⁺ content and estrogens. *J Am Soc Nephrol* 17(4):1035–1043
- Günther T, Ising H, Mohr-Nawroth F, Chahoud I, Merker HJ (1981) Embryotoxic effects of Mg²⁺ deficiency and stress on rats and mice. *Teratology* 24(2):225–233
- Hruby A, Ngwa JS, Renström F, Wojczynski MK, Ganna A, Hallmans G, Houston DK, Jacques PF, Kanoni S, Lehtimäki T, Lemaitre RN, Manichaikul A, North KE, Ntalla I, Sonestedt E, Tanaka T, van Rooij FJ, Bandinelli S, Djoussé L, Grigoriou E, Johansson I, Lohman KK, Pankow JS, Raitakari OT, Riserus U, Yannakoulia M, Zillikens MC, Hassanali N, Liu Y, Mozaffarian D, Papoutsakis C, Syvänen AC, Uitterlinden AG, Viikari J, Groves CJ, Hofman A, Lind L, McCarthy MI, Mikkilä V, Mukamal K, Franco OH, Borecki IB, Cupples LA, Dedoussis GV, Ferrucci L, Hu FB, Ingelsson E, Kähönen M, Kao WH, Kritchevsky SB, Orho-Melander M, Prokopenko I, Rotter JJ, Siscovick DS, Witteman JC, Franks PW, Meigs JB, McKeown NM, Nettleton JA (2013) Higher Mg²⁺ intake is associated with lower fasting glucose and insulin, with no evidence of interaction with select genetic loci, in a meta-analysis of 15 CHARGE Consortium Studies. *J Nutr* 143(3):345–353
- Hurley LS, Cosens G, Theriault LL (1976) Teratogenic effects of Mg²⁺ deficiency in rats. *J Nutr* 106(9):1254–1260
- Komiya Y, Runnels LW (2015) TRPM channels and magnesium in early embryonic development. *Int J Dev Biol* 59(7–9):281–288
- Leck I (1974) Causation of neural tube defects: clues from epidemiology. *Br Med Bull* 30(2):158–163
- Makrides M, Crowther CA (2001) Magnesium supplementation in pregnancy. *Cochrane Database Syst Rev* (4):CD000937. doi:10.1002/14651858.CD000937.pub2
- Mandiracioglu A, Ulman I, Lüleci E, Ulman C (2004) The incidence and risk factors of neural tube defects in Izmir, Turkey: a nested case-control study. *Turk J Pediatr* 46(3):214–220
- Meyer TE, Verwoert GC, Hwang SJ, Glazer NL, Smith AV, van Rooij FJ, Ehret GB, Boerwinkle E, Felix JF, Leak TS, Harris TB, Yang Q, Dehghan A, Aspelund T, Katz R, Homuth G, Kocher T, Rettig R, Ried JS, Gieger C, Prucha H, Pfeuffer A, Meitinger T, Coresh J, Hofman A, Sarnak MJ, Chen YD, Uitterlinden AG, Chakravarti A, Psaty BM, van Duijn CM, Kao WH, Witteman JC, Gudnason V, Siscovick DS, Fox CS, Köttgen A (2010) Genetic factors for osteoporosis consortium; meta-analysis of glucose and insulin related traits consortium. Genome-wide association studies of serum magnesium, potassium, and sodium concentrations identify six Loci influencing serum magnesium levels. *PLoS Genet* 6(8). doi:10.1371/journal.pgen.1001045
- Miao C, Jiang Q, Li H, Zhang Q, Bai B, Bao Y, Zhang T (2016) Mutations in the motile cilia gene *dnaaf1* are associated with neural tube defects in humans. *G3 (Bethesda)*. doi:10.1534/g3.116.033696
- Romani AM (2011) Cellular magnesium homeostasis. *Arch Biochem Biophys* 512(1):1–23
- Romero JR, Castonguay AJ, Barton NS, Germer S, Martin M, Zee RY (2010) Gene variation of the transient receptor potential cation channel, subfamily M, members 6 (TRPM6) and 7 (TRPM7), and type 2 diabetes mellitus: a case-control study. *Transl Res* 156(4):235–241
- Saraç M, Özel ŞK, Kazez A (2007) Spina bifida: current situation in Eastern Anatolia. *Çocuk Cerrahisi Dergisi* 21(3):116–119
- 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 (2002) Hypomagnesemia with secondary hypocalcemia is caused by mutations in TRPM6, a new member of the TRPM gene family. *Nat Genet* 31(2):166–170
- Schlingmann KP, Konrad M, Seyberth HW (2004) Genetics of hereditary disorders of magnesium homeostasis. *Pediatr Nephrol* 19(1):13–25
- 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 (2005) Novel TRPM6 mutations in 21 families with primary hypomagnesemia and secondary hypocalcemia. *J Am Soc Nephrol* 16(10):3061–3069
- Semczuk M, Semczuk-Sikora A (2001) New data on toxic metal intoxication (Cd, Pb, and Hg in particular) and Mg status during pregnancy. *Med Sci Monit* 7(2):332–340
- Shuen AY, Wong BY, Wei C, Liu Z, Li M, Cole DE (2009) Genetic determinants of extracellular Mg²⁺ concentration: analysis of multiple candidate genes, and evidence for association with the estrogen receptor alpha (ESR1) locus. *Clin Chim Acta* 409(1–2):28–32
- Shuttleff DB, Graaf WD (2001) Overview of clinical issues in the management of myelomeningocele. In: Sarwark JF, Lubicky J (eds) *Caring for the child with spina bifida*, 1st edn. American Academy of Orthopedic Surgeons, Illinois
- Song Y, Hsu YH, Niu T, Manson JE, Buring JE, Liu S (2009) Common genetic variants of the ion channel transient receptor potential membrane melastatin 6 and 7 (TRPM6 and TRPM7), Mg²⁺ intake, and risk of type 2 diabetes in women. *BMC Med Genet* 10:4
- Takaya J, Yamato F, Kaneko K (2006) Possible relationship between low birth weight and Mg²⁺ status: from the standpoint of “fetal origin” hypothesis. *Magnes Res* 19(1):63–69
- van Rooij IA, Vermeij-Keers C, Kluijtmans LA, Ocké MC, Zielhuis GA, Goorhuis-Brouwer SM, van der Biezen JJ, Kijpers-Jagtman AM, Steegers-Theunissen RP (2003) Does the interaction between maternal folate intake and the methylenetetrahydrofolate reductase polymorphisms affect the risk of cleft lip with or without cleft palate? *Am J Epidemiol* 157(7):583–591
- Venu L, Padmavathi IJ, Kishore YD, Bhanu NV, Rao KR, Sainath PB, Ganeshan M, Raghunath M (2008) Long-term effects of maternal magnesium restriction on adiposity and insulin resistance in rat pups. *Obesity (Silver Spring)* 16(6):1270–1276
- Walder RY, Landau D, Meyer P, Shalev H, Tsolia M, Borochowitz Z, Boettger MB, Beck GE, Englehardt RK, Carmi R, Sheffield VC (2002) Mutation of TRPM6 causes familial hypomagnesemia with secondary hypocalcemia. *Nat Genet* 31(2):171–174
- Walder RY, Yang B, Stokes JB, Kirby PA, Cao X, Shi P, Searby CC, Husted RF, Sheffield VC (2009) Mice defective in *Trpm6* show embryonic mortality and neural tube defects. *Hum Mol Genet* 18(22):4367–4375
- Woudenberg-Vrenken TE, Sukinta A, van der Kemp AW, Bindels RJ, Hoenderop JG (2011) Transient receptor potential melastatin 6 knockout mice are lethal whereas heterozygous deletion results in mild hypomagnesemia. *Nephron Physiol* 117(2):p11–p19

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