Common single nucleotide polymorphisms in transient receptor potential melastatin type 6 increase the risk for proton pump inhibitor-induced hypomagnesemia: a case–control study

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Objective Proton pump inhibitors (PPIs) are effective drugs for the treatment of gastric acid-related disorders. Serious adverse events are rare for PPIs, but recent data suggest that PPIs cause hypomagnesemia. The aim of this study was to estimate the frequency of PPI-induced hypomagnesemia and to define the risk factors for its development.

Materials and methods A total of 133 chronic users of PPIs were enrolled and patients were distinguished on the basis of their serum Mg²⁺ concentrations. Common single nucleotide polymorphisms (SNPs) in the candidate gene, transient receptor potential melastatin type 6 (TRPM6), were screened.

Results Seventeen out of 133 patients had PPI-induced hypomagnesemia. The duration of PPI use was longer in those with hypomagnesemia (7.7 vs. 5.2 years). Two common SNPs in TRPM6 (rs3750425 and rs2274924) increased the risk for PPI-induced hypomagnesemia by 5.8-fold.

Conclusion We found hypomagnesemia in 13% of PPI users. SNPs in TRPM6 drive the risk of developing hypomagnesemia during chronic PPI use. Pharmacogenetics and Genomics 00:000–000 Copyright © 2016 Wolters Kluwer Health, Inc. All rights reserved.

Keywords: hypertension, hypomagnesemia, proton pump inhibitors, TRPM6

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Received 6 June 2016 Accepted 11 November 2016

Introduction

The advent of proton pump inhibitors (PPIs) as potent acid-suppressive drugs has revolutionized the management of acid-related diseases. Millions of individuals take PPIs on a continuous or a long-term basis. PPIs interfere with electrolyte absorption and the literature contains a few cases with reversible hypocalcemia and hypokalemia, but also hypomagnesemia. Several meta-analyses confirm the notion that PPI intake increases the risk for hypomagnesemia [1,2]. This effect is specific for PPIs and absent with the use of other gastric-suppressing agents such as histamine-2 receptor antagonists [3]. PPI-induced hypomagnesemia occurs after prolonged long-term PPI use, but resolves within days following interruption [3].

The frequency of and the risk factors for PPI-induced hypomagnesemia are not well understood. Hypomagnesemia is often not recognized in clinical practice as patients gradually develop nonspecific symptoms such as muscle cramps and fatigue [4]. PPI-induced hypomagnesemia is probably caused by gastrointestinal loss as renal retention is unaffected [5,6]. PPIs may increase the local pH in the colon, which may reduce magnesium (Mg²⁺) absorption. In the colon, transient receptor potential melastatin type 6 (TRPM6) is the main gate keeper of Mg²⁺ absorption [7]. TRPM6-mediated Mg²⁺ transport is influenced by local pH changes, epidermal growth factor, estrogens, ATP, and insulin [8–13]. Interestingly, common single nucleotide polymorphisms (SNPs) in TRPM6 have been found to be associated with gestational diabetes and type-2 diabetes mellitus, especially with low Mg²⁺ intake [10,14]. We hypothesized that SNPs in the TRPM6 gene constitute a bona fide risk factor for the development of hypomagnesemia in PPI users.

This study aims, therefore, to gain a better understanding of the risk factors that are associated with hypomagnesemia among PPI users. Specifically, we were interested
in determining whether the presence of TRPM6 SNPs modifies the risk for PPI-induced hypomagnesemia. To this end, we prospectively compiled a cohort of PPI users in the Netherlands and collected demographical, genetic, and clinical data.

Materials and methods

Ethics statement
The study was carried out according to the declaration of Helsinki. All patients provided written informed consent. Before starting, the study protocol was approved by the local ethics committee (METC Arnhem Nijmegen, The Netherlands). All study investigators had access to the study data, reviewed, and approved the final manuscript.

Patient identification
The pool of patients eligible for study entry included patients aged greater than or equal to 18 years who were actively using PPIs at outpatient visits at one of the participating centers: Radboud University Medical Center (Nijmegen, The Netherlands), Canisius Wilhelmina Ziekenhuis (Nijmegen, The Netherlands), and Bernhoven Ziekenhuis (Oss, The Netherlands). Patients were excluded if they were alcohol abuse, uncontrolled diabetes mellitus, extremes of BMI (BMI <16 or >35), and/or short-bowel disease. Hypomagnesemia was defined as having a serum Mg²⁺ concentration less than 0.7 mmol/l according to reference values of the Dutch Federation for Clinical Chemistry [15]. PPI-induced hypomagnesemia was confirmed by one dechallenge–rechallenge cycle and these patients were designated as cases. Patients without a PPI-induced hypomagnesemia served as controls.

Laboratory procedures
Serum Mg²⁺ was determined using a Hitachi autoanalyzer according to the manufacturer’s protocol (Abbott Diagnostics, Ottignies/Louvain-La-Neuve, Belgium).

Clinical risk profiling
Clinical data were obtained using retrospective patient file analysis. The following patient characteristics and disease-specific factors were selected as potential determinants for the phenotype under study: sex, age, BMI, type of PPI, dosage, indication, and length of use. Given that hypertension, diabetes mellitus, and the use of diuretics have been previously linked to hypomagnesemia, patients were screened for these parameters.

Genetic risk profiling
DNA isolation was performed on freshly collected EDTA whole-blood samples using the HP-PCR Template Preparation kit (Roche Applied Science, Penzberg, Germany). In addition, DNA was obtained from 14 patients who were previously diagnosed with PPI-induced hypomagnesemia in our medical center. PCR was performed using primers that covered exons 26–27 of TRPM6 (NG_017036; Supplementary Table 1, Supplemental digital content 1, http://links.lww.com/FPC/B130). Each targeted SNP (dbSNP identifiers: rs45616231, rs3750425, rs2274924 and rs2274925) site on PCR amplicons was sequenced using an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, California, USA).

Statistics
Differences in serum Mg²⁺ between subgroups were tested using a Student t-test, including Welch’s correction, where applicable. Pearson χ²-test was used to identify possible risk factors for hypomagnesemia. Hardy–Weinberg equilibrium for each SNP studied was calculated. SNP frequencies were determined for the four studied SNPs and compared between cases and controls using Pearson’s χ²-test. Fisher’s exact test was used where appropriate [16]. Haplotype views were generated with genotype data from the four SNPs using Haplovieview (Broad Institute, Cambridge, Massachusetts, USA). The relative risk attributed to each haplotype was calculated as an odds ratio with a 95% confidence interval using the most common haplotype as a reference. Statistical significance was defined as P value less than 0.05.

Results

Cohort
One hundred and thirty-three PPI users who visited the outpatient clinics and fulfilled the inclusion criteria were recruited (Fig. 1). The most common indications for PPI use in the total cohort included gastroesophageal reflux (48%), antacids/protectant (28%), and gastrenteritis (18%). The type of PPI used in our population was omeprazole (41%), esomeprazole (29%), pantoprazole (27%), rabeprazole (2%), and lansoprazole (1%). The percentage of patients using each type of PPI, dosage, indication, and length of use was significantly correlated with lower serum Mg²⁺ concentration (r = −0.16, P < 0.05). Duration of PPI use was longer in patients with PPI-induced hypomagnesemia (7.7 years) versus those with normomagnesemia (5.2 years; P < 0.05). The type of PPI used in our population was omeprazole (41%), esomeprazole (29%), pantoprazole (27%), rabeprazole (2%), and lansoprazole (1%). The mean serum Mg²⁺ concentration was 0.79 ± 0.01 mmol/l (Table 1). Some 13% of the patients had Mg²⁺ levels below the threshold (<0.70 mmol/l), with a mean serum Mg²⁺ concentration of 0.61 ± 0.02 mmol/l, whereas the mean serum Mg²⁺ concentration of normomagnesemic PPI users was 0.82 ± 0.01 mmol/l (Fig. 2).

Clinical risk profiling
Duration of PPI use was longer in patients with PPI-induced hypomagnesemia (7.7 years) versus those with normomagnesemia (5.2 years; P < 0.05). Duration of PPI use was significantly correlated with lower serum Mg²⁺ levels (r = −0.16, P < 0.05). Age, sex, and BMI were equally distributed between the groups. We evaluated factors that affect the risk for PPI-induced hypomagnesemia using χ² testing and binary logistic regression analysis. Univariate analysis showed that hypertension was more frequently present in patients with PPI-induced hypomagnesemia compared with PPI users with normal Mg²⁺ levels (P < 0.05, Table 1).

Genetic risk profiling
Two SNPs in exons 26 and 27 of TRPM6 have been identified previously to reduce the insulin-stimulated
activity of the channel [10]. To identify whether TRMP6 SNPs constitute a genetic risk factor for PPI-induced hypomagnesemia, an extended cohort of 147 PPI (133 from the case–control study plus 14 patients with a known PPI-induced hypomagnesemia; 31 low Mg²⁺; 116 normal Mg²⁺) users were sequenced for TRPM6 SNPs located to exons 26 and 27: rs45616231, rs3750425, rs2274924, and rs2274925 (Fig. 3). We found that SNPs rs3750425 and rs2274924 were significantly enriched among PPI-induced hypomagnesemia patients ($P < 0.01$, Supplementary Table 2, Supplemental digital content 2, http://links.lww.com/FPC/B131).

**Table 1 Clinical demography of patients using proton pump inhibitors**

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Low Mg²⁺  ($n=17$)</th>
<th>Normal Mg²⁺  ($n=116$)</th>
<th>Total ($n=133$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (range) (years)</td>
<td>62 (46–83)</td>
<td>60 (20–90)</td>
<td>60 (20–90)</td>
</tr>
<tr>
<td>Sex (male:female) (%)</td>
<td>41 : 59</td>
<td>44 : 56</td>
<td>44 : 56</td>
</tr>
<tr>
<td>BMI*</td>
<td>27.2</td>
<td>27.7</td>
<td>27.7</td>
</tr>
<tr>
<td>Serum Mg²⁺ (Mg²⁺) (mmol/l)</td>
<td>0.61 ± 0.02</td>
<td>0.82 ± 0.01</td>
<td>0.79 ± 0.01</td>
</tr>
<tr>
<td>PPI use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration (years)</td>
<td>7.7 ± 1.1</td>
<td>5.2 ± 0.4</td>
<td>5.5 ± 0.4</td>
</tr>
<tr>
<td>Type</td>
<td>rs: 0.29 p: 0.18</td>
<td>rs: 0.29 p: 0.27</td>
<td>rs: 0.29 p: 0.27</td>
</tr>
<tr>
<td>Dose (mg/d)</td>
<td>49.4 ± 5.2</td>
<td>46.3 ± 1.7</td>
<td>46.7 ± 1.7</td>
</tr>
<tr>
<td>Indication (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reflux/dyspepsia</td>
<td>9 (53)</td>
<td>55 (47)</td>
<td>64 (48)</td>
</tr>
<tr>
<td>Protectant</td>
<td>6 (35)</td>
<td>31 (27)</td>
<td>37 (28)</td>
</tr>
<tr>
<td>Gastritis</td>
<td>1 (6)</td>
<td>23 (19)</td>
<td>24 (18)</td>
</tr>
<tr>
<td>Barrett/esophagitis</td>
<td>2 (12)</td>
<td>9 (8)</td>
<td>11 (8)</td>
</tr>
<tr>
<td>Comorbidities (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>13 (77)*</td>
<td>51 (44)</td>
<td>64 (48)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>5 (29)</td>
<td>15 (13)</td>
<td>20 (15)</td>
</tr>
<tr>
<td>Diuretic use</td>
<td>5 (29)</td>
<td>22 (19)</td>
<td>27 (20)</td>
</tr>
</tbody>
</table>

PPI, proton pump inhibitor.

*Mean BMI.

*Percentage of PPI-type: esomeprazole (e), omeprazole (o), pantoprazole (p), rabeprazole (r), and lansoprazole (l). Pearson’s $\chi^2$-test was used to identify possible risk factors for hypomagnesemia.

*$P < 0.05$ was considered statistically significant.

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To examine the copresence of the risk polymorphisms, a linkage analysis was carried out. Both SNPs were linked in $\pm 78\%$ of the alleles across the sequenced genomic region (Fig. 3). To determine the effects of coinheritance of both SNPs, odds ratios were calculated for the risk of PPI-induced hypomagnesemia (Table 2). PPI users who carry both SNPs have a 5.8-fold increased risk of developing PPI-induced hypomagnesemia ($P < 0.01$). The presence of the single rs3750425 SNP constitutes a 2.4-fold increased risk, although this result fell short of significance ($P = 0.08$).

### Table 2: Common SNPs in TRPM6 increase the risk for hypomagnesemia

<table>
<thead>
<tr>
<th>Haplotypes</th>
<th>Cases</th>
<th>Controls</th>
<th>OR (95% CI)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAGG</td>
<td>39</td>
<td>199</td>
<td>2.38 (0.91–6.22)</td>
<td>0.08</td>
</tr>
<tr>
<td>CGGT</td>
<td>7</td>
<td>15</td>
<td>5.83 (2.00–17.02)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TGAC</td>
<td>8</td>
<td>7</td>
<td>1.021 (0.12–8.98)</td>
<td>0.56</td>
</tr>
<tr>
<td>TGGC</td>
<td>1</td>
<td>5</td>
<td>10.21 (0.90–115.33)</td>
<td>0.08</td>
</tr>
<tr>
<td>TAGT</td>
<td>2</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>TGGT</td>
<td>–</td>
<td>3</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

The relative risk associated with haplotype single nucleotide polymorphism (SNP) alleles was estimated as odds ratio (OR) with a 95% confidence interval (CI) using a wild-type haplotype as a reference. The four haplotype SNPs are arranged according to their chromosomal sequence position on the coding strand and represented as a four-way letter code with position $1 = rs2274925$, $2 = rs2274924$, $3 = rs3750425$, and $4 = rs45616231$ in agreement with Supplementary Table 2. Bold values denote $P = 0.0012$.

**Discussion**

Our study presents the first prospective systematic analysis of hypomagnesemia among PPI users. We found that 13% of regular PPI users had hypomagnesemia ($<0.7 \text{mmol/l}$) and that this is independent of type-2 diabetes mellitus and the use of diuretics. Our study also establishes that TRPM6 SNPs are a risk factor for PPI-induced hypomagnesemia.

We found that the haplotype containing two common TRPM6 SNPs (rs3750425 and rs2274924) increases the risk for PPI-induced hypomagnesemia by 5.8-fold. Indeed, these SNPs have previously been shown to be associated with hypomagnesemia in diabetes mellitus [10,14]. Both identified SNPs cause a change at the amino acid level [p.Val1393Le, (rs3750425) and p.Lys1584Glu, (rs2274924)] that renders the channel insensitive for insulin stimulation [10]. Carriers of the SNPs in TRPM6 will have an impaired insulin response and as a consequence a reduced intestinal Mg$^{2+}$ absorption capacity. Especially when the maximal Mg$^{2+}$ absorption capacity is required, for instance, when Mg$^{2+}$ intake is low or PPIs further impair Mg$^{2+}$ absorption, hypomagnesemia may develop. This explains why TRPM6 SNPs carriers are at greater risk for the development of hypomagnesemia. TRPM6 genotyping provides a novel approach to identify patients at risk for PPI-induced hypomagnesemia.

Serum Mg$^{2+}$ levels are not measured regularly in clinical practice and as a result hypomagnesemia is commonly overlooked in PPI users. Some patients may have hypomagnesemia without suffering from hypomagnesemia-related symptoms. As serum Mg$^{2+}$ measurements are often not available, retrospective analyses carry the risk of selection bias. However, the frequency in our cohort (13%) that was studied prospectively is comparable with recently reported data from retrospective cohort studies (range 5–13%) [18–20]. This proportion is considerably higher than that found in the general population, where a prevalence of 2–4% is usually observed [18,21–23].

Duration of PPI use may be an important determinant for PPI-induced hypomagnesemia. In our cohort, the duration of PPI use was longer in patients with hypomagnesemia than normomagnesemic patients. This suggests that hypomagnesemia only occurs when the body Mg$^{2+}$ stores, such as bone, are depleted. Indeed, animal and human studies show that bone mass only reduces after long-term Mg depletion [24]. Our findings are supported by a recent systematic review showing that hypomagnesemia arises after an average of 5.5 years of PPI use [3]. Early detection and treatment of PPI-induced hypomagnesemia is important as it may increase the risk for fracture and cardiac arrhythmias [25,26].

Although several studies indicate that diuretics may be important contributors to hypomagnesemia in PPI users [18,27,28], we found that the use of diuretics was not associated with hypomagnesemia in our cohort. It could
be reasoned that only specific types of diuretics such as thiazide-diuretics drive the risk for PPI-induced hypomagnesemia [29]. The limited number of patients using diuretics in our cohort precluded a subtype analysis that would address this point. The presence of hypertension is correlated with PPI-induced hypomagnesemia. Mg^{2+} supplementation is generally associated with a significant decrease in blood pressure [6,30–32].

The strength of our study is its prospective nature as most population studies on PPI-induced hypomagnesemia have been carried out with a retrospective analysis. Given that Mg^{2+} measurements are not part of routine biochemical testing, retrospective analysis carries the risk of preselection bias. We estimated the frequency of PPI-induced hypomagnesemia in an outpatient setting, which fuels the view that our results are generalizable to other populations. Our study is subject to several limitations. A limitation of the study is the candidate-based approach of our SNP screening. It is possible that genome-wide screening of the complete genome might enable the identification of other loci that affect the risk. However, the number of (confirmed) causative genes governing traits of biomedical importance such as hypomagnesemia that have been confirmed is probably small. The list of candidate genes is exhaustive. Another limitation is the absence of measurements of serum Mg^{2+} concentrations in these patients before starting PPI treatment. However, challenge–rechallenge studies have been carried out previously and show that PPI use is causal for hypomagnesemia in these patients [3].

Conclusion
Our study shows that PPI-induced hypomagnesemia is a common phenomenon in PPI users and that SNPs in TRPM6 drive its risk. Given that millions of individuals are dependent on PPI use worldwide, our findings indicate that PPI-induced hypomagnesemia may affect large patient populations.

Acknowledgements
The authors thank the contributing physicians of the Radboud university medical center, including F. Hoentjen MD, M. Goerres MD, J. Kersten MD, and E. Klappe MD. They also thank Dr A. Lameris, Dr M. Blanchard, L. Bernts, and D. Viering for their (technical) support and expertise.

This study was funded through a grant from the Radboud University Medical Center and further supported by grants from the Netherlands Organization for Scientific Research (VICI 016.130.668) and the EURenOmics project from the European Union Seventh Framework Programme (FP7/2007–2013, agreement no. 305608)

Conflicts of interest
There are no conflicts of interest.

References


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