

# Association study in Mexican patients with thyrotoxic hypokalemic periodic paralysis

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**Abstract.** Hypokalemic periodic paralysis type 1 (OMIM; HOKPP1) and type 2 (OMIM; HOKPP2) are diseases of the muscle characterized by episodes of painless muscle weakness, and is associated with low potassium blood levels. Hyperthyroidism has been associated with thyrotoxic periodic paralysis (TTPP) (OMIM; TTPP1 and TTPP2), and genetic susceptibility has been implicated. In the present study, the clinical and epidemiological characteristics of patients with TTPP are described, together with their association with

genetic variants reported previously in other populations. A prospective and a retrospective search of the medical records of patients who attended the emergency department at the Hospital Universitario 'Dr. Jose E. Gonzalez' in Monterrey, Nuevo León, Mexico, and were diagnosed with TTPP was performed. A total of 16 gene variants in the genes *MUC1*, *CACNA1S*, *KCNE3* and *SCN4A*, and nine ancestry informative markers (AIMs), were analysed by Multiplex TaqMan™ Open Array assay, and a genetic association study was performed. A total of 11 patients were recruited, comprising nine males and two females (age range, 19-52 years) and 64 control subjects. Only two cases (18%) had a previous diagnosis of hyperthyroidism; the rest were diagnosed subsequently with Graves' disease. Based on the analysis, two DNA variants were found to potentially confer an increased risk for TTPP: *SIPRI* rs3737576 [odds ratio (OR), 4.38; 95% confidence interval (CI), 1.08-17.76] and AIM rs2330442 (OR, 4.50; 95% CI, 1.21-16.69), and one variant was suggested to be possibly associated with TTPP, namely *MUC1* rs4072037 (OR, 3.08; 95% CI, 0.841-1.38). However, there were no statistically significant associations between any of the 24 DNA variants and TTPP in a population from northeast Mexico.

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*Abbreviations:* AIM, ancestry informative marker; AFR, African [1,000 genomes project super population]; AMR, ad mixed American [1,000 genomes project super population]; *CACNA1s*, voltage-gated calcium channel  $\alpha 1$  subunit S gene; CLM, Colombians from Medellin, Colombia [1,000 genomes project population]; EAS, East Asian [1,000 genomes project super population]; EUR, European [1,000 genomes project super population]; HOKPP1, hypokalemic periodic paralysis type 1; HOKPP2, hypokalemic periodic paralysis type 2; *KCNE3*, voltage-gated potassium channel subfamily E regulatory subunit 3 gene; MXL, Mexican ancestry from Los Angeles USA [1,000 genomes project population]; *MUC1*, mucin 1, cell surface associated; *SCN4A*, voltage-gated sodium channel  $\alpha$  subunit 4 gene; SAS, South Asian [1000 genomes project super population]; TTPP, thyrotoxic periodic paralysis

*Key words:* hypokalemic periodic paralysis, thyrotoxic hypokalemic periodic paralysis, *MUC1*, association study, genetic variant, *CACNA1s*, *KCNE3*, *SCN4A*

## Introduction

Periodic paralysis is a characteristic of neuromuscular diseases of paroxysmal muscle weakness of the limbs. Common causes of this condition are thyrotoxic periodic paralysis types 1 and 2 (TTPP1 and TTPP2 OMIM #188580 and #613239, respectively) (1), which are considered a type of endocrine channelopathy. The acute presentation of flaccid muscular palsy associated with any cause of thyrotoxicosis that produces hypokalaemia is the primary characteristic of TTPP (2). Although TTPP is more common in Asian populations, a significant increase in the number of cases worldwide has been reported, and even though the incidence of hyperthyroidism is higher in women, TTPP is more prevalent in men (3,4). The first clinical manifestation consists of recurrent episodes of muscle weakness, which can vary from a low grade to the most severe forms, with complete flaccid palsy (4). Proximal muscles of the lower limbs are more prone to be associated with paralysis, with no sensitive involvement. Precipitating

factors for acute episodes are high consumption of simple carbohydrates, alcohol intake and intense exercise (4). Thyroid function testing is a valuable tool to distinguish TTPP (4). In the majority of the cases of TTPP, hyperthyroidism is caused by Graves' disease, which is also the leading cause of hyperthyroidism worldwide (4). However, TTPP may also be associated with other causes of hyperthyroidism, including functional thyroid nodules, toxic multinodular goitre and factitious thyrotoxicosis (5). Gene variants have been reported to be associated with thyrotoxic hypokalemic periodic paralysis (THPP1) [voltage-gated calcium channel  $\alpha 1$  subunit S (*CACNA1s*) gene], THPP2 [voltage-gated potassium channel subfamily J member 18 (*KCNJ18*) gene], hypokalemic periodic paralysis type 1 (HOKPP1; *CACNA1S* gene), and HOKPP type 2 (HOKPP2) [voltage-gated sodium channel  $\alpha$  subunit 4 (*SCN4A*) gene] (1). The voltage-gated calcium channel  $\alpha 1C$  subunit (*CACNA1C*) gene is associated with HOKPP1 (OMIM, #170400) and TTPP1 (OMIM, #188580) (1), and has been studied in patients with TTPP (1). Variants in other genes that encode for muscle ion channels have also been investigated. In a patient with TTPP of Portuguese descent, an R83H variant in the voltage-gated potassium channel subfamily E regulatory subunit 3 gene (*KCNE3*) (6). Since TTPP patients have an increased level of ATPase activity, genes that encode  $\alpha$  and  $\beta$  adrenergic receptors have been analysed, although no associations have been identified in these cases (6-9). Another study showed that 10 out of 30 Caucasian or Brazilian patients with TTPP possessed a variant in the *KCNJ18* gene (associated with TTPP2), an inwardly rectifying potassium channel expressed in skeletal muscles that is transcriptionally regulated by thyroid hormone (10).

Although other ethnicities are affected, TTPP is most prevalent in individuals of Asian and, secondly, of Latin American descendant (4). To the best of our knowledge, there are no previous studies that have been performed on Mexican patients. The aim of the present study was to describe the clinical and epidemiological characteristics of patients with TTPP. An association study was performed with variants in the *CACNA1s*, mucin 1 (*MUC1*), *KCNE3* and *SCN4A* genes, as well as in ancestry informative markers (AIMs) and Sphingosine 1 Phosphate Receptor 1 (*S1PR1*) ancestry genes, in a population from northeast Mexico. In total, 9 AIMs were included to analyse population structure and identify outlier subjects. The use of AIMs in population studies can identify population heterogeneity in complex populations and may reduce type I and type II errors in multifactorial diseases and multifactorial traits (11,12).

## Materials and methods

**Ethics.** The present study was performed at the Genetics and Endocrinology Departments of the University Hospital 'Dr José Eleuterio González' Universidad Autónoma de Nuevo León (UANL) (Monterrey, México), with the approval of the Ethics Committee (approval no. EN13004), University Hospital 'Dr José Eleuterio González', UANL.

**Design of study and subjects.** Between May 2012 and December 2014, the prospective recruitment of north-eastern Mexican patients newly diagnosed with TTPP, admitted through the emergency department at the Hospital Universitario

'Dr Jose E. Gonzalez' in Monterrey, Nuevo León, Mexico was performed, as well as a retrospective search of medical records of patients whom had previously been diagnosed with TTPP and had attended the endocrinology outpatient clinic. All subjects accepted and signed informed consent forms. Northeastern Mexican patients, of either sex and all ages were invited to participate in the present study. A total of 11 patients were recruited (9 males and two females; age range, 19-52 years; median, 31 years). A clinical evaluation was performed for each patient, which included medical history, thyroid function test, assessment of the blood electrolyte levels, and the type of treatment received. A venous blood sample was obtained and placed in an EDTA vial for DNA extraction. A total of 64 Northeastern Mexican control subjects, including 26 females and 38 males (age range, 19-52 years; median, 41.4 years) were obtained from the DNA bank of the Genetics department at the University Hospital 'Dr. José Eleuterio González' (11,12).

**Laboratory tests.** The thyroid profile [thyroid-stimulating hormone (TSH), free thyroxine (FT4), total triiodothyronine (T3T) and total thyroxine (T4T)] were determined by electrochemiluminescence using a Cobas e411 Analyzer (Roche Diagnostics) and T3 uptake was assessed by chemiluminescence (using an IMMULITE 1000 Immunoassay system; MyIMSA Solutions, Ltd.; Siemens Healthineers). The levels of serum and urinary electrolytes were measured using an ion-selective method (Dx 800 Beckman Coulter Hematology Analyzer; Beckman Coulter, Inc.). Blood and urinary samples were taken as routine at the University Hospital 'Dr. José Eleuterio González', and assessed according to the manufacturer's protocol.

**DNA extraction.** DNA extraction was performed using 200  $\mu$ l EDTA treated whole blood samples, using a QIAamp<sup>®</sup> Blood Mini kit on the automated QIAcube system (Qiagen GmbH). Purified DNA was collected, diluted to a concentration of 50 ng/ $\mu$ l [determined by measuring the absorbance (A) at  $A_{260/280}$  and  $A_{260/230}$  to be 1.6 to 1.9], and stored at -20°C until further use (11,12).

**Open array reactions.** Open array was performed using the TaqMan<sup>®</sup> assay (Applied Biosystems; Thermo Fisher Scientific, Inc.), and genotypes were captured using the OpenArray<sup>®</sup> NT Genotyping system (Applied Biosystems; Thermo Fisher Scientific, Inc.). Non-template control (process control) was used in each plate assay. DNA samples (50 ng/ $\mu$ l each sample) and TaqMan<sup>®</sup> OpenArray Master mix (Applied Biosystems; Thermo Fisher Scientific, Inc.) were mixed in a 384 well plate and transferred to the OpenArray plate using an autoloader. The OpenArray plate was subsequently filled with immersion fluid, and sealed with glue. Multiplex TaqMan<sup>®</sup> assay reactions were performed in a Dual Flat Block GeneAmp PCR system 9700 (Applied Biosystems; Thermo Fisher Scientific, Inc.). The PCR cycling conditions were as follows: Initial denaturation at 93°C for 10 min; followed by 50 cycles at 95°C for 45 sec, 94°C for 13 sec and 53°C for 14 sec; and a final step at 25°C for 2 min. Samples were stored at 4°C until further use (11,12).

**Genotype analysis.** Genotype analysis was analysed using the TaqMan<sup>®</sup> Genotyper Software version 1.0, according to

the manufacturer's protocol (Applied Biosystems; Thermo Fisher Scientific, Inc.). The accuracy of the genotyping was assessed by comparison with 15 samples, all of which had been genotyped three times, resulting in 45 comparisons per single nucleotide polymorphism (SNP).

**Genotype association test.** Genetic data were analysed using the Golden Helix SNP & Variation Suite version 8.7 program (Golden Helix, Inc.), with the *Homo sapiens* GRCh37/hg19 genome assembly. The 24 SNPs were analysed to detect possible deviations from the Hardy-Weinberg equilibrium (HWE) using a Fisher's exact test ( $P < 0.01$  was considered to indicate a statistically significant difference) (11). Association studies were performed using the dominant genetic model. Fisher's exact test was used to estimate P-values, odds ratios (ORs), and 95% confidence intervals (CIs). Fisher's exact test P-values, corrected using the Bonferroni method, and false discovery rates (FDRs) were calculated to exclude spurious associations.  $P < 0.05$ , corrected by Bonferroni's method and FDR, was considered to indicate a statistically significant difference (11).

**Results**

**Clinical characteristics.** A total of 11 northeastern Mexican patients with THPP were evaluated. Hypokalaemia was detected at the onset of paralysis symptoms. Of the 11 patients, 10 were recruited during the acute onset of symptoms, and a further patient through a retrospective search of cases. The majority of the cases were male (82%). Hypokalaemia was associated with the severity of the clinical manifestations, which varied from muscle weakness to muscular palsy. No family cause of hyperthyroidism was identified, instead all cases were sporadic cases. Table I shows the laboratory findings, including the low urinary and serum potassium values (range: 15-30 mM/l, and 1.2-2.4, mM/l). The thyroid hormones profile revealed a  $TSH \leq 0.01$  in all patients, with significant increases observed for T3 Cap, T3T, FT4 and T4T. Of these, 2 patients disclosed a precipitant factor in the hours prior to the event, one patient had had a meal abundant in carbohydrates, and the other patient had engaged in vigorous physical activity. Additionally, 4 patients had a history of similar episodes, and received treatment with potassium replacement in other hospitals, where the cause of the hypokalaemia was not determined, entailing further hypokalemic crisis. The remaining 5 patients developed hyperkalaemia as a side effect of routinely receiving potassium replacement. After the diagnosis of Graves' disease as the cause of the hypokalaemia, the 11 patients received <sup>131</sup>I-iodine, which halted the hypokalemic crisis (Table I).

**Genotype analysis.** The allelic and genotype frequencies, and HWE P-values calculated for the 24 SNPs, in cases and in control subjects, and are presented in Table II. These results show that all the control subjects and patients were in HWE ( $P > 0.01$ ). The associations of the 24 gene variants were analysed; 15 variants were from genes associated with electrolyte membrane transport, and had been previously reported to be associated with THPP in other populations: *CACNA1S* (13); rs12139527, rs1546416, rs3767500, rs3818873, rs1998721, rs4915212, rs9427714, rs734881, rs4951629, rs12561765 and rs2296383, *KCNE3* (6); rs17215437 and *SCN4A* (14,15); and

Table I. Clinical and biochemical characteristics of the patients with THPP.

Patient	Age, years	Sex (M/F)	Family history	Recruitment condition	Antecedent of similar episodes	Trigger	TSH, (0.27-4.2 uIU/ml) <sup>c</sup>	T4L, (0.93-1.7 ng/dl) <sup>c</sup>	T3T, (80-200 ng/dl) <sup>c</sup>	T4T, (5.1-14.1 ug/dl) <sup>c</sup>	T3 uptake, (24-35%) <sup>c</sup>	K <sup>+</sup> urinary, (40-80 mmol/l) <sup>c</sup>	Na <sup>+</sup> urinary, (40-50 mmol/l) <sup>c</sup>	K <sup>+</sup> blood, (3.6-5.1 mmol/l) <sup>c</sup>
1	31	F	Hypothyroidism	Acute	Yes	-	<0.005 <sup>b</sup>	5.9	452	23.3	40	20	75	2.4
2	36	M	Negative	Acute	No	Food intake	<0.005 <sup>b</sup>	3.5	234	19.6	39	22	100	1.51
3	52	M	Negative	Acute	No	-	<0.005 <sup>b</sup>	2.26	211	18	45	25	86	2.1
4	19	M	Hyperthyroidism	Acute	Yes	-	0.01 <sup>a</sup>	3.57	125	20.2	40.2	26	131	2.1
5	40	M	Hypothyroidism	Acute	No	-	<0.005 <sup>b</sup>	4.14	350	19.76	37.5	30	100	2.0
6	22	M	Negative	Acute	No	-	<0.005 <sup>b</sup>	6.11	651	24.86	39.5	26	234	1.4
7	22	M	Negative	Retrospective Search	No	Physical activity	<0.005 <sup>b</sup>	3.4	210	19.3	40	29	153	1.8
8	34	M	Hypothyroidism	Acute	Yes	-	<0.005 <sup>b</sup>	3.1	263	15.15	42	15	172	1.2
9	20	M	Hypothyroidism	Acute	Yes	-	<0.005 <sup>b</sup>	3.14	260.2	17.53	36.5	17	88	1.6
10	23	F	Hypothyroidism	Acute	No	-	<0.005 <sup>b</sup>	2.38	388	16.94	42	18	148	1.9
11	43	M	Neoplasm	Acute	No	-	<0.005 <sup>b</sup>	2.99	205.8	19.51	41	20	78	1.6

<sup>a</sup> $P < 0.05$ , <sup>b</sup> $P < 0.01$ . <sup>c</sup>Physiological range. THPP, thyrotoxic hypokalemic periodic paralysis; M, male; F, female; TSH, thyroid-stimulating hormone; FT4, free thyroxine; T3T, total triiodothyronine; T4T, total thyroxine.

rs2070720, rs2058194 and rs9894841. Additionally, 9 AIM variants: *SIPRI* (rs3737576), *ATF3* (rs4951629), phospholipase 5 (*PLD5*; rs316873), elastin (*ELN*; rs868005 and rs2239691), lysine demethylase 2A (*KDM2A*; rs11227699), *AMZ2P1*; *GNAI3* (rs11652805, rs2330442 and rs10954737); and the *MUC1* gene (rs4072037) were also included (16).

**Genotype association test.** Based on the 24 SNPs, only two genetic variants were predicted to confer a risk for TTPP with a significant OR (Table II): *SIPRI* rs3737576 (T allele; OR, 4.38; 95% CI, 1.08-17.76, C allele; OR, 0.23; 95% CI, 0.06-0.92), and the AIM rs2330442 (OR, 4.50; 95% CI, 1.21-16.69, A allele; OR, 0.22; 95% CI, 0.06-0.82, G allele). The variant *MUC1* rs4072037 (OR, 3.08; 95% CI, 0.84-11.38) could confer risk to TTPP. After applying the Bonferroni and FDR corrections to the P-values, statistical significance was no longer detected.

## Discussion

TTPP is a rare disease, its prevalence among patients with thyrotoxicosis in the US is estimated to be between 0.1-0.2%, and the incidence in Japanese and other Asian populations has been declining in the last 10 years (17). The majority of the information regarding the pathology of TTPP has been obtained through case reports, predominantly from Asian patients (17). Due to the number of TTPP patients diagnosed in the Hospital Universitario 'Dr Jose E. Gonzalez' between May 2013 and December 2014, the present study aimed to determine common precipitating risk factors and to identify genetic variants associated with this disorder in a northeastern Mexican population. All patients had symptoms of severe hypokalaemia and flaccid muscle palsy, particularly of the lower limbs at their admission to the emergency department. Hypokalaemia was caused by a massive shift in potassium concentration from extracellular to intracellular space. Increased excretion is the most common mechanism underlying hypokalaemia, and thus it should be determined whether potassium replacement may exacerbate the condition, causing arrhythmias or even death, following correction of hypokalaemia (18). A careful clinical evaluation of patients with hypokalaemia should be made to eliminate the possibility of thyrotoxicosis as the cause of the electrolyte alterations.

Genetic predisposition in hypokalemic periodic paralysis has been investigated, and several gene variants have been reported to be associated with TTPP. Gene variants in genes associated with ion channel transport are predominantly associated with TTPP, such as the *CACNA1C* gene, which is involved calcium transport and is associated with TTPP in the Chinese population (14,19). Furthermore, variants in genes associated with potassium channels, such as the *KCNE3* gene that encodes muscle ion channels, have been identified in a patient of Portuguese descent (6), and the *KCNJ18* (Kir2.6) gene has been identified in Brazilian patients (10). In the present study; however, no such associations were identified between these genes and TTPP in our population.

Previously, it has been shown that *MUC1* regulates the renal calcium channel TRPV5, and has a putative protective role against calcium nephrolithiasis via increasing urinary calcium reabsorption (20). Homeostasis of calcium and potassium are connected; therefore, this may underlie

the interaction and association between genetic variants of *MUC1* with hypokalaemia. The C allele in rs4072037 of the *MUC1* gene is associated with a decrease in magnesium levels (21,22). A previous study reported that hypomagnesaemia is associated with hypokalaemia, which is mediated by stimulation of the renal outer medullary potassium channels, resulting in increased potassium excretion (23). Previously published clinically relevant data concerning the association between potassium and magnesium showed that >50% of cases of clinically significant hypokalaemia is accompanied by concomitant magnesium deficiency, and is clinically most frequently observed in individuals receiving loop or thiazide diuretic therapy. Concomitant magnesium deficiency has long been appreciated to aggravate hypokalaemia. Hypokalaemia associated with magnesium deficiency is often refractory to treatment with potassium (24).

The T allele (rs3737576) of the *SIPRI* gene had a lower frequency in Latino American populations (AMR), and has a higher frequency in Mexicans from Los Angeles (MXL) and Peruvians of Lima Peru (PEL) when compared with other worldwide populations (Jan2020.archive.ensembl.org/Homo\_sapiens/Variation/Population?db=core;r=1:101243507-101244507;v=rs3737576;vdb=variation;vf=2090687) (25). The genotype TT (rs3737576) was identified more frequently in the cases of the AMR and MXL populations when compared with controls, which suggested that ancestry may be a relevant component in assessing the risk of TTPP. Thus, TTPP replication association studies in AMR populations are required to corroborate the preliminary findings of this pilot study. The *SIPRI* gene has been shown to inhibit kidney epithelial-mesenchymal transition initiated by ischemia/reperfusion injury via the phosphoinositide 3 kinase/Akt pathway, and reperfusion can result in hyperkalaemia (26).

AIM rs2330442 is a human-specific segregation variant (16) (A low-risk allele, and G risk allele, in the present study). Allele A was found to have a lower frequency in cases and in the AFR super population when compared with controls, AMR, East Asian (EAS), South Asian (SAS) and EUR super populations and MXL population (Jan2020.archive.ensembl.org/Homo\_sapiens/Variation/Population?db=core;r=7:42339972-42340972;v=rs2330442;vdb=variation;vf=17272273) (25). TTPP occurs more frequently in young Asian and Latin American males compared with Caucasians or Africans (8,27). The rs2330442 variant (Allele A, OR, 4.50; 95% CI, 1.21-16.69) is located between two enhancer sequences in ENSR00001124514 (inactive in the adrenal gland) and ENSR00001124515 (interesting repressed in the adrenal gland; ENSEMBL; Jan2020.archive.ensembl.org/Homo\_sapiens/Location/View?db=core;r=7:42340222-42340723;v=rs2330442;vdb=variation;vf=17272273). The rs2330442 variant is included inside as a constrained element (a functional element in a reference genome; a conserved sequence across 100 eutherian mammal species) (28).

The present study is the first to have reported on the potential genetic associations of patients with in a northeastern Mexican cohort, to the best of our knowledge. The allelic frequency of DNA variants with the risk associated with TTPP in cases from northeastern Mexico was assessed; however, none of the variants were significantly associated, and a significant risk with TTPP was not identified.

Table II. SNPs analysed in patients with thyrotoxic hypokalemic periodic paralysis and controls, tested under the dominant genetic model.

Gene	SNP	Localization	Fisher's Exact test			OR (95% CI)	Allele frequency		Genotype frequency		Fisher's HWE P-value	
			P-value	Bonferroni corrected P-value	False discovery rate		Cases (n)	Controls (n)	Cases (n)	Controls (n)	Cases	Controls
<i>SIPRI</i> (AIM)	rs3737576	1:101244007	0.055	1.000	0.660	C: 0.23 (0.06-0.92)	C: 0.352 (45)	C/C: 0.167 (2)	C/C: 0.109 (7)	0.010	0.618	
						T: 4.38 (1.08-17.76)	T: 0.648 (83)	C/T: 0.083 (1)	C/T: 0.484 (31)			
								T/T: 0.750 (9)	T/T: 0.406 (26)			
<i>MUC1</i>	rs4072037	1:155192276	0.114	1.000	0.908	C: 3.08 (0.84-11.38)	C: 0.230 (28)	C/C: 0.250 (3)	C/C: 0.066 (4)	0.577	0.569	
						T: 0.32 (0.09-1.20)	T: 0.770 (94)	C/T: 0.417 (5)	C/T: 0.328 (20)			
								T/T: 0.333 (4)	T/T: 0.607 (37)			
<i>CACNA1S</i>	rs12139527	1:201040054	0.339	1.000	1.000	-	G: 0.073 (9)	G/G: 0.000 (0)	G/G: 0.000 (0)	1.000	0.538	
						-	A: 1.000 (24)	G/A: 0.000 (0)	G/A: 0.145 (9)			
								A/A: 1.000 (12)	A/A: 0.855 (53)			
<i>CACNA1S</i>	rs1546416	1:201040428	1.000	1.000	1.000	G: 0.92 (0.26-3.20)	G: 0.281 (36)	G/G: 0.083 (1)	G/G: 0.125 (8)	0.700	0.069	
						A: 1.09 (0.31-3.80)	A: 0.719 (92)	G/A: 0.333 (4)	G/A: 0.313 (20)			
								A/A: 0.583 (7)	A/A: 0.563 (36)			
<i>CACNA1S</i>	rs3767500	1:201053906	0.530	1.000	1.000	C: 0.57 (0.16-2.00)	C: 0.357 (45)	C/C: 0.167 (2)	C/C: 0.159 (10)	0.171	0.281	
						G: 1.75 (0.50-6.11)	G: 0.643 (81)	C/G: 0.250 (3)	C/G: 0.397 (25)			
								G/G: 0.583 (7)	G/G: 0.444 (28)			
<i>CACNA1S</i>	rs3818873	1:201054308	1.000	1.000	1.000	C: 0.78 (0.21-2.87)	C: 0.203 (26)	C/C: 0.083 (1)	C/C: 0.016 (1)	0.402	0.205	
						T: 1.28 (0.35-4.71)	T: 0.797 (102)	C/T: 0.250 (3)	C/T: 0.375 (24)			
								T/T: 0.667 (8)	T/T: 0.609 (39)			
<i>CACNA1S</i>	rs1998721	1:201075620	1.000	1.000	1.000	A: 1.03 (0.30-3.53)	A: 0.300 (39)	A/A: 0.083 (1)	A/A: 0.108 (7)	0.977	0.497	
						G: 0.97 (0.28-3.32)	G: 0.700 (91)	A/G: 0.417 (5)	A/G: 0.385 (25)			
								G/G: 0.500 (6)	G/G: 0.508 (33)			
<i>CACNA1S</i>	rs4915212	1:201077077	1.000	1.000	1.000	-	T: 0.023 (3)	T/T: 0.000 (0)	T/T: 0.000 (0)	1.000	0.849	
						-	C: 1.000 (24)	T/C: 0.000 (0)	T/C: 0.046 (3)			
								C/C: 1.000 (12)	C/C: 0.954 (62)			
<i>CACNA1S</i>	rs9427714	1:201077983	1.000	1.000	1.000	G: 0.78 (0.15-4.03)	G: 0.109 (14)	G/G: 0.000 (0)	G/G: 0.016 (1)	0.753	0.764	
						A: 1.27 (0.25-6.54)	A: 0.891 (114)	G/A: 0.167 (2)	G/A: 0.188 (12)			
								A/A: 0.833 (10)	A/A: 0.797 (51)			

Table II. Continued.

Gene	SNP	Localization	Fisher's Exact test			OR (95% CI)	Allele frequency		Genotype frequency		Fisher's HWE P-value	
			P-value	Bonferroni corrected P-value	False discovery rate		Cases (n)	Controls (n)	Cases (n)	Controls (n)	Cases	Controls
CACNA1S	rs734881	1:201081156	1.000	1.000	1.000	T 1.19 (0.34-4.17)	T: 0.219 (28)	T/T: 0.000 (0)	T/T: 0.063 (4)	0.362	0.493	
						C 0.84 (0.24-2.94)	C: 0.792 (19)	T/C: 0.417 (5)	T/C: 0.313 (20)			
								C/C: 0.583 (7)	C/C: 0.625 (40)			
CACNA1S	rs2296383	1:201091737	0.545	1.000	1.000	A 0.65 (0.19-2.27)	A: 0.338 (44)	A/A: 0.000 (0)	A/A: 0.154 (10)	0.362	0.157	
						G 1.54 (0.44-5.34)	G: 0.792 (19)	A/G: 0.417 (5)	A/G: 0.369 (24)			
								G/G: 0.583 (7)	G/G: 0.477 (31)			
CACNA1S	rs12561765	1:201104563	0.684	1.000	1.000	T 0.36 (0.04-3.08)	T: 0.100 (13)	T/T: 0.000 (0)	T/T: 0.000 (0)	0.880	0.370	
						C 2.75 (0.33-23.27)	C: 0.958 (23)	T/C: 0.083 (1)	T/C: 0.200 (13)			
								C/C: 0.917 (11)	C/C: 0.800 (52)			
ATF3 (AIM)	rs4951629	1:212613541	0.323	1.000	1.000	C 0.48 (0.14-1.66)	C: 0.411 (51)	C/C: 0.083 (1)	C/C: 0.145 (9)	0.977	0.435	
						T 2.10 (0.60-7.33)	T: 0.708 (17)	C/T: 0.417 (5)	C/T: 0.532 (33)			
								T/T: 0.500 (6)	T/T: 0.323 (20)			
PLD5 (AIM)	rs316873	1:242179202	0.533	1.000	1.000	T 1.71 (0.47-6.26)	T: 0.375 (9)	T/T: 0.083 (1)	T/T: 0.123 (8)	0.397	0.619	
						C 0.58 (0.16-2.13)	C: 0.625 (15)	T/C: 0.583 (7)	T/C: 0.415 (27)			
								C/C: 0.333 (4)	C/C: 0.462 (30)			
(AIM)	rs2330442	7:42340472	0.024*	0.584	0.584	A 0.22 (0.06-0.82)	A: 0.208 (5)	A/A: 0.083 (1)	A/A: 0.169 (11)	0.402	0.591	
						G 4.50 (1.21-16.69)	G: 0.792 (19)	A/G: 0.250 (3)	A/G: 0.523 (34)			
								G/G: 0.667 (8)	G/G: 0.308 (20)			
ELN	rs868005	7:74030784	0.518	1.000	1.000	C 0.52 (0.13-2.11)	C: 0.125 (3)	C/C: 0.000 (0)	C/C: 0.031 (2)	0.621	0.524	
						T 1.92 (0.47-7.80)	T: 0.875 (21)	C/T: 0.250 (3)	C/T: 0.359 (23)			
								T/T: 0.750 (9)	T/T: 0.609 (39)			
ELN	rs2239691	7:74054839	1.000	1.000	1.000	T 0.80 (0.22-2.94)	T: 0.167 (4)	T/T: 0.000 (0)	T/T: 0.015 (1)	0.488	0.215	
						C 1.25 (0.34-4.59)	C: 0.833 (20)	T/C: 0.333 (4)	T/C: 0.369 (24)			
								C/C: 0.667 (8)	C/C: 0.615 (40)			
(AIM)	rs10954737	7:83903731	0.762	1.000	1.000	C 1.28 (0.37-4.44)	C: 0.333 (8)	C/C: 0.083 (1)	C/C: 0.123 (8)	0.665	0.491	
						T 0.78 (0.23-2.73)	T: 0.667 (16)	C/T: 0.500 (6)	C/T: 0.400 (26)			
								T/T: 0.417 (5)	T/T: 0.477 (31)			

Table II. Continued.

Gene	SNP	Localization	Fisher's Exact test			OR (95% CI)	Allele frequency		Genotype frequency		Fisher's HWE P-value	
			P-value	Bonferroni corrected P-value	False discovery rate		Cases (n)	Controls (n)	Cases (n)	Controls (n)	Cases	Controls
<i>KDM2A</i> (AIM)	rs11227699	11:67131021	0.497	1.000	1.000	A: 2.00 (0.40-10.04)	A: 0.458 (11)	A: 0.444 (56)	A/A: 0.083 (1)	A/A: 0.175 (11)	0.077	0.461
						G: 0.50 (0.10-2.51)	G: 0.542 (13)	G: 0.556 (70)	A/G: 0.750 (9)	A/G: 0.540 (34)		
<i>KCNE3</i>	rs17215437	11:74457316	1.000	1.000	1.000	-	T: 0.000 (0)	T: 0.015 (2)	T/T: 0.000 (0)	T/T: 0.000 (0)	1.000	0.900
						-	C: 1.000 (24)	C: 0.985 (128)	T/C: 0.000 (0)	T/C: 0.031 (2)		
<i>SCN4A</i> ; <i>CD79B</i>	rs2070720	17:63941413	1.000	1.000	1.000	C: 1.19 (0.34-4.17)	C: 0.375 (9)	C: 0.341 (43)	C/C: 0.167 (2)	C/C: 0.143 (9)	0.700	0.351
						T: 0.84 (0.24-2.92)	T: 0.625 (15)	T: 0.659 (83)	C/T: 0.417 (5)	C/T: 0.397 (25)		
<i>SCN4A</i>	rs2058194	17:63942988	1.000	1.000	1.000	C: 1.17 (0.28-4.84)	C: 0.542 (13)	C: 0.484 (62)	C/C: 0.333 (4)	C/C: 0.250 (16)	0.577	0.622
						T: 0.85 (0.21-3.51)	T: 0.458 (11)	T: 0.516 (66)	C/T: 0.417 (5)	C/T: 0.469 (30)		
<i>SCN4A</i>	rs9894841	17:63960325	0.545	1.000	1.000	C: 0.65 (0.19-2.27)	C: 0.333 (8)	C: 0.346 (45)	C/C: 0.250 (3)	C/C: 0.169 (11)	0.030	0.078
						T: 1.54 (0.44-5.34)	T: 0.667 (16)	T: 0.654 (85)	C/T: 0.167 (2)	C/T: 0.354 (23)		
<i>AMZ2P1</i> ; <i>GNAJ3</i> (AIM)	rs11652805	17:64991033	0.744	1.000	1.000	C: 0.70 (0.17-2.85)	C: 0.125 (3)	C: 0.169 (22)	C/C: 0.000 (0)	C/C: 0.015 (1)	0.621	0.447
						T: 1.43 (0.35-5.84)	T: 0.875 (21)	T: 0.831 (108)	C/T: 0.250 (3)	C/T: 0.308 (20)		

\*P<0.05. SNP, single nucleotide polymorphism; localization, localization in the Genome Reference Consortium Human Build 38 patch release 10 (GRCh38.p10); FDR, false discovery rate; OR, odds ratio; CI, confidence interval; HWE, Hardy-Weinberg Equilibrium; Chr, chromosome; S1PR1, Sphingosine-1-Phosphate Receptor 1; MUC1, Mucin 1, Cell Surface Associated; CACNA1S, Calcium Voltage-Gated Channel Subunit  $\alpha 1$  S; ATF3, Activating Transcription Factor 3; PLD5, Phospholipase D Family Member 5; ELN, Elastin; KDM2A, Lysine Demethylase 2A; KCNE3, Potassium Voltage-Gated Channel Subfamily E Regulatory Subunit 3; SCN4A, Sodium Voltage-Gated Channel Alpha Subunit 4; AIM, ancestry informative marker.



The C allele rs3737576 of the *SIPRI* gene (chromosome 1) had a lower frequency in the AMR super population when compared with the controls of the present study and AMR super population. The AIM rs2330442 (chromosome 7), allele G has been shown to have a higher frequency in cases and the AFR super population when compared with controls, AMR, SAS, EAS and EUR super populations, and the MXL population (25).

TTPP has been reported to occur with differential frequency in other populations, identified more frequently in male Latino American and Asian populations. The Mexican population is a model for genetic studies due to the great ethnic diversity and complexity within native and Mestizo populations (29). Additional TTPP replication association studies in AMR populations are required to corroborate the preliminary findings in the present pilot study. Full sequencing of the *CACNA1s*, *MUC1*, *KCNE3* and *SCN4A* genes would also be advantageous as it may be used to identify novel mutations and variations that are associated with TTPP. Additionally, exome and whole-genome studies may be necessary to investigate the above genes and metabolic pathways involved in magnesium and potassium transport and homeostasis.

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### Availability of data and materials

The datasets used and/or analysed during the present study are available from the corresponding author on reasonable request.

### Authors' contributions

MABM, HLGB, JZVP, RMCF, FJLG and LEMG designed the study. MABM, FJLG and JZVP contributed to the recruitment of the patients and performed the medical examinations. MABM and HLGB performed the bioinformatics, statistical, and genetics analysis. All authors read and approved the final manuscript.

### Ethics approval and consent to participate

The present study was approved by the Ethics Committee of University Hospital 'Dr. José Eleuterio González', UANL (approval no. EN13004). All patients provided written informed consent.

### Patient consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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