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Association between *CACNG2* polymorphisms (rs4820242, rs2284015 and rs2284017) and chronic peripheral neuropathic pain risk in a Mexican population

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Abstract. – OBJECTIVE: In animal models and humans, mutations in voltage-dependent calcium channel gamma-2 subunit gene *(CACNG2)* have been associated with neuronal hyperexcitability, including neuropathic pain. The objective of this study was to determine the allelic and genotypic frequencies of *CACNG2* polymorphisms (rs4820242, rs2284015 and rs2284017) and their association with the risk of chronic peripheral neuropathic pain (CPNP) in the Mexican population.

PATIENTS AND METHODS: Single nucleotide polymorphisms (SNPs) were determined by real-time PCR, and allelic and genotypic frequencies were compared between healthy Mexican subjects and CPNP patients. The risk of association of *CACNG2* SNPs with the presence of CPNP and its characteristics was evaluated.

RESULTS: The allele G (OR 2.08, $p = 0.01$) of rs2284015 was observed as a risk factor for developing CPNP. The allele A of rs4820442 showed a risk of association with a history of surgery (OR 3.92, $p = 0.04$), radiculopathy (OR 4.29, $p = 0.0001$), bilateral presentation of pain (OR 3.15, $p = 0.003$), and neuropraxia (OR 0.36, $p = 0.01$). The allele C of rs2284015 was associated with an increased risk of burning and allodynia. In the analysis of the association of genotype frequencies and inheritance patterns, as well as in the analysis of interaction with sex, a modification of risk was observed.

CONCLUSIONS: The allele G of rs2284015 and the AGC haplotype of *CACNG2* rs4820242, rs2284015 and rs2284017, regardless of sex and etiology could contribute to the risk of CPNP. Certain alleles and genotypes could constitute severity markers in CPNP with sex-biased effects; however, further studies are required to confirm these observations.

Key Words:
Pain, Neuropathic, Chronic pain, *CACNG2*, Nucleotide polymorphism, Single.

Introduction

Chronic neuropathic pain is considered a public health problem because of its great impact on the social, economic and healthcare systems. It is estimated that it affects between 6.9% and 10% of the world's population[1,2].

There are large individual differences in sensitivity and tolerance to pain sensation in patients with apparently similar conditions[3,4]. These differences may be related to genetic factors[4] as more than 150 genes involved in pain mechanisms have been proposed[5].

Studies in animal models of neuroma have allowed the identification of a quantitative trait locus (QTL) associated with predisposition to pain. This QTL has been mapped in a 25 cM interval on chromosome 15[6,7]. Using recombinant progeny testing and recombinant inbred segregation test, the location of the pain-associated QTL was narrowed to an interval of 4.2 Mb. 155 genes have been cataloged in this region. By bioinformatic analysis, and whole-genome expression analysis for the L5 dorsal root ganglion, voltage-dependent calcium channel gamma-2 subunit (*CACNG2*) was one of the genes proposed as relevant for the study of pain[8].

CACNG2 codes for 323 amino acids voltage-dependent calcium channel gamma-2 subunit

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or transmembrane α-amino-3-hidroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) regulatory protein gamma-2 (TARP γ-2) and is a subunit of the auxiliary channel that increases neuronal conductance and excitability.

CACNG2 regulates both the traffic of AMPAR towards the neuronal membrane and the synaptic cleft, as well as the kinetics of the AMPAR[9]. The functional role of the gene in pain processing was evidenced electrophysiologically and behaviorally in a stargazer mouse model, a type of *CACNG2* hypomorphic mutant that is more susceptible to developing neuropathic pain after nerve injury[8].

In humans, mutations in *CACNG2* are strongly associated with epilepsy. Like neuropathic pain, epilepsy is the result of neuronal hyperexcitability[10]. Nissenbaum et al[11] and Borstov et al[12] identified the association of the homozygous ACC haplotype of *CACNG2* gene single nucleotide polymorphisms (SNPs) rs4820242, rs2284015 and rs2284017 with the tendency to develop neuropathic pain after breast cancer surgery [Odds ratio (OR) 1.65, $p = 0.001$ and OR 1.40, $p = 0.033$, respectively]. Bortsov et al[12] did not observe an association of the ACC haplotype with the risk of post-herniotomy chronic pain in male patients (OR 0.97, $p = 0.90$), but they did observe an association in the opposite direction (OR 0.55, $p = 0.024$) of that reported by Nissenbaum et al[8] for rs2284015.

The objective of this study was to determine the allelic and genotypic frequencies of *CACNG2* polymorphisms (rs4820242, rs2284015 and rs2284017) and their association with the risk of chronic peripheral neuropathic pain (CPNP) in the Mexican population.

Patients and Methods

Study Design

A case-control study was performed after approval of the local Research and Ethics Committee (registration number 15/18) at the Instituto Nacional de Rehabilitación "Luis Guillermo Ibarra Ibarra" (INR LGII), Mexico City, Mexico. All procedures were in accordance with the 1964 Declaration of Helsinki and its later amendments. Participants provided a written informed consent.

Enrollment and Study Population

All cases and controls were recruited from March of 2019 to August of 2020. Blood samples were obtained after written informed consent was provided. All participants were interviewed to obtain clinical and demographic data.

Only Mexican origin individuals between 18 and 75 years of age were included. Mexican origin was defined as individuals who self-reported both themselves, and their ancestors for at least 3 generations, as being born in Mexico. Samples from relatives of any of the participants were not included.

Cases included 75 Mexican patients diagnosed with CPNP according to the International Association for the Study of Pain (IASP) classification of chronic pain for the 11th revision of the International Classification of Diseases and Related Health Problems (ICD) of the World Health Organization (WHO)[13]. These patients were referred from different hospitals in Mexico to the INR Pain Clinic, and all patients completed pain assessments.

Cases with pain of oncological origin, history of recent surgery (≤ 3 months) or psychiatric pathology were excluded.

Controls were 75 healthy Mexican individuals who denied having a personal or family history of CPNP.

Chronic Peripheral Neuropathic Pain

Each case was assessed by an experienced pain management specialist. History related to the presence of CPNP, syndromic diagnosis, location and time of evolution of pain were obtained. Pain intensity was measured by the Visual Analogue Scale (VAS)[14]. Neurological examinations focused on sensitive, motor, and sympathetic pain were performed.

The Douleur Neuropathique 4 (DN4) questionnaire was used to discriminate neuropathic pain from other painful states[15,16]. This questionnaire evaluates 10 items grouped in 4 sections. A score of 1 is given to each positive item and a score of 0 to each negative item. The total score was calculated as the sum of the 10 items and the cut-off value for the diagnosis of neuropathic pain was a score ≥ 4[16].

The Leeds Assessment of Neuropathic Symptoms and Signs (LANSS) Pain Scale was used to differentiate patients with neuropathic pain from those with nociceptive pain[17]. Total score was calculated as the sum of the descriptive and sensory examinations. Maximum score is 24, which consists of 16 points for sensory descrip-

tion and eight points for sensory dysfunction. A cut-off value ≥ 12 indicated that possible neuropathic mechanisms may contribute to the patient's pain[17].

The test of Daniels and Worthingham (D&W) was used to assess muscle strength[18]. The numerical grading system from 5 to 0 was used, including: 5 - actively move through full passive range with maximum resistance at end resistance, 4 - actively move through full passive range with some manual resistance at the end of range, 3 - actively move through full passive range against gravity, 2 - actively move through full passive range with gravity eliminated, 1 - flicker of contraction on palpation or partial range with gravity eliminated, and 0 - no contraction[18].

Electrodiagnostic studies included nerve conduction studies (NCS) and needle electrode examination (electromyography-EMG) and were performed to determine the syndromic diagnosis, as well as the site and degree of peripheral nerve injury using the Seddon classification[19,20]. Seddon classification includes three types of nerve injury: neuropraxia, axonotmesis and neurotmesis. Neuropraxia is the first degree of nerve injury and is characterized by temporary conduction block with demyelination at the affected site. Axonotmesis is the second degree of nerve injury and is characterized by axonal loss with preservation of the connective tissue layers. Neurotmesis is the third degree and the most serious nerve injury, where the nerve is physically divided[19,21].

Finally, a three-step analgesic ladder was used to assess adequate pain relief. Anticonvulsants, tricyclic antidepressants, and serotonin-norepinephrine reuptake inhibitors were placed in the first step, tramadol was added in the second step, and higher potency opioids were added in the third step[22-24].

Allelic and Genotypic Frequencies Previously Reported

The description of each SNP included in the study, the allelic and genotypic frequency reported in the Mexican population in the PAGE study[25], and worldwide in the 1,000 Genomes project[26], are shown in Table I.

The PAGE study was designed to characterize the genetic architecture of complex traits among underrepresented minority populations through large scale genetic and epidemiological research[25]. The 1,000 Genomes project was created to show a catalog of human genetic variation, using voluntarily donated samples from people in different parts of the world who self-declared as healthy[26].

Real-Time Polymerase Chain Reaction (qPCR) Genotyping of CACNG2 Polymorphisms

Genomic DNA was extracted from 5 ml of whole blood containing EDTA by the sucrose gradient method[27]. The quantity and quality of the DNA were analyzed using the NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) at 260 and 280 nm. DNA samples were stored at -20ºC until use. All genotyping was done blindly to clinical information.

Genotyping of three different *CACNG2* (rs4820242, rs2284015 and rs2284017) was performed using the allelic discrimination. SNPs were analyzed using predesigned Applied Biosystems TaqMan SNP Genotyping assays (rs4820242, assay ID C___2504978_20: TAAAAGTGATCTGAGGGCAATGGCA[A/G]TCCTTGTGCTGTTGGGAAGGGGAGG; rs2284015, assay ID C___2462885_1: TCCCTCCATCCATCTAACTAAAATC[C/G]AAGGAAAGCAAATTGCCACTATGAT; rs2284017,

Table I. Reported characteristics of the selected single nucleotide polymorphisms.

CACNG2 gene			Allele and genotype frequency in Mexican (the PAGE study)		1,000 genomes[26]
Position	SNP ID	Consequence	RA (F)	AA (F)	MA (F)
chr22:36586628	rs4820242	Intron variant	G (0.3034)	A (0.6965)	G (0.3784)
chr22:36700528	rs2284015	Intron variant	C (ND)	G (ND)	G (0.2955)
chr22:36700882	rs2284017	Intron variant	T (0.5535)	C (0.4464)	C (0.4223)

CACNG2: calcium voltage-gated channel auxiliary subunit gamma 2; PAGE: Population Architecture using Genomics and Epidemiology; SNP: single nucleotide polymorphism; RA: reference allele; F: frequency; AA: alternate allele; MA: minor allele; G: guanine; A: adenine; C: cytosine; ND: not described; T: thymine.

assay ID C___215960016_10: TAGACCATCGCTCAATGAAGTCACT[C/T]AGCTATTCAAAACATGTTCAGCCAT; Thermo Fisher Scientific Inc., Waltham, MA, USA).

The qPCR amplifications for each SNP were carried out in a total of 5 µL reaction containing 18 ng of genomic DNA, 2.5 µl of 2x Applied Biosystems TaqMan Universal PCR Master Mix (Thermo Fisher Scientific Inc., Waltham, MA, USA) and 0.12 µl of the specific 40x TaqMan SNP assay. qPCR was performed on an Applied Biosystems Step One Plus instrument (Thermo Fisher Scientific Inc., Waltham, MA, USA) according to the manufacturer´s recommendations. Negative non-template-controls were included in every run. Genotypes were determined using the Applied Biosystems Step One/Step One Plus Software v2.3. Inconclusive genotypes were repeated.

Statistical Analysis

Select Statistics Services software (Exeter, United Kingdom) was used, taking into account the frequency of the minor allele (MA) reported in the PAGE study, and in the 1,000 Genomes project in healthy populations, for each polymorphism studied with a relative precision of 50%, 95% confidence interval, and OR of 2 and 1:1 ratio of cases and controls. A total sample size of 72 is estimated to demonstrate statistical significance.

Data were entered and analyzed using Statistical Package for the Social Sciences (SPSS) software v.25.0 (IBM Corp., Armonk, NY, USA). The differences in demographic variables between cases and controls, as well as in clinical variables of patients with CPNP according to the presence of reference or alternative alleles, were examined using the Mann-Whitney U test and Pearson's Chi-square test with Yates's correction for continuity (two-tailed).

Allele frequencies were obtained by direct counting. The Hardy-Weinberg equilibrium was tested by Chi-square goodness of fit test, to compare the observed genotype frequencies to the expected frequencies among control subjects. OR and 95% confidence intervals (CI) were calculated. The association analysis by OR and binary logistic regression analysis between the studied groups were performed using the SNPStats: the web tool for SNP analysis and VassarStats. A two-tailed $p < 0.05$ was considered statistically significant.

Results

Characteristics of Study Participants

Comparative demographic data of CPNP patients and controls, and clinical characteristics of CPNP patients are shown in Table II. All cases presented neuropathic pain corroborated by scores on the LANSS and DN4 scales, as well as some type of nerve injury demonstrated by electrodiagnosis (neuropraxia 61.3%, axonotmesis 29.3% and neurotmesis 9.4%). In some cases, they presented motor alterations, with D&W test grade 3 in 6.7% and grade 4 in 14.7%. 13 cases had a history of spinal surgery, of which 92.3% had failed back surgery syndrome.

The remaining 62 cases had histories of lumbar spinal stenosis (38.7%), spinal disc herniation (32.3%), complex regional pain syndrome type II (11.3%), cervical spinal stenosis (9.7%), postherpetic neuralgia (6.5%), and peripheral psoriatic neuropathy (1.6%). The most frequent characteristics in patients with CPNP were radiculopathy (80%), lumbar location (64%) and unilateral pattern of pain (52%).

Allodynia and burning, hypoesthesia and paresthesia, as well as pricks and cramps frequently occurred together (54.7%, 44% and 48% respectively). 94.7% of the cases were receiving analgesics from the first and second step.

Allele and Genotype Frequencies of Case and Control Group

The allelic frequencies of rs4820242, rs2284015, and rs2284017 were found in Hardy-Weinberg equilibrium. The rs4820242 and rs2284017 did not show significant differences between CPNP patients and control group. However, rs2284015 was significantly different between cases and controls (Table III).

Allele G (OR 2.08, 95% CI 1.21-3.57, $p = 0.01$), as well as genotypes GG (codominant model, OR 5.56, 95% CI 1.11-27.73, $p = 0.026$ and recessive model, in age and sex adjusted analysis OR 13.93, 95% CI 1.68-115.47, $p = 0.01$) and CG/GG (dominant model, OR 2.17, 95% CI 1.12-4.19, $p = 0.02$) of rs2284015 were observed as risk factors for developing CPNP (Table III and Table IV).

The interaction analysis of sex with respect to the genotype of rs2284015 and rs2284017 (crude and adjusted for age) for the risk of CPNP did not show statistical significance ($p > 0.05$), regardless of the inheritance model. The AA genotype of rs4820242 showed statistical significance in the sex interaction analysis for the risk of CPNP

Table II. Demographic and clinical characteristics of study participants.

Variable	CPNP	Controls	*p*-value
Age (years)			**0.001**
Median (IR)	51 (19)	31 (9)	
Sex, n (%)			0.17
Female	53 (70.7)	45 (60.0)	
Male	22 (29.3)	30 (40.0)	
History of surgery			
Yes	13 (17.3)		
No	62 (82.6)	75 (100)	
Syndromic diagnosis, n (%)			
Radiculopathy	60 (80.0)		
Neuropathy	15 (20.0)		
Location, n (%)			
Lumbar	48 (64.0)		
Cervical	6 (8.0)		
Cervical and thoracic limbs	5 (6.7)		
Lumbar and pelvic limbs	16 (21.3)		
Laterality, n (%)			
Unilateral	39 (52)		
Bilateral	36 (48)		
VAS without treatment (score)			
Median (IR)	7 (3)		
VAS with treatment (score)			
Median (IR)	2 (2)		
Positive symptoms			
Allodynia	8 (10.7)		
Burning	17 (22.7)		
Allodynia and burning	41 (54.7)		
None	9 (12.0)		
Negative symptoms			
Hypoesthesia	19 (25.3)		
Paresthesia	18 (24.0)		
Hypoesthesia and paresthesia	33 (44)		
None			
Other symptoms			
Prick	20 (26.7)		
Cramps	10 (13.3)		
Prick and cramps	36 (48.0)		
None	9 (12.0)		
DN4 (score)			
Median (IR)	9 (2)		
LANSS (score)			
Median (IR)	24 (5)		
Seddon classification, n (%)			
Neuropraxia	46 (61.3)		
Axonotmesis	22 (29.3)		
Neurotmesis	7 (9.4)		
D&W test (Grades), n (%)			
Grade 0	0 (0.0)		
Grade 1	0 (0.0)		
Grade 2	0 (0.0)		
Grade 3	5 (6.7)		
Grade 4	11 (14.7)		
Grade 5	59 (78.6)		
Analgesic ladder (steps)			
First	18 (24.0)		
Second	53 (70.7)		
Third	4 (5.3)		

CPNP: chronic peripheral neuropathic pain; IR: interquartile range; VAS: visual analog scale; DN4: Douleur Neuropathique 4; LANSS: Leeds Assessment of Neuropathic Symptoms and Signs; D&W: Daniels and Worthingham ($p < 0.05$ marked in bold).

Table III. *CACNG2* rs4820242, rs2284015, and rs2284017 allelic frequencies and HWE in study participants.

Allele	Controls 2n = 150 (%)	CPNP 2n = 150 (%)	OR (95% CI)	*p*-value	*p*-value HWE
			rs4820242		
A	104 (0.69)	105 (0.7)	1.03 (0.63-1.69)	1.0	1.0
G	46 (0.31)	45 (0.3)	0.97 (0.59-1.59)		
			rs2284015		
C	123 (0.82)	103 (0.69)	**0.48 (0.28-0.83)**	0.01	0.66
G	27 (0.18)	47 (0.31)	**2.08 (1.21-3.57)**		
			rs2284017		
C	91 (0.61)	98 (0.65)	1.22 (0.76-1.95)	0.47	1.0
T	59 (0.39)	52 (0.35)	0.82 (0.51-1.31)		

HWE: Hardy-Weinberg equilibrium; CPNP: chronic peripheral neuropathic pain; OR: odds ratio; CI: confidence interval; A: adenine; C: cytosine; T: thymine; G: guanine ($p < 0.05$ marked in bold).

Table IV. *CACNG2* rs4820242, rs2284015, and rs2284017 genotypic frequencies and models of inheritance associated with CPNP.

Model	Genotype	Controls n = 75 (%)	CPNP n = 75 (%)	Crude analysis		Adjusted by age + sex	
				OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Codominant	AA	36 (48.0)	37 (49.3)	1.00	0.99	1.00	0.83
	AG	32 (42.7)	31 (41.3)	0.94 (0.48-1.85)		1.18 (0.45-3.09)	
	GG	7 (9.3)	7 (9.3)	0.97 (0.31-3.05)		0.72 (0.14-3.57)	
Dominant	AA	36 (48.0)	37 (49.3)	1.00	0.87	1.00	0.89
	AG/GG	39 (52.0)	38 (50.7)	0.95 (0.50-1.80)		1.07 (0.43-2.65)	
Recessive	AA/AG	68 (90.7)	68 (90.7)	1.00	NA	1.00	0.61
	GG	7 (9.3)	7 (9.3)	1.00 (0.33-3.00)		0.67 (0.14-3.15)	
Overdominant	AA/GG	43 (57.3)	44 (58.7)	1.00	0.87	1.00	0.65
	AG	32 (42.7)	31 (41.3)	0.95 (0.49-1.81)		1.24 (0.49-3.15)	
Log-additive	-	-	-	0.97 (0.59-1.58)	0.9	0.96 (0.48-1.91)	0.9
rs2284015							
Codominant	CC	50 (66.7)	36 (48.0)	1.00	**0.026**	1.00	**0.013**
	CG	23 (30.7)	31 (41.3)	1.87 (0.94-3.73)		2.18 (0.74-6.40)	
	GG	2 (2.7)	8 (10.7)	**5.56 (1.11-27.73)**		**18.71 (2.11-165.79)**	
Dominant	CC	50 (66.7)	36 (48.0)	1.00	**0.02**	1.00	**0.03**
	CG/GG	25 (33.3)	39 (52.0)	**2.17 (1.12-4.19)**		**2.99 (1.08-8.26)**	
Recessive	CC/CG	73 (97.3)	67 (89.3)	1.00	0.043	1.00	**0.01**
	GG	2 (2.7)	8 (10.7)	4.36 (0.89-21.26)		**13.93 (1.68-115.47)**	
Overdominant	CC/GG	52 (69.2)	44 (58.7)	1.00	0.17	1.00	0.35
	CG	23 (30.7)	31 (41.3)	1.59 (0.81-3.12)		1.61 (0.59-4.44)	
Log-additive	-	-	-	**2.07 (1-19-3.59)**	**0.0077**	**3.09 (1.33-7.16)**	**0.0058**
rs2284017							
Codominant	CC	29 (38.7)	30 (40.0)	1.00	0.33	1.00	0.38
	CT	33 (44.0)	38 (50.7)	1.11 (0.56-2.22)		0.53 (0.19-1.47)	
	TT	13 (17.3)	7 (9.3)	0.52 (0.18-1.49)		0.44 (0.10-2.04)	
Dominant	CC	29 (38.7)	30 (40.0)	1.00	0.87	1.00	0.17
	CT/TT	46 (61.3)	45 (60.0)	0.95 (0.49-1.82)		0.51 (0.19-1.36)	
Recessive	CC/CT	62 (82.7)	68 (90.7)	1.00	0.15	1.00	0.53
	TT	13 (17.3)	7 (9.3)	0.49 (0.18-1.31)		0.65 (0.16-2.56)	
Overdominant	CC/TT	42 (56.0)	37 (49.3)	1.00	0.41	1.00	0.37
	CT	33 (44.0)	38 (50.7)	1.31 (0.69-2.49)		0.66 (0.26-1.68)	
Log-additive	-	-	-	0.82 (0.51-1.31)	0.4	0.62 (0.30-1.28)	0.19

CPNP: chronic peripheral neuropathic pain; OR: Odds ratio; CI: confidence interval; A: adenine; C: cytosine; T: thymine; G: guanine. ($p < 0.05$ marked in bold).

in the codominant model (female OR 1.0, male OR 0.33, 95% CI 0.11-0.94, $p = 0.043$). However, when adjusting for age, it did not retain statistical significance.

In the haplotype analysis of rs4820242, rs2284015 and rs2284017, the ACC haplotype was the most frequent (29.38%). Only the AGC haplotype (frequency of 13.2%), with respect to the ACC haplotype adjusted for age and sex, was observed as a risk factor for CPNP (OR 6.63, CI 95% 1.33-33.11, $p = 0.023$). The interaction analysis of sex with respect to haplotypes for the risk of CPNP did not show statistical significance.

Allele and Genotype Frequencies Stratified by Characteristics of CPNP

Significant differences were observed when performing the association analysis of allele frequencies with each of the characteristics of CPNP, comparing rs4820242 stratified by history of surgery, syndromic diagnosis, laterality and neuropraxia, and comparing rs2284015 stratified by positive symptoms ($p < 0.05$). No significant differences were observed when comparing allele frequencies of rs2284017 stratified by characteristics of CPNP (Table V).

The AG/GG genotypes of rs4820242 in the dominant model showed significant differences in frequency when stratified by history of laterality surgery and pain, as well as lower risk of association with respect to AA genotype. Genotypes with at least one G allele, regardless of the inheritance pattern, were associated with a lower frequency and risk of association of radiculopathy *vs.* neuropathy with respect to genotypes without G allele (Figure 1 and Table VI).

The GG genotype of rs2284015 in the recessive model showed significant differences in frequency when stratified by history of surgery, and a higher risk of association with respect to CC/CG geno-

Table V. *CACNG2* rs4820242 and rs2284015 allelic frequencies and HWE in CPNP patients.

rs4820242					
Allele	**Non-surgical 2n = 124 (%)**	**Surgical 2n = 26 (%)**	**OR (95% CI)**	***p*-value**	***p*-value HWE**
A G	82 (0.66) 42 (0.34)	23 (0.88) 3 (0.12)	**3.92 (1.11-13.83)** **0.25 (0.07-0.89)**	**0.04**	1.0
	Neuropathy 2n = 30 (%)	**Radiculopathy 2n = 120 (%)**			
A G	13 (0.43) 17 (0.57)	92 (0.77) 28 (0.23)	**4.29 (1.86-9.92)** **0.23 (0.10-0.53)**	**0.0001**	1.0
	Unilateral 2n = 78 (%)	**Bilateral 2n = 72 (%)**			
A G	46 (0.59) 32 (0.41)	59 (0.82) 13 (0.18)	**3.15 (1.48-6.69)** **0.31 (0.14-0.67)**	**0.003**	1.0
	Non-neuropraxia 2n = 58 (%)	**Neuropraxia 2n = 92 (%)**			
A G	42 (0.72) 16 (0.28)	63 (0.68) 29 (0.32)	**0.36 (0.17-0.75)** **2.71 (1.31-5.61)**	**0.01**	1.00
rs2284015					
	No positive symptoms 2n = 68 (%)	**Burning and allodynia 2n = 82 (%)**	**OR (95% CI)**	***p*-value**	***p*-value HWE**
C G	40 (0.59) 28 (0.41)	63 (0.77) 19 (0.23)	**2.32 (1.09-2.15)** **0.43 (0.21-0.87)**	**0.02**	0.79

HWE: Hardy-Weinberg equilibrium; CPNP: chronic peripheral neuropathic pain; OR: odds ratio; CI: confidence interval; A: adenine; C: cytosine; T: thymine; G: guanine ($p < 0.05$ marked in bold).

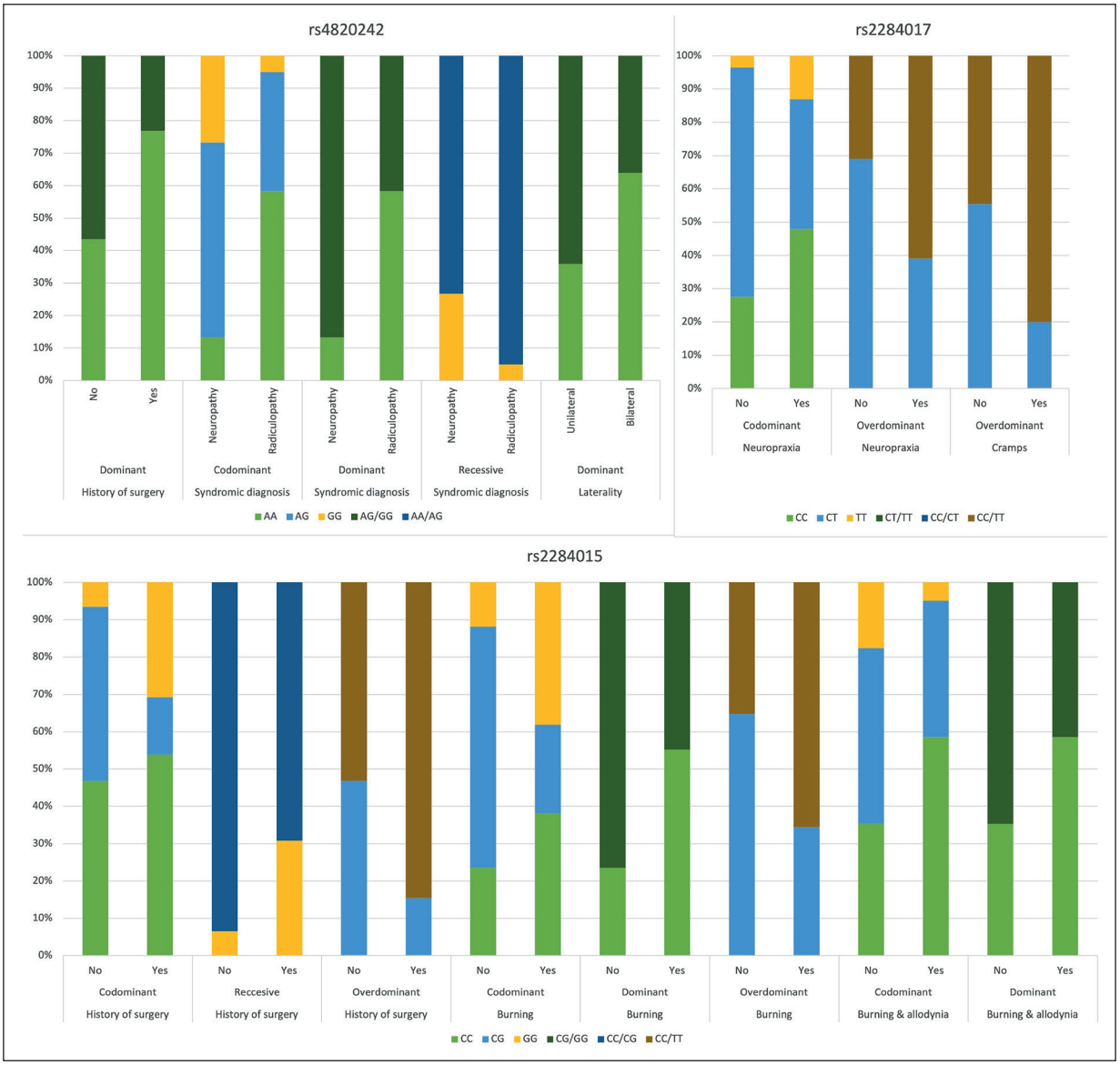

Figure 1. Genotype distribution of *CACNG2* rs4820242, rs2284015 and rs2284017 classified by clinical characteristics of CPNP ($p < 0.05$).

types. The CG/GG genotype in dominant model and CG in the over-dominant model showed differences in frequency when stratified by burning, as well as lower risk of association with respect to CC or CC/GG genotypes, respectively. The CG/GG genotypes in the dominant model showed differences in frequency when stratifying by joint presentation of burning and allodynia, as well as lower risk of association with respect to CC genotype (Figure 1 and Table VI).

The CT genotype of rs2284017 in codominant and over-dominant models showed differences in frequency when stratifying by neuropraxia and cramps, as well as a lower risk of association with respect to CC and CC/TT genotypes, respectively (Figure 1 and Table VI).

Regarding the interaction analysis of sex and the characteristics of CPNP, the AA genotype of rs4820242 was observed as a factor that reduces the risk of allodynia in males (OR 0.10, 95% CI 0.02-0.65, $p = 0.027$). The CC genotype of rs2284015 was observed to increase the risk of hypoesthesia (OR 31.96, 95% CI 2.92-349.89, $p = 0.0013$), and reduces the risk of neuropraxia (OR 0.10, 95% CI 0.01-0.68, $p = 0.015$) in age-adjusted males. The CT genotype rs2284017 was

Table VI. *CACNG2* rs4820242, rs2284015, and rs2284017 genotypic frequencies and models of inheritance associated with the clinical characteristics of CPNP.

rs4820242							
Model	**Genotype**	**Non-surgical n = 62 (%)**	**Surgical n = 13 (%)**	**Crude analysis**		**Adjusted by age + sex**	
				OR (95% CI)	***p*-value**	**OR (95% CI)**	***p*-value**
Dominant	AA	27 (43.5)	10 (76.9)	1.00	**0.025**	1.00	**0.018**
	AG/GG	35 (56.5)	3 (23.1)	**0.23 (0.06-0.92)**		**0.19 (0.04-0.84)**	
Log-additive	-	-	-	**0.25 (0.07-0.91)**	**0.015**	**0.20 (0.05-0.83)**	**0.009**
		Neuropathy n = 15 (%)	**Radiculopathy n = 60 (%)**				
Codominant	AA	2 (13.3)	35 (58.3)	1.00	**0.0018**	1.00	**0.022**
	AG	9 (60.0)	22 (36.7)	**0.14 (0.03-0.71)**		**0.13 (0.03-0.71)**	
	GG	4 (26.7)	3 (5.0)	**0.04 (0.01-0.34)**		**0.04 (0.00-0.33)**	
Dominant	AA	2 (13.3)	35 (58.3)	1.00	**0.001**	1.00	**0.0014**
	AG/GG	13 (86.7)	25 (41.7)	**0.11 (0.02-0.53)**		**0.11 (0.02-0.54)**	
Recessive	AA/AG	11 (73.3)	57 (95)	1.00	**0.021**	1.00	**0.024**
	GG	4 (26.7)	3 (5)	**0.14 (0.03-0.74)**		**0.15 (0.03-0.77)**	
Log-additive	-	-	-	**0.20 (0.07-0.55)**	**5 e-04**	**0.19 (0.07-0.55)**	**6 e-04**
		Unilateral n = 39 (%)	**Bilateral n = 36 (%)**				
Dominant	AA	14 (35.9)	23 (63.9)	1.00	**0.015**	1.00	**0.025**
	AG/GG	25 (64.1)	13 (36.1)	**3.16 (1.23-8.12)**		**3.10 (1.13-8.50)**	
Log-additive	-	-	-	**3.34 (1.48-7.56)**	**0.017**	**3.42 (1.42-8.23)**	**0.003**
rs2284015							
Model	**Genotype**	**Non-surgical n = 62(%)**	**Surgical n = 13 (%)**				
Codominant	CC	29 (46.8)	7 (53.9)	1.00	0.02	1.00	**0.005**
	CG	29 (46.8)	2 (15.4)	0.29 (0.05-1.49)		**0.16 (0.003-0.98)**	
	GG	4 (6.5)	4 (30.8)	4.14 (0.83-20.79)		4.33 (0.70-26.94)	
Recessive	CC/CG	58 (93.5)	9 (69.2)	1.00	**0.022**	1.00	**0.017**
	GG	4 (6.5)	4 (30.8)	**6.44 (1.36-30.47)**		**7.95 (1.46-43.19)**	
Overdominant	CC/GG	33 (53.2)	11 (84.6)	1.00	0.028	1.00	**0.0048**
	CG	29 (46.8)	2 (15.4)	0.21 (0.04-1.01)		**0.11 (0.02-0.65)**	
		Non-burning n = 17 (%)	**Burning n = 58 (%)**				
Codominant	CC	4 (23.5)	32 (55.2)	1.00	0.054	1.00	**0.018**
	CG	11 (64.7)	20 (34.5)	0.23 (0.06-0.81)		**0.16 (0.04-0.62)**	
	GG	2 (11.8)	6 (55.2)	0.38 (0.06-2.53)		0.34 (0.05-2.51)	
Dominant	CC	4 (23.5)	32 (55.2)	1.00	**0.019**	1.00	**0.007**
	CG/GG	13 (76.5)	26 (44.8)	**0.25 (0.07-0.86)**		**0.19 (0.05-0.69)**	
Overdominant	CC/GG	6 (35.3)	38 (65.5)	1.00	**0.027**	1.00	0.0084
	CG	11 (64.7)	20 (34.5)	**0.29 (0.09-0.89)**		**0.20 (0.06-0.70)**	
		No positive symptoms n = 34 (%)	**Burning and allodynia n = 41 (%)**				
Codominant	CC	12 (35.3)	24 (58.5)	1.00	0.062	1.00	**0.054**
	CG	16 (47.1)	15 (36.6)	0.47 (0.17-1.26)		0.39 (0.13-1.15)	
	GG	6 (17.6)	2 (4.9)	0.17 (0.03-0.95)		**0.16 (0.03-0.97)**	
Dominant	CC	12 (35.3)	24 (58.5)	1.00	**0.044**	1.00	**0.029**
	CG/GG	22 (64.7)	17 (41.5)	**0.39 (0.15-0.99)**		**0.33 (0.12-0.92)**	
Log-additive	-	-	-	**0.43 (0.21-0.90)**	**0.019**	**0.39 (0.18-0.87)**	**0.016**
rs2284017							
Model	**Genotype**	**Non-neuropraxia n = 29 (%)**	**Neuropraxia n = 46 (%)**				
Codominant	CC	8 (27.6)	22 (47.8)	1.00	**0.031**	1.00	**0.039**
	CT	20 (69.0)	18 (39.1)	**0.33 (0.12-0.92)**		**0.33 (0.11-0.94)**	
	TT	1 (3.5)	6 (13)	2.18 (0.23-21.04)		2-04 (0.21-20.07)	
Overdominant	CC/TT	9 (31)	28 (60.9)	1.00	**0.011**	1.00	**0.014**
	CT	20 (69)	18 (39.1)	**0.29 (0.11-0.77)**		**0.29 (0.11-0.80)**	
		Non-cramps n = 65 (%)	**Cramps n = 10 (%)**				
Overdominant	CC/TT	29 (44.6)	8 (80)	1.00	0.032	1.00	**0.023**
	CT	36 (55.4)	2 (20)	0.20 (0.04-1.02)		**0.18 (0.03-0.94)**	

CPNP: chronic peripheral neuropathic pain; OR: odds ratio; CI: confidence interval; A: adenine; C: cytosine; T: thymine; G: guanine ($p < 0.05$ marked in bold).

observed to increase the risk of motor alterations (OR 12.01, 95% CI 1.32-109.63, $p = 0.0004$) in age-adjusted females.

The CG genotype rs2284015 in women showed a mean of 3.11 ± 0.48 with a difference of 1.17 (95% CI 0.30-2.04, $p = 0.0082$) in pain intensity. The GG genotype rs4820242 in men showed a mean of 8.33 ± 0.33 with a difference of -1.00 (95% CI -2.10 - -0.10, $p = 0.014$) for the DN4 scale score.

Discussion

Chronic neuropathic pain is caused by a lesion or disease of the somatosensory nervous system, which has a great impact on the health-related quality of life, sleep, mood and anxiety[4], and is one of the main non-fatal causes of seeking medical service worldwide[28].

The minimum time during which pain must be present to be considered chronic is 3 months[13]. Pain can be spontaneous or evoked, with an increased response to a painful stimulus (hyperalgesia) or a painful response to normally non-painful stimulus (allodynia).

Diagnosis requires a history of nervous system injury or disease, and neuroanatomically logical or plausible pain distribution[13]. Pain may be the most prominent symptom or the only manifestation of neurological disease. Even in patients with the same underlying cause of neuropathic pain, different signs and symptoms may be present. Negative symptoms indicate reduced impulse conduction in neural tissues, such as hypoesthesia or anesthesia and weakness. Positive symptoms reflect an abnormal level of excitability in the nervous system of pain, paresthesia, dysesthesia, and spasms[29], so some or all of them must be present and compatible with the innervation of the affected nerve structure[13].

Chronic neuropathic pain can be caused by injury or disease of the peripheral (CPNP) or central (CCNP) somatosensory nervous system. Some types of neuropathic pain that are included in peripheral nerve injuries are trigeminal neuralgia, pain secondary to peripheral nerve injury of trauma or surgery, painful polyneuropathy caused by metabolic, autoimmune, familial, or infectious diseases, environmental, occupational, and neurotoxic drugs, postherpetic neuralgia, painful radiculopathy, and other unspecified causes such as carpal tunnel syndrome[13].

Chronic neuropathic pain is a multifactorial entity, in which imbalance between excitatory and inhibitory somatosensory signaling, ion channel alterations, and variability in the way pain messages are modulated in the central nervous system are implicated[30]. Therefore, various molecular aspects involved in its pathophysiology are under study. In the future, the results of these investigations could allow the identification of predictive, diagnostic and prognostic biomarkers, as well as the development of new diagnostic procedures and personalized interventions.

To date, various genetic and non-genetic factors involved in the susceptibility to developing CPNP have been identified. Regarding genetic factors, mutations in the sodium channel protein type 9 subunit alpha (*SCN9*) gene have been described as the cause of congenital sensitivity to pain with anhidrosis, erythromelalgia and paroxysmal extreme pain, and monogenic rare diseases associated with chronic neuropathic pain[4]. Therefore, it has been proposed that variants in genes involved in the pain pathway, such as *CACNG2*, could contribute to the susceptibility of developing neuropathic pain[4].

The homozygous ACC haplotype of *CACNG2* rs4820242, rs2284015 and rs2284017 constitutes a risk factor for chronic neuropathic pain in women undergoing mastectomy[8,11,12], while the C allele of rs2284015 is a factor that reduces the risk of neuropathic pain in men undergoing herniotomy[12]. The genetic structure can have variations in different populations. The demographic and clinical characteristics of a group of Mexican patients with CPNP were described. The allele and genotypic frequencies of *CACNG2* rs4820242, rs2284015 and rs2284017 between patients with CPNP and healthy controls were compared, and the risk of association of the 3 SNPs with the presence of CPNP and its different characteristics were evaluated.

Unlike previously reported studies, our study included CPNP cases of both sexes, with surgical and non-surgical histories associated with pain. CPNP was more often observed in females with an average age of 51 years, a syndromic diagnosis or radiculopathy and more frequently affecting lumbar area and extremities, like that previously reported in the international literature[30]. Although there were no differences in the frequency of men and women in the group cases and controls, there were differences in age, thus all statistical tests were adjusted for age and sex.

In this study, none of the women had a history of mastectomy and none of the men had a history

of herniotomy; however, both men and women had CPNP related to different degenerative alterations or spinal surgery.

Regarding the allelic frequencies of the 3 SNPs, it was observed that they were in Hardy-Weinberg equilibrium. In the controls, the frequency of the minor allele (MA) in rs4820242 and rs2284017 was like that reported in the Mexican population in the PAGE study and in the international 1,000 Genomes project. However, the allelic frequency of rs2284015 had not been specifically reported in the Mexican population, and in this study, it was considerably lower than that reported in the 1,000 Genomes project.

Our results show that the allele G of rs2284015, like that reported by Bortsov et al[12] in men, and the AGC haplotype of rs4820242, rs2284015 and rs2284017, increase the risk of presenting CPNP in both sexes.

However, an association between the ACC haplotype with the risk of CPNP was not found, nor was an interaction with sex demonstrated. Thus, the differences in women found with the reports by Nissenbaum[11] et al[8] could be related to sexual dimorphism[31]. Variability in sexual dimorphism is equivalent to the interaction of genotype by sex and can occur if QTL or SNP affects only one sex (sex-specific effects), affects both sexes but to a different magnitude (sex-biased effects) or affects both sexes even if in opposite directions (sex-antagonistic effects)[32,33]. Important differences in different species, and in humans, have been reported[34-36] concerning sensitivity to pain and analgesics, as well as differing susceptibility to developing chronic pain in males and females.

This variability in sexual dimorphism may be due to different gene expressions as a consequence of changes in the cellular and hormonal environment of the two sexes[31,36]. Thus, it is possible that the allele C of rs2284015 or ACC haplotype contribute to chronic pain in women undergoing mastectomy. Meanwhile, the allele G of rs2284015 or AGC haplotype, in the opposite sense to that reported in women undergoing mastectomy, and independent of sex and etiology, could contribute to the risk of CPNP.

However, this possibility must be validated in a study with a larger number of patients that analyzes the participation of other potential genetic and non-genetic risk factors for CPNP, as well as their interaction with sex. The comprehensive assessment of pain and the use of clinical scales with greater objectivity than the use of cold or heat tolerance tests allowed us to analyze the association between most of the clinical characteristics of pain in patients with CPNP and allelic and genotypic variants of *CACNG2*.

Although the sample size was too small to give definitive results on the possible association of each clinical characteristic of CPNP with each SNP, or even the haplotype, significant differences could be demonstrated in the allelic and genotypic frequency of SNPs according to certain antecedents or clinical features.

The allele A of rs4820442 did not increase the risk of neuropathic pain as reported in other studies[8,11,12]. However, a higher risk association of this allele in a homozygous state with a history of surgery, radiculopathy and bilateral presentation of pain was observed. Regarding the allele G of rs2284015, in addition to increasing the risk of neuropathic pain, in a homozygous state it was associated with a history of surgery and regardless of their status (homo or heterozygous), with less frequency of positive symptoms of CPNP such as burning or allodynia. The C allele of rs2284017 also showed no risk association with CPNP; however, when its association with clinical characteristics was analyzed, in heterozygous state, a risk reduction in neuropraxia and cramps was observed. The observed risk associations may be related to the etiology of de CPNP, but they could also constitute markers of severity.

Finally, when sex interaction analysis was carried out in the association of the characteristics of the CPNP with the different genotypes of the SNPs, a modification of risk dependent on sex was observed, which could suggest sex-biased effects.

Our study has some limitations. For example, the sample size calculation to identify differences in allele frequencies between CPNP and healthy controls was carried out considering the allele frequencies reported in other populations with CPNP and in the healthy Mexican population. However, the sample size must be increased to evaluate the association of all the clinical characteristics of CPNP with the allelic frequencies of the SNP analyzed. In view of the multifactorial nature of CPNP, it is important to consider other risk factors that have been associated with its development. The matching of cases and controls based on their risk factors or the stratified/multivariated analysis with a larger sample size would allow us to confirm the contribution of the *CACNG2* SNPs to the risk of CNPN.

Conclusions

The results show that the allele G of rs2284015 and the AGC haplotype of *CACNG2* rs4820242, rs2284015 and rs2284017, in the opposite sense of that reported in women undergoing mastectomy, independent of sex and etiology, could contribute to the risk of CPNP. We also observed significant differences in the allelic and genotypic frequency of SNPs according to certain antecedents or clinical characteristics. Interaction with sex was observed in some of the associations, so that certain alleles and genotypes could constitute severity markers in CPNP with sex-biased effects; however, it is necessary to carry out a study with a greater number of controls and patients.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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