Genetic Variations of OPRM1, OPRK1, and COMT Genes and Their Possible Associations with Oral Pain in a Population from Argentina

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Aims: To analyze in a population from Argentina the variation of three genes involved in the control of pain pathways—two genes that code for opioid receptors (OPRM1 and OPRK1) and COMT, which codes for an important enzyme in the control of neurotransmission—and to evaluate the associations of these genes with oral pain and the need for analgesics in the population under study. **Methods:** A total of 134 volunteer donors from the city of Resistencia and 27 donors from the Wichí community for comparison were analyzed for 13 single nucelotide polymorphisms (SNPs) and 1 insertion/deletion (Indel) localized in the three genes using polymerase chain reaction-restriction fragment length polymorphism or standard PCR and electrophoresis. All 134 individuals from Resistencia provided biologic samples for DNA analysis, and a subset (n = 81) agreed to answer a questionnaire for an association analysis. Statistical tests for a possible association between genetic variation and self-reported ethnic origin, oral pain, and need for analgesic drugs were performed. **Results:** Significant differences were found when the study population was compared to populations from other continents, as well as between the two studied populations ($P < .05$). A positive association was suggested for the COMT gene from Resistencia with both oral pain intensity and analgesic requirements. **Conclusion:** The admixture process that occurred in the past of Resistencia probably contributed to a genetic differentiation in this population, and this genetic variation might influence phenotypic expressions of pain perception and analgesic requirements. *J Oral Facial Pain Headache 2018;32:367–374. doi: 10.11607/ofph.1902*

Keywords: *admixed population, pain genetics, pain perception, single nucleotide polymorphisms*

Pain is a personal and complex experience considered to reflect both emotional and sensory elements.[1,2] Sensitivity to pain is influenced by psychologic, cultural, and genetic factors, which vary between individuals. The heritability of pain sensitivity has been proven in twin studies reporting significant results for experimental pain responses.[3] Among the genetic factors influencing pain sensitivity, there are several genes that are involved in the control of pain signaling, such as the genes coding for opioid receptors and the genes coding for enzymes that control neurotransmission. The study of these genes can be an important approach to understanding the molecular basis of pain perception.[4,5]

All endogenous and external opioid substances interact with opioid receptors, although the analgesic power of these substances seems to be related to chemical structure and affinity to specific sites of binding.[6,7] There are different types and subtypes of human opioid receptors, but the most important for pain sensitivity is the mu (μ) receptor in its variants 1 and 2, although a role has also been reported for the kappa (κ) receptor.[8] The human gene OPRM1 encodes the μ opioid receptor (MOR). Several pharmacologic studies have shown that morphine and other related, commonly used drugs act preferentially in activating MOR.[9,10] The OPRM1 gene presents several mutations that can alter the expression and/or function of MOR, so it is important to understand its genetic variation.[5,10,11] Another gene, OPRK1, encodes the κ opioid receptor (KOR), which is mainly activated by the binding of the peptide

Fig 1 Geographic location of the two samples analyzed for this study. The Wichí people included in this study live dispersed in the northwestern area of the province of Chaco.

dynorphin. Allelic variations in OPRK1 can also alter the function or expression of the receptor, even when they are located in introns.[12–15] Both the OPRM1 and OPRK1 genes are polymorphic[8] and exhibit different distributions of variants among populations of the world.[12,16–18] The information available on such variation mainly comes from populations from North American, European, and to a lesser extent, Asian countries,[17,19] while little information is available for populations from Central and South American countries.[20,21] Yet another gene, the gene for catechol-O-methyltransferase (COMT), also contributes to the variability in pain and the response to opioids. Different studies have shown an association between certain COMT polymorphisms and morphine requirements,[22–24] cold pain sensitivity,[25] and acute[26] and chronic pain.[27–30]

The human population of Chaco, a province from northeast Argentina, is heterogenous and presents an unequal distribution of native people and immigrants. The capital city of Resistencia was established at the end of the 19th century to promote European immigration and agricultural colonization.[31,32] Although very little is known about the genetic variation in the population from this city, it has been suggested that the people living in Resistencia share a common genetic background with native communities living in the vicinity.[33] Moreover, a previous report on another locality from the province of Chaco, Misión Nueva Pompeya, estimated a native contribution of 25% in uniparental markers for the urban people living there.[34] In fact, Native American people from different communities live nearby several cities from the province of Chaco, keeping their original seminomadic habits. Among them, the Wichí (Mataco-Wichí) are the most numerous, and together with Qom and Mocoví communities make the province of Chaco the one with the highest number of living Native American people in Argentina. The composition of the people who arrived at the province of Chaco and their subsequent admixture with individuals of native origin are the main features that make the population in this province somewhat unique.[32] Data available from northeastern Argentinian populations show differences in genetic background for both noncoding regions of the genome and for coding genes related to pain sensitivity when compared to other populations of the world.[18] A previous study on women belonging to different Native American communities living in the phytogeographic region of Gran Chaco, within which the province of Chaco is included, showed a wide range of pain responses at the moment of infant delivery among Wichí, Chané, and Guaraní communities.[35] Differing genetic variability can generate unequal phenotypic effects in different populations, particularly in those that have experienced a recent admixture process.[36]

The aim of this study was to analyze in an Argentinian population the variations of OPRM1 and OPRK1 genes of the endogenous opioid system and the related gene COMT, as well as to evaluate the associations of these genes with oral pain and the need for analgesics in the population under study.

Materials and Methods

Study Group

Adults attending one public hospital (Hospital Central de Odontología) and two private hospitals (Sanatorio Frangioli and Sanatorio Güemes) from Resistencia were asked to participate in the study. A total of 134 donors provided biologic samples for obtaining DNA and were included in the allele and genotype frequency study. Additionally, 81 of the volunteers agreed to take part in the association study by answering a questionnaire.

For comparison, a small sample of 27 Native American Wichí people from the same province were also included in this study. The estimated total Wichí population living in the province of Chaco is around 4,600 people.[37] Despite its limited number, the sample offered an approach to assess the genetic variability of this neighboring population living in the same province. The geographic locations of both populations are detailed in Fig 1. This study was part of a project previously approved by the Ethics

Table 1 Reference Number, Nucleotide and Amino Acid Changes, and Location of Markers Analyzed for OPRM1, OPRK1, and COMT Genes

Gene/marker	Change	Amino acid change	Chromosome location
OPRM1			
rs1799972	C–T	Ala→Val	Exon 1. 154360696
rs1799971	A–G	Asn→Asp	Exon 1. 154360797
rs17174794	C–G	Ser→Cys	Exon 2. 154411110
rs2075572	C–G	–	Intron 2. 15412004
rs540825	T–A	His→Gln	Exon X. 154414446
rs562859	T–C	–	Exon X. 154414573
OPRK1			
rs35566036	Ins–Del	–	Promoter. 54328138: 54328137
rs3808627	C–T	–	Promoter. 54327355
rs6985606	T–C	–	Intron 2. 54323669
COMT			
rs740603	A–G	–	Intron 1. 19945177
rs6269	A–G	–	Intron 2. 19949952
rs4633	C–T	–	Exon 3. 19950235
rs4818	G–C	–	Exon 4. 19951207
rs4680	A–G	Val→Met	Exon 4. 19951271

C = cytosine; T = thymine; A = adenine; G = guanine; Ins–Del = insertion–deletion; Ala = alanine; Val = valine; Asn = asparagine; Asp = aspartic acid; Ser = serine; Cys = cysteine; His = histidine; Gln = glutamine; Met = methionine.

Committee of Instituto Multidisciplinario de Biología Celular, and all donors gave written consent to participate in the study.

DNA Extraction and Genotyping

DNA was obtained from saliva by a protocol of proteinase K lysis followed by extractions with lithium chloride and chloroform-isoamyl alcohol, as previously reported.[38] In addition, 81 of the donors from Resistencia answered a survey about ancestry and, among other questions, how intensely they perceived oral pain during dental appointments. This was indicated on a numeric rating scale (NRS) from 0 to 10, where 0 represented no pain and 10 the worst pain imaginable.[39,40] The respondents also indicated whether they needed analgesics for oral pain relief.

Six single nucleotide polymorphisms (SNPs) of the OPRM1 gene, two SNPs and one insertion/deletion (Indel) of the OPRK1 gene, and five SNPs of COMT were analyzed. Table 1 shows the markers analyzed in detail. The primer sequences and the cycling conditions for OPRM1 were obtained from the following papers: Gelernter et al[41] for rs1799971 and rs1799972; Bergen et al[42] for rs17174794 and rs2075572; and Smith et al[43] for rs540825 and rs562859. The primers for the OPRK1 indel (rs35566036) were obtained from Edenberg et al,[14] and allele-specific primers for OPRK1 SNPs were designed in the present authors' laboratory according to the GenBank sequence NM_000912.4 as follows: Fw: 5′-TTCCTTGCGTTCTCTTCCATTCC-3′, RvG: 5′-GAGGCCCGGGTAAGGGGAG-3′, and RvA: 5′-TGAGGCCCGGGTAAGGTGAA-3′ for rs3808627; and Fw: 5′-CTTCATCTTTCAAGCACAGC-3′, RvA: 5′-AATGAAGAGACTGACGATCA-3′, and RvG: 5′-AATGAAGAGACTGAGG ATCG-3′ for rs6985606. For the COMT gene, primers and cycling conditions can be found elsewhere.[20,21,28,44]

Statistical Analyses

Allele and genotype frequencies, Hardy-Weinberg equilibrium, and fixation index (FST) were estimated using the program Arlequin 3.5.[45] For population comparisons, four populations from the 1000 Genomes Project database[46] were considered: Mexican (Mexican ancestry from Los Angeles, California, USA); European (residents of Utah, USA sampled by the Centre d′Etude du Polymorphisme Humain, with Northern and Western European ancestry); Japanese (Tokyo, Japan); and Yoruba (Ibadian, Nigeria). The results were also compared to data from Argentina obtained in the authors' laboratory, including Wichí people from the same province (obtained in the present study) and previous data on OPRM1 for the population of the neighboring province, Corrientes.[18]

As stated above, 81 participants agreed to answer a questionnaire for an association analysis. The associations of ancestry and/or genetic polymorphisms with variation in pain sensitivity were analyzed by conducting a comparative, prospective, and transversal design for this purpose.

The 81 samples were genetically studied, and information was gathered from the survey about sensitivity to oral pain stimuli and the need for analgesic drugs. Two independent variables were ancestry (South American Gran Chaco native/non-native) and genetic variation of OPRM1, OPRK1, and COMT. The polymorphisms were first analyzed separately

Table 2 Genotype Frequencies of the Analyzed Polymorphisms in the Population of Resistencia (n = 134) and in the Wichí Community (n = 27)

Gene/marker	Genotype	Resistencia	Wichí
OPRM1			
rs1799972	CC	0.984	1
	CT	0.016	0
	TT	0	0
rs1799971	AA	0.606	0.769
	AG	0.345	0.192
	GG	0.049	0.039
rs17174794	CC	0.976	1
	CG	0.024	0
	GG	0	0
rs2075572	CC	0.160	0.269
	CG	0.480	0.539
	GG	0.360	0.192
rs540825	TT	0.550	0.769
	TA	0.383	0.154
	AA	0.067	0.077
rs562859	TT	0.556	0.615
	TC	0.379	0.308
	CC	0.065	0.077
OPRK1			
rs35566036	Del/Del	0.740	0.615
	Del/Ins	0.240	0.346
	Ins/Ins	0.020	0.039
rs6985606	CC	0.544	0.346
	CT	0.387	0.539
	TT	0.069	0.115
	CC	0.621	0.385
rs3808627	CT	0.334	0.423
	TT	0.045	0.192
COMT			
rs740603	AA	0.198	0.864
	AG	0.528	0.136
	GG	0.274	0
rs6269	AA	0.417	0.769
	AG	0.444	0.231
	GG	0.139	0
rs4633	CC	0.278	0.185
	TC	0.583	0.482
	TT	0.139	0.333
rs4818	CC	0.111	0.792
	GC	0.407	0.208
	GG	0.482	0
rs4680	AA	0.148	0.154
	AG	0.537	0.500
	GG	0.315	0.346

and then analyzed together for each gene. The level of oral pain on a scale from 0 to 10 during the appointment with the dentist and the need for pain relievers related to oral pain were alternatively chosen as the dependent variable. The data were analyzed with the statistical software Medcalc[14] and VCCstat 2.0. Frequency and/or percentage distributions of each sample were set for every polymorphism. For measures on an ordinal scale, the number of cases, the minimum and maximum values, the median, and the standard deviation (SD) were computed. When necessary, significance tests were performed (analysis of variance [ANOVA], chi-square, Fisher test, Kruskal-Wallis test). The level of significance was set at $P \leq .05$.

Results

Population Study

Genotype frequencies for the OPRM1, OPRK1, and COMT polymorphisms are detailed in Table 2. All of them fit the Hardy-Weinberg equilibrium ($P > .05$). The less variable SNPs for the Resistencia population were OPRM1 rs1799972 and rs17174794 (no homozygotes were found for the less frequent allele), while the highest heterozygosity values were found for COMT rs740603, rs4633, and rs4680. Some particular alleles were not found among the Wichí individuals (Table 2).

Linkage disequilibrium was observed for the Resistencia population for four markers of OPRM1 (rs1799971, rs2075572, rs540825, rs562859), for the Indel of OPRK1 to both OPRK1 SNPs, and for pairs of markers of COMT, except for rs740603–rs4680 and rs740603–rs6269 ($P < .05$) (Table 3). Linkage disequilibrium was not calculated for the Wichí population given the small number of individuals tested.

In order to explore a possible substructuring of the sample of Resistencia, data were compared from the public hospital to data from private institutions. There were nonsignificant values for all three genes ($P > .05$). There was also no sex difference ($P > .05$).

As expected, the comparisons between the present results and those reported for populations from Asian (Japanese) and African (Yoruba) continents showed the most different values for the three genes. Moreover, significant differences were observed in the comparison of the Resistencia population to European and Mexican populations, which are more closely related in ancestry. The individuals of the Wichí population from the same province (Table 4) also showed some differences for the OPRK1 and COMT genes in comparison to the Resistencia population (data not shown), while the data on OPRM1 markers from the neighboring province of Corrientes were not significant (FST Resistencia-Corrientes = 0.08; $P = .279$).

Association Study

In the Resistencia population, the Kruskal-Wallis test showed no association between ancestry (South American Gran Chaco native or non-native) and sensitivity to oral pain stimuli ($P = .64$). Also, the analysis of associations between each genetic polymorphism and sensitivity to oral pain/analgesic requirements

Table 3 Linkage Disequilibrium Between Polymorphisms for OPRM1, OPRK1, and COMT

OPRM1	rs1799972	rs1799971	rs17174794	rs2075572	rs540825	rs562859
rs1799972	*	–	–	–	–	–
rs1799971	–	*	–	+	+	+
rs17174794	–	–	*	–	–	–
rs2075572	–	+	–	*	+	+
rs540825	–	+	–	+	*	+
rs562859	–	+	–	+	+	*
OPRK1	**rs35566036**	**rs6985606**	**rs3808627**			
rs35566036	*	+	+			
rs6985606	+	*	–			
rs3808627	+	–	*			
COMT	**rs740603**	**rs6269**	**rs4633**	**rs4818**	**rs4680**	
rs740603	*	–	+	+	–	
rs6269	–	*	+	+	+	
rs4633	+	+	*	+	+	
rs4818	+	+	+	*	+	
rs4680	–	+	+	+	*	

+ = markers are linked ($P < .05$); – = markers are not linked ($P > .05$).

Table 4 Fixation Index Values for Resistencia Population Compared to Data from the 1,000 Genomes Project

Gene/marker	European Americana	MXL	CEU	JPT	YRI
OPRM1					
rs1799972	–	–0.00503	0.00503	0.00503	0.50831**
rs1799971	–	–0.00503	0.01336	0.13770**	0.22111**
rs17174794	–	0.01005	–0.00401	0.01005	0.01005
rs2075572	–	0.05474**	–0.00250	0.11194**	0.06151**
rs540825	–	0.02482*	–0.00344	0.12621**	0.18873**
rs562859	–	0.00372	0.02518*	0.10410**	0.02518**
OPRK1					
rs35566036[a]	0.04780*	–	–	–	–
rs6985606	–	0.33907**	0.46735**	0.30366**	0.00503
rs3808627	–	0.27301**	0.18635**	0.34966**	0.00503
COMT					
rs740603	–	0.04420**	–0.00125	0.00719	0.02746**
rs4633	–	–0.00291	–0.00308	0.05029**	0.03133**
rs6269	–	0.01423	0.00812	–0.00457	–0.00484
rs4818	–	0.32842**	0.11110**	0.17749**	0.42182**
rs4680	–	–0.00426	–0.00101	0.03553**	0.01952*

[a]Data obtained from Edenberg et al.[14]

MXL = Mexican ancestry from Los Angeles, California USA; CEU = Residents of Utah, USA with North and Western European ancestry; JPT = Japanese in Tokyo, Japan; YRI = Yoruba in Ibadian, Nigeria. *$P < .05$. ** $P < .01$.

Table 5 Fisher Exact Test for COMT Gene Polymorphisms for Phenotypic Expressions Under Study at Dental Appointment

Phenotype	Marker	Test	df	*P* value
Pain intensity	rs740603	Kruskal-Wallis = 4,959	2	.0838
Requirement for analgesia	rs4680	$\chi^2 = 5.892$	2	.0526
	rs740603	$\chi^2 = 4.685$	2	.0961

df = degrees of freedom. Only positive results are shown.

did not show significant values, although the analysis of the COMT gene resulted in three cases that bordered on statistical significance (Table 5).

When the association analysis was performed combining all markers together for each gene, 78 participants were included in the COMT analysis because of missing data in 3 out of 81 samples. There were associations between three different COMT genotypes (5 SNPs) and sensitivity to oral pain (Table 6). Furthermore, a positive association occurred between the COMT gene and a low requirement for analgesia to relieve oral pain for the

Table 6 Association Analysis (Nonparametric Mann-Whitney) Between COMT Gene and Intensity of Pain During Dental Appointment

	GG/GG/CC/CC/GG		GG/GG/CT/GC/GA		GG/AG/CT/GC/GA	
Statistics	Low pain (n = 74)	High pain (n = 4)	Low pain (n = 76)	High pain (n = 2)	Low pain (n = 73)	High pain (n = 5)
Mean ± SD	4 ± 3	6 ± 1	4 ± 3	0 ± 0	4 ± 3	1 ± 2
Median (min–max)	4 (0–10)	6 (5–7)	4 (0–10)	0 (0–0)	4 (0–10)	0 (0–3)

Order of COMT polymorphisms in the three genotypes: rs740603/rs6269/rs4633/rs4818/rs4680. Only significant results are shown. SD = standard deviation.

genotype rs740603 GG/rs6269 AG/rs4633 CT/rs4818 GC/rs4680 GA (Fisher exact test, $P = .009$).

Although the number of individuals included in this study was limited, significant differences were found when compared to other populations, and positive associations for the COMT gene with oral pain and with the need for pain relievers were found in the population of Resistencia.

Discussion

The genetic variation contributing to pain perception and analgesia is likely less predictable in admixed populations than in those of European or African origin. Thus, it is important to assess such variation in order to have a better approach to analgesic treatments.

In this study, population parameters of OPRM1, OPRK1, and COMT genes were analyzed in relation to oral pain sensitivity and analgesic requirements in the Resistencia population. All markers were polymorphic for this population and in agreement with the Hardy-Weinberg equilibrium. Therefore, the absence of population substructuring allowed the association tests to be performed.

It is interesting to note that, when comparing the present results to those of four other populations in the world, several markers showed significant differences in genotype frequencies. As expected, there were marked differences in the comparison to Asian and African populations. In particular for rs1799971, the G allele is not present in the African Yoruba described in the HapMap source. It has been suggested that this allele could have arisen after the human out-of-Africa migration event,[17] thus increasing interpopulation divergence. On the other hand, the present results showed more affinity with European and Mexican populations, as seen in previous reports,[9,47] although significant differences were also found in these two latter comparisons.

The Argentinian population studied is mostly composed of people with both immigrant and native ancestors and, to a lesser extent, people descending directly from immigrants or from native ancestors. During the past century, immigrants came mainly from Europe—particularly from Italy and Spain—together with some minorities, such as Japanese people from Asia and Jewish people from Europe. However, the flow of immigrants during the last decades has mainly come from Latin American countries such as Bolivia, Paraguay, and Peru, all of which still preserve an important native component in their respective populations.[48,49] More recently, some immigrants came also from distant places in the world, such as South Korea, China, Senegal, and Cape Verde, living together in the same territory. Therefore, the European contribution to the local genetic background is progressively reducing, while contributions from different locations in South America and other continents are becoming more evident.[50] Although the number of individuals from the neighboring Wichí population included in this study was small and they live in the same province as the population studied, some differences were found in genotype frequencies compared to the Resistencia population. These native people live in isolation, maintaining a very low level of genetic flow with urban communities, in the Chaco province and in other provinces of Argentina.[51,52] The Wichí people from Chaco currently live in community settlements around urban areas, and a genetic drift process can be acting on them, thus generating the observed population differentiation.[52–56] Nevertheless, further studies including a higher number of such individuals will be needed to allow definitive conclusions.

When compared to an admixed population from the same region, the population of Resistencia showed results for OPRM1 that were similar to a previous report from the neighboring province of Corrientes.[18] Since the capital cities of Chaco and Corrientes provinces are only separated by 20 km, a migration of persons may be maintained for several reasons, such as work or university studies, thus reducing the possibility of population differentiation. This suggests that the populations from northeastern Argentina have a particular identity as a result of admixture of native and non-native genetic components, thus differentiating them from other populations of the world.

The genetic influence of the polymorphisms studied on pain perception and analgesic requirements has been proven to act in different ways depending on the genetic background of the population studied.[21,57] Therefore, the particular variability observed in these populations may affect the prevalence of the above-mentioned phenotypes.

In this study, separated markers did not show statistical significance for oral pain phenotype or analgesic requirements, except for a tendency observed in the COMT gene for rs740603 and rs4680 variants.

When the polymorphisms within each gene were considered together, significant values were found for an influence of COMT gene on both pain intensity and analgesic requirements. However, some of the genotypes influencing these phenotypes were different from the genotypes previously described for other populations.[21] Unexpectedly, some of the genotypes showing an association were heterozygous. It is more likely that a homozygous genotype can show an association with a determined phenotype or characteristic, while heterozygous genotypes are supposed to give rise to an intermediate phenotype. The large variance of admixture observed among populations from Argentina might be a confounding factor for accurate genotype-phenotype linking, since individual Amerindian contribution has been reported to vary between 1.5% and 84.5% in Argentinian subjects.[58] However, since the number of individuals included in the present analysis might be a limitation for obtaining accurate estimations, further studies including a larger number of samples are needed.

The present results suggest that the current variation of the Resistencia population may be a consequence of the particular process of admixture that occurred in its past. This variation might modify the effect of genetic polymorphisms that affect pain perception and analgesic requirement phenotypes, which runs a risk for unnecessary pain when analgesic treatments are generalized based on data from other populations.

Conclusions

This is the first report of a pain genetics study from the country of Argentina. Although the present study's conclusions are preliminary given the limited number of individuals investigated, it could be a starting point for an extended analysis within Chaco province and in other populations from Argentina. The future inclusion of a higher number of individuals will allow for more definitive results that focus on understanding the different genotype-phenotype relationships and consider in greater depth the possible influence of other factors.

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