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COL1A1 Polymorphisms in Mexican Patients with Otosclerosis. Case-Control Study

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Abstract

Objective: To determine if there is association between otosclerosis and polymorphisms of *COL1A1* in Mexican patients.

Methods: A case-control study was done with 90 cases with confirmed otosclerosis diagnosis during surgical treatment and with 135 controls.

The sample size was calculated with the programs: Sample Size OCCE and Quanto. For all patients, medical history was collected, and complete audiological studies were done: otoscopy, audiometry, tympanometry and stapedial reflex. The rs2586498 (A/G), rs1107946 (G/T), rs2141279 (C/T), rs 1800012 (G/T) SNPs in *COL1A1* gene, were analyzed. The odds ratios were obtained and the logistic regression analyses were carried out.

Results: About half of the patients have family history of otosclerosis (56%) and in almost all cases (91%) the affection was bilateral. All the polymorphisms were in Hardy-Weinberg equilibrium and none showed association with otosclerosis in Mexican patients.

Conclusions: Our results suggest that polymorphisms of the *COL1A1* gene do not contribute significantly to the genetic risk for otosclerosis in our population. None of the analyzed polymorphisms revealed statistically significant associations with the disease. However, it is necessary to study other polymorphisms in the same gene and in others.

Keywords: Otosclerosis; *COL1A1*; Polymorphisms; Mexican patients

Introduction

Otosclerosis is an illness that affects the otic capsule, caused by changes in bone metabolism. It is one of the most common causes of conductive hearing loss in adults and is progressive [1]. It is the cause of 5-9% of the cases with hearing loss and of 18-22% of the conductive type [2]. Otosclerosis is more frequent in women (female: male ratio of 2:1) [3].

The way in which otosclerosis develops is still unclear, considering the genetic aspects, otosclerosis could be considered multifactorial and genetically heterogenous, but it could also be, in less cases, secondary to an autosomal dominant inheritance with reduced penetrance [4]. Until now, 8 loci have been associated with the autosomal dominant otosclerosis, OTSC1 on chromosome 15q25-q26, OTSC2 on chromosome 7q34-q36, OTSC3 on chromosome 6p21.3-p22.3, OTSC4 on chromosome16q22.1-q23.1, OTSC5 on chromosome 3q22-q24, OTSC7 on chromosome 6q13-q16.1, OTSC8 on chromosome 9p13.1-q21.11 and finally OTSC10 (1q411-44) [2,5-12]. In its clinical form, hearing loss, and tinnitus are the main features, the patients can also suffer vertigo [12-14]. Hearing loss is usually of the conductive type, but about 10% of the patients develop sensorineural hypoacusis usually in the advanced cochlear form of the disease, however, some patients can debut with it [5,13,14].

COL1A1 was the first gene in which association with otosclerosis was sought. Important also is the fact that this gene is also associated with osteoporosis in which there is usually conductive hearing loss such as in osteogenesis imperfecta [15-17]. Both diseases, osteogenesis imperfecta and osteoporosis, were related to *COL1A1* gene and both have hearing loss, more important in some cases of osteogenesis imperfecta because this disease is a mendelian one, and osteoporosis is multifactorial [18,19]. Some studies have demonstrated there is

association between some polymorphisms and otosclerosis, especially in Caucasian patients [20]. Other genes have also been involved, such as TGFB1 [21] and RELN [22]. The aim of this study was to determine if there is association between otosclerosis and polymorphisms rs1107946, rs2586498, rs2141279 and rs1800012 of *COL1A1*. At this moment there are few studies and there is a controversy about this association.

Material and Methods

Patients

A case-control study was done with 90 cases of confirmed otosclerosis diagnosed during surgical treatment and with 135 controls. The sample size was estimated with the following programs: Sample Size OCCE and Quanto. We used the minor alelic frequencies for each polymorphism. The statistic power was 80% and the significance was 5%. The result was different for each polymorphism, so we used the largest sample size, in such a way that all the polymorphisms were included.

For all patients, medical history data was collected, and complete audiological studies were done: otoscopy, audiometry, tympanometry and stapedial reflex. All the studies were conducted according to the principles expressed in the Declaration of Helsinki and the Mexican General Health Law, the project was authorized by the INRLGII ethics committee and the patients signed the informed consent (Number: 83/15).

The controls were studied with audiometry to be sure they did not have any clinical finding of otosclerosis.

Variables such as age, gender, inbreeding, consanguinity, age at the beginning of otosclerosis, one or both sides affected, number of family members affected if there were, history of having had measles and hormone intake (in women), were collected through a questionnaire. We selected homogeneous groups based on their site of provenance and their Spanish derived last names. All individuals were unrelated and with at least three generations of Mexican mestizo ancestry, who were born in central states of Mexico.

Genotyping

A peripheral blood sample was obtained from each participant to extract DNA using the Puregene DNA extraction kit (Qiagen, Minneapolis, MN, USA). The rs2586498 (A/G), rs1107946 (G/T), rs2141279 (C/T), rs 1800012 (G/T) SNPs in COL1A1 gene, were analyzed. Genotyping was performed by real-time PCR using specific TaqMan probes following the conditions recommended by the manufacturer (Applied Biosystems, Foster city, CA, USA). Briefly, each 25µl PCR reaction contained 1X TaqMan PCR master mix, specific probe at 100nm, 900nm of each primer and 25ng of genomic DNA. Samples were run in 48-well plates in a StepOne™ Real Time PCR System (Applied Biosystems). Cycling conditions included an initial denaturation step at 95°C for 10 min, followed by 40 cycles at 92°C for 15 sec and then 60°C for 1 min.

Statistical analysis

Descriptive analysis was performed with central tendencies, mean, mode and median for quantitative variables and frequencies for qualitative. For comparative analysis of quantitative variables, aT-student test was carried out and for qualitative variables, the X^2 test.

The odds ratios were obtained, and the logistic regression models were carried out with the variables in which bivariate analysis the p result was <0.1. A p <0.05 was considered significant. The Hardy-Weinberg equilibrium was assessed for all the analyzed polymorphisms (POPGEN32 ver. 1.31, University of Alberta and Center for International Forestry Research, Edmonton, Canada).

Results

56% of the patients have a family history of otosclerosis, the rest were isolated cases. From the 90 patients, 70% were female and 30% male and in the controls 76% and 24% respectively. The mean age was 47.63 ± 9.8 years (23-65) in cases and 41.27 ± 12.05 years (24-75) in controls. The disease was bilateral in 82 cases (91%) and unilateral in 8 (9%), left side more frequently involved. The disease started before the age of 40 in 85% of the cases and the mean time of evolution was 14.12 ± 9.7 years. The air-bone gap we found was 27.58 ± 9.1 dB (11.25-53.33).

All the polymorphisms were in Hardy-Weinberg equilibrium. The allelic and genotypic frequencies were very similar in cases and in controls [Table 1]. None of the polymorphisms showed association with otosclerosis in Mexican patients [Table 2].

We found no association between the background information of measles and hormone intake (in women) with otosclerosis. None of the patients reported consanguinity or inbreeding.

Discussion

Otosclerosis is a disabling disease because it produces hearing loss. The involvement of some genesis unquestionable as has been demonstrated in familial studies with more than one member affected per family and in monozygotic twins studies [13,15,23]. Otosclerosis

Table1: Allelic and genotypic distribution of *COL1A1* SNP polymorphisms in cases with otosclerosis and in controls.

SNP	Genotype/Alele	Cases (%) N=90	Controls (%) N=135	p HW
rs 1107946	GG	19(21.11)	28(20.74)	0.061
	GT	59(65.55)	98(72.59)	
	TT	12(13.33)	9(6.66)	
	G*	97(53.88)	154(57.03)	
	T	83(46.11)	116(42.96)	
rs 2141279	CC	59(65.55)	87(64.44)	0.11
	CT	31(34.44)	48(35.55)	
	TT	0	0	
	C*	149(82.77)	222(82.22)	
	T	31(17.22)	48(17.77)	
rs 2586498	GG	63(70)	100(74.07)	0.088
	AG	27(30)	35(25.92)	
	AA	0	0	
	G	153(85)	235(87.03)	
	A*	27(15)	35(12.96)	
rs 1800012	GG	71(78.89)	105(77.78)	0.881
	GT	19(21.11)	28(20.74)	
	TT	0	2(1.48)	
	G	161(89.44)	238(88.15)	
	T*	19(10.56)	32(11.85)	

SNP: Single Nucleotide Polymorphism. HW: Hardy Weinberg equilibrium.*Ancestral allele.

Table 2: Association of *COL1A1* gene polymorphisms and otosclerosis in Mexican patients.

SNP	Alternative name	Gene position	p X^2	OR (CI 95%)
rs1107946	Pcol2	5' Up stream	0.51	0.88(0.59-1.31)
rs2141279		Intron 15	0.87	1.04 (0.61-1.76)
rs2586498	Int5/rs7406586	Intron 5	0.53	0.84(0.47-1.50)
rs1800012	Sp1	Intron 1(+11126)	0.84	0.94(0.49-1.79)

SNP: Single Nucleotide Polymorphism; p X^2: p value from X^2; OR: Odds ratio; CI: confidence interval

is considered a bone disorder; because of this, some genes involved in bone metabolism are considered excellent candidates to investigate their possible association with otosclerosis. That is the case of *COL1A1* in which many polymorphisms have been studied. Three polymorphisms we studied were implicated in otosclerosis in other populations, for example rs2586498 in Americans [15], rs2141279 in Belgian-Dutch population [16], rs1800012 in USA Caucasian [20] [Table 3].

In general, different studies show that the association is mild when present; this supports the idea that otosclerosis is a complex disease with many genes involved; reflects genetic heterogeneity and/or the presence of other factors. The rs1107946 polymorphism has been associated with osteoporosis in Mexican women [24]. However, association with otosclerosis has not been found either in Caucasian patients from USA and Germany [20], or in our Mexican patients. The study conducted with Spanish population did not show any association with *COL1A1* and *COL1A2*, but another type of polymorphisms were studied, not SNPs [25].

The epigenetic and many environmental factors have been involved. It is also important to note that not all populations are the same due to the different genetic variants they have; this origin the distinct prevalence estimated between populations. Familiar cases show there is a great heritability of this disease [26-28]. Apparently, our patients did not present association between measles and the risk of developing otosclerosis as the Japanese patients that Komune, et al. studied [29]. There are studies that show association, considering the immunological response induced by persistent measles virus infection as one of the causes of otosclerosis [30]. Since 1930 many authors have said there is an association with the occurrence or aggravation of otosclerosis related with hormone intake or pregnancy, however there is controversy about this [14,26,30,31]. The patients of our sample did not show association with measles, however, we cannot exclude this possibility because we did not study the tissue obtained in surgery, no other study was done and data about measles and hormone intake were collected only by questioning the patients. We did not have information about vaccination in patients or controls, but due to their age it is possible that more controls than cases had measles vaccine, since Mexico started using it in 1970 [32].

Because of the important structural role of *COL1A1* in bone and the previous association of *COL1A1* rs1800012 and rs1107946 polymorphisms with osteoporosis in Mexican women [24], we thought these polymorphisms could be associated with otosclerosis. However, in our sample of Mexican patients association with otosclerosis has not been found. The same lack of association occurred in Caucasian patients from USA and Germany [20] and in a study conducted with Spanish population which did not show any association with *COL1A1* and *COL1A2* polymorphisms [25].

Table 3: Association of *COL1A1* polymorphisms and otosclerosis in different populations.

	Americans McKenna et al. (1998)[15]	Belgian-Dutch Schrauwen et al. (2012)[16]	White Americans Chen et al. (2007)[18]	Mexican This study (2019)
rs2586498	Yes			No
rs2141279		Yes		No
rs1800012			Yes	No
rs1107946				No

This lack of association of the *COL1A1* rs1800012 and rs1107946 polymorphisms with otosclerosis was unexpected due to the mentioned close association between osteoporosis and hearing loss, but we must take into consideration that these and other polymorphisms have showed association with osteoporosis risk condition in one anatomical area but not in other, such as hip and spine, even in the same sample [33,34]. Additionally, a weakness of this study was no taking into account the genetic stratification of Mexican population, which could mask the associations of the polymorphisms with otosclerosis or on the other hand, this could give rise to spurious associations.

Conclusion

In conclusion our study shows that the *COL1A1* polymorphisms analyzed in this sample of Mexican patients with otosclerosis seem not to be associated with the disease. However, it is necessary to corroborate these findings and study other polymorphisms in the same gene and in others.

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The authors declare there is not any conflict of interest.

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