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Vitamin D receptor gene polymorphisms and susceptibility for primary osteoarthritis of the knee in a Latin American population

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Abstract

Background: Primary Osteoarthritis (OA) of the knee is a multifactorial disease that has an important genetic component, and several genes have been associated with its development. The vitamin D receptor has a role in skeletal metabolism that suggests a relationship with OA. The aim of this study was to analyze the association of Vitamin D receptor gene (*VDR*) polymorphisms in Mexican Mestizo patients.

Methods: A case-control study was conducted in which 107 cases with primary OA of the knee and 114 controls were included. Cases were patients > 40 years of age with a Body mass index (BMI) of ≤27 and a radiological score for OA of the knee of ≥2. Controls were subjects > 40 years of age with a radiological score of < 2. *VDR* polymorphisms rs1544410, rs7975232, and rs731236 were analyzed by means of restriction endonucleases, and logistic regression was developed to evaluate risk magnitude.

Results: A significantly increased risk was found of nearly two-fold for the allele T and TT genotypes of rs731236, independently of other well recognized risk factors.

Conclusions: The rs731236 polymorphism is associated with the risk of primary OA of the knee in Mexican Mestizo population.

Keywords: Osteoarthritis, Vitamin D receptor gene, Polymorphism, Mexican Mestizo population, Association study

Background

Osteoarthritis (OA) is the most frequent form of arthritis and is a leading cause of musculoskeletal disability worldwide. The World Health Organization (WHO) estimates that approximately 10% of the world's population aged ≥60 years have symptomatic OA and that it is the fourth leading cause of Years lived with disability (YLD) [1, 2]. OA can occur in any joint, but the knee is the most common site involved; in fact, it is considered that 6% of adults can be affected and that this is one of the most common reasons for total joint replacement [3–5]. OA is characterized by progressive degeneration of articular cartilage in synovial joints, resulting in joint space narrowing, osteophyte formation, and subchondral sclerosis, which is clinically translated as pain and joint stiffness [4, 5].

OA is a multifactorial disease in which genetics and environmental factors, such as aging, gender, obesity, significant trauma, occupation, and sports activities, among others, are strongly related with its development [4, 5]. It is classified as primary when no discernible cause is evident and secondary when a triggering factor is apparent. Primary OA possess a strong genetic component, as demonstrated by several twin and family studies, which have demonstrated 39–65% heritability (h^2) and an increased risk for OA of up to 14-fold in first-degree relatives of probands with OA [6, 7]. On the other hand, genetic association studies have demonstrated that primary OA is associated with several genes related to different molecular pathways or classes of molecules such as inflammation, Extracellular matrix

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(ECM) molecules, Wnt signaling, Bone morphogenetic proteins, proteases or their inhibitors, and genes related with modulation of osteocyte or chondrocyte differentiation [6, 7].

The Vitamin D receptor plays an important role in skeletal metabolism because this acts as an important regulator of calcium metabolism and bone cell function; therefore, its abnormalities are probably related with OA [8]. The vitamin D receptor gene (*VDR*) is located on chromosome 12q13.11, contains 11 exons, and spans approximately 75 kb. The gene contains several polymorphisms, and three have been frequently studied for determining an association in OA: rs1544410 and rs7975232 in intron 8, and the synonymous variant rs731236 in exon 9 [9–11]. With regard to primary OA of the knee, some reports have shown an association in the presence of these *VDR* polymorphisms [12–14], however, this has not always been confirmed [15–18]. A meta-analysis on the three most frequently studied *VDR* polymorphisms in OA analyzed Asian and European studies; however, the results showed no association in all study subjects, as well as by stratification by ethnicity [19]. An updated meta-analysis, showed a significant association between the A allele and AA genotype of the rs7975232 with OA in Asian population, but not in the whole population [20]. Because genetic associations could vary among populations and because there are no association studies on *VDR* and OA in Latin-American populations, our aim was to analyze the association of the three *VDR* polymorphisms in Mexican Mestizo patients.

Methods

Subjects

We conducted a case-control study whose protocol was approved by the Ethics and Investigation Committee of the National Rehabilitation Institute, a tertiary-care referral center in Mexico City. All of the participants were recruited at the Articular Rehabilitation Clinic and were of Mexican Mestizo origin, the latter defined as a person born in Mexico, with a Spanish-derived last name, and with a family of Mexican ancestors back to the third generation [21]. Cases included persons aged > 40 years with a clinical diagnosis of OA and a radiologic score of ≥2 for OA of the knee, with a Body mass index (BMI, kg/m^2) of ≤27, with no history of serious injuries or knee surgeries, and with no other articular diseases. Controls were subjects aged > 40 years without a clinical diagnosis of OA of the knee, with a radiologic score of < 2, and with no history of serious knee injuries or diseases of the joints. All controls arrived at the clinic mainly due to orthopedic problems, such as shoulder lesions or fractures, or orthopedic problems not involving serious knee damage. Radiological evaluation of all participants was performed by a sole trained observer who was blinded to the patients' diagnosis. Grading of OA was assessed using a 5-point scale according to the Kellgren-Lawrence radiographic-classification grading method in anteroposterior weight-bearing and lateral x-rays of the knees [22]. To perform a more efficient classification of cases and controls and to identify possible co-variables or confounders, all study subjects were interviewed by application of a questionnaire designed specifically for this study in order to collect information regarding general, occupational, and sports activities, possible knee injuries, and clinical manifestations of OA, among others.

Genotyping

After obtaining signed informed consent, a 5-ml blood sample was drawn from each patient into tubes containing EDTA. Peripheral blood mononuclear cells were isolated, and DNA was extracted utilizing a salting out method. The genotype for three polymorphisms of the *VDR* was determined by Polymerase-chain-reaction (PCR) amplification and enzymatic digestion of the products using the primer pair listed previously [23]. The forward primer was the same for all three polymorphisms: 5′-CAACCAAGACTACAAGTACCGCGTCAGTGA-3′. For rs7975232 and rs731236 polymorphisms, the reverse primer was 5′-CACTTCGAGCACAAGGGGCGTTAGC-3′; and for rs1544410 was 5′-AACCAGCGGGAAGAGGTCAAGGG-3′. PCR was performed with a Gene Amp PCR system 9700 PE Applied Biosystems under standard conditions. Briefly, for fragment amplification 1X buffer solution was used (KCl 50 mM, Tris-HCl 20 mM pH 8.4), 0.6 mM DNTP, 0.5 μM of each primer, 4 mM MgCl2, 2.5 U Taq polymerase, and 250 ng genomic DNA, for a final volume reaction of 50 μL. A thermal profile was optimized as follows: 94 °C for 5 min for initial denaturation, followed by 28 cycles at 94 °C for 1 min, at 65 °C for 1 min, at 72 °C for 1 min, and 5 min at 72 °C for final extension. Subsequently, one microgram of the PCR product was digested with an excess of the endonucleases under conditions specified by the supplier (New England Biolabs, Inc., Beverly, MA, USA) and was electrophoresed on 1.5% ethidium- stained agarose gels. Information of Single Nucleotide Polymorphism (SNP) and product size after digestion with endonucleases is shown in Table 1.

Statistical analysis

Comparisons of continuous variables were tested by the Student *t* test, and corrected chi-squared statistics (x^2) were applied for categorical variables. Uni- and multivariate non-conditional logistic regression analyses were conducted to estimate probability for developing OA, comparing genotypes as main effect; Odds ratios and 95% Confidence intervals [OR (95% CI)] were reported. Alpha level was 0.05. Hardy-Weinberg equilibrium (HWE) was assessed for *VDR* polymorphisms by means of the chi-squared test, and the

Table 1 Information of the three single nucleotide polymorphisms of VDR gene

SNP	Alleles	Location	Change	Enzyme	Fragments (bp)		Frequencies Cases, controls	HWE
rs1544410	A/G	Intron 8	None	BsmI	G: 650/175	A: 825	0.615, 0.576	< 0.05
rs7975232	A/C	Intron 8	None	ApaI	C: 1700/300	A: 2000	0.528, 0.500	< 0.05
rs731236	C/T	Exon 9	Ile352=	TaqI	C: 1800/200	T: 2000	0.835, 0.759	0.28

SNP: rs numbers were taken from NCBI dbSNP (http://www.ncbi.nlm.nih.gov/SNP)
HWE *p* value in the control group

STATA ver.10.0 statistical software package and Haplo View 4.0 were utilized for calculations.

Results

The characteristics of the study population are shown in Table 2. We observed statistically significant differences in mean age and in previous sport-activity frequency ($p = 0.00001$ and 0.004; respectively).

The SNPs rs1544410 and rs7975232 were not in HWE (Table 1) and their allelic and genotypic frequencies did not showed significant differences between the study groups (Tables 1 and 3). Only rs731236 was in HWE, and its C and T alleles showed statistically significant differences [OR (95% CI) = 0.6 (0.4–1.0) and 1.6 (1.0–2.6); respectively]. In regard to its genotypes, CT genotype suggested a protective factor [OR (95%CI) = 0.5 (0.3–0.9)], and TT genotype exhibited an increased risk with an OR (95%CI) of 1.96 (1.1–3.4) (Table 3). For multivariate analysis two models were constructed, and the risk trends for CT and TT genotypes were maintained when these results were adjusted for gender, age, BMI, and previous sport activity (Table 4).

Table 2 General characteristics of the study population

	Cases ($n = 107$)	Controls ($n = 114$)	*p*
Age (mean ± SD years)	57.6 ± 8.9	51.7 ± 8.7	0.00001
BMI (mean ± SD kg/m^2)	26.4 ± 2.8	25.7 ± 3.4	0.08
Gender, females (*n*, %)	86 (81.1)	95 (83.3)	0.66
Smoking (*n*, %)	21 (26.9)	11 (26.83)	0.9
Alcoholism (*n*, %)	22 (28.2)	10 (24.4)	0.6
Occupational activity (n, %)			
Current (*n*, %)	9 (8.4)	11 (9.6)	0.7
Previous (*n*, %)	11 (10.3)	14 (12.3)	0.6
Sports activity			
Current (*n*, %)	18 (16.8)	29 (25.4)	0.12
Previous (*n*, %)	51 (47.7)	76 (66.7)	0.004
Kellgren-Lawrence grading			
Grade 0, *n* (%)	0	70 (61.4)	
Grade 1, *n* (%)	0	44 (38.6)	
Grade 2, *n* (%)	38 (35.5)	0	
Grade 3, *n* (%)	46 (42.9)	0	
Grade 4, *n* (%)	23 (21.5)	0	

Discussion

VDR polymorphisms are probably among those most studied for a genetic association in OA; however, there is no consistency in the results [12–18]. Even in the meta-analyses, there is no agreement in their findings [19, 20]. Those studies could entertain some limitations, because of the absence of analysis by OA site, since it is important to

Table 3 Allelic and genotype association testing results of rs1544410, rs7975232, and rs731236 in VDR for Mexican cases with osteoarthritis of the knee and controls

SNP	Cases ($n = 107$) *n* (%)	Controls ($n = 114$) *n* (%)	OR (CI 95%)[a]	*p*
rs1544410				
Allele				
A	82 (38.0)	97 (43.0)	0.8 (0.6–1.2)	0.4
G	132 (62.0)	131 (57.0)	1.1 (0.8–1.8)	0.4
Genotype				
AA	4 (3.7)	9 (7.9)	0.4 (0.1–1.5)	0.2
GA	74 (69.2)	79 (69.3)	0.9 (0.5–1.8)	0.9
GG	29 (27.1)	26 (22.8)	1.2 (0.6–2.3)	0.5
rs7975232				
Allele				
G	112 (52.0)	115 (50.0)	1.1 (0.7–1.6)	0.7
T	102 (48.0)	113 (50.0)	0.9 (0.6–1.4)	0.7
Genotype				
GG	17 (15.9)	16 (14.0)	1.1 (0.5–2.4)	0.7
GT	78 (72.9)	83 (72.8)	1.0 (0.5–1.8)	0.9
TT	12 (11.2)	15 (13.2)	0.8 (0.3–1.9)	0.7
rs731236				
Allele				
C	35 (16.0)	55 (24.0)	0.6 (0.4–1.0)	0.04
T	179 (84.0)	173 (76.0)	1.6 (1.0–2.6)	0.04
Genotype				
CC	3 (2.8)	3 (2.6)	1.1 (0.2–5.4)	0.9
CT	29 (27.1)	49 (42.9)	0.5 (0.3–0.9)	0.01
TT	75 (70.1)	62 (54.4)	1.9 (1.1–3.4)	0.02

[a]Unadjusted Odds ratios and 95% Confidence intervals [OR (95% CI)]

Table 4 Multivariate analysis results of rs731236 for Mexican cases with osteoarthritis of the knee and controls

Genotype	OR (CI 95%)[a]	*p*	OR (CI 95%)[b]	*p*
CC	0.9 (0.1–6.0)	0.9	1.1 (0.2–6.7)	0.9
CT	0.5 (0.3–0.9)	0.03	0.5 (0.3–0.9)	0.03
TT	1.9 (1.1–3.4)	0.03	1.8 (1.02–3.3)	0.04

[a]adjusted by gender, age, BMI
[b]adjusted by gender, age, BMI, previous sport activities

consider that associations in OA appear to be joint-specific, as suggested by association studies [6, 24] and supported by functional analyses [25]. Therefore, the genetic associations in OA of the knee should be analyzed independently of other anatomic sites. Our findings suggest that there is an association between the rs731236 polymorphism and knee OA in this Mexican Mestizo population, conferring an increased risk of nearly two-fold in the presence of T allele and TT genotype independently of other well recognized risk factors such as age, gender, BMI, and sport activities.

The rs731236 is a synonymous polymorphism located in the coding sequence and exerts no effect in the encoded protein [10, 11]. However, a functional effect of this polymorphism is suggested by studies in which homozygous TT were associated with low *VDR* messenger RNA (mRNA) levels, and with low serum vitamin D level in some types of cancer [26, 27]. Interestingly, Subjects with low serum levels of vitamin D had a 3-fold increased risk for progression of OA of the knee [28]. Moreover, the activity of Matrix metalloproteinases (MMP) in the growth plate chondrocyte is regulated by vitamin D [29], and low levels of vitamin D increase MMP activities [30], which contributes to cartilage degradation, the hallmark in OA [4, 5].

On the other hand, it is possible that the rs731236 reflects a real association with other genes located in the same chromosomal region, such as Collagen type II alpha 1 chain (*COL2A1)*, which is localized at a distance of 20 kb upstream [31]. Indeed, previously an association of *COL2A1* polymorphisms with knee OA was observed [32]. Or Histone deacetylase 7 (*HADC7),* at 10 kb downstream, which although there are not association studies with *HDAC7* polymorphisms, its increased expression in the cartilage of patients with OA suggests that it may contribute to cartilage degradation [33].

VDR polymorphisms have been analyzed in different ethnic groups and differences in allele frequencies have been noted [11]; this could be due to population stratification and could explain the inconsistency in the results. For case-control genetic-association studies, this is a matter of concern because it has been suggested that the existence of genetic subgroups in a population may lead to spurious associations [34]. To control this genetic confounder, ancestry informative markers are suggested to be analyzed to avoid bias [35]. Mexican Mestizos are an admixed population [36], therefore, a possible weakness in this work is that ethnicity was determined only by self-reported family ancestry and by family history. However, the degree to which population stratification has caused confounding remains controversial [37, 38], and it has been suggested that this should not be a major confounder and that self-reported ethnicity may be sufficient to resolve that bias [38, 39]. In fact, it have been demonstrated that self-identified ethnicity correlate well with ancestral markers [40, 41]. Additionally, it is also important to consider that our controls were originated from the same geographic regions of the country as that of cases, and that the allele associated with primary OA of the knee follows HWE. This may indicate that allele frequency is not affected by inbreeding, mutation, natural selection, migration, or even population stratification [42]. Thus, spurious associations resulting from the presence of genetically different strata in our study sample are unlikely.

We are aware that being a hospital-based case-control study, it is exposed to incurring in selection bias. Therefore, to assess cases with primary OA of the knee as close as possible, the variables considerably associated with the development of secondary OA were strictly controlled during study-subject recruitment. Therefore, we think that the possibility of selection bias in our sample is low. Additionally, other potential confounders were controlled during statistical analyses, through multivariate analysis because this protects against population structure and limits the number of false positives [43]. In that sense, variables with significant differences during univariate analysis did not exhibit an effect on risk magnitude during multivariate analysis.

We recognize that our main limitation comprises sample size. However, we attempted to increase our internal validity by strictly controlling possible confounder variables through selection criteria and multivariate analysis, and we consider that our cases are truly primary OA of the knees. Finally, it is important to consider that association studies in diverse ethnic groups worldwide, especially those with a complex admixture of ancestral populations such as Latin-American populations, are a powerful resource for analyzing the genetic bases of complex diseases [44]; therefore, genetic association studies in Latino Americans would provide a better appreciation of the genetic contributions to primary OA.

Conclusions

According to the findings of this study, the rs731236 polymorphism is associated with the risk of primary OA of the knee in Mexican Mestizo population.

Abbreviations
BMI: Body mass index; *COL2A1*: Collagen type II alpha 1 chain gene; ECM: Extracellular matrix; *HADC7*: Histone deacetylase 7 gene; HWE: Hardy-Weinberg equilibrium; MMP: Matrix metalloproteinases; mRNA: messenger RNA; OA: Osteoarthritis; OR (95% CI): Odds ratios (95% Confidence intervals); PCR: Polymerase-chain-reaction; SNP: Single Nucleotide Polymorphism; *VDR*: Vitamin D receptor gene; WHO: World Health Organization; YLD: Years lived with disability

Authors' contributions
Concept and Study design: NCG-H, VMB-C and AM-D. Data acquisition: NCG-H, VMB-C, EM-H, CD-S. Data analysis, manuscript preparation: AM-D. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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