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Analysis of Polymorphisms in the *MATN3* and *DOT1L* Genes and CTX-II Urinary Levels in Patients with Knee Osteoarthritis in a Northeast Mexican-Mestizo Population

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Aims: To investigate the possible roles of the single nucleotide polymorphisms (SNPs) *MATN3* (rs77245812) and *DOT1L* (rs12982744) with susceptibility to knee osteoarthritis (KOA) among mestizos from the northeast region of Mexico. In addition, we analyzed the relationship of their urinary levels of carboxy terminal telopeptide of collagen type II (CTX-II) and the radiological grade of disease.
Materials and Methods: A total of 223 individuals from a Northeast Mexico Mestizo population were included in this study: 110 patients with primary KOA and 113 healthy controls. Genotyping of the *MATN3* (rs77245812) and *DOT1L* (rs12982744) SNPs was performed by real-time polymerase chain reaction.
Results: No association was found between the polymorphisms *MATN3* (rs77245812), *DOT1L* (rs12982744), and the risk of developing KOA (odds ratio [OR] = 1.33, 95% confidence interval [CI] = 0.42–6.48, $p = 0.621$) (OR = 2.03, 95% CI = 0.35–11.5, $p = 0.422$). However, urinary CTX-II levels were considerably higher by radiographic grade.
Conclusions: An increase in CTX-II per radiographic grade was observed in the case group, but no association was found between *MATN3* and *DOT1L* genes and the risk of KOA in Mexican mestizos.

Keywords: *MATN3, DOT1L*, CTX-II, knee osteoarthritis

Introduction

Primary knee osteoarthritis (KOA) is the most frequent arthritis and one of the main causes of musculoskeletal disability in adults (Reginster, 2002). KOA is characterized by the degeneration of the cartilage and a narrowing of the joint space, leading to formation of osteophytes and subchondral sclerosis that causes pain and stiffness (Sun *et al.*, 2007). Clinically, the most affected joint is the knee. The estimated prevalence of KOA is 30% in people older than 65 years of age, presenting also a significant morbidity (Felson *et al.*, 1987; Spector and Hart, 1992). KOA is a complex disease, in which both the etiology and its pathogenesis are not yet fully understood. The main risk factors related to the appearance and progression of KOA are aging, hormonal factor, genetics, obesity, traumatisms, and inflammation (Blagojevic *et al.*, 2010; Leung *et al.*, 2014; Silverwood *et al.*, 2015).

Matrilin is a protein encoded by the *MATN3* gene, which is present on the short arm of chromosome 2, 2p24-p23. It consists of von Willebrand factor A domains, epidermal growth factor-like domains, and a C-terminal coiled-coil domain. In cartilaginous, matrix interacts with collagen fibrils, multiple proteoglycans, and other glycoproteins (Deák *et al.*, 1999).

In humans, nonsynonymous mutations in the *MATN3* gene affecting the vWFA domain (typically β-pleated leaf) have been found in patients with multiple epiphyseal dysplasia and early-onset OA (Chapman *et al.*, 2001; Mostert *et al.*, 2003; Mabuchi *et al.*, 2004; Mäkitie *et al.*, 2004).

Mutations in *MATN3* have also been reported in other osteochondrodysplasias, such as in bilateral hereditary microepiphyseal dysplasia, spondyloepimetaphyseal dysplasia (Borochowitz *et al.*, 2004), and idiopathic OA of the hand (Stefánsson *et al.*, 2003). Matrilin-3 is an integral component of the human articular cartilage matrix and it has been

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postulated that expression of matrilin-3 in OA may be a cellular response to the modified microenvironment in the disease (Pullig *et al.*, 2002). These findings suggest that the strict regulation of matrilin-3 expression is essential for the maintenance of the microenvironment in the extracellular matrix of cartilage. Few studies have aimed to investigate the relationship between rs77245812 in *MATN3* and KOA.

There are also several Wnt signaling pathways in which the formation of cartilage and bone and the development of the synovial joint are found (Hartmann and Tabin, 2001). For instance, *DOT1L* is a histone methyltransferase that was first identified in *Saccharomyces cerevisiae* as a telomeric silencing disruptor (Singer *et al.*, 1998). Previous studies have identified variants of the *DOT1L* gene associated with hip OA and the width of the joint space in individuals of European ancestry (Castano Betancourt *et al.*, 2012). There has been much evidence linking OA pathogenesis and Wnt/b-catenin signaling cascade in developing chondrocytes, prompting many scientists to characterize Wnt as an important pathway in OA (Warde, 2011; Sassi *et al.*, 2014).

On the contrary, the determination of biomarkers in OA has been proven as a useful tool to evaluate joint injury, intervention procedures, research, and prognosis. One of the most recently studied biomarkers to evaluate the severity and degradation of cartilage is the carboxy terminal telopeptide of collagen type II (CTX-II), which is used in cartilage lesions because of its relationship with the radiological grade and the clinical parameters of OA (Henrotin *et al.*, 2016). Thus, the analysis of CTX-II levels seems to be an effective way to determine the collection of type II collagen and its relationship with the radiological grade of the disease (Mazières *et al.*, 2006).

Because of the bias in the inclusion criteria of patients with OA, that is, different sites of the joint affected by the disease, different classification, and stratification designs, the variability of the scales used in the radiographic evaluation and subjective scores in patient pain assessment, several association studies between single nucleotide polymorphisms (SNPs) and OA remain controversial (Brazier *et al.*, 1999; Sheehy *et al.*, 2015). In addition, it is convenient to explore the ethnic and geographical allelic distribution, which is extremely important to fully understand the effects of the genetic variants.

Recently, the prevalence of KOA in Mexican population has been reported ~17.6% (Macías-Hernández *et al.*, 2018). There is lack of studies that relate KOA with genetic polymorphisms in the Mexican mestizo population; thus, we report the results of a study on this genetically distinct human population given the high prevalence of the disease and the socioeconomic impact it has in Mexico. In this study, we investigate the possible role of SNPs such as *MATN3* (rs77245812) and *DOT1L* (rs12982744) and susceptibility to KOA. In addition, we analyzed the relationship of the urinary levels of CTX-II and the radiological grade of the disease in mestizos from the northeast region of Mexico.

Materials and Methods

Patients

An observational, analytical, case–control study was carried out. Patients recruited in this study were unrelated Mexican mestizos who settled in the northeastern region of Mexico for at least three generations, with a total of 223 people, 110 patients with primary OA knee, and 113 healthy controls matched by age and sex, who were recruited in the Orthopedic Department of the Social Security Hospital of the city of Gomez Palacio, Durango, Mexico. This study was carried out in accordance with the ethical guidelines of the Declaration of Helsinki and the letter of approval was obtained from the Institutional Ethics Committee of the Universidad Juárez del Estado de Durango before carrying out the study.

OA radiographic evaluation

The diagnosis of KOA was based on the criteria of the American College of Rheumatology (Altman *et al.*, 1986). Subsequently, to confirm the status of the cases and controls, a complete clinical history and physical examination was performed. In addition, for both groups, anteroposterior and lateral radiographs were made in 30-degree knee flexion. In addition, the Kellgren–Lawrence radiological criteria were used to classify the severity of the disease (Kellgren and Lawrence, 1957). The radiological grade was classified as mild (KL grade 2), moderate (KL grade 3), and severe (KL grade 4). Patients with inflammatory arthritis, post-traumatic arthritis, or some other rheumatic disease were excluded from the study. The control group was selected from the same hospital, among individuals for whom knee X-rays were requested for any other complaint but had no signs or symptoms of arthritis or joint disease. The study groups were interviewed to obtain demographic data and risk factors associated with the pathology.

DNA extraction

Blood samples were obtained by venous puncture and were collected in vacutainer tubes with ethylenediaminetetraacetic acid. DNA isolation was performed by the ''salting out'' method (Miller *et al.*, 1988). The optical density reading was performed at 260 and 280 nm with 1.8 μL ratios to measure DNA concentrations by spectrophotometry using the nanodrop 2000 equipment (NanoDrop, Berlin, Germany). The purified DNA had an average ratio OD 260/280 in the range of 1.8–1.9. Three microliters was added to the solution containing the DNA probe (10 ng) and adjusted to a final volume of 20 μL by adding nuclease-free water (all reagents from Applied Biosystems, Foster City, CA). Samples were preserved at −20°C until the genotype was determined.

DNA genotyping

The amplification of the polymorphism *MATN3* (rs77245812) and *DOT1L* (rs12982744) was determined by quantitative real-time polymerase chain reaction (qRT-PCR) using the Applied Biosystems 7300 Real-Time PCR System software (Thermo Fischer Scientific, Waltham, MA). Amplification reactions were carried out in each sample in duplicate on 96-well plates. The sequence primers used for genotyping analysis were 5′TCACGTCACTTCAGGCTGT3′ and 5′TGGGGTCACCATGTTCTC3′ for *MATN3*, and 5′CACTAGCCACTCGGCTGTGGGCACC3′ and 5′GACA TGTGGCTGGCGGGAAGGAGCT3′ for *DOT1L*. In an optical quality lid PCR tube, 10 μL of the Master Mix TaqMan Genotyping and 0.5 μL including the polymorphic sequence

and two probes labeled with 2′-chloro-7′-phenyl-1, 4-dichloro-6-carboxyfluorescein and 6-carboxyfluorescein were added to detect both alleles. Then, 5 μL was added to the solution containing the DNA probe (10 ng) and adjusted to a final volume of 20 μL by adding nuclease-free water (all reagents from Applied Biosystems). Real-time PCR thermocycling (Applied Biosystems) was carried out under the following conditions: a polymerase activation cycle of 95°C for 10 min, then 60 cycles of denaturation at 92°C during 15 s, and alignment/extension at 60°C for 1 min.

Positive and negative controls were included in the same plate to guarantee the validity and reproducibility of the method. Allelic discrimination was performed using the manufacturer's software system (Applied Biosystems).

CTX-II quantification

The urine samples corresponding to the first urination of the day were collected in a plastic container and stored at −80°C for further analysis. Measurements of urine concentrations (ng/L) of the CTX-II were quantified in both cases and controls. Urinary CTX-II concentrations were measured using an enzyme-linked competitive immunosorbent assay (ELISA) (US Biological Life Science, MA) following the manufacturer's instructions. Urinary CTX-II levels were adjusted for urinary creatinine concentrations (mM).

Statistical analysis

A descriptive analysis of the population under study was carried out to evaluate differences between groups; the chi-square test was used for categorical variables and the Mann–Whitney *U* test for nonparametric continuous variables. Allelic and genotypic frequencies were calculated by direct counting. In addition, Hardy–Weinberg equilibrium was performed for all SNPs. Genotypic frequencies were estimated under dominant, codominant, and recessive models. Binary logistic regression analysis was used to calculate odds ratios and their corresponding 95% confidence intervals. In addition, the genetic models were adjusted by body mass index (BMI). The median levels of CTX-II, BMI, glucose, cholesterol, and triglycerides were calculated by radiographic grade. In addition, a Spearman's correlation was carried out for BMI and CTX-II levels by radiographic grade. Statistical analysis was performed using the statistical program STATA 14 (Stata Corp., College Station, TX). The statistical significance was indicated by a value of $p < 0.05$.

Results

The general characteristics and clinical data of all the study groups are summarized in Table 1. Cases of KOA and healthy controls were matched by age ($p = 0.646$) and sex ($p = 0.736$), for which no statistical difference was found. When comparing the BMI, this was higher in KOA patients (median, 29.5 kg/m^2) than in controls (median, 27.05 kg/m^2) ($p = 0.003$). In addition, a statistically significant difference was found in concentrations of CTX-II in OA cases compared with controls (309.5 vs. 99.9 ng/mmol, respectively) ($p = 0.001$). The distribution of patients according to the degree of severity was as follows: grade 2 (46.3%), grade 3 (35.5%), and grade 4 (18.2%) based on the Kellgren–Lawrence radiological scale.

Table 1. Clinical Characteristics of Knee Osteoarthritis Patients and Control Subjects

Characteristics	*KOA patients* n = *110*	*Controls* n = *113*	p
Age (years)[a]	53.1 (44–62)	53.4 (45–62)	0.646
Sex (%)[b]			
Female	57 (51.8)	56 (49.5)	
Male[a]	53 (48.2)	57 (50.5)	0.736
BMI (kg/m^2)[a]	29.5 (25.7–31.9)	27.05 (24.9–30.3)	0.003[c]
CTX-II (ng/mmol)[a]	309.5 (249.7–384.9)	99.9 (93.7–105.7)	0.001[c]
Radiological grade (%)[d]			
KL 2	51 (46.3)		
KL 3	39 (35.5)		
KL 4	20 (18.2)		

[a]Median, IQR defined as the 25th to 75th percentile range. Mann–Whitney *U* test.
[b]Percentage (%), chi-square test.
[c]Significant if $p < 0.05$.
[d]Radiological grade (Kellgren and Lawrence).
BMI, body mass index; CTX-II, carboxy terminal telopeptide of collagen type II; IQR, interquartile range; KL, Kellgren–Lawrence; KOA, knee osteoarthritis.

Polymorphisms in *MATN3* (rs77245812) and *DOT1L* (rs12982744) were in Hardy–Weinberg equilibrium ($p > 0.05$). No significance association was found with KOA for rs77245812 and rs12982744 SNPs (Table 2).

Regarding the evaluation of biochemical parameters of KOA and radiographic grade, only urinary levels of CTX-II were associated with the severity of the disease ($p = 0.001$) as given in Table 3. BMI was not associated with KOA radiographic grades ($p = 0.232$); however, after performing a Spearman's correlation BMI was only correlated to KOA grade 3, $r = 0.428$, $p = 0.007$ (Fig. 1). We also analyzed a possible relationship between CTX-II urinary levels with the polymorphisms. As given in Table 4, urinary levels of CTX-II were higher in the CT genotype and CC genotype (202.2 and 215.2 ng/mmol) of the *MATN3 rs77245812* and *DOT1L rs12982744* genes, respectively; however, no statistical significance was found ($p = 0.64$ and $p = 0.80$, respectively).

Discussion

Articular cartilage contains a considerable amount of *MATN3*. The expression of protein is necessary for homeostasis of extracellular matrix (Klatt *et al.*, 2011). The relevance of *MATN3* in cartilage homeostasis has also been studied in the past. Although the role of *MATN3* for the risk to OA is not clear, some studies have shown the importance of variations in the *MATN3* gene region as a risk factor for OA (Pullig *et al.*, 2002, Otten *et al.*, 2005; Diab *et al.*, 2017).

In a Genome-Wide Association Analysis study, the association of *MATN3* with hand OA in a population of Iceland was demonstrated (Stefánsson *et al.*, 2003). On the contrary, a study suggested the relationship of *MATN3* with lumbar disk degeneration and hand OA (Min *et al.*, 2006). Of interest, in a Caucasian population no association between *MATN3* and KOA was found (Pullig *et al.*, 2007). In addition, in a study by Diab *et al.* (2017), in Egyptian patients, *MATN3* gene was associated with risk and severity of knee OA. Gu

Table 2. Association Analysis of Polymorphisms rs77245812 and rs12982744 with the Risk of Knee Osteoarthritis

Genotype	*KOA patients* n = *110 (%)*	*Controls* n = *113 (%)*	*OR (95% CI)*	p
MATN3 rs77245812				
Dominant				—
CC	55 (50)	63 (55.7)	1	
CT+TT	55 (50)	50 (44.3)	1.26 (0.74–2.13)	0.39
Recessive				
CC+CT	103 (93.6)	107 (94.6)	1	—
TT	7 (6.4)	6 (5.4)	1.21 (0.39–3.72)	0.737
Codominant				
CC	55 (50)	63 (55.7)	1	—
CT	48 (43.6)	44 (38.9)	1.24 (0.72–2.15)	0.424
TT	7 (6.4)	6 (5.4)	1.33 (0.42–6.48)	0.621
HWE (*p*-value)	0.413	0.635		
DOT1L rs12982744				
Dominant				
GG	56 (50.9)	57 (50.4)	1	—
GC+CC	54 (49.1)	56 (49.5)	0.98 (0.58–1.65)	0.944
Recessive				
GG+GC	106 (96.3)	111 (98.2)	1	—
CC	4 (3.7)	2 (1.8)	2.09 (0.37–11.6)	0.399
Codominant				
GG	56 (50.9)	57 (50.4)	1	—
GC	50 (45.5)	54 (47.7)	0.94 (0.55–1.60)	0.827
CC	4 (3.6)	2 (1.9)	2.03 (0.35–11.5)	0.422
HWE (*p*-value)	0.073	0.007		

$n = 223$.
Binary logistic regression analysis.
CI, confidence interval; HWE, Hardy–Weinberg equilibrium; OR, odds ratio.

et al. (2012) found an association between *MATN3* gene polymorphism and KOA in Chinese Han population. Contrary to Safia *et al.* and Gu *et al.*, no association was found for the *MATN3* rs77245812 and KOA in our study.

The disruptor of telomeric silencing 1-like (*DOT1L*) gene is involved in epigenetic regulation of gene transcription. Genome-wide association studies showed that *DOT1L* gene may protect against OA (Castano Betancourt *et al.*, 2012). *DOT1L* suppresses Wnt signaling by inhibiting the activity of sirtuin-1 (SIRT1), an important regulator of gene transcription. Inhibition of SIRT1 protects against OA triggered by loss of *DOT1L* activity (Silvia Monteagudo *et al.*, 2017).

For *DOT1L* rs12982744, a previous meta-analysis showed an association between the SNP and hip OA in men (Evangelou *et al.*, 2013) and rs12982744 was strongly associated with the width of the joint space and OA of the hip (Castano

Table 3. Concentrations of Biochemical Parameters in Knee Osteoarthritis Patients by Radiographic Grade

	KL 2 n = *51 (46.3%)*	*KL 3* n = *39 (35.5%)*	*KL 4* n = *20 (18.2%)*	p
CTX-II Median (p25–p75)	210.7 (108.9–259.35)	314.8 (293.4–358.1)	478.8 (442.5–502.6)	0.001*
Glucose Median (p25–p75)	90.3 (90–91)	90.9 (90.1–93)	90.4 (89–92.9)	0.59
Cholesterol Median (p25–p75)	121.6 (114.1–125.8)	116.2 (107.8–126)	113.6 (108.5–123.2)	0.14
Triglyceride Median (p25–p75)	121.9 (115.5–127.4)	119.6 (108.7–128.1)	121.2 (110.7–127.2)	0.68
BMI Median (p25–p75)	29.2 (25.7–31.5)	29.29 (25.7–31.2)	31.5 (26.2–34.7)	0.232

Kruskal–Wallis test and IQR defined as the 25th to 75th percentile range.
*Statistically significant if $p < 0.05$.

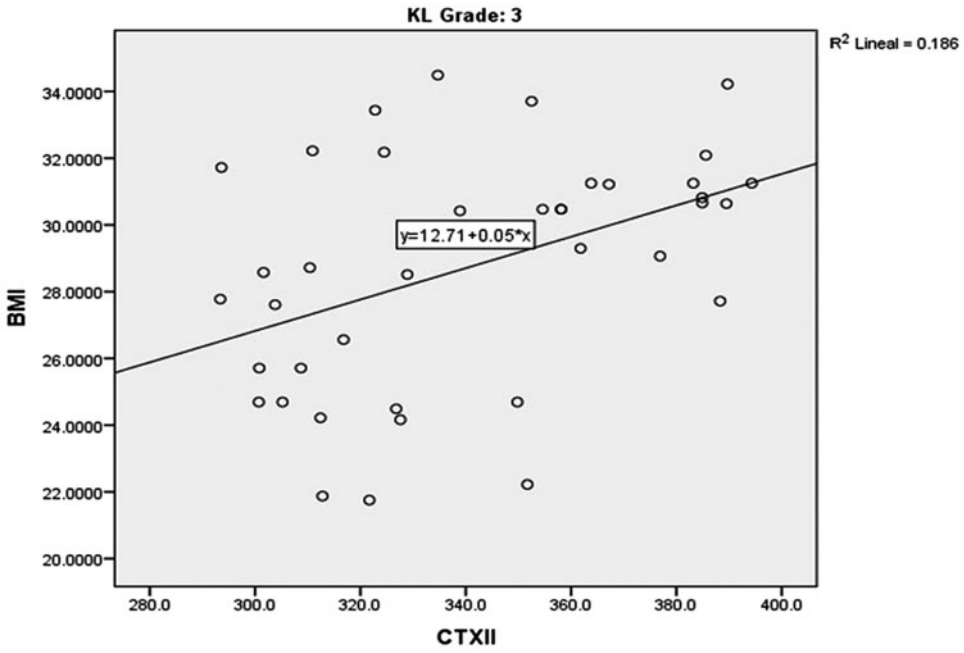

FIG. 1. Spearman's correlation between BMI and concentrations of CTX-II by radiological grade; $r = 0.428$, $p = 0.007$. BMI, body mass index; CTX-II, carboxy terminal telopeptide of collagen type II.

Betancourt *et al.*, 2012). A study conducted in the Han population of China also demonstrated a strong association of the rs12982744 polymorphism with KOA (Evangelou *et al.*, 2013; Zhou *et al.*, 2014). In relation to the results obtained for rs12982744, our study did not find a direct association with KOA in this Mexican mestizo population.

The success of many strategies to find genetic variants that underlie complex traits depends largely on how genetic variation is distributed among human populations (Bamshad *et al.*, 2004). It is important to consider that this study was carried out only in Mexican mestizos from the northeast region, and considering the high genetic variability of the populations of our country, it is essential to expand the description of the candidate genes for OA *MATN3* and *DOT1L* in individuals from central and southern Mexico.

In recent years, the search for biomarkers for OA has increased. Because of the fact that type II collagen is the most abundant protein in the extracellular matrix of cartilage, and that a major feature of this pathology is joint reduction, recent studies have suggested the use of biochemical markers of the decomposition of type II collagen, which represents one of the most attractive approaches to evaluate the progression of the disease. CTX-II, a specific biomarker for the rotation of type II collagen, is useful for diagnosing changes in cartilage, which could be useful for identifying patients with KOA with a high risk of rapid progression of joint damage (Garnero *et al.*, 2002). A previous study reported that CTX-II was effective for the diagnosis and monitoring of OA in murine models (Sarukawa *et al.*, 2010). In addition, CTX-II was also associated with the radiographic severity of OA in the hip and knee (Reijman *et al.*, 2004). Our results showed that in patients with KOA, there is a significantly higher concentration of urine CTX-II; also, a correlation was found between the concentrations of urine CTX-II and the severity of KOA evaluated radiographically, which were previously reported by other groups (Park *et al.*, 2015; Xin *et al.*, 2017). However, no association was found among *MATN3* and *DOT1L* polymorphisms and CTX-II in our study.

Table 4. Relationship Between Carboxy Terminal Telopeptide of Collagen Type II Urinary Levels and Polymorphisms

SNP/genotype	*CTX-II (ng/mmol) Median (IQR)*	p
MATN3 rs77245812		
CC	125.1 (96.5–312.4)	0.64
CT	202.2 (100.4–305.1)	
TT	201.8 (100.6–305.2)	
DOT1L rs12982744		
GG	203.5 (96.8–303.8)	0.80
GC	163.3 (100.2–325.2)	
CC	215.2 (97.9–338.9)	

Median (IQR: 25th to 75th quartile), Kruskal–Wallis test.
SNP, single nucleotide polymorphism.

As a hospital-based study, our study may present some limitations as inherent bias and small sample size may influence statistical power and affect the allele and genotype distribution. However, we tried to overcome these limitations. Specialized personnel validated the diagnosis of KOA and control subjects; also, the increased CTX-II concentrations in KOA subjects confirm correct selection of cases as annotated previously. Thus, the increased concentrations in CTX-II may be explained for some other risk factors, because no association was found with the polymorphisms of *MATN3* and *DOT1L.*

In conclusion, no association among *MATN3* and *DOT1L* genes and KOA risk in a population of northern Mexico was found in our study. These findings may suggest that KOA risk may be related to another possible etiology. Given the complexity in the etiology and physiopathology of disease and its genetic heterogeneity, we suggest a multicentric study with a larger simple size, which will be helpful to increase the confidence of associations.

Author Disclosure Statement

No competing financial interests exist.

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