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Original Article

Lack of association between *MMP-8* polymorphisms and osteoarthritis susceptibility in Chinese Han population

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Abstract: Research has showed that the expression of *MMP-8* was upregulated in osteoarthritis cartilage than in healthy controls. And one study has described that *MMP-8* polymorphisms might associated with decreased risk of osteoarthritis in a Finnish population. Thus, we analyzed whether these variants also contribute to osteoarthritis susceptibility in a Chinese Han population. Sequenom MassARRAY Assay was used to determine the genotype of 300 osteoarthritis patients and 428 healthy controls from Northwest China. Fisher's exact test/Chi-square test was used to compare the differences of the investigated SNPs (rs3740938, rs2012390, rs1940475, and rs11225395) allele and genotype distribution between osteoarthritis cases and controls. After that, the correlation between each SNP and osteoarthritis risk was assessed under multiple genetic models analysis and haplotype analysis. No significant difference was observed for the allele and genotype distribution of the four SNPs between cases and controls. Analysis of the genetic models and haplotype block also failed to reveal any significant association ($P>0.05$). Our present case-control study is the first to explore the potential correlation between *MMP-8* polymorphisms and osteoarthritis risk in a Chinese Han population. Though, negative results we got, further study is still required to validate the potential association in other populations and a larger sample size.

Keywords: Osteoarthritis, single nucleotide polymorphisms (SNPs), *MMP-8*, case-control study

Introduction

Osteoarthritis (OA) has been considered as a complicated degenerative joint disease mainly in the elderly, leading to progressive articular cartilage deterioration and loss and formation of osteophytes. The detrimental effects of trauma on a normal joint and the natural mechanical load on a maligned joint are the two fundamental mechanisms for the pathogenesis of OA [1]. And inflammatory factors, chemokines, and mediators that produced by chondrocyte and synovium playing a pivotal role in the development of OA [2-4]. Furthermore, genetic predispositions are also the potential risk factors for the initiation and progression of this disease [5-7].

OA affects the bone, cartilage, tendon, and synovium and causes the irreversible destruction of these structures. While bone and tendon are composed mainly of typeI collagen and cartilage is primarily made up of type II collagen and proteoglycan [8]. Matrix metalloproteinases (MMPs), produced by the stimulation of some inflammatory cytokines such as TNF-α and IL-1β, can degrade extracellular matrix [9]. Studies have shown that MMP-1 and MMP-13 have predominant role in OA because these enzymes can degrade collagen matrix compo-

Table 1. Age and gender characteristics of cases and controls in this study

Variable	Cases (n=300)	Controls (n=428)	*P* value
Gender			<0.050
Male	100 (33.3%)	197 (46.0%)	
Female	200 (66.7%)	231 (54.0%)	
Age, yr	60.64	52.71	<0.001

P values were calculated from Pearson Chi-square test.

nents of the affected joints [10-12]. Besides the two enzymes, MMP-8 also plays a part in OA through breaking down type I, II, and III collagen.

Several studies have demonstrated that *MMP-8* might involve in the pathogenesis of OA. George et al. did a mice model for human RA and observed that the levels of MMP-8 increased with the progression of collagen-induced arthritis in joint tissue compared with healthy mice [13]. Rose et al. found that the expression of *MMP-8* was upregulated in cartilage of OA patients than in controls, however, in OA synovium, no significant change was observed [14]. And AnnuNakki and colleagues indicated that *MMP-8* polymorphisms had suggestive association with OA risk, but their finding did not replicate in any other independent replication cohorts nor in a meta-analysis of them [15].

Whether polymorphisms of the *MMP-8* gene involved in the pathogenesis of OA is still poorly understood. Therefore, the objective of the present study was to investigated the potential association between *MMP-8* polymorphisms and OA risk through a case-control study in a Chinese Han population from Northwest China.

Materials and methods

Study subjects

300 OA patients and 428 healthy controls were enrolled in our present study (**Table 1**). All subjects were recruited at Honghui Hospital between 2013 to 2016. Inclusion and exclusion criteria were as follows: (1) Subjects are all ethnically homogeneous Chinese Han population from Northwest China. (2) The diagnosis of OA was satisfied with the criteria of the American College of Rheumatology [16]. (3) All subjects that suffered from rheumatoid arthritis or had severe articular trauma were excluded. This work was conformed to the Helsinki Declaration and approved by the ethics committee of Honghui Hospital and Northwest University. And all participants gave their written informed consent letter for the genetic analysis.

DNA isolation and genotyping

SNPs of the *MMP-8* gene that were used to investigate the association with OA susceptibility in the previous studies were selected for further genotyping. And the chosen SNPs were restricted with a minor allele frequency (MAF) >5% in the Chinese Han Beijing population. Peripheral blood sample was collected from each subject before they had received further hospitalization. The GoldMag-Mini Purification Kit (GoldMag Co. Ltd. Xi'an city, China) was used to isolate genomic DNA from leukocytes of the peripheral blood sample strictly following the manufacturer's instructions. DNA concentrations were determined by absorbance at 260 nm and 280 nm with the NanoDrop 2000 (Thermo Scientific, Waltham, MA). The PCR primers that were used for amplification as well as single base extension reactions were designed with Sequenom MassARRAY Assay Design 3.0 software [17] and were listed in **Table 2**. Subsequent SNPs genotyping were conducted by Sequenom MassARRAY RS1000 (Sequenom, San Diego, CA) in 384-well plate. Finally, Sequenom Typer 4.0 software (Sequenom) was used to carry out data management and analysis [17, 18].

Statistical analysis

All statistical analyses were performed using Microsoft Excel and SPSS 16.0 (SPSS, Chicago IL USA). Differences in gender and age between patients with OA and healthy controls were evaluated by Pearson Chi-square test. Deviation from Hardy-Weinberg equilibrium (HWE) of allele frequency of *MMP-8* rs3740938, rs2012390, rs1940475, rs11225394, and rs11225395 in controls was analyzed by the exact test. Chi-square test/Fisher's exact test was used to compare the differences of the investigated SNPs allele and genotype distribution between OA cases and controls. After that, the correlation between each SNP and OA risk was assessed under four genetic models:

Table 2. Summary of the primers used for the analysis of the *MMP-8* polymorphisms

SNP	First PCRP (5'→3')	Second PCRP (5'→3')	UEP SEQ (5'→3')
rs3740938	ACGTTGGATGGTCAGTAAGAGGAATCAAAG	ACGTTGGATGTGACATTTGATGCTATCAC	GATGCTATCACCACACT
rs2012390	ACGTTGGATGACTGTTTCTAGGTCACACCC	ACGTTGGATGTCAGGGAGAGGAAGCAATTC	gAAGCAAATGTGAGGAAGAT
rs1940475	ACGTTGGATGTTTGGGTTGAATGTGACGGG	ACGTTGGATGTAAAACCACCACTGTCAGGC	CTCCACAGCGAGGCTTTT
rs11225394	ACGTTGGATGCAATCTCAAACTAATCACCC	ACGTTGGATGTTAGGAAATAGTGTGGGTTG	AGTGTGGGTTGTTTTCTCTT
rs11225395	ACGTTGGATGAGAGCTGCTGCTCCACTATG	ACGTTGGATGGTTTAGAGAGACTGAGCTGG	gCTGAGCTGGGAGCTACTATA

SNPs, single-nucleotide polymorphisms; PCRP, PCR primer; UEP, Un-extended mini-sequencing primer.

Table 3. Main characteristics and allele frequencies of the *MMP-8* polymorphisms and odds ratio estimates for OA

SNP	Chromosome	Position	Allele	Minor allele frequency		HWE *P* value	OR (95% CI)	*P*[a]
				Case	Control			
rs3740938	11q22.2	102587062	A/G	0.238	0.224	0.781	1.09 (0.85-1.39)	0.512
rs2012390	11q22.2	102590777	G/A	0.279	0.255	1.000	1.14 (0.90-1.44)	0.293
rs1940475	11q22.2	102593248	T/C	0.373	0.357	0.345	1.07 (0.86-1.33)	0.536
rs11225394	11q22.2	102595413	T/C	0.092	0.097	0.038	0.94 (0.66-1.36)	0.757
rs11225395	11q22.2	102596480	A/G	0.368	0.350	0.288	1.08 (0.87-1.34)	0.484

HWE, Hardy-Weinberg equilibrium; OR: odds ratio; 95% CI: 95% confidence interval. [a]*P* values were calculated from Fisher's exact test/Chi-square test.

codominant, dominant, recessive, and additive model using PLINK software which available at http://pngu.mgh.harvard.edu/purcell/plink/. Finally, we used the Haploview software package (version 4.2) and the SHEsis software platform to evaluate and visualize patterns of linkage disequilibrium (LD) and haplotype construction [19]. The odd ratio (OR) and corresponding 95% confidence intervals (CI), calculated by multivariate unconditional logistic regression models with adjustment for gender and age, were used to assess the relationship between each SNP and OA susceptibility. Two-sided $P \leq 0.05$ was regarded statistically significant for statistical tests.

Results

300 OA patients and 428 healthy controls were enrolled in this study. In **Table 1**, significant differences were observed between OA cases and controls in terms of age ($P<0.001$) and gender ($P<0.050$). To eliminate influences of residual confoundings, the variable of age and gender were adjusted by multivariate unconditional logistic regression analysis.

The basic information on *MMP-8* rs3740938, rs2012390, rs1940475, rs11225394 and rs11225395 were listed in **Table 3**. Of these, four SNPs were all in line with Hardy-Weinberg equilibrium (HWE) in controls, with the exception of rs11225394 ($P=0.038$). Allele and genotype distribution of the SNPs between OA cases and controls showed no significant difference ($P>0.05$). Further multivariate unconditional logistic regression analysis with adjustment by age and gender also did not reveal any significant correlation between the four SNPs and OA risk in codominant, dominant, recessive and additive models with a value of $P>0.05$ (**Table 4**).

Furthermore, the association between *MMP-8* polymorphisms and OA risk were evaluated by haplotype analysis. The *MMP-8* linkage disequilibrium (LD) block showed statistically significant linkage between rs3740938, rs2012390, rs1940475, and rs11225395. Three haplotypes with frequencies of more than 0.05 in cases and controls were selected for further research (**Table 5**). Unfortunately, we found that the "AGTA" (adjusted OR=0.95, 95% CI: 0.70-1.27, $P=0.71$) and "GATA" (adjusted OR=0.81, 95% CI: 0.52-1.26, $P=0.35$) haplotypes were not associated with OA susceptibility compared with "GACG" haplotype.

Discussion

Genetic polymorphisms have resulted in the occurrence of biodiversity and could be altered

Table 4. Association between the four SNPs and OA risk in multiple genetic models

SNPs	Models	Genotype	Cases	Controls	Without adjustment		With adjustment	
					OR (95% CI)	P^a	OR (95% CI)	P^b
rs3740938 (G>A)	Codominant	GG	172	256	1.00		1.00	
		AG	113	151	1.11 (0.82-1.52)	0.78	0.91 (0.63-1.30)	0.87
		AA	15	20	1.12 (0.56-2.24)		0.96 (0.44-2.11)	
	Dominant	GG	172	256	1.00		1.00	
		AA+AG	128	171	1.11 (0.83-1.50)	0.48	0.91 (0.65-1.29)	0.61
	Recessive	GG+AG	285	407	1.00		1.00	
		AA	15	20	1.07 (0.54-2.13)	0.84	1.00 (0.46-2.16)	0.99
	Additive	-	-	-	1.09 (0.85-1.40)	0.51	0.94 (0.70-1.25)	0.67
rs2012390 (A>G)	Codominant	AA	151	238	1.00		1.00	
		GA	126	162	1.23 (0.90-1.67)	0.43	1.07 (0.75-1.53)	0.84
		GG	20	28	1.13 (0.61-2.07)		0.89 (0.44-1.77)	
	Dominant	AA	151	238	1.00		1.00	
		GG+GA	146	190	1.21 (0.90-1.63)	0.21	1.04 (0.74-1.47)	0.80
	Recessive	AA+GA	276	400	1.00		1.00	
		GG	20	28	1.03 (0.57-1.87)	0.92	0.86 (0.44-1.69)	0.66
	Additive	-	-	-	1.14 (0.90-1.45)	0.29	1.00 (0.76-1.32)	0.98
rs1940475 (C>T)	Codominant	CC	115	172	1.00		1.00	
		TC	146	206	1.06 (0.77-1.46)	0.81	1.00 (0.69-1.44)	0.79
		TT	39	50	1.17 (0.72-1.89)		0.84 (0.48-1.45)	
	Dominant	CC	115	172	1.00		1.00	
		TT+TC	185	256	1.08 (0.80-1.46)	0.61	0.96 (0.68-1.36)	0.83
	Recessive	CC+TC	261	378	1.00		1.00	
		TT	39	50	1.13 (0.72-1.77)	0.59	0.84 (0.50-1.39)	0.49
	Additive	-	-	-	1.07 (0.86-1.34)	0.53	0.94 (0.73-1.21)	0.61
rs11225395 (G>A)	Codominant	GG	116	175	1.00		1.00	
		AG	147	206	1.08 (0.78-1.48)	0.77	1.01 (0.71-1.46)	0.88
		AA	37	47	1.19 (0.73-1.94)		0.88 (0.50-1.55)	
	Dominant	GG	116	175	1.00		1.00	
		AA+AG	184	253	1.10 (0.81-1.48)	0.55	0.99 (0.70-1.40)	0.94
	Recessive	GG+AG	263	381	1.00		1.00	
		AA	37	47	1.14 (0.72-1.80)	0.58	0.88 (0.52-1.48)	0.62
	Additive	-	-	-	1.09 (0.87-1.36)	0.47	0.96 (0.74-1.24)	0.77

[a]*P* values were calculated from unconditional logistic regression analysis. [b]*P* values were calculated by unconditional logistic regression analysis with adjustments for age and gender.

slightly by geographic and ethnic distribution. Therefore, it is interesting to explore the correlation between the ethnic and geographic distribution of genetic polymorphisms and risk of diseases. In the present study, a case-control study of 300 OA patients and 428 healthy controls was designed to investigate the association of *MMP-8* polymorphisms with OA risk in a Chinese Han population of Northwest China. However, none of the genetic model analysis we used showed any significant association between *MMP-8* polymorphisms and OA susceptibility.

Articular cartilage deterioration is the hallmark of OA. The pivotal event of the irreversible articular cartilage damage in OA is the degradation of extracellular matrix (ECM). The increased and aberrant expression of MMP-8 can cleave type II collagen as well as other ECM and promote neutrophil migration which contributes to the development of local inflammation [20]. Meanwhile, in response to inflammatory insults, chondrocytes and neutrophils produce large amount of matrix-degrading enzymes, including MMPs. For example, IL-1, synthesized by chondrocytes, is capable of inducing the expression

Table 5. *MMP-8* haplotype frequencies and the association with OA susceptibility

Haplotype block	Haplotype frequencies		Without adjustment		With adjustment	
	Case	Control	OR (95% CI)	*P*[a]	OR (95% CI)	*P*[b]
GACG	0.627	0.643	1.00		1.00	
AGTA	0.238	0.224	1.10 (0.85-1.42)	0.46	0.95 (0.70-1.27)	0.71
GATA	0.092	0.103	0.91 (0.62-1.34)	0.64	0.81 (0.52-1.26)	0.35

[a]*P* values were calculated from unconditional logistic regression analysis. [b]*P* values were calculated by unconditional logistic regression analysis with adjustments for age and gender.

of MMPs and it localizes with MMP-1, MMP-8, MMP-13, and TNF-α [1]. The increased expression and activity of matrix-degrading enzymes are major contributors to articular cartilage degradation during osteoarthritis initiation and progression [21]. The above evidence justifies our present study for the association between *MMP-8* polymorphisms and OA susceptibility.

In the present study, we did not observe any significant correlation under the investigated genetic models between variants of *MMP-8* and OA risk. To our knowledge, only one study was committed to explore this potential association and found that the SNP rs1940475 of *MMP-8* was significantly associated with decreased risk of OA in a Finnish population [15]. Several reasons bring about this discrepancy. First, the allele and genotype frequency of the studied SNPs present regional disparities among the two groups. Second, the sample size of our cohort may not be large enough to achieve a convincing result. Third, differences in inclusion and exclusion criteria among patients and controls may affect study findings. And the number of the participants in Finnish study was also small (185 cases and 895 controls) and their findings did not replicate in OA replication study cohorts nor in their meta-analysis. Compared with MMP-1 and MMP-13, the most relevant factors in OA through regulating the process of collagen degradation [8], MMP-8 can not be considered as the major contributor in OA which may lead to our negative results.

Several limitations in our present study need to be addressed. First, the small sample size may not provide convincing evidence to reflect the real relationship between SNPs in the *MMP-8* gene and OA risk. Second, body mass is a crucial factor in the pathogenesis of OA [22], which was not analyzed due to lack of the corresponding clinical information. Third, the present study lacks functional assays to test whether these variants could affect the functions of the *MMP-8* gene in OA.

In conclusion, our present case-control study provides evidence that *MMP-8* polymorphisms are not associated with OA risk in a Chinese Han population from Northwest China. Given the fact that identification of candidate genes and SNPs would help elucidate the molecular pathogenesis of OA, and have the possibility to predict the risk of OA, further study is still required to validate the potential association in other populations and a larger sample size.

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Disclosure of conflict of interest

None.

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