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Original Article

IL1R2 polymorphisms and knee OA susceptibility in a Chinese Han population

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Abstract: Interleukin 1 (IL-1) is one of the most pivotal proinflammatory cytokines in the pathogenesis of osteoarthritis (OA) and its decoy receptor, IL-1R2, has also been demonstrated might involve in the development of OA in genetic association analysis and functional analysis. To evaluate the potential correlation of *IL1R2* polymorphisms with knee OA risk, Sequenom MassARRAY method was taken into use to determine the genotypes of 298 knee OA patients and 297 controls. The correlation between *IL1R2* variants and OA risk was examined by multivariate logistic regression analysis with adjustments for age and gender. Haplotype construction and analysis in *IL1R2* were also applied to detect the potential association. The heterozygous variant "GA" genotype of *IL1R2* rs3218977 was associated with increased risk of knee OA (adjusted OR=1.46, 95% CI: 1.01-2.11, P=0.045) after the adjustment. However, there was no statistically significant difference among any of the *IL1R2* haplotype frequencies in cases and controls. To our knowledge, the present study is the first to show the significant association between *IL1R2* polymorphisms and knee OA susceptibility in a Chinese Han population from Northwest China, though, it need further confirmation with a larger sample size combined with functional analysis.

Keywords: Osteoarthritis, *IL1R2*, rs3218977, polymorphisms, case-control study

Introduction

OA was once described as a non-inflammatory lesion due to the deficiency of robust aggregation and infiltration of inflammatory cells to the affected joint, however, in recent years, it is well-acknowledged that chronic and low-grade inflammatory response is implicated in this disease [1-4]. The chronic inflammation of OA leads to detrimental effects and results in tissue destruction and degradation, such as in articlar cartilage and synovial tissue, through enhancing the activity of nuclear factor-kappa B (NF-κB) and other pathways [5].

IL-1β, the active form of IL-1 in inflammation, as a pleiotropic cytokine, has been demonstrated to play influential roles during the development and progression of OA [6, 7]. IL-1β in combination with the membrane receptor IL-1R1 leads to the activation of the transcription factor NF-κB and MAPK [8, 9]. Activation of these pathways results in the expression of genes that encoding cytokines, chemokines, inflammatory mediators, and enzymes which involved in the pathogenesis of OA. *IL1R2*, located on 2q11.2, encodes cytokine receptor IL-1R2, whose extracellular domain shares considerable extent of sequence homology with that of IL-1R1, which result in the competition of them for the binding site of IL-1 [10]. IL-1R2 served as a decoy receptor of IL-1 with strong affinity, but cannot engage the subsequent signal transduction process which may generate an anti-inflammatory property [11].

Gene expression analysis conducted by Mukundan et al. did not observe mRNA expression of *IL1R2* in human OA-affected cartilage. And functional analysis in synovial cells and chondrocytes presented that soluble IL-1R2 was resistant to IL-1β-induced PGE_2, NO, IL-6, and IL-8

production which have been deostraed to be involved in inflammation and articular cartilage destruction [12]. The *IL1R2* gene locates in the *IL-1* gene cluster on chromosome 2q which has been reported to be associated with hand and knee OA risk [13, 14]. Annu Nakki et al. carried out a single nucleotide polymorphism (SNP) association analysis, but they did not find any significant polymorphism in *IL1R2* with severe hand OA risk in a Finnish population [15]. To date, it is still known that whether *IL1R2* polymorphisms are associated with knee OA susceptibility in Chinese Han population.

In the present study, we chose to explore six *IL1R2* polymorphisms (rs11674595, rs4851527, rs719250, rs3218896, rs3218977, and rs2072472) that have been investigated in some diseases, such as hand OA, ankylosing spondylitis, periodontitis, and endometriosis [15-18], and knee OA risk via a case-control study in a Chinese Han population from Northwest China.

Materials and methods

Study subjects

This study comprised a total of 298 knee OA cases (99 males and 199 females) and 297 controls (99 males and 198 females), derived from the Second Affiliated Hospital of Inner Mongolia Medical University, Hohhot and Honghui Hospital, Xi'an, from January 2013 to January 2016. Subjects in our study should satisfy the following criterias: (1) case-control individuals enrolled in this study were unrelated ethnic Han Chinese population from Northwest China. (2) cases with knee OA were newly diagnosed in accordance with the criteria of the Diagnostic and Therapeutic Criteria Committee of the American Rheumatism Association in 1986 [19]. (3) cases who had rheumatoid arthritis or a major knee trauma were excluded. To get allele frequencies and haplotypes for the *IL1R2* polymorphisms of controls, 297 individuals without a personal or family history of knee OA from physical examination center of the Second Affiliated Hospital of Inner Mongolia Medical University were selected. Informed consent was obtained from all subjects with appropriate ethics committee approval of the Second Affiliated Hospital of Inner Mongolia Medical University and Honghui Hospital. This work also conforms to all criteria described in the Helsinki Declaration.

SNPs selection and genotyping

Using HapMap database, we searched *IL1R2* SNPs which have been investigated in some diseases, such as hand OA, ankylosing spondylitis, periodontitis, and endometriosis, and restricted with minor allele frequencies >5% in the Chinese Han Beijing population to assure that the statistical power of the selected SNPs was large enough for data analysis. And six candidate polymorphisms (rs11674595, rs4851527, rs719250, rs3218896, rs3218977, and rs2072472) of *IL1R2* were selected and genotyped successfully.

GoldMag-Mini Purification Kit (GoldMag Co. Ltd. Xi'an, China) was used to isolate genomic DNA from leukocytes of blood samples which were drawn from all subjects. DNA concentrations were determined at a wavelength value of A260 and A280 nm by the NanoDrop 2000 (Thermo Scientific, Waltham, Massachusetts, USA). Sequenom MassARRAY Assay Design 3.0 Software was applied to design Multiplexed SNP MassEXTEND assay, and SNPs genotyping was conducted utilizing the Sequenom MassARRAY RS1000 (Sequenom, San Diego, CA) recommended by the manufacturer. In **Table 1**, we described the primers that were selected for the identification of the six SNPs. Data analysis was performed by using Sequenom Typer 4.0 Software (Sequenom Co. Ltd) [20].

Statistical analysis

We used Microsoft Excel and SPSS 16.0 (SPSS, Chicago IL USA) to carry out statistical analysis. To test for whether the selected SNPs deviated from Hardy-Weinberg equilibrium (HWE), observed genotype frequencies were compared with expected genotype frequencies. Pearson Chi-Square test/Fisher's exact test was applied to compare the genotype and allele frequencies of OA cases and controls. Using PLINK software (http://pngu.mgh.harvard.edu/purcell/plink/), dominant, recessive, and additive genetic models were used to assess odd ratios (ORs) and 95% confidence intervals (CIs) for SNPs main effects. Finally, SHEsis software platform was used for haplotype construction and analysis of linkage disequilibrium [21]. ORs and 95% CIs that calculated by multivariate unconditional logistic regression analysis with adjustments for age and gender were

Table 1. Primers that were selected for the identification of *IL1R2* SNPs

SNP	First PCRP (5'→3')	Second PCRP (5'→3')	UEP SEQ (5'→3')
rs11674595	ACGTTGGATGGAATCACTGGTGGGCTTATG	ACGTTGGATGAATGCAGATTCTCAGGTCGC	gggaCCAACCAGGACTTACTGAATC
rs4851527	ACGTTGGATGAAGGGCTTTGGAATCACCAG	ACGTTGGATGTGGCCGAGATCTTACAGCTA	CTTACAGCTAGTAAGCAGA
rs719250	ACGTTGGATGATCTGACACTCCAGTCTTTG	ACGTTGGATGATCCCAGGGAGAAAAGCAAC	gAGCTTGTACAAGTTTATGAA
rs3218896	ACGTTGGATGCTGCATGTGGATATGGTTTC	ACGTTGGATGCAAAAGGGCTTATGCCTTCC	CCCGCATACTCCAACTTC
rs3218977	ACGTTGGATGTGAGAACTCTGTGGGTTTCG	ACGTTGGATGACATCCAACAGTTTGGAATC	TGTGGTATGTGGGTCA
rs2072472	ACGTTGGATGCTTCGAAATACTCTGTCTGC	ACGTTGGATGTTCTAGAGGCCATGCGAAGA	gCTGCCTTGGGTCACT

SNP, single-nucleotide ploymorphism; PCRP, PCR primer; UEP, Un-extended mini-sequencing primer.

Table 2. Basic information and allele frequencies of *IL1R2* polymorphisms

SNP	Gene	Chromosome	Position	Allele	Minor allele frequency		HWE *P* value	OR (95% CI)	P[a]
					Case	Control			
rs11674595	*IL1R2*	2q11.2	102610992	C/T	0.232	0.208	0.600	1.15 (0.87-1.51)	0.322
rs4851527	*IL1R2*	2q11.2	102622376	A/G	0.285	0.286	0.118	0.99 (0.77-1.28)	0.971
rs719250	*IL1R2*	2q11.2	102623718	T/C	0.309	0.316	0.591	0.96 (0.75-1.23)	0.772
rs3218896	*IL1R2*	2q11.2	102631652	C/T	0.144	0.165	0.095	0.85 (0.62-1.17)	0.324
rs3218977	*IL1R2*	2q11.2	102641201	G/A	0.253	0.243	0.041	1.06 (0.81-1.37)	0.687
rs2072472	*IL1R2*	2q11.2	102643019	G/A	0.228	0.205	0.859	1.14 (0.87-1.51)	0.340

HWE: Hardy-Weinberg equilibrium; OR: odds ratio; 95% CI: 95% confidence interval. [a]*P* values were calculated from Pearson Chi-Square test.

Table 3. Association between rs3218977 and knee OA risk under multiple genetic models

SNPs	Models	Genotype	Cases	Controls	Logistic regression	
					OR (95% CI)	*P* values
rs3218977 (A>G)	Genotype	AA	167	176	1.00	
		GA	111	96	1.46 (1.01-2.11)	0.045*
		GG	20	24	0.77 (0.39-1.51)	0.451
	Dominant	AA	167	176	1.00	
		GG+GA	131	120	1.30 (0.92-1.84)	0.133
	Recessive	AA+GA	278	272	1.00	
		GG	20	24	0.68 (0.35-1.31)	0.245
	Additive	-	-	-	1.10 (0.84-1.44)	0.482

SNP: Single nucleotide polymorphism; OR: odds ratio; 95% CI: 95% confidence interval. *P* values were calculated by unconditional logistic regression analysis with adjustments for age and gender. *P≤0.05 indicates statistical significance.

used to evaluate the association between each SNP and knee OA susceptibility. Two-sided P≤0.05 was regarded as reaching the threshold of statistical significance for all statistical tests.

Results

The present study including 298 knee OA cases and 297 controls. The distribution of gender was similar between the two groups (P>0.05). And a significant difference in age distribution (60.60 years vs. 56.35 years) between cases and controls (P<0.001) was detected by Welch's *t* test. To eliminate influences of possible confounding, the variable of age and gender were adjusted in logistic regression analysis.

Basic information and allele frequencies of *IL1R2* polymorphisms were described in **Table 2**. None of the six SNPs in controls deviated significantly from HWE (P>0.01). Compared with the AA genotype of *IL1R2* rs3218977, the heterozygous variant GA genotype frequency in cases was significantly different from the controls (37.2% vs. 32.4%). In **Table 3**, the GA genotype of rs3218977 was associated with increased risk of knee OA (adjusted OR=1.46, 95% CI: 1.01-2.11, P=0.045) after adjustment by age and gender in multivariate unconditional logistic regression analysis. For the rest of *IL1R2* polymorphisms, neither allele nor genotype frequencies of them showed evidence for correlation to knee OA susceptibility.

Table 4. *IL1R2* haplotype frequencies and the association with knee OA risk

Haplotype block	Freq (case)	Freq (control)	Logistic regression	
			OR (95% CI)	P
GCTG	0.22	0.20	1.13 (0.86-1.48)	0.395
GTCA	0.14	0.16	0.87 (0.64-1.18)	0.363
GTTA	0.16	0.15	1.09 (0.79-1.51)	0.601
ACTA	0.28	0.28	1.01 (0.79-1.29)	0.956
GCTA	0.19	0.20	0.92 (0.70-1.22)	0.575

OR: odds ratio; 95% CI: 95% confidence interval. *P* values were calculated by unconditional logistic regression analysis with adjustments for age and gender.

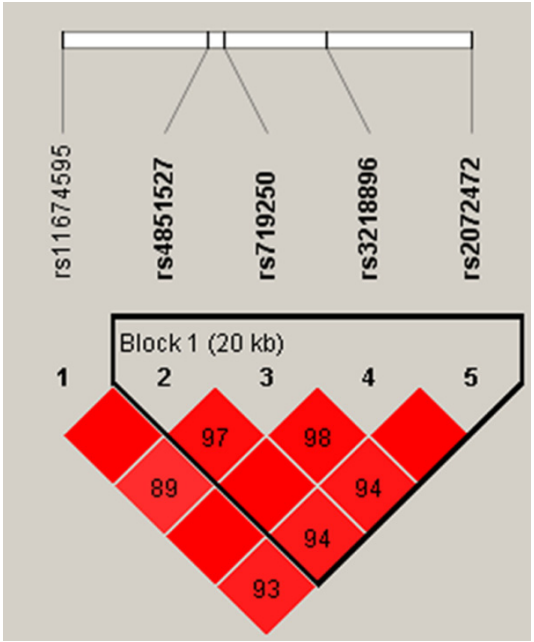

Figure 1. Haplotype block map for part of the SNPs in the *IL1R2* gene.

The minor allele of each polymorphism was considered as a risk allele compared with the wild-type allele. Then, we determined the possible associations of these polymorphisms with OA risk under dominant, recessive, and additive model analysis. However, we did not find any significant correlation between *IL1R2* poly morphisms and knee OA susceptibility in any genetic model after the adjustment (**Table 3**).

Finally, four *IL1R2* polymorphisms (rs4851527-rs719250-rs3218896-rs2072472) mapped to a 20kb LD block and showed a total of five haplotypes with frequencies of more than 0.05 in our subjects (**Table 4**). In **Figure 1**, the red squares of the *IL1R2* LD block presented significant linkage between the four SNPs. Unfortunately, there was no statistically significant difference among any of the *IL1R2* haplotype frequencies in cases and controls.

Discussion

Chronic inflammatory response has been correlated with the development and progression of OA. Inflammation is regulated by a series of cytokines, corresponding receptors, and downstream signaling pathways. IL-1 is one of the most pivotal proinflammatory cytokines in the pathogenesis of OA and its decoy receptor, IL-1R2, has also been demonstrated might implicate in the development of OA in genetic association analysis and functional analysis [12, 15]. In this investigation, a case-control study was designed to evaluate whether *IL1R2* polymorphisms were associated with knee OA risk. The heterozygous variant GA genotype of *IL1R2* rs3218977 was associated with increased risk of knee OA in a Chinese Han population from Northwest China.

Gene expression assays of human epithelial cells and OA-affected cartilage showed the sustained expression of IL-1 and IL-1R1, but not IL-1R2, a decoy receptor of IL-1. Function analysis also found that IL-1R2 could resist to IL-1β-induced IL-6, IL-8, NO, and PGE_2 production and inhibit IL-1-induced inflammatory process in human chondrocytes and synovial cells [12]. Therefore, we supposed that the decoy receptor was effective in restraining the effect of IL-1β in the pathogenesis of OA and served as a protective factor of this disease. Interestingly, we observed that *IL1R2* rs3218977 was associated with increased risk of knee OA in the present study. It is possible that rs3218977 exerts an inhibiting effect on this receptor expression, thereby contributing to a predisposition to knee OA.

Annu Nakki et al. provided the first exploration on the relationship between *IL1R2* polymorphisms and severe hand OA susceptibility in a Finnish population, however, they did not find significant association. In this study, we have exhibited for the first time that *IL1R2* polymorphisms were associated with increased risk of knee OA in a Chinese Han population. The following reasons may responsible for the different results of the two studies: the two groups

came from two ethnicities; the difference in sample size may lead to this distinction; the effect of genetic variants in the development of OA may vary between hand and knee joint [22].

Some potential limitations in this study should be specified successively. Firstly, the logistic regression analysis with adjustments only for age and gender, however, the variables of body mass and diabetes mellitus were not taken into consideration because of a lack of corresponding data from both cases with OA and controls [23, 24]. Secondly, only six SNPs with minor allele frequencies >5% in the Chinese Han Beijing population were selected for genotyping which may leave out some significant polymorphisms. Thirdly, the sample size (298 patients and 297 controls) in this study is small, therefore, a larger sample size will be more convincing.

To sum up, the present study is the first to demonstrate the significant association between *IL1R2* polymorphisms and knee OA susceptibility in a Chinese Han population from Northwest China, which may provide new therapeutic target in knee OA. And the present results need further confirmation with a larger sample size combined with functional analysis.

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Disclosure of conflict of interest

None.

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