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Original Article

IL1R1 polymorphisms are associated with ankylosing spondylitis in the Han Chinese population: a case-control study

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Abstract: Several studies have demonstrated that polymorphisms within the *IL-1* gene cluster are associated with the risk of ankylosing spondylitis (AS) in different populations. In this study, we desired to know whether *IL1R1*, a gene located in the *IL-1* gene cluster, is a susceptible gene for AS in a Northwest Chinese Han population. The Sequenom MassARRAY assay technique was used to determine the genotype of 267 AS patients and 297 controls from Northwest China. Genotype and allele distributions of the investigated *IL1R1* variants (rs10490571, rs12712127, rs956730, rs3917225, and rs3917318) were compared among the cases and controls using Chi-square/Fisher's exact tests. In addition, the associations of these polymorphisms with AS risk were also assessed under dominant, recessive, and additive genetic models using PLINK software. We found the minor G allele of rs3917225 was associated with an increased risk of AS (OR=1.39, 95% CI: 1.09-1.77, *P*=0.007). Significant association was also detected for rs956730 under the dominant model (OR=0.54, 95% CI: 0.30-0.96, *P*=0.032) and the additive model (OR=0.55, 95% CI: 0.34-0.90, *P*=0.016), adjusting for age and gender. This study is the first to demonstrate the significant association between *IL1R1* polymorphisms and AS susceptibility in a Northwest Chinese Han population.

Keywords: AS, *IL1R1*, genetic susceptibility, SNPs, case-control study

Introduction

Ankylosing spondylitis (AS) is a chronic autoimmune disease, which mainly affects the sacroiliac joints and spine, causing bone and joint erosion and even ankylosis. AS is highly hereditable with an approximate prevalence of three out of every 1000 adults in the Chinese population, and it often occurs in young men aged 20-30 with a higher family aggregation [1, 2]. The precise pathogenesis of AS has not been illustrated, but some investigations have suggested that hereditary factors are related to the predisposition of AS in the Han Chinese population. Common variants in *ETS1*, *ERAP1*, *IL12B*, *PTGER4*, *JARID1A*, and *JMY* may contribute to AS susceptibility [3-6].

It has been shown that Interleukin 1β (IL-1β), the active form of IL-1 in inflammation, as a pleiotropic cytokine might be involved in the active inflammation of AS. Chou et al. suggested that patients had increased production of IL-1β from peripheral blood mononuclear cells during active inflammation of AS [7]. Vazquez and colleagues showed that levels of IL-1β were higher in patients with AS than in healthy controls [8]. Single nucleotide polymorphism (SNP) markers within the *IL1* gene complex members (*IL1A*, *IL1B*, *IL1RN*) were found to be significantly correlated with AS in Taiwanese Chinese and in three Canadian populations [9, 10].

Although IL1 was thought to be correlated with AS, whether its receptor (IL1R1) was also implicated in the pathogenesis of AS remained unknown. Thus, the association of *IL1R1* polymorphisms with AS risk provides a new direction for further research. In this study, we randomly selected five *IL1R1* polymorphisms

Table 1. Primers that were used for identification of the five SNPs

SNP	First PCRP (5'→3')	Second PCRP (5'→3')	UEP SEQ (5'→3')
rs10490571	ACGTTGGATGTAGAAAGCTGGACACAGTGC	ACGTTGGATGCCTGGCTGCTTATCATACTC	AGGCAATGATACATGAACAATTC
rs12712127	ACGTTGGATGCTTCCACCTCTTTTGCACTC	ACGTTGGATGAAGAGGCAGAAAATGCACCG	gagACAGCTATGGATCAAGGTA
rs956730	ACGTTGGATGGGCTCAGGTTACCTCAATTC	ACGTTGGATGAGGCTCTTGTTCTCGTAACC	CCCTGGATATGCCTCTT
rs3917225	ACGTTGGATGAACACACCTCTGATACCTTG	ACGTTGGATGCAGCCTGACTAGTTCAACAC	gacCTAAATCCCAAGCTATTATTCAC
rs3917318	ACGTTGGATGGCCATACGGTTGTGAAAAGC	ACGTTGGATGGTCTGAAAAACAGGAAGCAC	GTAAGTAAAATTCTATTTATCATCATTC

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

Table 2. Distributions of age and gender in AS cases and controls

Variable	Cases (n=267)	Controls (n=297)	*P* value
Gender			< 0.001
Male	200 (74.9%)	99 (33.3%)	
Female	67 (25.1%)	198 (66.7%)	
Age, yr	31.58	56.35	< 0.001
Std. Deviation	12.28	9.22	

P values were calculated from Welch's *t* test and Pearson's χ^2 test.

(rs10490571, rs12712127, rs956730, rs3917225, and rs3917318) which have never been studied to determine whether this gene contributes to the development of AS in the Han Chinese population from Northwest China. These SNPs were chosen randomly which have not been reported to be associated with OA susceptibility.

Materials and methods

Subjects

This study was carried out in accordance with the Helsinki Declaration, and the research design was approved by the Ethics Committee of the Second Affiliated Hospital of Inner Mongolia Medical University. A total of 267 AS patients and 297 ethnically and geographically matched healthy controls were enrolled in the two hospitals from March 2013 to March 2016. The AS patients were diagnosed according to the modification of the 1984 New York criteria [11]. The controls were selected from a physical examination center and included individuals without a personal or family history of AS disorder. All the patients and controls provided their written informed consent after a full explanation for the genetic study.

DNA extraction and SNPs genotyping

Venous blood collection was conducted from all participants in the two hospitals. Using the GoldMag-Mini Purification Kit (GoldMag Co. Ltd. Xi'an, China), genomic DNA was extracted from leukocytes of the blood samples following a standard protocol. Then the DNA concentration was measured by the NanoDrop 2000 (Thermo Scientific, Waltham, Massachusetts, USA) at a wavelength value of A260 and A280 nm. Next, DNA samples were stored at -20°C before genotyping. We selected five *IL1R1* variants (rs10490571, rs12712127, rs956730, rs3917225, and rs3917318) with minor allele frequencies > 5% in the Chinese Han Beijing population in the HapMap database (http://www.hapmap.org) for genotyping. The five SNPs were chosen randomly and included those not reported to be associated with OA susceptibility. Sequenom MassARRAY Assay Design 3.0 Software was applied to design the Multiplexed SNP MassEXTEND assay. SNP genotyping was conducted with the Sequenom MassARRAY RS1000 (Sequenom, San Diego, CA). Finally, the data processing was performed by Sequenom Typer 4.0 Software (Sequenom Co. Ltd) [12, 13]. Primers used for the identification of the five SNPs are listed in **Table 1**.

Statistical analysis

All statistical analyses were performed using Microsoft Excel and SPSS 16.0 (SPSS, Chicago IL USA). Differences in age and gender among the AS patients and healthy controls were evaluated by Welch's *t* test and Pearson's χ^2 test, respectively. Deviation from the Hardy-Weinberg equilibrium (HWE) of allele frequency of *IL1R1* rs10490571, rs12712127, rs956730, rs3917225, and rs3917318 in controls was tested by the exact test. Genotype and allele distributions of each SNP were compared among the cases and controls using unconditional logistic regression analysis with or without adjustment for age and gender and Chi-square/Fisher's exact tests [14], respectively. The associations of these SNPs with AS risk were also assessed under dominant, recessive, and additive genetic models using

Table 3. Basic informations on candidate *IL1R1* polymorphisms

SNP	Gene	Chromosome	Position	Allele	Minor allele frequency		HWE *P* value	OR (95% CI)	*P*[a]
					Case	Control			
rs10490571	IL1R1	2q12.1	102717337	T/C	0.193	0.168	0.409	1.18 (0.87-1.60)	0.284
rs12712127	IL1R1	2q12.1	102726661	G/A	0.228	0.209	0.000	1.12 (0.84-1.49)	0.433
rs956730	IL1R1	2q12.1	102758116	A/G	0.232	0.268	0.769	0.83 (0.63-1.08)	0.170
rs3917225	IL1R1	2q12.1	102769302	G/A	0.414	0.337	1.000	1.39 (1.09-1.77)	0.007*
rs3917318	IL1R1	2q12.1	102792760	G/A	0.455	0.483	0.064	0.89 (0.71-1.13)	0.341

HWE: Hardy-Weinberg equilibrium; OR: odds ratio; 95% CI: 95% confidence interval. [a]*P* values were calculated from Pearson Chi-Square test. *$P \leq 0.05$ indicates statistical significance.

Table 4. Associations between *IL1R1* rs3917225 and rs956730 and AS susceptibility under multiple inheritance models

SNPs	Models	Genotype	Cases	Controls	Without adjustment		With adjustment	
					OR (95% CI)	*P* values	OR (95% CI)	*P* values
rs3917225 (A > G)	Genotype	AA	93	131	1.00		1.00	
		GA	127	132	1.36 (0.95-1.94)	0.029*	1.33 (0.72-2.44)	0.430
		GG	47	34	1.95 (1.16-3.26)		1.69 (0.72-4.00)	
	Dominant	AA	93	131	1.00		1.00	
		GG+GA	174	166	1.48 (1.05-2.08)	0.024*	1.41 (0.79-2.50)	0.240
	Recessive	AA+GA	220	263	1.00		1.00	
		GG	47	34	1.65 (1.03-2.66)	0.037*	1.46 (0.66-3.23)	0.360
	Additive	-	-	-	1.39 (1.09-1.76)	0.008*	1.31 (0.87-1.96)	0.190
rs956730 (G > A)	Genotype	GG	156	158	1.00		1.00	
		AG	98	119	0.83 (0.59-1.18)	0.380	0.59 (0.32-1.06)	0.051
		AA	13	20	0.66 (0.32-1.37)		0.26 (0.07-1.04)	
	Dominant	GG	156	158	1.00		1.00	
		AA+AG	111	139	0.81 (0.58-1.13)	0.210	0.54 (0.30-0.96)	0.032*
	Recessive	GG+AG	254	277	1.00		1.00	
		AA	13	20	0.71 (0.35-1.45)	0.340	0.33 (0.09-1.28)	0.096
	Additive	-	-	-	0.82 (0.63-1.08)	0.160	0.55 (0.34-0.90)	0.016*

P values were calculated by unconditional logistic regression analysis with or without adjustments for age and gender. *$P \leq 0.05$ indicates statistical significance.

PLINK software (http://pngu.mgh.harvard.edu/purcell/plink/) [15]. An odds ratio (OR) and 95% confidence intervals (CI) were used to evaluate the effect of each polymorphism and AS risk [16]. Statistical significance was set at a two-sided $P < 0.05$.

Results

A total of 267 AS patients and 297 controls were genotyped for *IL1R1* variants in the present study (**Table 2**). There were statistically significant differences between AS patients and controls in terms of age ($P < 0.001$) and gender ($P < 0.001$). So, unconditional logistic regression analysis with or without adjustment for age and gender was adopted to calculate the odds ratios. Besides *IL1R1* rs12712127, the other four SNPs were all in line with HWE in the controls ($P > 0.05$).

The minor allele frequencies of the *IL1R1* polymorphisms in cases and controls are listed in **Table 3**. A significant difference was observed in the rs3917225 allele distribution between the AS patients and the healthy controls (41.4% versus 33.7%). And this locus was significantly associated with an increased risk of AS (OR= 1.39, 95% CI: 1.09-1.77, P=0.007). Furthermore, significant association with increased AS susceptibility was also found in the "GG" genotype of rs3917225 when it was compared to the wild "AA" genotype (OR=1.95, 95% CI: 1.16-3.26, P=0.029). However, multivariate unconditional logistic regression analysis with adjustment by age and gender did not reveal any sig-

nificant correlation between the genotype of these SNPs and a risk for OA (**Table 4**).

Furthermore, the potential associations between these SNPs and AS susceptibility were also investigated under dominant, recessive, and additive genetic models. Significant evidence was detected for rs956730 under the dominant model (OR=0.54, 95% CI: 0.30-0.96, *P*=0.032) and the additive model (OR=0.55, 95% CI: 0.34-0.90, *P*=0.016) with adjustment by age and gender (**Table 4**). However, there was no significant difference after the Bonferroni correction.

Discussion

In this investigation, a case-control study was designed to investigate the association of *IL1R1* polymorphisms with AS risk in a Northwest Chinese Han population. Our findings showed that the gene of *IL1R1* was correlated with AS, and that rs3917225 and rs956730 might be risk associated SNPs that are involved in the pathogenesis of this disease, but the detailed mechanism is still not well established.

IL-1β has proinflammatory action, which is mediated by some transcriptional factors, such as Mitogen-activated protein kinase and nuclear factor kB [17, 18]. Studies also found that IL-1β could trigger the production of matrix metalloproteases with subsequent subchondral erosion and suppression of chondrocyte proteoglycan synthesis [19, 20]. Analyses of peripheral blood mononuclear cells by an ELISA test indicated an increase in IL-1β levels in AS patients compared with healthy controls [8]. Therefore, we speculated IL-1β might influence the pathogenesis of AS by activating inflammation and the subsequent cartilage lesion.

IL1R1, located in the *IL-1* gene cluster on chromosome 2q, which encodes cytokine receptor IL-1R1, which can combine with IL-1 on the cell surface and affect NF-κB signaling, leading to the up-regulation of inflammatory and immune gene expression [21]. And the degree of *IL1R1* expression on the cell surface affects the response of cells to IL-1 [22]. A genetic basis, such as *IL1R1* polymorphisms, may bring about interindividual differences in IL-1R1 receptor production. In the present study, we found *IL1R1* rs956730 was a protective factor for AS and rs3917225 was an increased risk factor for AS in a Northwest Chinese Han population, which may be because these SNPs affect the expression of the *IL1R1* gene and eventually influence the activity of the inflammatory and immune reactions.

This study, to our knowledge, is the first to present *IL1R1* polymorphisms and AS susceptibility in a Northwest Chinese Han population. Walter et al. indicated that SNPs within the *IL-1* gene cluster (*IL1A*, *IL1B*, *IL1RN*) are associated with susceptibility to AS in three Canadian populations. And one year later, Chou and colleagues also found significant correlations between the *IL-1* gene cluster polymorphisms and AS risk in Taiwanese Chinese. However, neither of the two studies has investigated the potential association between the SNPs of *IL1R1*, a gene also located in the *IL-1* gene cluster, and AS risk. In the present study, we found *IL1R1* polymorphisms are risk factors for AS in a Northwest Chinese Han population, which is in accordance with the findings of these two studies.

Several potential limitations must be considered when interpreting the results of this study. First, the sample size (267 AS patients and 297 controls) in this investigation is small, which may influence the stability of our results. Second, there may be other SNPs in this gene that are related to AS susceptibility but that were not evaluated for their potential associations.

To sum up, this study is the first to demonstrate the significant association between *IL1R1* polymorphisms and AS susceptibility in a Northwest Chinese Han population. Given that AS is a prevalent disease in young men worldwide, identifying potential predictive markers for this disease is of great significance for diagnosing and treating AS in the general population. Therefore, future studies should focus on the validation of this association in other populations, using a larger sample size.

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Disclosure of conflict of interest

None.

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