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JMY Polymorphism Is Related to Severity of Ankylosing Spondylitis in Chinese Han Patients

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Ankylosing spondylitis (AS) is a largely genetically determined autoimmune disease. *JMY* has recently been found to be associated with susceptibility to AS in patients of western European descent. We aimed to examine the influence of *JMY* polymorphisms on the severity of AS in the Chinese ethnic majority Han population. Blood samples were drawn from 396 Chinese Han AS patients whose duration of disease was about 9–12 years. Four tag single-nucleotide polymorphisms (tagSNPs) in *JMY* were selected and genotyped. Frequencies of different genotypes and clinical indexes about the severity of AS were analyzed. The rs2607142, rs16876619, and rs4704556 SNPs are related to BASFI. The rs2607142, rs4704556, and rs16876657 SNPs are related to BADAI. The rs4704556 and rs16876657 SNPs are related to mSASSS. *JMY* is related to the severity of AS in Chinese Han patients.

Introduction

Ankylosing spondylitis (AS) is a chronic inflammatory disorder characterized by inflammation in the spine and sacroiliac joints causing initial bone and joint erosion and subsequent ankylosis (Brown *et al*., 2002). Most patients develop first symptoms of AS when younger than 30 years of age (Braun and Sieper, 2007). Significant radiographic progression occurs in the first 10 years of disease, and more recent studies have shown that structural damage at initial presentation is the best predictor of further damage (Carette *et al*., 1983; Gran and Skomsvoll, 1997; van der Heijde, 2004).

AS patients' disease severity is largely genetically determined. There is an abundance of genetic studies on disease susceptibility; in contrast, few candidate gene association studies have been performed for disease severity or disease features in AS, that is, significant deformities and disabilities. The goal of this study is to find the gene related to the severity of AS, hence we can choose correct treatments.

JMY encodes for JMY (junction-mediating and regulatory protein), which is a transcription cofactor originally identified as a p300-binding protein that augments the p53 tumor suppressor response (Shikama *et al*., 1999). In genome-wide association studies, the rs16876657 SNP in *JMY* is found related to AS susceptibility in patients of western European descent (WTCCC and TAST, 2007; Pointon *et al*., 2011; TASC and WTCCC, 2011). We hypothesize that this gene may predict the severity of AS. In this study, we examined single-nucleotide polymorphisms (SNPs) in *JMY* in AS patients of the Chinese ethnic majority Han population and their clinical indices about the severity of AS.

Methods

Study population

In this work, 396 AS patients were recruited. All patients are Han Chinese HLA-B27-positive AS patients. They are treated by nonsteroidal anti-inflammatory drug routinely; no other treatments are used for patients. Among the AS patients, there are 345 male (87.1%) and 51 female (12.9%); the average age is 29.4 years (range 16 to 60 years). The average duration since AS diagnosis is 11.5 years (range 8 to 18 years) (Table 1). The diagnosis of AS has been made by experienced rheumatologists; all diagnoses satisfy the modified New York criteria (van der Linden *et al*., 1984). Subjects with inflammatory bowel disease, psoriasis, rheumatoid arthritis, or other autoimmune diseases are excluded from this study.

Basic data acquisition

The Bath AS function index (BASFI) and Bath AS disease activity index (BASDAI) are administered to the patients and the controls using questionnaires; these indexes are the most widely used tools for the assessment of AS functional status and activity (Calin *et al*., 1994; Garrett *et al*., 1994). The modified Stokes AS Spine Score (mSASSS) is a validated scoring

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Table 1. Demographic Data of Ankylosing Spondylitis Patients

Sex	
Male	345 (87.1%)
Female	51 (12.9%)
Age	29.4 ± 8.2
Duration of diagnosis	11.5 ± 2.1
BASFI	3.94 ± 1.45
BASDAI	5.60 ± 1.25
mSASSS	13.7 ± 15.0

Numerical values presented as mean ± standard deviation.
BASFI, bath ankylosing spondylitis function index; BASDAI, bath ankylosing spondylitis disease activity index; mSASSS, modified Stokes Ankylosing Spondylitis Spine Score.

system for quantification of chronic spinal changes (Baraliakos *et al*., 2009). Standard anteroposterior and lateral radiographs of the cervical and lumbar spine are obtained for each subject, and the lateral views are used to derive a mSASSS score for each patient (Creemers *et al*., 2005; Sieper *et al*., 2009). Three of the authors separately assigned the mSASSS scores and the average is used.

SNPs selection

JMY is on chromosome 5. The rs2607142, rs16876619, rs4704556, and rs16876657 SNPs are selected to serve as the multimarker tagging algorithm with criteria of r^2 more than 0.8 and for all SNPs with minor allele frequency more than 20%, the population is set as CHB (Chinese Han Beijing). We use the data download from Hapmap to select the tagSNPs randomly. Haploview 4.2 software (Broad Institute, Cambridge, MA) is used in this procedure. Figure 1 shows the position of each tagSNP.

DNA extraction and genotyping analysis

DNA was isolated from 2 mL whole blood samples using the AxyPrep Blood Genomic DNA Miniprep kit (Axygen Biosciences, Union City, CA). Detection of the SNPs was performed by the MassARRAY system (Sequenom, San Diego, CA). The chip-based matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry technology was used in this procedure (Tost and Gut, 2005).

Statistical analysis

The Hardy–Weinberg equilibrium is tested for the four tagSNPs. Numerical values are presented as mean ± standard deviation (Table1). All clinical indexes related to severity of AS for each genotype must undergo the Levene test. A p-value less than 0.05 indicates heterogeneity of variance, therefore, we use the Kruskal–Wallis test, a nonparametric test. A p-value less than 0.05 is considered a significant difference between the three genotypes, which means, this SNP is related to severity of AS (Table 2).

FIG. 1. Positions of each selected tagSNP on *JMY*. The rs2607142 SNP localizes between exon 1 and exon 2; the rs16876619 SNP localizes between exon 4 and exon 5; the rs4704556 SNP localizes between exon 6 and exon 7; and the rs16876657 SNP localizes on exon 7. SNP, single-nucleotide polymorphism.

Ethics statement

The blood samples of both AS patients and controls used in this study are part of the samples taken for diagnostic tests. During the collection and use of DNA samples, clinical data guidelines, regulations of the local Ethics Committee, and the Helsinki Declaration in 1975 are followed. Written informed consents were obtained from all the patients. The study procedure is approved by our Institutional Review Board. The full name of the IRB is ethics committee of Chinese PLA general hospital.

Results

Most of the Levene tests indicate heterogeneity of variance; therefore, we used the Kruskal–Wallis test. The rs2607142, rs16876619, and rs4704556 SNPs are related to BASFI. The rs2607142, rs4704556, and rs16876657 SNPs are related to BADAI. The rs4704556 and rs16876657 SNPs are related to mSASSS. The GG genotype in rs2607142 can enhance BASFI and BASDAI. The CC genotype in rs16876619 can enhance BASFI. The CC genotype in rs4704556 can enhance BASFI, BASDAI, and mSASSS. The AA genotype in rs16876657 can enhance BASDAI and mSASSS. *JMY* is related to severity of AS in Chinese Han patients. Figure 2 is a linkage disequilibrium (LD) map of these four SNPs. Nearby SNPs are positive for the statistical analysis due to high LD (Fig. 2). These SNPs from the same block have the same meaning, indicating a relationship in the severity of AS. The data from Hapmap indicate that these four SNPs are not in high LD. AS may have a much larger genetic component associated with *JMY*, not only limited to these four chosen SNPs. Combining all the above data, the CC genotype in rs4704556 is a newly found risk factor for severity of AS.

Discussion

JMY encodes a transcription cofactor that augments the p53 tumor suppressor response (Shikama *et al*., 1999). With its influence on p53, *JMY* can affect apoptosis during the DNA damage response (Coutts *et al*., 2007). *JMY* has been linked to AS in GWAS in patients of western European descent (WTCCC and TAST, 2007; Pointon *et al*., 2011; TASC and WTCCC, 2011). We focus on particular SNPs of this gene and the severity of AS in the Chinese Han population. We tried our best to limit the duration of disease to 10 years to avoid time effect. There is no difference in the duration of disease comparing each group (Table 2). Our results highlight that the *JMY* gene can affect the rate of disease progression.

In conclusion, in Chinese patients, *JMY* is related to severity of AS. Of clinical relevance, the specific SNPs in these genes can be used to guide genetic analysis and counseling, medical and surgical treatment options, and ultimate prognosis. Further studies are needed to elucidate the molecular roles these genes play in AS.

Table 2. Clinical Indices About Severity of Ankylosing Spondylitis

	Freq.[a]	*BASFI*	*BASDAI*	*mSASSS*	*Duration of disease*
rs2607142					
Levene test[b]		1.113E-13[c]	6.052E-12	2.727E-14	1.820E-6
AA[d]	80	3.635 ± 0.162[e]	3.775 ± 0.115	9.825 ± 1.520	11.475 ± 0.232
AG	200	3.788 ± 0.102	3.840 ± 0.073	10.890 ± 0.961	11.480 ± 0.147
GG	114	4.553 ± 0.135	4.298 ± 0.096	17.667 ± 1.274	11.526 ± 0.194
Kruskal-Wallis test		**0.003***[,f]	**0.038***	0.700	0.948
rs16876619					
Levene test		5.187E-9	3.247E-7	8.008E-9	1.041E-7
TT	46	3.717 ± 0.215	3.783 ± 0.153	11.609 ± 2.039	11.457 ± 0.306
CT	170	3.696 ± 0.112	3.824 ± 0.808	10.576 ± 1.061	11.553 ± 0.159
CC	178	4.315 ± 0.109	4.135 ± 0.078	14.865 ± 1.036	11.444 ± 0.155
Kruskal-Wallis test		**0.010***	0.215	0.303	0.839
rs4704556					
Levene test		4.340E-17	1.462E-11	5.237E-15	0.001
CC	82	4.817 ± 0.158	4.488 ± 0.112	20.683 ± 1.472	11.195 ± 0.232
CT	206	3.794 ± 0.099	3.864 ± 0.071	11.165 ± 0.929	11.597 ± 0.146
TT	106	3.687 ± 0.139	3.717 ± 0.099	9.226 ± 1.295	11.557 ± 0.204
Kruskal-Wallis test		**<0.001***	**<0.001***	**0.014***	0.296
rs16876657					
Levene test		0.173	0.299	0.003	0.022
GG	2	3.000 ± 1.054	3.000 ± 0.738	5.000 ± 9.830	12.000 ± 1.453
AG	52	3.923 ± 0.207	3.731 ± 0.145	9.154 ± 1.928	12.192 ± 0.285
AA	340	3.992 ± 0.081	4.000 ± 0.057	13.212 ± 0.754	11.382 ± 0.111
Kruskal-Wallis test		0.371	**0.011***	**<0.001***	0.138
ANOVA		0.618[g]	0.097		

[a]Frequencies of each genotype.
[b]Levene test is used to test homogeneity of variance. *p*-Value less than 0.05 indicates heterogeneity of variance, therefore we use Kruskal-Wallis test, a non-parametric test.
[c]*p*-Value for Levene test.
[d]All clinical indices are analyzed due to the genotype of each SNPs.
[e]The mean ± deviation of each index is calculated for different genotype.
[f]*p*-Value for Kruskal-Wallis test.
[g]*p*-Value for ANOVA.
*Means significant difference between different genotypes.

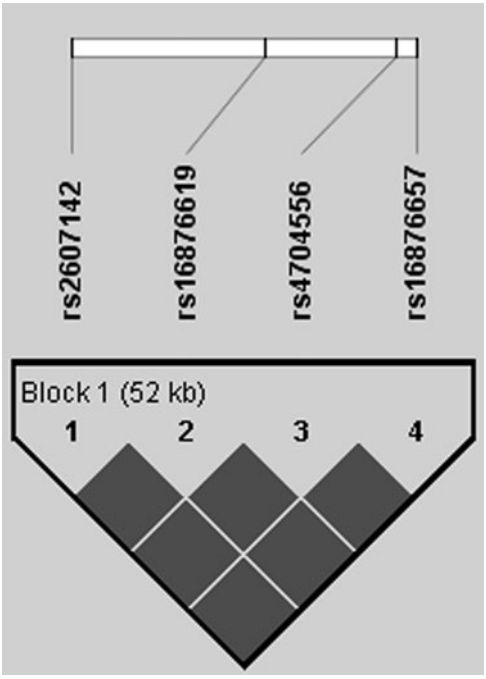

FIG. 2. Linkage disequilibrium (LD) map of all AS patients. Darker color indicates higher LD and lighter color indicates less LD. Numbers in the squares indicate the correlation coefficient (R2) value.

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Author Disclosure Statement

No competing financial interests exist.

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