Original Article

Association between \textit{ERAP1} gene polymorphisms and ankylosing spondylitis susceptibility in Han population

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Abstract: Purposes: The present study was designed to investigate the relationship between endoplasmic reticulum amino peptidase 1 (\textit{ERAP1}) gene polymorphisms and ankylosing spondylitis (AS) in Han population of Shaanxi province. Methods: 100 AS patients and 100 healthy people were enrolled in present study as case and control groups respectively, and the control group was matched with the case group by age and gender. \textit{ERAP1} gene rs27434 and rs7711564 polymorphisms were test by TaqMan probe genotyping method. SHEsis software was used to operate linkage disequilibrium (LD) and haplotype analysis between the two single nucleotide polymorphisms (SNPs). \chi^2 test was employed to compare the differences of the genotype, allele and haplotype frequencies between the case and control groups. Results: In \textit{ERAP1} rs27434 and rs7711564 polymorphisms, the frequencies of AA and CC genotypes in case group were significantly higher compared to those in control group (\(P=0.036\); \(P=0.039\)), and so were the frequencies of A and C alleles (OR=1.589, 95% CI=1.070-2.359, \(P=0.028\); OR=1.535, 95% CI=1.021-2.308, \(P=0.050\)). Linkage disequilibrium test and haplotype analysis of the alleles of the two SNPs showed that the frequency of A-C haplotype was higher in case group than that in control group (\(P=0.005\)), which indicated that A-C might be the susceptible haplotype to AS. Conclusions: \textit{ERAP1} gene rs27434 and rs7711564 polymorphisms may increase the risk of AS.

Keywords: \textit{ERAP1}, polymorphism, ankylosing spondylitis, haplotype

Introduction

Ankylosing spondylitis (AS) is a chronic systemic inflammatory disease with inflammations in sacroiliac joints and spine attachment points as the main symptom. AS is one of the sero-negative spondyloarthopathies, mainly involve spine part and sacroiliac joints, lead to poker spine and fibrosis of spine, then give rise to varying degrees of complications including eyes, lungs, muscle and bone lesions. Its incidence in China is about 0.26% and the ratio of male to female is about 4~10:1 [1]. The pathogenesis of AS is still not clear and may be associated with genetic and environmental factors. Previous studies show that human leukocyte antigen (HLA) is the main AS-affected region [2], and HLA-B27 accounts for 16% of the total genetic factors [3], thus indicating that there may be other susceptible genes that play important roles in the onset of AS [4, 5].

It is believed that AS is a polygenic disease caused by the combined influence of environmental and genetic factors. As reported in literatures, there are more than 20 genes related to AS risk, and one of them is endoplasmic reticulum amino peptidase 1 (\textit{ERAP1}) [6, 7]. \textit{ERAP1} gene encodes an aminopeptidase linked to endoplasmic reticulum, and participates in regulating the concentration and amount of cytokines, membrane-bound cytokine receptors and soluble cytokine receptors. Many researchers suggested that \textit{ERAP1} gene implicated in the pathogenesis of AS [8, 9]. However, inconsistent conclusions have been obtained among different races or regions by researches about the correlation of \textit{ERAP1} gene with AS risk [10].

In order to investigate the relationship between \textit{ERAP1} polymorphisms and the risk of AS in Chinese, \textit{ERAP1} gene rs27434 and rs7711564
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**Materials and methods**

**General data of subjects**

100 AS patients aged 18-50 (67 men and 33 women, mean age 27.9) as cases were recruited from the Rheumatism Departments of The Second Hospital Affiliated to Zhejiang University School of Medicine. They were diagnosed with AS after routine examinations, X-ray, CT and nuclear magnetic resonance examinations according to the Modified New York Criteria (1984) for AS [11]. Disease courses of the patients were 1~15 years. All the cases did not get radiation or chemotherapy treatments before blood sampling. Meanwhile, 100 healthy controls (71 males and 29 females) were recruited from the physical examination departments of the same hospitals during the same period. The age range and the average age of the control subjects were 18~50 and 27.6 respectively. No differences in age and sex existed between the two groups. All the subjects were unrelated Han populations who lived in Zhejiang region within three generations, and signed informed consent. Sample collection was carried out in accordance with the National Human Genome Research Ethics Guidelines. Our study was conducted under the permission from the Ethics Committee of The Second Hospital Affiliated to Zhejiang University School of Medicine.

**Extraction of genomic DNA**

6 ml fasting venous blood from each subject in morning was put into ethylenediamine tetraacetic acid dipotassium salt (EDTA-K2) anticoagulation tube for full anticoagulation after uniform mixing to avoid false-positive result. The genomic DNA in the peripheral whole blood was extracted with DNA extraction kit. DNA concentration and purity were detected according to instructions.

**Genotyping of ERAP1 rs27434 and rs7711564 polymorphisms**

Primer sequences of ERAP1 rs27434 and rs7711564 polymorphisms were designed with Primer Premier 5.0 software and synthesized by Shanghai GeneCore BioTechnologies Co., Ltd. The forward and reverse primer sequences are shown in **Table 1**. The Taqman genotyping method was applied to analyze the two single nucleotide polymorphisms (SNPs). Taqman probes (ABI company) for rs27434 and rs7711564 polymorphisms were respectively C_794791_10 and C_3056830_10. 10 µL PCR reaction system included 5 µL 2×Master Mix, 0.25 µL 40×probe, 10 ng DNA and the rest volume of sterile water. Reaction conditions were as follows: 40 cycles of predenaturation at 95°C for 10 minutes, denaturation at 92°C for 15 seconds and annealing at 60°C for 1 minute; and finally extension at 40°C for 5 minutes. In order to test the genotyping results, we randomly selected 5% of the samples for sequencing test, and each 96-pore reaction plate contained the standard samples of 3 known genotypes (wild homozygote, mutant homozygote and heterozygote) as known controls. The above process was conducted in ABI 7500 Real Time PCR system and the results were analyzed by SDS1.4 software.

**Statistical analysis**

Genotype and allele frequencies of the two SNPs were calculated by direct calculation. χ² test was used to compare the differences of genotype and allele frequencies of the two SNPs between case and control groups. The calculation was performed with SPSS 18.0 software, and there existed statistical significance only when \( P<0.05 \). Linkage disequilibrium (LD) and haplotypes of the two SNPs were analyzed with SHEsis software (statistical significance with \( P<0.05 \)). Odds ratios (ORs) and 95% confidence intervals (95% CIs) were used to represent the relative risk of AS.
Results

Genotype and allele frequencies of ERAP1 rs27434 and rs7711564 polymorphisms in cases and controls

Genotype distribution of ERAP1 gene rs27434 and rs7711564 polymorphisms is shown in Table 2. Case subjects had obviously higher frequencies of AA and CC genotypes in these two polymorphisms than the control subjects. The differences were statistically significant ($P<0.05$), showing that AA and CC were the susceptible genotypes to AS (OR=2.323, 95% CI=1.075-5.019; OR=3.173, 95% CI=1.077-9.343). In addition, compared to the control subjects, the cases had apparently higher frequencies of rare alleles A and C ($P<0.05$), suggesting that rare alleles A and C were closely related to AS onset (OR=1.589, 95% CI=1.070-2.359; OR=1.535, 95% CI=1.021-2.308).

LD test and haplotype analysis

LD test and haplotype analysis of the alleles of ERAP1 rs27434 and rs7711564 polymorphisms showed $D^*=0.83$ and $r^2=0.43$, which suggested that LD existed between rs27434 and rs7711564 polymorphisms. Based on the frequencies of these haplotypes, the distribution of every single haplotype was calculated, and the differences in haplotype distribution between the two groups were compared (Table 3).

Analysis of haplotype frequencies of ERAP1 rs27434 and rs7711564

LD analysis of the alleles of ERAP1 rs27434 and rs7711564 polymorphisms revealed that these two SNPs formed 4 haplotypes (Table 3). There existed significant differences in the distribution of A-C haplotype between the cases and the controls ($P<0.05$), so A-C haplotype might correlate with the enhanced risk of AS (OR=2.082, 95% CI=1.253-3.459).

Discussion

Ankylosing spondylitis (AS) is a multigenic disease. The clinical classification and diagnosis thereof are not very clear. The onset of AS is concealed and symptoms of it are also changeable. In addition, early symptoms of AS such as lumbosacral and lower back pains and morning stiffness are less likely to attract patients’...
attention [12]. As a result, when inflammatory changes of bilateral sacroiliac joints can be clinically shown on X-ray films (grade II or above), the best time for treatment of AS may have been missed. Therefore, early diagnosis, prevention and treatment of AS are extremely significant. In recent years, it has become the most promising molecular genetics research method to investigate the genetic polymorphisms of candidate genes of AS onset. Genetic polymorphism or gene polymorphism is a phenomenon where two or more discontinuous variants, genotypes or alleles often simultaneously occur in a same biological population. Gene polymorphism varies with different races and regions, and the genetic distribution of populations in different regions is not completely identical, thus leading to frequency deviation in statistics.

ERAP1 is an amino peptidase induced by γ-interferon and involves in trimming HLA class I-binding precursors. Through gene sequence analysis, we can know that ERAP1 gene is located on chromosome 5q15 with a total length of 47379 bp and adjacent to LNPEP gene, containing 27 exons and encoding 941 amino acids [13]. Proteins encoded by ERAP1 gene mainly serve as molecular rulers for antigen peptides and shed enzymes, facilitating the recognition of natural killer cells and specific CD8+ T cells [14, 15]. ERAP1 can also block the combination of tumor necrosis factors (TNFs), interleukin-1 (IL-1) and IL-6 with target cells by degrading receptors of proinflammatory cytokines on cell membrane such as tumor necrosis factor receptor 1 (TNFR1), interleukin-1 receptor 2 (IL-1R2) and IL-6R [16]. Furthermore, the ERAP1 gene can process peptides with optimal length to form new HLA-I molecules like HLA-B27 [17]. AS has specific immunoregulatory defects and the pathogenesis of it is an inflammatory reaction involving many cytokines. So it is believed that ERAP1 gene take part in the occurrence of AS. Hereafter, various studies have been carrying out in order to confirm the hypothesis. In 2007, in the first genome-wide association study, ERAP1 gene was found to be the susceptible gene of AS. This conclusion was confirmed in multiple populations afterwards [18-24]. Because AS is a complex disease and different populations have different genetic structures, it is critical to further verify this conclusion in various populations. Therefore, we analyzed ERAP1 rs27434 and rs7711564 polymorphisms in both case group and control group in Chinese.

Our study showed that there existed an association of ERAP1 gene polymorphisms with AS risk. AA genotype and A allele of rs27434 associated with 2.323 and 1.589 times increased risk of AS. At the same time, rs7711564 polymorphism CC genotype and C allele could increase the AS risk about 3.173 and 1.535 folds, respectively. The same conclusion was obtained among other studies [25-27]. In addition, LD analysis indicated that A-G haplotype of ERAP1 rs27434 and rs7711564 polymorphisms had the frequencies of 32.00% and 19.00% in cases and controls respectively, with statistical significance (P<0.05). The result indicated A-G haplotype might increase the risk of AS with the OR of 2.082.

The above listed data of our study demonstrated that the ERAP1 gene plays an important role in the occurrence of AS. Because the sample size was small, genotype distributions of rs7711564 were deviate from Hardy-Weinberg equilibrium (HEW) both in case and control groups. Therefore, our results should be settled rationally. Additionally, the pathogenesis of AS is still unclear up to now. So a well designed further study is necessary.

Disclosure of conflict of interest

None.

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References


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Pazar B, Safrany E, Gergely P, Szanto S, Szekanecz Z and Poor G. Association of ARTS1 gene polymorphisms with ankylosing spondylitis in the Hungarian population: the rs27044 variant is associated with HLA-B*2705 sub-type in Hungarian patients with ankylosing spondylitis. J Rheumatol 2010; 37: 379-384.
