Interleukin-18 Polymorphisms Impose Considerable Impacts on Ankylosing Spondylitis Occurrence

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Abstract

**Background:** This research was conducted to explore the genetic association of interleukin-18 (*IL-18*) polymorphisms in promoter with ankylosing spondylitis (AS) in Chinese population using a case-control design.

**Methods:** Polymerase chain reaction (PCR) and sequencing were used to complete the genotyping of *IL-18* polymorphisms in 146 AS patients and 134 healthy controls. The genotype distribution of polymorphism was detected the status of Hardy-Weinberg equilibrium (HWE). The genotype and allele frequencies difference between the two groups was compared by chi-square test. Odds ratio (OR) with 95% confidence interval (95%CI) was calculated to express the association strength of AS. The linkage disequilibrium of *IL-18* polymorphisms was investigated in AS occurrence by Haploview.

**Results:** TT genotype and T allele of rs1946518 showed the significantly higher frequencies in AS patients than that of the controls \((P=0.042, 0.026)\), which indicated that rs1946518 was obviously associated with AS risk (TT vs. GG: OR=1.993, 95%CI=1.021-3.891; T vs.G: OR=1.460, 95%CI=1.046-2.038). However, no significant association was found between rs187238 and AS. Moreover, the strong linkage disequilibrium was found and haplotype rs1946518T-rs187238C was a susceptible factor of AS (OR=1.638, 95%CI=1.017-2.639).

**Conclusion:** *IL-18* rs1946518 polymorphism may contribute to the susceptibility of individuals to AS in Chinese population, but not another polymorphism rs187238. The interaction of the two polymorphisms should be considered in AS etiology.

Background

Ankylosing spondylitis (AS) is known as a common chronic inflammatory disorder and mainly infringes on spine, involved sacroiliac and surrounding joints [1]. It affects 0.1%-1.4% of population worldwide, especially males with the age of 20–40 years old [2]. The occurrence of AS can lead to the loss of work productivity and money, decrease the life quality, but its pathogeneisis is still unclear explicitly. According to the previous studies, AS is defined as a complex multifactorial diseases, referring to genetic and environmental factors [3–5]. Familial and twins studies report that genetic factors account for over 90% of AS susceptibility [6, 7]. *Human leukocyte antigen (HLA)-B27* is considered as the important susceptible gene to AS, but it only contributes 16% of AS genetic risk [8]. So more studies should be conducted to explain AS etiology.

Interleukin-18 (*IL-18*), also called as interferon-γ (IFN-γ) inducible factor, is a new-found cytokine recently, belonging to IL-1 superfamily [9]. It is produced by activated monocytes, macrophages and dendritic cells [10]. *IL-18* can strongly induce immune cells to release multiple cytokines such as IFN-γ, TNF-β, increase the expression of FasL and enhance the cytotoxicity of natural killer (NK) cells, which plays the important role in immune regulation, anti-infection and chronic inflammatory diseases [11]. In previous studies, *IL-18* is found to be associated with rheumatoid arthritis (RA) and [12, 13]. Recently, the study reports that
serum IL-18 level in AS patients is obviously higher than that of the controls [14]. It may play an important role in the onset of AS.

With the development of molecular biological technique, single nucleotide polymorphism (SNP) in gene has been becoming the popular means of exploring disease susceptibility. IL-18 is encoded by IL-18 gene located on chromosome 11q22.2-22.3 [15], including multiple SNPs, which two polymorphisms in promoter region are widely studied in several diseases. In the present study, we explored the association of IL-18rs1946518 (-607G/T) and rs187238 (-137G/C) polymorphisms in promoter with AS susceptibility in a Chinese Han population and would hope to provide some clues for looking for susceptible population of AS through the detection of gene polymorphisms.

Materials And Methods

Subject selection

In the research, 128 AS patients and 134 healthy people were enrolled as the case and control groups respectively during from June, 2014 to September, 2016. AS patients were confirmed by clinical features and CT or MRI examination in the Department of Rheumatism of Daping Hospital according to the modification of the New York criteria for AS in 1984 [16]. Their age range was 17-53 years old. These AS patients would be excluded with immune diseases, chronic diseases and tumors. In the meanwhile, the controls were selected from the physical examination center of the same hospital with AS patients for regular health check-up and they were healthy with the age of 21-56 years old. The controls were required without any history of immune inflammatory diseases. Moreover, the controls had to frequency-match with the cases by age and sex.

All subjects were Chinese Han population and they were not any blood relationship each other. The study was supported by the Research Ethics Committee of Daping Hospital and all subjects. Before collecting blood sample, written informed consents were signed by each subject.

DNA extraction

The subject was collected 2ml fasting peripheral venous blood in the early morning and put it into specific vacuum tubes with EDTA2Na, stored at -80°C. Then blood genomic DNA was extracted by conventional phenol-chloroform extraction and ethanol precipitation. The quality and concentration of DNA were detected by 1.0% agarose gel electrophoresis (AGE) and NanoDrop 2000 ultramicrospectrophotometer.

The genotyping of IL-18 polymorphisms
In this study, the methods of polymerase chain reaction (PCR) and sequencing were used to complete the genotyping of *IL-18* rs1946518 and rs187238 polymorphisms. PCR primers sequences were designed by Primer 5.0 software and synthesized by Invitrogen (Shanghai). The detailed primer sequences were:

rs1946518: 5'-TGCTGTATCAGATGCAAGCC-3' and 5'-CTCTCCCCAAGCTTACTTTC-3';
5'-AATAAAGTGGCAGAGGAT-AC-3' and 5'-ACAGAGCCCCAACTTTTACG-3' for rs187238. PCR system was a volume of 20μl mixture, containing 20ng genomic DNA, 10μl 2×PCR Mix, each 0.5μl of upstream and downstream primers and added ddH_2O to the final volume. The whole PCR procedure was as follows: 95℃ predegeneration 2min, 40 cycles of 95℃ degeneration 30s, 56℃ annealing 60s, 72℃ extension 30s, and final extension at 72℃ for 5min. PCR amplification products were detected by 1.0% AGE. Eligible PCR products were sent to Shanghai Sangon Biotech Co. Ltd for sequencing so as to determine the genotype of *IL-18* polymorphisms in case and control groups.

**Statistical analysis**

We obtained the genotype and allele frequencies of *IL-18* polymorphisms by direct counting. The genotype distribution of each polymorphism in the control group was checked whether was consistent with Hardy-Weinberg equilibrium (HWE). The genotype and allele frequencies were measured the significant difference between the two groups by χ² test. The calculation of odds ratio (OR) with 95% confidence interval (95%CI) was to express the risk intensity of AS caused by *IL-18* polymorphisms. The relevant data were conducted by SPSS 18.0 software. What's more, the linkage disequilibrium of *IL-18* two polymorphisms in this study was explored by Haploview software. *P*<0.05 is defined as the significant difference between compared two groups.

**Results**

**The basic characteristics of subjects**

The demographic information of subjects in the case and control groups was displayed in Table 1. The mean age in the two group was respectively 34.65 ± 10.63 and 35.46 ± 9.52 years old. The males and females in the case group was 89 and 57, the number in the control group was 75 and 63. The significant difference was not found between the two groups between the two groups (*P*> 0.05). The disease duration of AS patients was 6.32 ± 4.51 years. One third of AS patients were smokers and the percentage in controls was nearly 25% (34.93% & 24.64%), the difference was not significant (*P* = 0.082). Drinking was not a risk factor of AS in this population, either (*P* = 0.575). However, the positive rate of HLA-B27 in AS patients was 87.67%, 16.67% in controls, so HLA-B27 was an independent influence factor of AS (*P*< 0.01).
Table 1
The basic information of subjects in the case and control groups

| Characteristic         | Case, n = 146 | Control, n = 138 | P   |
|------------------------|--------------|-----------------|-----|
| Age (year)             | 34.65 ± 10.63 | 35.46 ± 9.52    | 0.436 |
| Gender (male/female)   | 89/57        | 75/63           | 0.260 |
| Disease duration (year)| 6.32 ± 4.51  |                 |      |
| Smoking (yes/%)        | 51 (34.93)   | 34 (24.64)      | 0.082 |
| Drinking (yes/%)       | 38 (26.03)   | 31 (22.46)      | 0.575 |
| HLA-B27 (positive/%)   | 128 (87.67)  | 23 (16.67)      | <0.01|

HLA-B27: human leukocyte antigen-B27

The distribution difference of genotype and allele in IL-18 polymorphisms between the two groups

The genotype and allele frequencies of IL-18 rs1946518 and rs187238 polymorphisms in promoter in the case and control groups were showed in Table 2. The genotype distribution of two polymorphisms in the control group conformed to HWE (P = 0.839 and 0.294 respectively), so our selected group was the typical Mendelian population. For rs1946518 polymorphisms, its GG, GT, TT genotype frequencies were 21.92%, 50.00% 28.08% in AS patients and 31.34%, 48.51%, 20.15% in controls respectively. TT genotype of rs1946518 was significantly higher frequency in AS patients than that of the controls (P = 0.042), similarly, T allele frequency of rs187238 was also obviously different between the two groups (53.08% & 44.40%, P = 0.026). So rs1946518 was significantly associated with the increased risk of AS (TT vs. GG: OR = 1.993, 95%CI = 1.021–3.891; T vs. G: OR = 1.460, 95%CI = 1.046–2.038). However, there was no significant difference between the case and control groups in genotype or allele frequency of rs187238 (P > 0.05), suggesting it might not contribute the independent risk of AS.
Table 2
The genotype and allele frequencies of \textit{IL-18} polymorphisms in two study groups

| Genotype/allele | Case, n = 146 (\%) | Control, n = 134 (\%) | OR (95%CI) | \(P\) | \(P_{HWE}\) |
|-----------------|--------------------|------------------------|------------|------|--------|
| rs1946518       |                    |                        |            |      |        |
| GG              | 32 (21.92)         | 42 (31.34)             | Ref.       | -    | 0.839  |
| GT              | 73 (50.00)         | 65 (48.51)             | 1.474 (0.835–2.603) | 0.180 |        |
| TT              | 41 (28.08)         | 27 (20.15)             | 1.993 (1.021–3.891) | 0.042 |        |
| G               | 137 (46.92)        | 151 (55.60)            | Ref.       |      |        |
| T               | 155 (53.08)        | 117 (44.40)            | 1.460 (1.046–2.038) | 0.026 |        |
| rs187238        |                    |                        |            |      | 0.294  |
| GG              | 98 (67.12)         | 101 (75.37)            | Ref.       |      |        |
| CG              | 41 (28.08)         | 29 (21.64)             | 1.458 (0.840–2.530) | 0.180 |        |
| CC              | 7 (4.80)           | 4 (2.99)               | 1.649 (0.521–5.218) | 0.390 |        |
| G               | 237 (81.16)        | 231 (86.19)            | Ref.       |      |        |
| C               | 55 (18.84)         | 37 (13.81)             | 1.424 (0.912–2.225) | 0.119 |        |

The haplotype analysis of \textit{IL-18} two polymorphisms in AS occurrence

The linkage disequilibrium of \textit{IL-18} rs1946518 and rs187238 polymorphisms was investigated and the strong linkage disequilibrium was found by Haploview. Three haplotypes were detected in our study population, that is rs1946518G-rs187238G, rs1946518T-rs187238G and rs1946518T-rs187238C. Their frequency information was showed in Table 3. Haptype rs1946518T-rs187238C carriers were significantly more in AS patients than that of the controls (18.83\% & 13.81\%, \(P=0.041\)) and it was a risk factor of AS in this study group (OR = 1.638, 95%CI = 1.017–2.639).
Table 3
The haplotype analysis of *IL-18* polymorphisms in promoter in AS occurrence

| Rs1946518-rs187238 | Case, 2n = 292 (%) | Control, 2n = 268 (%) | OR (95%CI) | P     |
|---------------------|--------------------|------------------------|------------|-------|
| G-G                 | 137 (46.92)        | 151 (56.34)            | Ref.       | -     |
| T-G                 | 100 (34.25)        | 80 (29.85)             | 1.378 (0.948–2.003) | 0.093 |
| T-C                 | 55 (18.83)         | 37 (13.81)             | 1.638 (1.017–2.639) | 0.041 |

Discussion

In the present study, *IL-18* rs1946518 and rs187238 polymorphisms in promoter region was investigated the association with AS susceptibility in Chinese Han population. The results showed that TT genotype of rs1946518 was significantly associated with the elevated risk of AS and T allele was also significantly higher frequency in AS patients than that of the controls, so rs1946518 was the risk factor of AS. But rs187238 didn't show any significant association with AS occurrence risk in genotype or allele and it was not an independent susceptible factor to AS. In addition, the strong linkage disequilibrium was found between the two polymorphisms and the carriage of rs1946518T-rs187238C haplotype contributed to the risk of AS in this study population. This study is the first time to reveal the impact of *IL-18* polymorphisms on AS occurrence risk in Chinese population.

IL-18 is firstly found to induce T helper type 1 (Th1) and NK cells produce IFN-γ. Its precursor protein in humans consists of 193 amino acids without signal peptide and biological activity. IL-18 is a novel cytokine with the similar structure of IL-1 and the analogous biological function of IL-12 [17]. Inactive IL-18 protein is cleaved by Caspase-1 to acquire activity and Caspase-1 has been found to regulate autoinflammation in the occurrence of rheumatic diseases, including AS [18]. In publication, IL-18 can facilitate Th1 cells to secret a number of cytokines, including IFN-γ, IL-2, GM-CSF and so on, and promote the proliferation of Th1 cells, too [19]. However, the balance level of Th1/Th2 cytokines play the key role in AS [20, 21].

Moreover, as a kind of proinflammatory cytokine, IL-18 is recently found to act on articular hondrocytes and synovial tissues, regulate some cell response and participate in joint inflammation and damage [22]. For example, *IL-18* gene promoter (−607A/C and −137C/G) polymorphisms are explored the role in RA based on a meta-analysis, the results show that −607A/C polymorphism may contribute to the risk of RA [23]. But the relative studies referring to IL-18 and AS are few. Fortunately, published article records that serum IL-18 level in AS patients is obviously higher than that of the controls [24]. IL-18 is also significantly associated with ESR, CRP, BASFI and BASMI which are as indicators of disease activity for AS [25], and also shows the positive association with TNF-α level in AS. We guess that IL-18 level not only reflects disease activity of AS patients, but participants in regulating some inflammatory cytokines expression such as TNF-α so as to affect AS development. Another article speculates that increased IL-18
induce Th1 proliferation to produce more IFN-γ, which leads to the imbalance of Th1/Th2 and damaged cytokine network, and then the accumulation of adverse factors causes AS.

It is useful to explore the association of gene polymorphisms with disease for early diagnosis and pathogenesis explanation. IL-18 encoding gene \textit{IL-18} contains 6 exons and 5 introns and have been discovered a number of SNPs, which rs1946518 and rs187238 in promoter region are the most common SNPs in \textit{IL-18}. rs1946518 (-607G/T) have been reported to be associated with promoter activity \cite{26} and it may alter the expression of \textit{IL-18} mRNA to cause a series of pathological changes in body and involves in AS etiology. Rs187238 is the another functional polymorphism which affects transcriptional activity of \textit{IL-18} \cite{15}. However, so far, no study reports the association of \textit{IL-18} polymorphisms with AS. So this study is necessary and meaningful.

\section*{Conclusions}

In conclusion, \textit{IL-18} rs1946518 polymorphism may involve in the occurrence of AS, but not rs187238. The interaction of the two polymorphisms in this study is also detected in AS and rs1946518T-rs187238C haplotype may be a risk factor of AS. Some limitations should be not ignored, including relative small sample size, only one Han population and selection criteria of subjects, environmental factors. Additionally, the exact mechanism of \textit{IL-18} in AS occurrence is also unclear. Therefore, more studies with well-design are needed to verified the results in this study and explore the mechanism of \textit{IL-18} polymorphisms in AS in the future.

\section*{Abbreviations}

interleukin-18 (\textit{IL-18})

ankylosing spondylitis (AS)

Polymerase chain reaction (PCR)

Hardy-Weinberg equilibrium (HWE)

Odds ratio (OR)

95\% confidence interval (95\%CI)

interferon-γ (IFN-γ)

natural killer (NK)

rheumatoid arthritis (RA)

single nucleotide polymorphism (SNP)
Declarations

Ethics approval and consent to participate

This study was supported by the Ethics Committee of Daping Hospital and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

The subjects had been informed the objective. Certainly, written consents were signed by every subject in this study.

Consent for publication

We obtaining permission from participants to publish their data.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Not applicable.

Authors’ contributions

W.G. design of the work; H.W. the acquisition, analysis, Y.W. interpretation of data; Q.D. the creation of new software used in the work; Z.W. have drafted the work or substantively revised it. All authors read and approved the final manuscript.

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