Interleukin-18 Polymorphisms Impose Considerable Impacts on Ankylosing Spondylitis Occurrence

Wenlong Gou

Daping Hospital

Hai Wang

Daping Hospital

Yu Wang

Daping Hospital

Quanyin Du

Daping Hospital

Ziming Wang (✉ hgngks@126.com)

Daping Hospital <https://orcid.org/0000-0002-3532-8642>

Research article

Keywords: Interleukin-18, Ankylosing spondylitis, Polymorphism, Haplotype

Posted Date: June 15th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-34936/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

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 pathogenesis 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-18* rs1946518 (-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'-CTCTCCCAAGCTTACTTTC-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 ddH₂O to the final volume. The whole PCR procedure was as follows: 95°C predegeneration 2min, 40 cycles of 95°C degeneration 30s, 56 °C annealing 60s, 72°C extension 30s, and final extension at 72°C 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 χ^2 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 *IL-18* polymorphisms in two study groups

Genotype/allele	Case, n = 146 (%)	Control, n = 134 (%)	OR (95%CI)	<i>P</i>	<i>P</i> _{HWE}
rs1946518					0.839
GG	32 (21.92)	42 (31.34)	Ref.	-	
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 *IL-18* two polymorphisms in AS occurrence

The linkage disequilibrium of *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. Hapotype 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)	<i>P</i>
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 secrete 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 *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 *IL-18*. rs1946518 (-607G/T) have been reported to be associated with promoter activity [26] and it may alter the expression of *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 *IL-18* [15]. However, so far, no study reports the association of *IL-18* polymorphisms with AS. So this study is necessary and meaningful.

Conclusions

In conclusion, *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 *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 *IL-18* polymorphisms in AS in the future.

Abbreviations

interleukin-18 (*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)

agarose gel electrophoresis (AGE)

polymerase chain reaction (PCR)

T helper type 1 (Th1)

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.

Funding

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.

Acknowledgements

Not applicable.

References

1. Ghasemi-Rad M, Attaya H, Lesha E, Vegh A, Maleki-Miandoab T, Nosair E, Sepehrvand N, Davarian A, Rajebi H, Pakniat A *et al*: **Ankylosing spondylitis: A state of the art factual backbone**. *World journal of radiology* 2015, **7**(9):236-252.
2. Tsui FW, Tsui HW, Akram A, Haroon N, Inman RD: **The genetic basis of ankylosing spondylitis: new insights into disease pathogenesis**. *The application of clinical genetics* 2014, **7**:105-115.
3. Lindstrom U, Forsblad-d'Elia H, Askling J, Kristensen LE, Lie E, Exarchou S, Jacobsson L: **Perinatal characteristics, older siblings, and risk of ankylosing spondylitis: a case-control study based on national registers**. *Arthritis research & therapy* 2016, **18**:16.
4. Sparks JA, Costenbader KH: **Genetics, environment, and gene-environment interactions in the development of systemic rheumatic diseases**. *Rheumatic diseases clinics of North America* 2014, **40**(4):637-657.

5. Ranganathan V, Gracey E, Brown MA, Inman RD, Haroon N: **Pathogenesis of ankylosing spondylitis - recent advances and future directions.** *Nature reviews Rheumatology* 2017, **13**(6):359-367.
6. Brown MA, Laval SH, Brophy S, Calin A: **Recurrence risk modelling of the genetic susceptibility to ankylosing spondylitis.** *Annals of the rheumatic diseases* 2000, **59**(11):883-886.
7. Brown MA, Kennedy LG, MacGregor AJ, Darke C, Duncan E, Shatford JL, Taylor A, Calin A, Wordsworth P: **Susceptibility to ankylosing spondylitis in twins: the role of genes, HLA, and the environment.** *Arthritis and rheumatism* 1997, **40**(10):1823-1828.
8. Chen WC, Wei JC, Lu HF, Wong HS, Woon PY, Hsu YW, Huang JD, Chang WC: **rs657075 (CSF2) Is Associated with the Disease Phenotype (BAS-G) of Ankylosing Spondylitis.** *International journal of molecular sciences* 2017, **18**(1).
9. Krumm B, Meng X, Xiang Y, Deng J: **Identification of small molecule inhibitors of Interleukin-18.** *Scientific reports* 2017, **7**(1):483.
10. Wawrocki S, Druszczyńska M, Kowalewicz-Kulbat M, Rudnicka W: **Interleukin 18 (IL-18) as a target for immune intervention.** *Acta biochimica Polonica* 2016, **63**(1):59-63.
11. Novick D, Kim S, Kaplanski G, Dinarello CA: **Interleukin-18, more than a Th1 cytokine.** *Seminars in immunology* 2013, **25**(6):439-448.
12. Li LL, Deng XF, Li JP, Ning N, Hou XL, Chen JL: **Association of IL-18 polymorphisms with rheumatoid arthritis: a meta-analysis.** *Genetics and molecular research : GMR* 2016, **15**(1).
13. Angelo HD, Gomes S, II, Oliveira RD, Louzada-Junior P, Donadi EA, Crovella S, Maia MM, de Souza PR, Sandrin-Garcia P: **Interleukin-18, interleukin-12B and interferon-gamma gene polymorphisms in Brazilian patients with rheumatoid arthritis: a pilot study.** *Tissue antigens* 2015, **86**(4):276-278.
14. Przepiera-Bedzak H, Fischer K, Brzosko M: **Serum Interleukin-18, Fetuin-A, Soluble Intercellular Adhesion Molecule-1, and Endothelin-1 in Ankylosing Spondylitis, Psoriatic Arthritis, and SAPHO Syndrome.** *International journal of molecular sciences* 2016, **17**(8).
15. Buraczynska M, Ksiazek K, Zukowski P, Grzebalska A: **Interleukin-18 gene polymorphism and risk of CVD in older patients with type 2 diabetes mellitus.** *Diabetes research and clinical practice* 2016, **121**:178-183.
16. van der Linden S, Valkenburg HA, Cats A: **Evaluation of diagnostic criteria for ankylosing spondylitis. A proposal for modification of the New York criteria.** *Arthritis and rheumatism* 1984, **27**(4):361-368.
17. Afonina IS, Muller C, Martin SJ, Beyaert R: **Proteolytic Processing of Interleukin-1 Family Cytokines: Variations on a Common Theme.** *Immunity* 2015, **42**(6):991-1004.
18. Winkler S, Hedrich CM, Rosen-Wolff A: **[Caspase-1 regulates autoinflammation in rheumatic diseases].** *Zeitschrift fur Rheumatologie* 2016, **75**(3):265-275.
19. Xu MH, Yuan FL, Wang SJ, Xu HY, Li CW, Tong X: **Association of interleukin-18 and asthma.** *Inflammation* 2017, **40**(1):324-327.
20. Wang J, Zhao Q, Wang G, Yang C, Xu Y, Li Y, Yang P: **Circulating levels of Th1 and Th2 chemokines in patients with ankylosing spondylitis.** *Cytokine* 2016, **81**:10-14.

21. Wen JT, Zhang DH, Fang PF, Li MH, Wang RJ, Li SH: **Role of Th1/Th2 cytokines in the diagnosis and prognostic evaluation of ankylosing spondylitis.** *Genetics and molecular research : GMR* 2017, **16**(1).
22. Dai SM, Matsuno H, Nakamura H, Nishioka K, Yudoh K: **Interleukin-18 enhances monocyte tumor necrosis factor alpha and interleukin-1beta production induced by direct contact with T lymphocytes: implications in rheumatoid arthritis.** *Arthritis and rheumatism* 2004, **50**(2):432-443.
23. Cai LP, Zhou LJ, Lu SY, Liang YE, Chen XY, Liu L, Lin J: **Association of IL-18 promoter gene polymorphisms with rheumatoid arthritis: a meta-analysis.** *Molecular biology reports* 2014, **41**(12):8211-8217.
24. Sari I, Kebapcilar L, Taylan A, Bilgir O, Kozaci DL, Yildiz Y, Yuksel A, Gunay N, Akkoc N: **Fetuin-A and interleukin-18 levels in ankylosing spondylitis.** *International journal of rheumatic diseases* 2010, **13**(1):75-81.
25. Przepiera-Bedzak H, Fischer K, Brzosko M: **Extra-Articular Symptoms in Constellation with Selected Serum Cytokines and Disease Activity in Spondyloarthritis.** *Mediators of inflammation* 2016, **2016**:7617954.
26. Yang B, Feng W, Li Y, Shi Y, Cai B, Liao Y, Zhang J, Huang Z, Wang L: **Interleukin 18 -607 A/C Gene Polymorphism is Associated With Susceptibility to IgA Nephropathy in a Chinese Han Population.** *Applied immunohistochemistry & molecular morphology : AIMM* 2017, **25**(10):725-730.