Original Article

The Impact of Functional Promoter Variants in Interleukin-18 on Susceptibility to Rheumatoid Arthritis in Iranian Population

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Abstract

Background: Interleukin-18 (IL-18) is recognized for its pro-inflammatory properties and plays a central role in the progression of rheumatoid arthritis (RA). The specific single-nucleotide polymorphisms (SNPs), rs1946518 (-607C>A) and rs187238 (-137G>*C*), that are found in the IL-18 promoter region can potentially impact the expression of the IL-18 gene. This study aimed to investigate the correlation between these two polymorphisms and RA in the Iranian population.

Materials and Methods: In this study, we conducted a case–control analysis with a total of 275 subjects consisting of 135 patients with RA and 140 controls. The high-resolution melting (HRM) method, performed through real-time polymerase chain reaction, was utilized for genotyping these polymorphisms.

Results: Regarding the rs1946518 polymorphism, the frequency of AA and CA genotypes and allele A was significantly greater in the control group compared to the RA group (AA vs CC; OR: 0.42; 95%CI [0.198-0.872], CA vs CC; OR: 0.57; 95%CI [0.324-1.001], A vs C; OR: 0.58; 95%CI [0.401-0.836] ($P < 0.05$). There was no statistically significant difference in the frequency of genotypes and allele frequencies between the control and patient groups in terms of the rs187238 polymorphism ($P > 0.05$). The level of both the C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) was notably elevated in the patient group with CC genotype in rs1946518 ($P < 0.05$).

Conclusion: In the rs1946518 polymorphism, the AA and AC genotypes and the A allele demonstrated protective effects in RA. Besides, the CC genotype was associated with some laboratory characteristics in the RA group.

Keywords: Autoimmune, interleukin-18, polymorphism, rheumatoid arthritis

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Introduction

The prevalence of rheumatoid arthritis (RA) has remained relatively stable within the range of 0.5% to 1% across diverse populations. Notably, specific populations exhibit a higher prevalence, while others display lower rates, demonstrating the influence of genetic factors on the progression of RA.[1,2] Although the precise origins of RA are not yet fully understood, it appears that a combination of genetic and environmental factors may play a role in autoantibody production.[3,4]

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Immunomodulating molecules including cytokines secreted by immune cells are among the critical mediators associated with the development of RA, some of which may serve as potential targets for future therapeutic interventions.[5,6] As a pro-inflammatory cytokine with pleiotropic effects, interleukin 18 (IL-18) is known as an interferon-γ-inducing factor that has a part in regulating both the innate and adaptive immune systems and is synthesized by different types of cells encompassing macrophages, possibly lymphocytes, dendritic cells, and non-immune cells.[7,8] It stimulates IFN-γ and exerts various biological effects including a crucial role in immune responses driven by T helper 1 (Th1) cells.[9] IL-18, on its own, is not directly engaged in the growth of Th1; however, it has a function in the triggering of Th1 cells through interleukin 12.[10] In T cells, IL-12 and IL-18 work together to trigger IFN-γ, and IL-18 enhances the expression of the IFN-γ receptor in cells that produce gamma interferon.[8]

The association between the level of interleukin-18 and several autoimmune diseases including systemic lupus erythematosus (SLE), multiple sclerosis, myasthenia gravis, and RA has been revealed.[11-13] The concentration of interleukin 18 rises in the synovial serum, synovial tissue, and synovial fluid of individuals with RA.[14] In joints affected by RA, IL-18 plays a role in inflammation by promoting the extravasation of leukocytes. Potentially, IL-18 contributes to the expansion of inflammatory tissue by promoting angiogenesis and inducing cell movement.[15]

The concentration of IL-18 in joints' synovial tissues is linked to the magnitude of IL-1β, TNF-α, and the intensity of inflammation. IL-18, IL-1β, or TNF-α induce the formation of osteoclasts by promoting a rise in receptor activator of nuclear factor-κB ligand (RANKL) production from T cells in the synovial inflammation of patients with RA through indirect mechanisms.[16]

Numerous studies have demonstrated the significant influence of the -607C/A (rs1946518) and -137G/C (rs187238) variants on the expression levels of IL-18, particularly in association with different diseases.[17-19] The presence of rs1946518C allele and rs187238G allele in the promoter of the IL-18 gene facilitates the attachment of transcription factors and; consequently, elevates the magnitude of IL-18 mRNA.[20] Due to the diverse genetic backgrounds across populations, we conducted the first investigation into the correlation between rs1946518 and rs187238 and susceptibility to RA in the Iranian population. To explore the correlation between these variants and some clinical characteristics such as disease activities, we examined the interplay between some laboratory factors and these two polymorphisms.

Materials and Methods

This case–control study conducted 275 participants consisting of 135 patients with RA and 140 healthy individuals. RA diagnosis was based on the American College of Rheumatology (ACR) 2010 criteria.[21] The control individuals were age- and gender-matched to the case group. All patients and healthy controls were recruited from the rheumatology clinics and inpatient wards at Imam Reza Hospital of Aja University of Medical Sciences, Tehran, Iran. The individuals in the control group had no signs and a history of autoimmune and autoinflammatory diseases. We employed a written questionnaire to collect patient data, including age, gender, age at disease onset, body mass index (BMI), blood pressure, and family background of RA and associated disorders. Laboratory parameters, such as serum concentrations of C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), white blood count (WBC), hemoglobin, platelets (PLT), creatinine, and other relevant factors, were assessed. The research ethics committee of AJA University approved the study (with the reference number IR.AJAUMS.REC.1401.193).

SNP selection and genotyping

After conducting a comprehensive review of studies, we selected two variants that impact the transcription of the IL-18 gene. The two genetic variations, namely rs1946518 and rs187238, are located within specific sites for binding transcription factors, such as cAMP response element-binding proteins (CREB) and histone H4 gene transcription factor 1 (H4TF-1). These binding sites are situated in the IL-18 promoter region.[20]

The quality and quantification of the DNA samples that were extracted were assessed using a UV-Vis spectrophotometer and gel electrophoresis. The genotyping of rs1946518 and rs187238 SNPs was performed using the polymerase chain reaction high-resolution melting (PCR-HRM) method which was described in previous studies.[22,23] PCR was carried out utilizing the forward and reverse primers outlined in Table 1 to amplify fragments containing rs1946518 and rs187238 in the IL-18 gene promoter sequence.

Statistical analyses

We undertook statistical analysis using SPSS 25. Logistic regression was utilized to compute odds ratios, confidence intervals, and *P* values to evaluate the association between genotype and RA risk. For laboratory tests and other clinical characteristics, we calculated *P* values using Student's *t*-test or Chi-square, with a level of significance at $P < 0.05$.

Results

Demographic and laboratory features

The mean age of the subjects was 46.67 ± 10.10 years for the patients and 45.27 ± 12.41 years for the control group. Table 2 provides a summary of the baseline characteristics of the study participants. The patient and control groups did not show significant differences in mean age (*P*: 0.307) or sex (*P*: 0.896), indicating that the matching between these groups was well-suited. In the RA group, 28 individuals had a positive family history, whereas the individuals in the healthy group did not have a history of this disease in their families. The patient group had a significantly higher BMI than the

Table 1: Primer sequences for the amplification of fragments around the two polymorphisms of the IL-18 gene

SNP ID	Primer sequence	PCR product length (bp)	Annealing temperature
rs1946518	F: GCCACACGGATACCATCATTAG	104	59°C
	R: TGCCCTCTTACCTGAATTTTGG		
rs187238	F: TGGCAGAGGATACGAGTAC	149	59°C
	R: GGACTAAGGAGGTGCTTTC		

Table 2: Baseline characteristics of rheumatoid arthritis (RA) patients and control subjects who participated in this study

Characteristics	Patients	Controls	*P*
Total number	135	140	--
Age at sampling	46.67±10.10	45.27±12.41	0.307
Gender *n* (%)			
Male	41 (30.4%)	44 (31.4%)	
Female	94 (69.6%)	96 (68.6%)	0.896
Age of onset	40.86±9.81	--	--
BMI	26.30±2.65	24.57±3.02	<0.001*
SBP	123.18±12.33	121.17±9.64	0.135
DBP	78.25±8.49	78.85±8.36	0.557
Positive family history *n* (%)	28 (20.7%)	0	<0.001*

**P*<0.05. RA: Rheumatoid arthritis; BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure

control group ($P < 0.001$). However, no significant difference was observed in blood pressure between the control group and RA patients ($P > 0.05$).

In terms of laboratory tests, the RA patients showed significantly higher levels of ESR, CRP, and creatinine compared to the control group ($P < 0.001$). On the other hand, hemoglobin levels in the RA group were notably lower than those in the control group ($P < 0.001$). The RA group also had a significantly higher WBC count and triglyceride levels than the control group ($P < 0.05$). No statistically significant difference was observed between the control and patient groups in the results of other laboratory tests [Table 3].

The rs1946518 polymorphism (-607C/A)

There was a statistically significant increase in the frequency of CA and AA genotypes in the control group compared to the RA group ($P < 0.05$). The genotypes CC, CA, and AA were observed with frequencies of 53.33%, 33.34%, and 13.33% in the RA group. In comparison, the control group exhibited frequencies of 37.14%, 40.72%, and 22.14% for the same genotypes, respectively. The combined genotypic frequencies of CA+AA (CA+AA vs. CC) in the dominant model showed a decreased risk associated with RA (*P*: 0.007) [Table 4]. The analysis of allele distribution revealed a significant increase in the frequency of the A allele in the control group compared to the patient group (*P*: 0.002). Moreover, stratified analysis did not reveal any statistically significant association between SNP genotypes and sex, age of onset, and creatinine subgroups in patients ($P > 0.05$).

The average levels of ESR and CRP in the patient group showed statistically significant differences when categorized by genotype ($P < 0.001$). Individuals with the CC genotype exhibited greater levels of CRP and ESR in the RA group compared to those with the CA+AA genotype ($P < 0.05$) [Table 5].

The rs187238 polymorphism (-137 G/C)

No statistically significant disparity was observed in the frequency of rs187238 genotypes between individuals with RA and the control group. In the control group, the frequencies of GG, GC, and CC genotypes were 52.86%, 37.86%, and 9.28%, respectively, while in the RA patient, the frequency of these genotypes was 58.52%, 36.30%, and 5.18%, respectively.

Our assessment of different inheritance models for the rs187238 polymorphism showed that the genotype frequencies did not indicate an increased or decreased risk of RA under the dominant/recessive models ($P > 0.05$). In our analysis of allele distribution, we found that the G allele was more frequent in (76.67%) RA group in contrast to the control group (71.79%), but this difference was not statistically significant (*P*: 0.205) [Table 4].

Regarding the age of onset and gender, as well as the levels of ESR, CRP, and creatinine, no statistically significant association was identified in the genotype-based classification for assessing the risk of RA in the patient group ($P > 0.05$) [Table 5].

Discussion

The association between cytokine gene polymorphisms and various autoimmune diseases including RA has been demonstrated in numerous studies.[24-26] The expression of interleukin 18 protein and mRNA was revealed to be elevated in the synovial tissue of individuals diagnosed with RA.[27] One research illustrated that IL-18 can induce proinflammatory cytokine generation including IL-1β and TNFα in both macrophages and monocytes.[28] A study found that IL-18 in the synovial tissue of individuals suffering from RA is closely linked with IL-1β and TNFα.[29]

Researchers conducted a study to investigate the involvement of interleukin-18 in synovial inflammation in patients with RA. They examined mice lacking IL-18 (IL-18-/-) and induced arthritis using collagen.

Mice lacking interleukin-18 gene demonstrate reduced rates of incident RA and reduced severity of illness compared to normal mice. This was accompanied by decreased joint inflammation and damage, highlighting the crucial involvement of IL-18 in the progression of inflammatory arthritis.[30] Substituting

a cytosine nucleotide with adenine at position rs1946518 disrupts the binding site for CREB and negatively affects the transcription of the IL-18 gene. Furthermore, the replacement of guanine with cytosine at position rs187238 affects the binding region of H4TF-1, leading to diminished promoter activity caused by the presence of the C allele.[31] A study showed that the patient group had elevated levels of IL-18 in the serum compared to the control group. However, no significant difference was found among the different genotypes.[32] During the investigation of promoter activity about the IL-18 gene G to C polymorphism at position rs187238, variations in the expression of interleukin 18 mRNA were detected, depending on the specific allele type.[20]

In peripheral blood mononuclear cells (PBMCs) activated by LPS, individuals carrying the C allele at position rs187238 exhibited reduced levels of IL-18. Conversely, at position rs1946518 in LPS-stimulated PBMCs, individuals with the A allele showed increased production of IL-18 versus healthy control comparison group.[33] Individuals with chronic hepatitis C virus infection who had the GG genotype at position rs187238 and CC genotypes at position rs1946518 showed increased spontaneous production of IL-18.[34] Cavalcante *et al*.[35] studied the Brazilian population with type 2 diabetes. They found that individuals carrying the rs187238 position CC genotype exhibited increased serum concentrations of IL-18. Arimitsu and coworkers found that monocytes with the genotype G/G at position rs187238 have a greater capacity for IL-18 production in contrast to those with the G/C genotype.[36]

Our work is the first study for exploring the connection between IL-18 variants, namely rs1946518 and rs187238, and their association with susceptibility to RA in Iranian subjects. Our findings revealed a higher frequency of the A allele and AC

Table 3: Laboratory characteristics of patients with rheumatoid arthritis (RA) and the control group

	Patients (135)	Controls (140)	*P*
ESR (mm/h)	38.45±21.34	16.11±6.89	<0.001*
CRP (mg/l)	17.24±12.55	4.5414±2.90	<0.001*
White blood cell (10^9/1)	7.35±2.90	6.61±1.41	0.001*
Hemoglobin	12.46±1.09	14.18±1.60	<0.001*
PLT (10^9/1)	265.86±70.55	248.89±66.69	0.041*
Creatinine (mg/dL)	1.03±0.19	0.86±0.17	<0.001*
BUN	17.11±4.73	16.30±3.86	0.123
FBS	97.82±16.24	94.01±22.24	0.107
HDL	49.28±7.93	50.53±10.94	0.279
LDL	109.50±29.25	104.99±30.24	0.210
TG	171.90±45.60	156.52±56.97	0.014*

*P<0.05. RA=Rheumatoid arthritis; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; BUN: Blood urea nitrogen; PLT: Platelet; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; TG: Triglyceride; FBS: Fasting blood sugar; SD: Standard deviation

Table 4: Association between genotypes and allele frequency of IL-18 polymorphisms with rheumatoid arthritis (RA) risk

Genotype group	Patients (n=135) n (%)	Controls (n=140) n (%)	OR (95%CI)	*P*
rs1946518				
CC	72 (53.33%)	52 (37.14%)	Reference	---
CA	45 (33.34%)	57 (40.72%)	0.57 (0.324-1.001)	0.044*
AA	18 (13.33%)	31 (22.14%)	0.42 (0.198-0.872)	0.017*
Dominant inheritance				
CC	72 (53.33%)	52 (37.14%)	Reference	---
CA + AA	63 (46.67%)	88 (62.86%)	0.51 (0.310-0.860)	0.007*
Recessive inheritance				
AA	18 (13.33%)	31 (22.14%)	Reference	0.060
CA + CC	117 (86.67%)	109 (77.86%)	1.84 (0.938-3.718)	
Allele				
C	189 (70.00%)	161 (57.50%)	Reference	---
A	81 (30.00%)	119 (42.50%)	0.58 (0.401-0.836)	0.002*
rs187238				
GG	79 (58.52%)	74 (52.86%)	Reference	---
GC	49 (36.30%)	53 (37.86%)	0.86 (0.508-1.474)	0.610
CC	7 (5.18%)	13 (9.28%)	0.50 (0.161-1.454)	0.234
Dominant inheritance				
GG	79 (58.52%)	74 (52.86%)	Reference	---
GC + CC	56 (41.48%)	66 (47.14%)	0.79 (0.479-1.316)	0.395
Recessive inheritance				
CC	7 (5.18%)	13 (9.28%)		
GC + GG	128 (94.81%)	127 (90.72%)	0.53 (0.174-1.500)	0.246
Allele				
G	207 (76.67%)	201 (71.79%)	Reference	---
C	63 (23.33%)	79 (28.21%)	0.77 (0.517-1.156)	0.205

*P<0.05

Table 5: Association of IL-18 polymorphisms with various parameters of rheumatoid arthritis (RA)

	rs1946518		*P*
	CC (*n*: 72)	CA + AA (*n*: 63)	
Age of onset	40.13±9.19	41.69±10.50	0.359
Sex			
Males	21 (29.17%)	20 (31.75%)	0.445
Females	51 (70.83%)	43 (68.25%)	
ESR (mm/h)	44.98±22.91	31.00±16.65	<0.001*
CRP (mg/l)	20.62±14.74	13.36±7.93	0.001*
Creatinine (mg/dL)	1.03±0.20	1.02±0.18	0.670
	rs187238		*P*
	GG (*n*: 79)	GC + CC (*n*: 56)	
Age of onset	40.74±11.20	41.03±7.54	0.858
Sex			
Males	22 (27.85%)	19 (33.93%)	
Females	57 (72.15%)	37 (66.07%)	0.285
ESR (mm/h)	38.67±22.16	38.16±20.34	0.892
CRP (mg/l)	16.29±10.93	18.56±14.53	0.303
Creatinine (mg/dL)	1.03±0.18	1.03±0.21	0.994

*P<0.05. ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; SD: Standard deviation

and AA genotypes at the rs1946518 polymorphism among healthy individuals when compared to those carrying the C allele ($P < 0.01$). Similarly, the existence of individuals with the CA + AA genotype (dominant model) was higher among control subjects than those with RA [Table 4].

Mihailova *et al*.[37] found that the rs1946518 CA position likely influences susceptibility to RA. However, in contrast to our study, they observed a greater distribution of the genotype CA in the control group. A previous investigation indicated that the rs187238 polymorphism might confer a risk for RA.[38] Nevertheless, this correlation was not evident in the Iranian population [Table 4].

A cohort study in Caucasians showed that the haplotype of rs1946518C/rs187238C is more common among RA patients of German and Scottish descent compared to healthy persons, suggesting that SNPs play a vital role in the genetic basis of RA development.[39] Sivalingam *et al*.[31] concluded that the frequency of the AA genotype is higher in the healthy group at position rs1946518 in comparison with the patient group. Their findings suggest that the presence of the AA genotype is linked to reduced interleukin 18 transcription and provides protection against RA. This is important because elevated expression of interleukin 18 protein can trigger an inflammatory response.

In a study conducted by Shi *et al*.,[40] a notable disparity in allelic frequency and genotype distribution at position rs1946518 of IL-18 was observed between the control group and the patient group. However, there were no statistically significant differences in genotype distribution and allelic frequency at position rs187238 of IL-18. Our results are in line with this finding, indicating that the presence of the C allele in patients is associated with a heightened susceptibility to RA, while the presence of the A allele provides greater protection against the disease. Differences in findings regarding the relationship between RA and these two polymorphisms have been observed across various populations, indicating some variations in results. Rueda *et al*.[41] reported that the rs1946518 and rs187238 polymorphisms are not linked with susceptibility to RA. Pawlik and colleagues[42] demonstrated that these two variants do not affect the activity of RA, joint involvement, or extra-articular features in the Polish population. Similarly, Ying *et al*.[32] did not demonstrate a connection between the rs1946518 SNP and RA disease. In a meta-analysis study, no correlation was detected between autoimmune diseases and the rs1946518 polymorphism in the promoter of the IL-18 gene. Research on RA with a limited sample size may lack the statistical capability to detect minor effects.[43] Divergent outcomes in distinct ethnic groups could be linked to variations in both genetic and environmental influences.

Among Japanese individuals with sarcoidosis, the genetic SNP of IL-18 at rs1946518 with the C allele may represent a hereditary risk factor.[44] Among the healthy group, the presence of rs1946518 AA genotypes was observed in relation to type 1 diabetes. Conversely, at position rs187238, the GC genotype frequency was raised, and the GG genotype frequency declined.[39] A study on asthmatic children from Egypt examined the gene polymorphism of IL-18 at position rs1946518C/A. The study found no significant differences in allele and genotype frequency between asthma patients and the control group. However, individuals with the AA mutant genotype had a higher mean value of IgE in comparison with the control group.[45]

Our patients with the CC genotype at rs1946518 exhibited elevated levels of CRP and ESR in RA, indicating the presence of inflammatory processes and the active stage of the illness. According to a study, there is an indication for RA where the CC genotype in the rs1946518 SNP shows a correlation with elevated levels of ESR and CRP. Conversely, the lowest levels of ESR and CRP are observed in the AA and AC genotypes among individuals in the RA group.[46] Pawlik *et al*.[47] provide evidence that in the rs1946518 SNP, the presence of CC and AC genotypes is associated with erosive disease, as opposed to the AA genotype. However, further investigations are required to thoroughly examine the relationship between the CC genotype and predisposition to RA.

The rs1946518 polymorphism shows that the AA and AC genotypes, as well as the A allele, have a protective effect against RA. On the other hand, the CC genotype is associated with higher levels of ESR and CRP in the RA group. In conclusion, rs1946518 polymorphism demonstrated protective effects in RA and was correlated with some clinical characteristics of this diseases. Due to conflicting findings in studies, differences in genetic backgrounds, and existing controversies, it is strongly recommended that future studies be conducted in other populations.

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Ethics approval and consent to participate

This study was approved by the ethics committee of AJA university of medical sciences with the reference number IR.AJAUMS.REC.1401.193.

Informed consent was obtained from all subjects. Also, all authors agree to publish this manuscript in your valuable journal.

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Conflicts of interest

There are no conflicts of interest.

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