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Importance of STAT3 Polymorphisms on the Risk and Clinical Characteristics of Rheumatoid Arthritis

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ABSTRACT

Signal transducer and activator of transcription 3 (*STAT3*) has been introduced as one of the critical genetic factors in the pathogenesis of rheumatoid arthritis (RA). Single nucleotide polymorphisms (SNPs) in microRNA binding sites, known as miRSNPs, are a class of common variants in the 3′ untranslated regions of genes targeted by miRNAs. miRSNPs unbalance gene expression by disrupting the binding regions of microRNAs. In this study, we intended to evaluate the association of two miRSNPs with the risk of RA development and its clinical features.

We studied 120 Iranian patients with RA and 125 non-RA subjects as controls. The genotypes and alleles of rs1053005 and rs1053023 in each individual were assessed by the high-resolution melting method.

The distribution of *STAT3* variants did not differ markedly in RA patients compared to healthy controls. Stratification analysis revealed that rs1053005 was linked with a higher concentration of C-reactive protein and an increased erythrocyte sedimentation rate, two indicators of inflammation and disease activity in RA patients. The rs1053023 variant was correlated with higher levels of creatinine as an indicator of renal involvement.

Our data demonstrate an association between *STAT3* variants and clinical characteristics of RA, such as disease activity and probably kidney impairment. However, we did not observe a significant relationship between the two targeted variants and a predisposition to RA.

Keywords: MicroRNAs; Rheumatoid arthritis; Single nucleotide polymorphisms; *STAT3*

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INTRODUCTION

Signal transducer and activator of transcription 3 (*STAT3*) is a transcription factor that demonstrates critical roles in the pathogenesis of autoimmune diseases

via the mediation of T helper 17 (Th17) cell differentiation, interleukin 6 (IL-6) signaling pathway, and an increase in the concentration of IL-17.[1-3] The increased level of activated STAT3 (phosphorylated [p]-STAT3) has been shown in patients with systemic lupus erythematosus (SLE) who had no renal damage. This trend has been especially detected in patients with lupus nephritis and other chronic kidney diseases.[4-7] Similarly, in patients with rheumatoid arthritis (RA), the upregulation of *STAT3* mRNA and p-STAT3 in different cell types, including synovial fluid T cells, monocytes, and peripheral blood T cells, has been reported. Also, a direct relationship between STAT3 levels and the production of IL-6 and IL-17 has been revealed.[8-10] STAT3 is recognized as a critical element in RA pathogenesis. It is involved in the disease activity and severity through the induction of production of proinflammatory cytokines, migration of lymphocytes and synoviocytes, and survival of synovial fibroblasts.[4,11-14] Regarding the association of *STAT3* inhibitors with the inhibition of arthritis and joint erosion, some studies have suggested *STAT3* as a potential target for RA treatment.[15-17]

Some studies have provided evidence that microRNAs (miRNAs) play a major role in the regulation of *STAT3* expression.[18,19] The seed regions of miRNAs bind to the 3′ untranslated regions (3′UTRs) of target mRNAs to suppress their expression, either with or without mRNA degradation.[20,21] Germline variants in the 3′UTRs region could lead to dysregulation of gene expression through complete disruption of the binding region or formation of a new miRNA binding site and subsequent increase or decrease in the miRNA-to-mRNA binding efficiency. These variants could directly change the sequence of the miRNA binding sites or impact the secondary structure and thermodynamic features of other flanking binding locations.[22-24]

Given the role of STAT3 in immune function and its dysregulation in autoimmune disorders, especially RA, it could be predicted that single-nucleotide polymorphisms (SNPs) in miRNA binding regions (miRSNPs) in *STAT3* might implicate susceptibility to RA.

In our previous report, we showed the impact of miRSNPs in the *STAT3* gene on the occurrence and clinical characteristics of SLE.[25] Accordingly, in the current study, with regards to the similar pathogenic molecular mechanism of SLE and RA, we chose two miRNA binding site variants in the *STAT3* sequence (rs1053005 and rs1053023) to determine the possible association between *STAT3* miRSNPs and predisposition to RA in an Iranian population. Moreover, we assessed the relationship between these miRSNPs in the *STAT3* gene and some clinical parameters of RA in the patients.

MATERIALS AND METHODS

Sample Collection

In the current case-control research, we enrolled 120 patients with RA from the Rheumatology Department of Alzahra Hospital, Isfahan, Iran, who fulfilled the 2019 American College of Rheumatology (ACR) criteria for RA.[26] The control group comprised 125 disease-free, unrelated individuals recruited from the same medical center. All the subjects were of Iranian ethnicity, and age and gender were matched between the two groups.

The Research Ethics Committee of Isfahan University of Medical Sciences approved this study (ID number: IR.MUI.MED.REC.1400.096), and informed consent was obtained from all participants. The study participants' demographic factors and laboratory findings were documented using a structured questionnaire. Finally, 3 ml of whole blood was collected from each subject and was frozen at −20°C until further experiments.

miRSNP Selection and Genotyping

As previously stated,[25] we selected the genetic variants by literature review and exploring SNP, miRNA, and miRSNPs databases. Accordingly, two SNPs in the 3′UTR of *STAT3* (rs1053005 and rs1053023) were selected to study their association with a predisposition to RA development.

DNA was isolated from blood samples of patients and healthy subjects by the DNA Extraction Kit (GeNetBio, Korea) based on the manufacturer's protocol. The rs1053005 and rs1053023 variants were genotyped using the high-resolution melting (HRM) method by employing HOT FIREPol EvaGreen HRM Mix (no ROX) (Solis BioDyne, Tartu, Estonia) and a Rotor-Gene 6000™ (Corbett Research, Mortlake, New South Wales, Australia). The primer sequences and HRM protocol have been mentioned previously.[25]

Logistic regression analysis was accomplished to investigate the association between genotypes and RA and to calculate specific odds ratios (OR), 95% confidential intervals (CI), and *p* values. For

demographic, clinical, and laboratory characteristics, *p* values were calculated using the independent sample t-test, the Chi-squared test, or the Mann–Whitney U test. SPSS 25.0 (SPSS Statistics 25, Armonk, NY, IBM Corp.) was used for statistical analyses, and p<0.05 was considered significant.

RESULTS

Of the patients, 36 (30%) were males, and 84 (70%) were females. In comparison, the control group consisted of 41 (32.8%) men and 84 (67.2%) women. The mean age of disesase onset was 41.18±10.26. Subjects with RA had an elevated body mass index (BMI) compared to controls (26.09±2.50 vs. 24.18±3.29; $p<0.001$). Twenty (16.7%) of patients showed a history of immune-related diseases. The complete demographic characteristics are shown in Table 1. Considering the laboratory data, RA cases presented with significantly increased serum levels of the following: erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), blood urea nitrogen (BUN), white blood cell (WBC) count, creatinine, and triglyceride ($p<0.05$). However, the mean level of hemoglobin was higher in healthy individuals than in RA cases (p<0.001). Table 2 details laboratory features in RA and non-RA participants.

STAT3 Genotypes and RA Risk

The frequencies of the AA, GG, and AG genotypes of rs1053005 in RA cases were 46.6%, 26.7%, and 26.7%, respectively, whereas, in the control group, these frequencies were 47.2%, 31.2%, and 21.6%.

Furthermore, the frequencies of the G and A alleles in controls were 37.2% and 62.8%, respectively, while these numbers in RA participants were 40% and 60%, respectively. No statistically significant difference was observed between different genotypes and the risk of RA development. Moreover, combined genotype analysis showed similar results between the case and control groups (AG+GG vs. AA; $p=0.999$).

Given genotype distributions and allele frequencies, rs1053023 was not associated with the risk of RA. The genotype distributions and allele frequencies in RA and control subjects were, respectively, as follows: AA (50% vs. 55.2%), AG (24.2% vs. 28%), GG (25.8% vs. 16.8%) genotypes, and A (62.08% vs. 69.2%) and G (37.92% vs. 30.8%) alleles. The distribution of alleles and genotypes for rs1053005 and rs1053023 are shown in Table 3.

Genotype Stratification Analysis

Stratification analysis revealed that the mean levels of CRP, ESR, and hemoglobin are significantly different in RA patients with various genotypes of rs1053005 ($p<0.05$). Based on the stratification data for rs1053005, the A allele (AA and AG genotypes) is associated with increased levels of CRP and ESR. In the stratification based on the rs1053023 polymorphism, the G allele is associated with increased creatinine ($p<0.001$). The mean levels of creatinine in RA subjects with AA, AG, and GG genotypes were 0.89±0.12, 1.12±0.12, and 1.20±0.16, respectively. The details of the findings obtained by stratification analysis for the rs1053005 and rs1053023 polymorphisms in the RA group are documented in Table 4.

Table 1. Baseline characteristics of rheumatoid arthritis patients and controls

Characteristics	Patients	Controls	*p*
Total Number	120	125	--
Current Age	47.41±10.30	45.36±12.49	0.162
Gender n (%)			
Male	36 (30%)	41 (32.8%)	
Female	84 (70%)	84 (67.2%)	0.637
Age of Onset	41.18±10.26	--	--
Positive Family History n (%)	20 (16.7%)	0	--
SBP	122.70±12.48	120.96±9.60	0.222
DBP	78.66±7.744	78.56±8.29	0.917
BMI	26.09±2.50	24.18±3.29	<0.001*

*p value < 0.05. RA: rheumatoid arthritis; SBP: systolic blood pressure; DBP: diastolic blood pressure; BMI: body mass index.

Table 2. Laboratory results of rheumatoid arthritis cases and controls

	Patients (120)	Controls (125)	*p*
ESR (mm/h)	37.47±22.33	15.55±6.81	<0.001*
CRP (mg/L	16.63±12.91	4.52±2.78	<0.001*
White Blood Cells (10^9/L)	7.30±2.17	6.57±1.35	0.002*
Hemoglobin	12.42±1.06	14.19±1.65	<0.001*
PLT (10^9/L)	264.35±72.29	250.91±65.66	0.129
Creatinine (mg/dL)	1.03±0.19	0.85±0.17	<0.001*
BUN	17.26± 4.79	16.04± 4.02	0.032*
FBS	96.83±15.78	92.99±21.57	0.114
HDL	49.39±7.62	50.47±10.93	0.369
LDL	109.88±28.86	106.36±30.85	0.358
TG	170.02±47.67	155.96±58.67	0.041*

*p value < 0.05. RA: rheumatoid arthritis; SD: standard deviation; 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.

Table 3. Association between genotypes and allele frequency of *STAT3* polymorphisms with the risk of rheumatoid arthritis

Genotype Group	Patients (n = 120) n (%)	Controls (n = 125) n (%)	OR (95%CI)	*p*
rs1053005				
AA	56 (46.6%)	59 (47.2%)	Reference	--
AG	32 (26.7%)	39 (31.2%)	0.86 (0.45-1.63)	0.652
GG	32 (26.7%)	27 (21.6%)	1.24 (0.63–2.46)	0.524
Combined Genotype				
AA	56 (46.7%)	59 (47.2%)	Reference	--
AG+GG	64 (53.4%)	66 (52.8%)	1.02 (0.59-1.74)	0.999
Allele				
A	144 (60%)	157 (62.8%)	Reference	--
G	96 (40%)	93 (37.2%)	1.12 (0.76-1.64)	0.577
rs1053023				
AA	60 (50%)	69 (55.2%)	Reference	--
AG	29 (24.2%)	35 (28%)	0.95 (0.49-1.81)	0.999
GG	31 (25.8%)	21 (16.8%)	1.69 (0.84-3.45)	0.139
Combined Genotype				
AA	60 (50%)	69 (55.2%)	Reference	--
AG+GG	60 (50%)	56 (44.8%)	1.23 (0.72-2.10)	0.444
Allele				
A	149 (62.08%)	173 (69.2%)	Reference	--
G	91 (37.92%)	77 (30.8%)	1.37 (0.92-2.03)	0.106

RA: rheumatoid arthritis

Table 4. Association of *STAT3* polymorphisms with various parameters of rheumatoid arthritis

	rs1053005			
	AA (n = 56)	**AG (n = 32)**	**GG (n = 32)**	***p* value**
Age of Onset	42.33±9.80	41.53±9.88	38.81±11.29	0.295
ESR (mm/h)	45.08±25.31	34.68±20.35	26.93±11.71	0.001*
CRP (mg/l)	21.22±15.63	14.93±8.47	10.30±7.24	<0.001*
Creatinine (mg/dL)	1.016±0.17	1.02±0.20	1.04±0.21	0.789
Hemoglobin	12.57±1.04	12.62±1.05	11.95±.98	0.013*
BMI	26.02±2.46	26.63±2.43	25.67±2.62	0.30
	rs1053023			
	AA (n = 60)	**AG (n = 29)**	**GG (n = 31)**	***p* value**
Age of onset	40.45±9.41	39.48±10.22	44.19±11.50	0.152
ESR (mm/h)	39.70±23.82	37.55±22.59	33.09±18.90	0.413
CRP (mg/L)	16.07±10.71	15.80±11.91	18.47±17.27	0.654
Creatinine (mg/dL)	0.89±0.12	1.12±0.12	1.20±0.16	<0.001*
Hemoglobin	12.52±0.93	12.22±1.11	12.42±1.24	0.481
BMI	26.39±2.70	25.82±2.40	25.75±2.07	0.411

*p value < 0.05. RA: rheumatoid arthritis; SD: standard deviation; ESR: erythrocyte sedimentation rate; CRP: C‑reactive protein; SD: standard deviation; BMI: body mass index.

DISCUSSION

Previous studies have demonstrated that STAT3 plays a pivotal role in B cell function, differentiation of T cells into T follicular helper and Th17 cells, plasma cell differentiation, and production of IL-6, IL-17, and antibodies.[1,2,27] One study showed that germline gain of function mutations could lead to multiorgan autoimmune manifestations.[28] The positive effect of using *STAT3* inhibitors has been described in several experimental models of autoimmune disorders such as inflammatory bowel diseases, psoriasis, SLE, and RA.[29-32] As overexpression of *STAT3* plays an active role in the pathogenesis of RA, this gene has been suggested as a promising target for the treatment of RA.[15,33] Moreover, STAT3 is related to disease severity, activity, joint erosion, and renal involvement in RA and SLE through modulation of synovial fibroblasts, IL-6, and IL-17.[5,13,34,35] Convincing lines of evidence have indicated that abnormal expression of *STAT3* might be involved in the pathogenesis, severity, and activity of autoimmune disorders, especially RA.

miRSNPs are a class of common variants that are located in the 3′-UTR region of target genes and could impair the expression of target genes by disrupting the binding locations of miRNAs.[23,36] In our previous study, we demonstrated that rs1053023 (A>G) and rs1053005 (A>G) are two miRSNPs that are positioned near the miRNA response elements of *STAT3*.[25,37] Given the similarities between SLE and RA in terms of etiopathogenesis and molecular mechanisms, we evaluated the correlations between these variants and the risk of RA. However, our findings indicated that rs1053005 and rs1053023 were not associated with susceptibility to RA.

The distribution of rs1053005 and rs1053023 in *STAT3* did not differ markedly between RA cases and controls (Table 3). However, in a previous work, we showed that rs1053023 was related to an increased risk of SLE.[25] Moreover, Davidson et al, reported that rs1053005 is significantly correlated with an augmented risk of ankylosing spondylitis, an inflammatory autoimmune disease common in the Chinese population.[38] Moreover, another study reported that the GG genotype and G allele in rs1053005 could increase the risk of type 1 diabetes mellitus by 2.93 and 1.79 folds, respectively.[37] Similar to our results, Stypinska et al. showed that rs1053005 is not connected with RA risk in the Polish population. In contrast, they reported that the rs1026916 AA genotype is associated with a higher risk of RA and the rs2293152 polymorphism is associated with RA severity.[39] The current study results show that these two miRSNPs probably have no similar effects on the pathogenesis of RA or SLE. However, similar to the findings of our previous study, these variants were correlated with some laboratory parameters of disease activity and severity. Stratification analysis revealed that rs1053005 was associated with

higher concentrations of CRP and ESR as markers of inflammation and disease activity in RA patients.[40-42] (Table 4). In the previous report, we also found that rs1053005 is correlated with disease activity indices in SLE patients, including CRP, complement component 3 (C3), C4, and anti-double-stranded DNA.[25] In a previous work on SLE subjects, we found that rs1053023 was correlated with renal involvement, whereas, in the current study, this variant only exhibited a correlation with high levels of creatinine.

Unfortunately, in this study, we did not have data on kidney damage in RA patients. However, the association of rs1053023 with increased creatinine corresponded with the findings in SLE patients. These results agreed with previous data that showed *STAT3* is considerably overexpressed in patients with lupus nephritis compared to normal subjects and even in SLE patients without renal involvement.[5] Besides, *STAT3* inhibition or deficiency might potentially suppress lupus nephritis in mouse models.[32,43]

In conclusion, in the present study, for the first time, we assessed the association between rs1053005 and rs1053023 in the *STAT3* gene. We demonstrated that these common variants were correlated with disease activity and kidney involvement in Iranian RA subjects. We acknowledge some probable limitations in the statistical validity of the present study: the small population size may preclude definite conclusions; therefore, further association studies in populations with larger sample sizes will help to confirm the observed associations. Additionally, it is recommended that future studies evaluate the possible effects of these miRSNPs on the expression of the *STAT3* gene and response to STAT3-inhibitor therapies.

STATEMENT OF ETHICS

The present study was confirmed (approval code: IR.MUI.MED.REC.1400.096) by the ethics committee of Isfahan University of Medical Sciences.

FUNDING

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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