Introduction

Systemic lupus erythematosus (SLE) is a heterogeneous autoimmune disease affecting several systems and organs in the body. The association of STAT4 transcription factor with SLE risk remains unclear.

Objectives: The aim of this study was to investigate the association of STAT4 gene polymorphism (rs7574865) with the incidence of SLE.

Patients and Methods: One hundred and sixty participants (80 patients with SLE and 80 healthy individuals) were included in this study. Gene analysis was conducted by amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) in peripheral blood samples.

Results: Fifty-seven percent (n=45) of patients with SLE had SLE disease activity index (SLEDAI) above six and had active disease. In the SLE group, the frequency of G and T alleles were 81% and 19%, respectively. Moreover, 72.50% (n=58) of patients carried the GG genotype, 17.5% (n=14) had the GT genotype and 10.1% (n=8) carried the TT genotype. There was no significant difference between allele frequency and genotypic distribution for rs7574865 polymorphism (P > 0.05) between SLE and control groups. Significant differences were observed between the distribution of genotypes and clinical manifestations including leukopenia (P = 0.04), pulmonary (P = 0.01) and ophthalmic (P = 0.049) problems. The T allele with an odd ratio of 1.47 and confidence interval of 0.80 to 2.6 could increase the risk of SLE; however, it was not statistically significant (P = 0.20).

Conclusion: The T allele and TT genotype of the STAT4 rs7574865 polymorphism could increase the risk of lupus; however, these observations were not statistically significant.

Key point

Studying population differences in genetic susceptibility factors helps us to expand our understanding of the disease mechanisms. In this study, we found that the distribution of GT and TT genotypes was higher in SLE patients. In addition, there were significant differences between the distribution of genotypes and clinical manifestations including leukopenia, pulmonary and ophthalmic problems.
exert a significant role in their pathogenesis. However, the molecular mechanisms by which these variants contribute to the autoimmune disease are unidentified. A variation of STAT4 gene (a single-nucleotide polymorphism (SNP) located in the 3rd intron; rs7574865) probably affects the expression of this protein at both translation and splicing levels (6).

**Objectives**

In SLE patients, the rs7574865 SNP has been connected with a severe disease phenotype and cardiovascular morbidity (8,9). Studying population differences in genetic susceptibility factors help us to expand our understanding of the disease mechanisms and accordingly develop clinical intervention for the complex SLE disease. In this study, we aimed to investigate the association of the STAT4 gene polymorphism (rs7574865) with the incidence of SLE.

**Patients and Methods**

**Subjects**

In this cross-sectional study, 80 SLE patients were enrolled. The included patients had over 15 years old without underlying diseases. Patients with diabetic nephropathy or other rheumatic diseases such as rheumatoid arthritis were excluded from the study. In addition, 80 healthy controls were subjected as normal control for comparing the results. After recording clinical information, disease activity was determined based on Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2k) criteria in which organic brain syndrome, psychosis, seizures, visual disturbances, neurological disorders, vasculitis, cerebrovascular accidents and lupus headache was considered eight points. Accordingly arthritis, myositis, urinary casts, hematuria, proteinuria, considered four points; pericarditis, pleurisy, mucosal ulcers, alopecia, rash, low-complement levels and increased DNA binding was considered two points; while fever, thrombocytopenia and leukopenia got one point. Based on the Anić study (10), score higher than six is considered as active SLE.

**DNA extraction and qualification**

Whole blood sample (2 mL) was collected from SLE patients and healthy individuals in the same condition and prepared for DNA extraction. DNA extraction was conducted for each sample using magnetic nanoparticles (ZiAViZ kit, Iran). Qualification of samples were conducted for each sample using magnetic nanoparticles and prepared for DNA extraction. DNA extraction was conducted for each sample using magnetic nanoparticles (ZiAViZ kit, Iran). Qualification of samples were conducted for each sample using magnetic nanoparticles and prepared for DNA extraction.

**DNA amplification**

DNA amplification was conducted by amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) for genotyping using thermocycler. DNA was amplified in a 25-μL PCR resection including 5 μL 10× PCR buffer, 0.75 μL MgCl2 50 mM, 0.5 μL dNTP, 0.625 μL specific primer 10 mM (Table 1), 1-2 μL DNA solution, 2.5 U Taq DNA polymerase and DEPC up to 25 μL. Prepared samples were incubated at 94˚C for two minutes and 30 cycles in 94˚C for 30 seconds, 57˚C for 30 seconds, 72˚C for 40 seconds. At last, the samples were incubated for five minutes at 72˚C.

**Data analysis**

Quantitative variables were expressed as mean ± standard deviation or median (min-max) based on normal data distribution. Qualitative variables were expressed as frequency (percentage). Chi-square test was employed to compare the observed genotypes with the expected values. Genotype and allele frequencies in both healthy and SLE groups were calculated and the effect of each genotype and allele for SLE risk were assessed by chi-square test and logistic regression with odds ratio (OR) and 95% confidence interval (CI). Data was analyzed using SPSS software version 23.0. In evaluating the results, a \( P \) value < 0.05 was considered meaningful.

**Results**

This study was conducted on eighty SLE patients; 87.7% of whom were female (n=71). Moreover, eighty healthy controls were included in the study to compare the obtained results. It was observed that the mean age of patients was 37.91 ± 11.75 years and the mean age of healthy individuals was 52.4 ± 6.54 years.

The median disease activity based on SLEDAI criteria among SLE patients was 8 (min 0-max 22). Our study showed that the SLEDAI scores of 45 patients (57%) were higher than six and they had active disease. Twenty-five patients had proteinuria and renal involvement and 5% of them had proteinuria in the nephrotic range and 12 patients (14.8%) had hematuria. The median of serum creatinine in the SLE group was 1.07 (0.06-7.6) mg/dL which was significantly different from the control group (\( P = 0.01 \)). It was also observed that twenty patients (45%) with active SLE had significant renal involvement (\( P = 0.005 \)). In addition, 84.6% (66 patients) and 67.1% (53 patients) had positive antinuclear antibody (ANA) and
anti-double stranded DNA (anti-dsDNA), respectively. The involvement of different organs and tissues among SLE patients was represented in Table 2. The most common clinical manifestations were anemia (66.7%) and rash (53.2%).

In SLE patients, the frequency of the G and T alleles were 81% and 19%, respectively. There were no statistically significant differences in the frequency of G and T alleles between SLE patients and controls ($P=0.207$; Figure 1A). In addition, 72.50% of SLE patients ($n=58$) had GG genotype, 17.5% of them ($n=14$) had GT genotype, and 10.1% of them ($n=8$) had TT genotype. There was no significant difference in genotype distribution of the rs7574865 polymorphism between the SLE and control groups ($P=0.566$; Figure 1A).

The genotype distribution (GG, GT and TT) of rs7574865 polymorphism was evaluated based on the clinical variables. There was no significant difference between the distribution of genotypes and positive complement, anti-dsDNA, C-reactive protein (CRP), ANA and proteinuria ($P\geq 0.461$, Table 2). The organ involvement and genotype distribution of rs7574865 polymorphism genotypes were also investigated. There were significant differences between the genotype distribution of rs7574865 and leukopenia ($P=0.045$) and pulmonary involvement ($P=0.011$) with ophthalmic ($P=0.049$) involvement. However, the genotype distribution of rs7574865 was not statistically affected other clinical manifestations ($P\geq 0.461$, Table 2). The results of logistic regression analysis showed that the frequency of the T allele in the lupus group was higher compared to the control group (19% versus 13%) and with OR=1.47 and confidence interval of 0.80 to 2.68, it increased the risk of lupus, however, it was not statistically significant ($P=0.208$). Similarly, the TT (OR=1.73, CI 0.58-5.68; $P=0.33$) and GT (OR=1.26, CI 0.54-2.64; $P=0.58$) genotypes increased the risk of SLE. The higher frequent distribution of GT and TT genotypes in the lupus group than the healthy group was not statistically significant. Furthermore, GT+TT genotype could increase the risk of SLE (OR=1.43, CI 0.69-2.9; $P=0.33$).

| Variables          | Genotype frequencies, n (%) | Total | $P$ value* |
|--------------------|----------------------------|-------|------------|
| Gender             |                            |       |            |
| Men                | GG 6 (76%)                 | GT 2 (25%) | TT - | 8 (10%) | 0.555 |
|                    | Women 51 (64.4%)           | 12 (15.2%) | 8 (10.1%) | 72 (90%) |      |
| CRP                | Positive 26 (32.9%)        | GT 6 (7.6%) | TT 5 (6.3%) | 37 (46.8%) | 0.634 |
|                    | Negative 31 (39.2%)        | 8 (10.1%) | 3 (3.8%) | 42 (53.2%) |      |
| ANA                | Positive 49 (62.8%)        | GT 11 (14.1%) | TT 6 (7.6%) | 66 (84.6%) | 0.787 |
|                    | Negative 8 (10.1%)         | 3 (3.8%) | 1 (1.3%) | 12 (15.4%) |      |
| anti-dsDNA         | Positive 40 (50.6%)        | GT 9 (11.4%) | TT 4 (5.1%) | 53 (67.1%) | 0.508 |
|                    | Negative 17 (21.5%)        | 5 (6.3%) | 4 (5.1%) | 26 (32.9%) |      |
| Blood complement   | Positive 30 (38%)          | GT 7 (9%) | TT 6 (7.6%) | 41 (54.4%) | 0.461 |
|                    | Negative 27 (34.2%)        | 7 (9%) | 2 (2.6%) | 36 (45.6%) |      |
| Proteinuria        | Positive 19 (24.4%)        | GT 4 (5.1%) | TT 2 (2.6%) | 25 (31.6%) | 0.861 |
|                    | Negative 38 (48.7%)        | 10 (12.8%) | 6 (7.6%) | 54 (68.4%) |      |

CRP: C-reactive protein.
**Table 3.** The correlation between genotype distribution of STAT4 gene polymorphism and clinical manifestations in SLE patients

| Diagnostic markers            | GG   | GT   | TT   | Total | P value* |
|-------------------------------|------|------|------|-------|----------|
| Leukopenia Yes                 | 17   | -    | 1    | 18    | 0.045    |
| No                            | 41   | 14   | 7    | 62    | 0.107    |
| Hematuria Yes                  | 6    | 3    | 3    | 12    | 0.461    |
| No                            | 52   | 11   | 5    | 68    | 0.517    |
| Anemia Yes                     | 37   | 10   | 7    | 54    | 0.227    |
| No                            | 19   | 4    | 1    | 24    | 0.249    |
| Thrombocytopenia Yes           | 15   | 2    | 3    | 20    | 0.187    |
| No                            | 42   | 11   | 5    | 58    | 0.685    |
| CNS involvement Yes            | 7    | -    | -    | 7     | 0.049    |
| No                            | 50   | 14   | 8    | 72    | 0.980    |
| Dermatological involvement Yes | 29   | 10   | 3    | 42    | 0.980    |
| No                            | 28   | 4    | 5    | 37    | 0.517    |
| Hematological problems Yes     | 19   | 3    | 4    | 26    | 0.011    |
| No                            | 38   | 11   | 5    | 53    | 0.011    |
| Vasculitis Yes                 | 2    | 1    | -    | 3     | 0.045    |
| No                            | 55   | 13   | 8    | 76    | 0.685    |
| Ophthalmic involvement Yes     | -    | 1    | 1    | 2     | 0.049    |
| No                            | 57   | 16   | 7    | 77    | 0.980    |
| Musculoskeletal involvement Yes| 15   | 5    | 2    | 21    |          |
| No                            | 42   | 10   | 6    | 58    |          |
| Pulmonary involvement Yes      | -    | -    | 1    | 1     |          |
| No                            | 57   | 17   | 7    | 78    |          |

CNS: Central nervous system.
* Data presented as number (percentage). P value < 0.05 was considered as significant result.

**Discussion**

In this study, the association of the STAT4 rs7574865 polymorphism with SLE was investigated. About 57% of patients with lupus had the active disease (SLEDAI >6). In the SLE group, the frequencies of G and T alleles were 81% and 19%, respectively. In addition, 17.5% and 10.1% of patients had GT and TT genotypes, respectively. The T allele increased the risk of lupus; however, it was not statistically significant. The higher distribution of GT and TT genotypes, carrying at least one T allele in SLE patients was not statistically significant. No significant difference was observed between lupus and control groups regarding allelic frequency and genotype distribution of rs7574865 polymorphism.

Studies indicate that expression of the TNFSF4 and STAT4 genes along with IL-10 and TNF-α levels can be useful as biomarkers for assessing the SLE activity and managing treatment protocols (11). The STAT4 haplotype has been reported to be linked with an increased risk of SLE and rheumatoid arthritis, indicating a common pathway for these diseases (8).

A pervious meta-analysis on forty studies (2012) revealed that the STAT4 rs7574865 T allele increases vulnerability to primary Sjögren’s syndrome, autoimmune thyroid, inflammatory bowel diseases, type 1 diabetes, SLE and rheumatoid arthritis (7). Moreover, the STAT4 gene polymorphisms are associated with susceptibility to the SLE in different populations. The TT genotype of STAT4 rs7574865 polymorphism was significantly associated with an increased risk of SLE in the Japanese population (12). In a Chinese population, the T allele of this polymorphism was a risk allele in the SLE (13).

Another meta-analysis on 24 research papers from the different populations including 17 389 lupus patients and 29 073 controls showed a significant relationship between T allele and risk genotypes (TT, TG and TT + TG) of rs7574865 polymorphism and susceptibility to the SLE. Subgroup analysis of this study showed an association between rs7574865 polymorphism and ethnicities; the rs7574865 T allele has significant correlation with SLE in the Asian population (14). The genetic contribution of this gene to the SLE may be higher in the Japanese population than in European Americans (15). The results of these studies suggest that STAT4 acts as a genetic risk factor in the pathogenesis of SLE. Likewise, we found that the distribution of GT and TT genotypes and the frequency of the T allele were higher in SLE patients compared to controls. Moreover, statistically insignificantly, the studied polymorphism could increase the risk of lupus in adults. However, the mechanism of this gene to increase the risk of SLE is still unknown. In addition, the question of how STAT4 polymorphisms regulate this complex disease needs to be clarified. It should be noted that in some ethnic populations and autoimmune diseases STAT4 rs7574865...
Systemic lupus erythematosus does not have a role in susceptibility, suggesting that some of the SLE susceptibility genes might be population-specific (16).

The SLE patients demonstrate different clinical phenotypes. The STAT4 SNPs are correlated with definite clinical manifestations of SLE including presence of anti-dsDNA, an earlier onset of disease, a severer disease phenotype, and an increased risk for lupus nephritis (LN) and stroke (9, 17, 18). In individuals with STAT4 risk allele, an increased IFN-γ production is induced by IL-12, speculating that the increased risk for LN might be driven by IFN-γ overproduction (19). Yang et al observed a strong association between SNP rs7574865 and anti-dsDNA positive antibodies in SLE patients (16). In addition, rs7574865 risk allele carriers had higher levels of C3, C4 and anti-dsDNA (17). Additionally, an increase in the frequency of the rs7574865 T allele was tested in SLE patients with rash, hematologic disorders, proteinuria, and anti-dsDNA-positive antibodies (20). Patients with the STAT4 CC genotype had significantly increased SLE activity index (SLEAI) and injury index compared to the GG genotype and these homozygous patients have a high risk for severe disease manifestations (21). El-Saadany et al found an association between STAT4 rs7574865 polymorphism and increased susceptibility to lupus. The frequency of STAT4 (TT) genotype in patients was much higher than control group. The T allele carriers had a higher risk of developing the disease and had lower complement (C3, C4), proteinuria and anti-dsDNA (22). In the present study, we found significant differences between the distribution of GG, GT, and TT genotypes and leukopenia, pulmonary and eye involvement. No significant correlation was observed for the rest of the clinical, demographic data and the manifestations of SLE. Further studies is necessary to these polymorphisms contributing in different clinical SLE phenotypes.

**Conclusion**

Overall, the T allele and TT genotype of the STAT4 rs7574865 polymorphism could increase the risk of developing lupus; however, this observation was not statistically significant, which may be due to the small sample size. Genotype distribution of the studied polymorphism could significantly impact leukopenia and pulmonary and ophthalmic problems.

**Limitations of the study**

Small sample size was the main limitation of this study, during the COVID-19 pandemic, patients did not agree to participate in this study.

**Authors’ contribution**

MRA and SZV designed the study. AK selected the patients. GB collected the samples. SM H and SSH conducted data analysis. SZV prepared the draft of the manuscript. MRA and SZV revised the manuscript. All authors read and confirmed the final version of the manuscript before submission.

**Conflicts of interest**

The authors declare that they have no competing interests.

**Ethical issues**

The research followed the tenets of the Declaration of Helsinki. Written consent forms were signed by all participants. This study was approved by ethics committee (Ethical code: IR.TBZMED.REC.1398.928) of Tabriz university of medical sciences. This study was extracted from residency thesis of Gholnaz Bayat at the Kidney Research Center at this university - (Thesis#63403). Additionally, the authors completely have observed the ethical issues including data fabrication, falsification, plagiarism, double publication misconduct, or submission and redundancy.

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**References**

1. Pan L, Lu MP, Wang JH, Xu M, Yang SR. Immunological pathogenesis and treatment of systemic lupus erythematosus. World J Pediatr. 2020;16:19-30. doi: 10.1007/s12519-019-00229-3.

2. Leffers HCB, Lange T, Collins C, Ulff-Møller CJ, Jacobsen S. The study of interactions between genome and exposome in the development of systemic lupus erythematosus. Autoimmun Rev. 2019;18:382-92. doi: 10.1016/j.autrev.2018.11.005.

3. Teruel M, Alarcón-Riquelme ME. The genetic basis of systemic lupus erythematosus: What are the risk factors and what have we learned. J Autoimmun. 2016;74:161-75. doi: 10.1016/j.
4. Mathur AN, Chang HC, Zisoulis DG, Stritesky GL, Yu Q, O'Malley JT, et al. Stat3 and Stat4 direct development of IL-17-secreting Th cells. J Immunol. 2007;178:4901-7. doi: 10.4049/jimmunol.178.8.4901.

5. Watford WT, Hissong BD, Bream JH, Kanno Y, Muul L, O'Shea JJ. Signaling by IL-12 and IL-23 and the immunoregulatory roles of STAT4. Immunol Rev. 2004;202:139-56. doi: 10.1111/j.0105-2896.2004.00211.x.

6. Korman BD, Kastner DL, Gregersen PK, Remmers EF. STAT4: genetics, mechanisms, and implications for autoimmunity. Curr Allergy Asthma Rep. 2008;8:396-403. doi: 10.1007/s11882-008-0077-8.

7. Liang YL, Wu H, Shen X, Li PQ, Yang XQ, Liang L, et al. Association of STAT4 rs7574865 polymorphism with autoimmune diseases: a meta-analysis. Mol Biol Rep. 2012;39:887-82. doi: 10.1007/s11033-012-1754-1.

8. Remmers EF, Plenge RM, Lee AT, Graham RR, Hom G, Behrens TW, et al. STAT4 and the risk of rheumatoid arthritis and systemic lupus erythematosus. N Engl J Med. 2007;357:977-86. doi: 10.1056/NEJMoa073003.

9. Svenungsson E, Gustafsson J, Leonard D, Sandling J, Gunnarsson I, Nordmark G, et al. A STAT4 risk allele is associated with ischaemic cerebrovascular events and anti-phospholipid antibodies in systemic lupus erythematosus. Ann Rheum Dis. 2010;69:834-40. doi: 10.1136/ard.2009.115535.

10. Anić F, Zuvic-Butorac M, Stamac D, Novak S. New classification criteria for systemic lupus erythematosus correlate with disease activity. Croat Med J. 2014;55:514-9. doi: 10.3325/cmj.2014.55.514.

11. Uzrail AH, Assaf AM, Abdalla SS. Correlations of Expression Levels of a Panel of Genes (IRF5, STAT4, TNFSF4, MEC2P, and TLR7) and Cytokine Levels (IL-2, IL-6, IL-10, IFN-γ, and TNF-α) with Systemic Lupus Erythematosus Outcomes in Jordanian Patients. Biomed Res Int. 2019;2019:1703842. doi: 10.1156/nejmod.2007.3.

12. Kiyohara C, Washio M, Horiuchi T, Tada Y, Asami T, Ide S, et al. Cigarette smoking. STAT4 and TNFRSF1B polymorphisms, and systemic lupus erythematosus in a Japanese population. J Rheumatol. 2009;36:2195-203. doi: 10.3899/jrheum.090181.

13. Li P, Cao C, Luan H, Li C, Hu C, Zhang S, et al. Association of genetic variations in the STAT4 and IRF7/KIAA1542 regions with systemic lupus erythematosus in a Northern Han Chinese population. Hum Immunol. 2011;72:249-55. doi: 10.1016/j.humimm.2010.12.011.

14. Wang JM, Xu WD, Huang AF. Association of STAT4 Gene Rs7574865, Rs10168266 Polymorphisms and Systemic Lupus Erythematosus Susceptibility: A Meta-analysis. Immunol Invest. 2021;50:282-94. doi: 10.1080/08820139.2020.1752712.

15. Kawasaki A, Ito I, Hikami K, Ohashi J, Hayashi T, Goto D, et al. Role of STAT4 polymorphisms in systemic lupus erythematosus in a Japanese population: a case-control association study of the STAT1-STAT4 region. Arthritis Res Ther. 2008;10:R113. doi: 10.1186/ar2516.

16. Yang W, Ng P, Zhao M, Hirankarn N, Lau CS, Mok CC, et al. Population differences in SLE susceptibility genes: STAT4 and BLK, but not PAK, are associated with systemic lupus erythematosus in Hong Kong Chinese. Genes Immun. 2009;10:219-26. doi: 10.1038/gene.2009.1.

17. Taylor KE, Remmers EF, Lee AT, Ortman WA, Plenge RM, Tian C, et al. Specificity of the STAT4 genetic association for severe disease manifestations of systemic lupus erythematosus. PLoS Genet. 2008;4:e1000084. doi: 10.1371/journal.pgen.1000084.

18. Bolin K, Sandling JK, Zickert A, Jönsen A, Sjöwall C, Svenungsson E, et al. Association of STAT4 polymorphism with severe renal insufficiency in lupus nephritis. PLoS One. 2013;8:e64450. doi: 10.1371/journal.pone.0064450.

19. Hagberg N, Joellson M, Leonard D, Reid S, Eloranta ML, Mo J, et al. The STAT4 SLE risk allele rs7574865[T] is associated with increased IL-12-induced IFN-γ production in T cells from patients with SLE. Ann Rheum Dis. 2018;77:1070-7. doi: 10.1136/annrheumdis-2017-212794.

20. Su Y, Zhao Y, Liu X, Guo JP, Jiang Q, Liu XY, et al. Variation in STAT4 is associated with systemic lupus erythematosus in Chinese Northern Han population. Chin Med J (Engl). 2010;123:3173-7.

21. Nageeb RS, Omran AA, Nageeb GS, Yousef MA, Mohammad YAA, Fawzy A. STAT4 gene polymorphism in two major autoimmune diseases (multiple sclerosis and juvenile onset systemic lupus erythematosus) and its relation to disease severity. Egypt J Neurol Psychiatr Neurosurg. 2018;54:16. doi: 10.1186/s41983-018-0011-5.

22. El-Saadany HM, Amer WH, Khalil HS, Gaber RA, Elshweikh SAI TER. Association of STAT4 polymorphism with susceptibility and severity of rheumatoid arthritis and systemic lupus erythematosus in Egyptian patients. The Egyptian Rheumatologist. 2016;38:21-7. doi: 10.1016/j.ejr.2015.04.003.