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Original Article

Association of STAT4 rs7582694 with susceptibility to systemic lupus erythematosus in population of Lorestan province, Iran

Gholam Reza Izadkhasti[1], Seyed Reza Kazeminezhad[1*], Mohammad Reza Akhoond[2]

[1]Department of Genetics, Faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran
[2]Department of Statistics, Faculty of Mathematical Sciences and Computer, Shahid Chamran University of Ahvaz, Ahvaz, Iran

*__Corresponding Author:__ Seyed Reza Kazeminezhad, Associate Professor, Tel/Fax: +98-613 3338965, Email: kazemi_reza@scu.ac.ir

Abstract

Background and aims: Systemic lupus erythematosus (SLE) is a polygenic, inflammatory disease with a complex genetic inheritance that affects almost all the organs and systems of the host body. According to studies, *STAT4* is a susceptible gene that can participate in the development of SLE in different populations. The aim of this study was to show the association between rs7582694 single nucleotide polymorphism with increased risk of SLE disease and two serological symptoms of the disease (i.e., anti-dsDNA and ANA) in the population residing in Lorestan province.

Methods: The present study was conducted as a case control research. In this study, the prevalence of *STAT4* gene G/C (rs7582694) single nucleotide polymorphism (SNP) in the patients with SLE (n=122) and in control group (n=127) was investigated among a sample population from Lorestan province. This SNP was genotyped based on using two methods including PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) and tetra-primer ARMS-PCR (amplification-refractory mutation system) methods.

Results: According to the obtained results, the frequency of minor allele C from this SNP (related allele with the disease) as compared to the major allele G (normal allele) was significantly higher in SLE patients than the controls. In addition, it showed a significant association (odds ratio [OR] = 1.623, 95% CI = 1.111-2.370, P = 0.012) with susceptibility to SLE. Moreover, a significant correlation (OR = 2.249, 95% CI = 1.031–4.904, P = 0.042) was found between the rs7582694 CC genotype and the risk of SLE in the population of Lorestan.

Conclusion: Overall, based on the results it can be concluded that there was a relationship between the *STAT4* gene G/C (rs7582694) SNP and the increased risk of SLE. However, no association was observed between the above-mentioned gene and anti-dsDNA or ANA that are some of the symptoms of SLE.

Keywords: SLE; Polymorphism; STAT4; rs7582694

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Introduction

Systemic lupus erythematosus (SLE) is a multifactorial, inflammatory autoimmune disease with a broad spectrum of clinical presentations, as well as antinuclear antibodies (ANA) and anti-dsDNA antibodies (1-3). The formation of SLE is due to the production of autoantibodies against host body antigens (4). The precipitation of complexes formed by this antibody and antigens in different body organs leads to the stimulation of the complement system, and then tissue inflammation and damage (5). Although the exact cause of SLE still remains unknown, according to some studies, the role of epigenetic, genetic, racial, hormonal, environmental, and immunological factors in the development of SLE has been approved (3,6,7). The SLE prevalence is higher in young women and is nine times more than men. However, the severity of the disease is higher in men as compared to women (3,4). Moreover, SLE concordance between the monozygotic twins is approximately 25%-50% while being nearly 5% in dizygotic twins. This confirms the role of genetic factors in susceptibility to SLE (8). Until now, various genes such as *HLA-DR*, *PTPN22*, *STAT4*, and others have been reported to be associated with the ability to develop the immune and inflammatory responses in SLE so that some of these loci also affect the severity of the disease in addition to susceptibility to the disease. For example, the *STAT4* gene which is a genetic risk factor for SLE is also associated with the severity of the disease (3,9). The STAT4 (signal transducer and activator of transcription 4) protein which belongs to the STAT proteins family includes STAT1, 2, 3, 4, 5a, 5b, and 6 members (10). The STAT proteins are activated through the JAK-STAT signaling pathway and mediate signaling of many inflammatory cytokines, grows factors, and hormones.

Therefore, they play an important role in the regulation of innate and acquired immune responses (11). Studies on JAK-kinase signaling pathway show the aberrant signaling of the STAT proteins in autoimmune and inflammatory disease such as SLE (12). Genome-wide association studies (GWAS) indicate that genetic diversity in the *STAT4* gene and also rs7582694 SNP in the third intron of this gene are a risk factor for SLE development. These intronic SNPs probably lead to the change of the gene transcription rate through involving in the binding of the transcription factors or histone proteins to their binding site (12-19). In this study, the *STAT4* gene G/C (rs7582694) SNP association was analyzed with the risk of SLE disease in individuals residing in Lorestan province. Furthermore, the relationship between this SNP and two serological criteria of SLE (i.e., production of ANA and anti-dsDNA antibodies) was investigated.

Materials and Methods

Control and case samples

This was a population-based case-control study including 127 healthy individuals with no family history of autoimmune diseases who were matched to the patient group in terms of age and sex. These individuals were selected as a control group. In addition, the patient group contained 122 participants with a record of SLE disease, each of which had at least four symptoms of ACR (American College of Rheumatology) criteria (20). The presence of both serological factors associated with the disease that is, ANA (antinuclear antibody) autoantibodies and anti-dsDNA was also determined by diagnostic tests in the patients. In this study, all the patients and healthy controls were selected from the population of Lorestan province.

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes using the salting-out method and was stored at –20°C until analyzed. Approximately 2 µL of this genomic DNA with 150 ng approximate concentration was used to genotype each sample. Identification of STAT4 G/C (rs7582694) polymorphism was performed employing two methods. The first method included polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). According to this method, PCR was first performed using the outer forward and reverse primers, as was used in previous studies (3). The processes carried out are shown in Table 1. In the PCR program, the temperatures of denaturation, annealing, and elongation were determined at 95, 54, and 72°C, respectively. The times of these steps were also set at 29, 29, and 20 seconds, respectively. In addition, the product of this reaction was 338bp long that was digested with the restriction enzyme TaaI/Hpy CH4III (produced by Thermo scientific, Lithuania Company) with recognition and cut site (ACN/GT). The *STAT4* gene C allele was cleaved into 258 and 80bp fragments, but G allele remained uncut. This polymorphism was also confirmed by the second method, namely, ARMS-PCR. In this way, four primers were used two of which (i.e., the outer forward and reverse) were the same primers applied in the PCR-RFLP method. The two other primers (i.e., inner forward and reverse) were also designed at the NCBI's Bioinformatics Database and then were blasted. The temperatures of denaturation, annealing, and elongation were determined at 95°C, 60°C, and 72°C, respectively in the PCR reaction program. In addition, the time of these steps was set at 29, 29, and 20 seconds, respectively. Based on this method, the 338bp product always exists. If there is STAT4 C allele, the 280bp excess product will exist, and if there is STAT4 G allele, the 105 bp product will exist. In both methods, DNA fragments were separated by electrophoresis in 2% agarose gel and were visualized by the safe stain (Sinaclon, Iran) staining under UV light (Figures 1 and 2).

Table 1. Characteristics of Forward and Reverse Primers in ARMS-PCR and PCR-RFLP Methods

Name	Primer Sequence	Tm
Outer Forward	5'-ATCCAACTCTTCTCAGCCCTT-3'	58.73
Outer Reverse	5'-TCATAATCAGGAGAGAGGAGT-3'	54.62
Inner Forward	5'-AATTCATGAAGGGATGACACATAG-3'	56.80
Inner Reverse	5'-CAAACATGCATAGGTTGCATACTG-3'	58.96

Tm, Temperature of melting.

Statistical analysis

The mean age distribution of the control and patient groups was evaluated employing the T-dual test. The sex distribution was also assessed in both groups using the chi-square test. Besides, the distribution of rs7582694 genotype was tested to examine the deviation from Hardy-Weinberg equilibrium applying the chi-square test in the

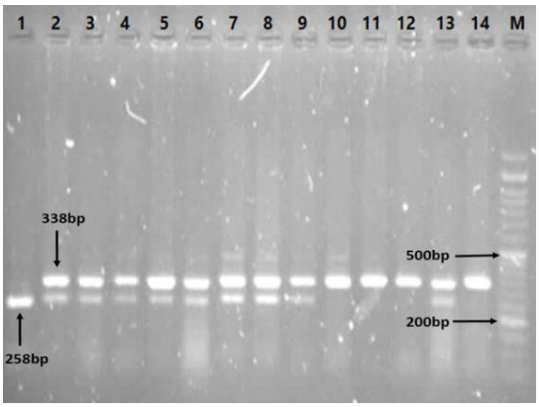

Figure 1. Electrophoresis of PCR-RFLP products on 2% agarose gel. Wells 10, 11, 12, and 14 show homozygous GG genotype; Wells 2-9, and 13 represent heterozygous GC genotype; and well 1 demonstrates the homozygous CC genotype; Besides, well M contains 50 bp marker.

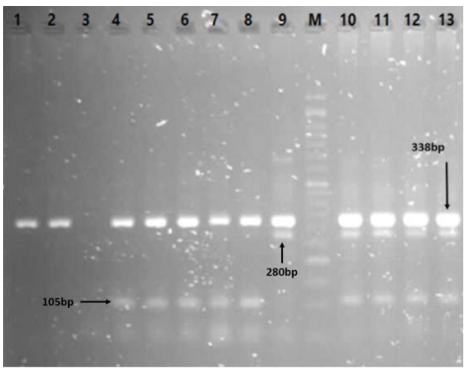

Figure 2. Electrophoresis of ARMS-PCR products on 2% agarose gel. Wells 4-8 show homozygous GG genotype; Wells 10-13 demonstrate heterozygous GC genotype ; Well 9 displays homozygous CC genotype; Well 3 denotes negative control (PCR mix without DNA); and well M contains 50 bp marker.

controls. Genotypic and allelic distribution between the patients and healthy controls was evaluated by chi-square and logistic regression test. Moreover, the odds ratio (OR) and 95% confidence intervals (CI) were calculated. The possible association of this SNP with two clinical manifestations was determined by chi-square test. Finally, a statistical analysis of the data was carried out using the SPSS (Statistical Package for the Social Sciences) software, version 18.

Results

In this study, rs7582694 SNP was genotyped in 122 patients and 127 healthy controls. The distribution of G/C genotype from the mentioned polymorphism did not show any significant deviation from Hardy–Weinberg equilibrium between the patients and control subjects (*P* value of 0.159 & 0.104, respectively). In fact, a balance was observed in both groups. The *t* test for both independent samples demonstrated that the age difference observed between the patient and control groups was statistically significant (*P* = 0.004). It means that the average age of the control group was higher than that of the patients. However, since this disease often occurs at an early age, it is concluded that the existing control samples are perfectly suited for comparison with the existing patients. Chi-square test indicated that there was no significant relationship between sex and disease incidence (*P* = 0.310). The result of *STAT4* gene G/C genotype in SLE patients and healthy controls was compared and studied (Table 2). The C allele frequency in the patients was significantly higher than that of the control group (OR = 1.623, 95% CI = 1.111-2.370, *P* = 0.012). Consequently, it can be inferred that the chance of disease with a C allele was 1/623 times more than G allele. According to the obtained results and also considering the GG genotype as a reference, it was concluded that the CC genotype (OR = 2.249, 95% CI = 1.031–4.904, *P* = 0.042) was associated with an increased risk of SLE. In spite of the higher frequency of heterozygote GC genotype in patients (40/99%) as compared to the control group (33.86%), this genotype was not correlated with an increased risk of SLE (OR = 1.619, 95% CI = 0.94–2.788, *P* = 0.082). Furthermore, based on the result of the Chi-square test, the probable association of this polymorphism with both ACR criteria for SLE (i.e., ANA & anti-dsDNA antibodies) was investigated. Nonetheless, no relationship was found between these polymorphisms and the above-mentioned criteria (Table 3).

Discussion

SLE is a polygenic and chronic disease with complex inheritance and various immunological abnormalities that can affect almost all the organs and tissues of the body such as joints, muscles, skeletons, central nervous system, skin, lungs, heart, kidneys, and digestive system (3,5,12). Prevalence of this disease is higher in African Americans, Hispanic, Afro (African)-Caribbean, native North American Indians, Indian, and Chinese in comparison with Europeans. Besides, SLE may have more severity so that African Americans rather than Europeans show four times higher SLE prevalence. The rate of disease in Afro-American blacks is higher than those of Afro-African. It is also higher among the urban population as compared to the rural community. Therefore, this topic

Table 2. Allele and genotype distribution of *STAT4* gene (rs7582694) in SLE patients and controls

SNP	Alleles/Genotypes	Patients No. (%)	Controls No. (%)	*P*	Odds ratio (95 % CI)
rs7582694	G	152(62.30)	185 (72.83)	–	1(referent)
	C	92 (37.70)	69 (27.17)	0.012	1.623(1.111–2.370)
	GG	51 (41.80)	71 (55.90)	–	1(referent)
	GC	50 (40.99)	43 (33.86)	0.082	1.619(0.94–2.788)
	CC	21 (17.21)	13 (10.24)	0.042	2.249(1.031–4.904)

Table 3. Frequencies of rs7582694 genotype with two serological features in SLE patients

Serological features	Frequency (%)	GG (%)	GC (%)	CC (%)	*P*
Anti-dsDNA	54 (47.4)	25 (46.3)	20 (37.0)	9 (16.7)	0.571
ANA	56 (49.6)	20 (35.7)	25 (44.6)	11 (19.6)	0.558

suggests the environment role in susceptibility to SLE (3,21). The STAT4 protein is a transcription factor that transmits the signals from the interleukin 12 (IL-12), and thus causes type II interferon (IFNII) and IFNα secretion in Th1 cells (13). Generally, loss of immune tolerance, increased antigenic load, T cell extra help, defective B cell suppression, and immune response shift from Th1 to Th2 cause an increase in B cell activation. This also causes the production of pathogens autoantibodies and eventually results in the prevalence of the disease symptoms (8,17). In many previous studies, several genes and loci have been identified that are predisposed to the SLE and other complicated autoimmune diseases. Genome-wide association studies have determined many genetic factors in this context. The *STAT4* gene is one of these genetic factors the association of which with SLE is taken into account very much. Several polymorphisms have been found in this gene, showing a high correlation with autoimmune diseases. Up to now, a number of studies have confirmed the association of rs7582694 SNP in this gene with several autoimmune diseases such as Sjögren's syndrome, autoimmune hepatitis, and lupus (3, 13, 22, 23). In the SNP database (dbSNP), the allele frequencies of G and C alleles of rs7582694 SNP are 0.73 and 0.27, respectively (based on the 1000 genomes project). The results obtained from the control group also showed the same amounts. However, in the patient group, the frequencies obtained included 0.62 and 0.38, respectively, which were significantly higher than those of the control group and SNP database (Table 2). The association study between the minor C allele of the *STAT4* gene rs7582694 polymorphism and the risk of SLE disease has not been so far carried out in Iran. In other words, the present study is the first one to be conducted in this regard among the population of Lorestan province. The results of the current study are in conformity with the findings of other similar studies conducted among Chinese, Korean, European, Finnish, Swedish, and Polish populations in which a significant correlation was found between this polymorphism and susceptibility to SLE disease (2,15,24-26). However, the results do not match the findings of a study carried out in this regard in Egypt (27). This seems to be due to the lower number of the patient and control samples participated therein. Moreover, the association between the rs7574865 polymorphism of this gene with susceptibility to SLE has been reported in Iran (18). The ANA antibodies are found to be the most recognized antibodies in SLE patients and are observed in more than 95% of the patients. These antibodies can bind to DNA, RNA, nuclear proteins, and protein/nucleus complexes (8). The results of the statistical analysis revealed that there was no significant relationship between anti-dsDNA and ANA with the type of the genotype (Table 3). Thus, since the association between this polymorphism and these autoantibodies has been proven in some studies (2,15), it seems that further studies are required in this respect among the population of Lorestan province, in particular, and across the population of Iran, in general.

Conclusion

Overall, the results of the present study showed that the *STAT4* gene (rs7582694) SNP was associated with the increased risk of SLE among the population of Lorestan province. Conversely, however, no relationship was observed between the two serological diagnostic criteria, namely, ANA and anti-dsDNA autoantibodies. Under this condition, the current study confirmed the findings of previous studies conducted on other populations. This indicates that STAT4 can be a necessarily predisposing gene which is commonly involved in eliminating the disease.

Conflict of interests

None.

Ethical considerations

Permission was obtained from the Ethics Committee of Lorestan University of Medical Sciences under the code of IR.LUMS.REC.1396.318. Then, following obtaining written consent from each of the individuals, their blood samples were collected from the patients in the medical diagnostic laboratory located in Khorramabad.

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References

1. Petri M, Orbai AM, Alarcon GS, Gordon C, Merrill JT, Fortin PR, et al. Derivation and validation of the Systemic Lupus International Collaborating Clinics classification criteria for systemic lupus erythematosus. Arthritis Rheum. 2012;64(8):2677-86. doi: 10.1002/art.34473.
2. Piotrowski P, Lianeri M, Wudarski M, Olesinska M, Jagodzinski PP. Contribution of STAT4 gene single-nucleotide polymorphism to systemic lupus erythematosus in the Polish population. Mol Biol Rep. 2012;39(9):8861-6. doi: 10.1007/s11033-012-1752-3.
3. Bertsias G, Cervera R, Boumpas DT. Systemic lupus erythematosus: pathogenesis and clinical features. EULAR textbook on rheumatic diseases. Geneva, Switzerland: European League Against Rheumatism; 2012:476-505.
4. Ngo ST, Steyn FJ, McCombe PA. Gender differences in autoimmune disease. Front Neuroendocrinol. 2014;35(3):347-69. doi: 10.1016/j.yfrne.2014.04.004.
5. Cojocaru M, Cojocaru IM, Silosi I, Vrabie CD. Manifestations of systemic lupus erythematosus. Maedica (Buchar). 2011;6(4):330-6.
6. Wang G, Tam LS, Kwan BC, Li EK, Chow KM, Luk CC, et al. Expression of miR-146a and miR-155 in the urinary sediment of systemic lupus erythematosus. Clin Rheumatol. 2012;31(3):435-40. doi: 10.1007/s10067-011-1857-4.
7. Lai NS, Koo M, Yu CL, Lu MC. Immunopathogenesis of systemic lupus erythematosus and rheumatoid arthritis: the role of aberrant expression of non-coding RNAs in T cells. Clin Exp Immunol. 2017;187(3):327-36. doi: 10.1111/cei.12903.
8. Mok CC, Lau CS. Pathogenesis of systemic lupus erythematosus. J Clin Pathol. 2003;56(7):481-90.

9. Shahrokhi SZ, Kazemi Nezhad SR, Baharvand Ahmadi S, Akhoond MR. Association Study of the PTPN22 Gene Polymorphisms with Systemic Lupus Erythematosus in Lorestan Province of Iran. Gene Cell Tissue. 2017;4(3):e12023. doi: 10.5812/gct.12023.
10. Lim CP, Cao X. Structure, function, and regulation of STAT proteins. Mol Biosyst. 2006;2(11):536-50. doi: 10.1039/b606246f.
11. Vinkemeier U, Moarefi I, Darnell JE Jr, Kuriyan J. Structure of the amino-terminal protein interaction domain of STAT-4. Science. 1998;279(5353):1048-52.
12. Goropevsek A, Holcar M, Avcin T. The Role of STAT Signaling Pathways in the Pathogenesis of Systemic Lupus Erythematosus. Clin Rev Allergy Immunol. 2017;52(2):164-81. doi: 10.1007/s12016-016-8550-y.
13. Gestermann N, Mekinian A, Comets E, Loiseau P, Puechal X, Hachulla E, et al. STAT4 is a confirmed genetic risk factor for Sjogren's syndrome and could be involved in type 1 interferon pathway signaling. Genes Immun. 2010;11(5):432-8. doi: 10.1038/gene.2010.29.
14. Demirci FY, Wang X, Kelly JA, Morris DL, Barmada MM, Feingold E, et al. Identification of a New Susceptibility Locus for Systemic Lupus Erythematosus on Chromosome 12 in Individuals of European Ancestry. Arthritis Rheumatol. 2016;68(1):174-83. doi: 10.1002/art.39403.
15. Sigurdsson S, Nordmark G, Garnier S, Grundberg E, Kwan T, Nilsson O, et al. A risk haplotype of STAT4 for systemic lupus erythematosus is over-expressed, correlates with anti-dsDNA and shows additive effects with two risk alleles of IRF5. Hum Mol Genet. 2008;17(18):2868-76. doi: 10.1093/hmg/ddn184.
16. Banchereau J, Pascual V. Type I interferon in systemic lupus erythematosus and other autoimmune diseases. Immunity. 2006;25(3):383-92. doi: 10.1016/j.immuni.2006.08.010.
17. Enevold C, Nielsen CH, Jacobsen RS, Hermansen ML, Molbo D, Avlund K, et al. Single nucleotide polymorphisms in genes encoding toll-like receptors 7, 8 and 9 in Danish patients with systemic lupus erythematosus. Mol Biol Rep. 2014;41(9):5755-63. doi: 10.1007/s11033-014-3447-4.
18. Mirkazemi S, Akbarian M, Jamshidi AR, Mansouri R, Ghoroghi S, Salimi Y, et al. Association of STAT4 rs7574865 with susceptibility to systemic lupus erythematosus in Iranian population. Inflammation. 2013;36(6):1548-52. doi: 10.1007/s10753-013-9698-8.
19. Sutton C, Brereton C, Keogh B, Mills KH, Lavelle EC. A crucial role for interleukin (IL)-1 in the induction of IL-17-producing T cells that mediate autoimmune encephalomyelitis. J Exp Med. 2006;203(7):1685-91. doi: 10.1084/jem.20060285.
20. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum. 1997;40(9):1725. doi: 10.1002/1529-0131(199709)40:9<1725::aid-art29>3.0.co;2-y.
21. Lau CS, Yin G, Mok MY. Ethnic and geographical differences in systemic lupus erythematosus: an overview. Lupus. 2006;15(11):715-9. doi: 10.1177/0961203306072311.
22. Li X, Chen H, Cai Y, Zhang P, Chen Z. Association of STAT4 and PTPN22 polymorphisms and their interactions with type-1 autoimmune hepatitis susceptibility in Chinese Han children. Oncotarget. 2017;8(37):60933-40. doi: 10.18632/oncotarget.17458.
23. Migita K, Nakamura M, Abiru S, Jiuchi Y, Nagaoka S, Komori A, et al. Association of STAT4 polymorphisms with susceptibility to type-1 autoimmune hepatitis in the Japanese population. PLoS One. 2013;8(8):e71382. doi: 10.1371/journal.pone.0071382.
24. Namjou B, Sestak AL, Armstrong DL, Zidovetzki R, Kelly JA, Jacob N, et al. High-density genotyping of STAT4 reveals multiple haplotypic associations with systemic lupus erythematosus in different racial groups. Arthritis Rheum. 2009;60(4):1085-95. doi: 10.1002/art.24387.
25. Luan H, Li P, Cao C, Li C, Hu C, Zhang S, et al. A single-nucleotide polymorphism of the *STAT4* gene is associated with systemic lupus erythematosus (SLE) in female Chinese population. Rheumatol Int. 2012;32(5):1251-5. doi: 10.1007/s00296-010-1767-9.
26. Hellquist A, Sandling JK, Zucchelli M, Koskenmies S, Julkunen H, D'Amato M, et al. Variation in STAT4 is associated with systemic lupus erythematosus in a Finnish family cohort. Ann Rheum Dis. 2010;69(5):883-6. doi: 10.1136/ard.2009.112284.
27. El Bassuonia MA, Mohamed DF, Radwana WM, Hasab El-Naby AS. Contribution of the *STAT4* gene single nucleotide polymorphism to systemic lupus erythematosus. Menoufia Med J. 2016;29(2):373-8. doi: 10.4103/1110-2098.192430.