Menoufia Medical Journal

PRINT ISSN: 1110-2098 - ONLINE ISSN: 2314-6788

journal hompage: www.menoufia-med-j.com

Volume 29 | Issue 2 Article 31

6-1-2016

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Recommended Citation

Hasab El-Naby, Ahmed S.; Mohamed, Dalia F.; El Bassuonia, Maha A.; and Radwana, Wafaa M. (2016) "Contribution of the STAT4 gene single nucleotide polymorphism to systemic lupus erythematosus," *Menoufia Medical Journal*: Vol. 29: Iss. 2, Article 31.
DOI: https://doi.org/10.4103/1110-2098.192430

Contribution of the *STAT4* gene single nucleotide polymorphism to systemic lupus erythematosus

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Received 02 September 2014
Accepted 16 November 2014

Menoufia Medical Journal 2016, 29:373–378

Objectives
We investigated the prevalence of the *STAT4* G > C (rs7582694) polymorphism in patients with systemic lupus erythematosus (SLE) and controls in a sample of the Egyptian population.
Background
STAT4 has been found to be a susceptible gene in the development of SLE in various populations.
Participants and methods
The presence of the *STAT4* G>C (rs7582694) polymorphism was determined by PCR-RFLP.
Results
There was an insignificant difference between SLE patients and controls in their phenotypes ($P > 0.05$). Also, the distribution of the polymorphism among the SLE patients in terms of different symptoms and the anti-dsDNA titer showed an insignificant difference ($P > 0.05$).
Conclusion
There is no association between the *STAT4* G>C (rs7582694) polymorphism and susceptibility for SLE in the population of Egypt that may be different from other populations in geographic location, together with the racial and ethnic differences, and also environmental factors, with differences in lifestyle.

Keywords:
polymorphism, systemic lupus erythematosus, *STAT4*

Menoufia Med J 29:373–378

1110-2098

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder characterized by the development of an immune response directed against any part of the host body. The course of SLE is unpredictable, with periods of remission and flare-ups. Moreover, this autoimmune disorder is highly heterogeneous, with various clinical manifestations and biosynthesis of a broad array of autoantibodies. The occurrence of SLE is nine times more frequent in premenopausal women than in men [1].

It is known that environmental factors together with genetic components are involved in the abnormal immune responses and pathogenesis of SLE [2,3]. Flare-ups of SLE can be triggered by various environmental components, such as exposure to ultraviolet light, drugs, chemicals, and viral infections [4]. Candidate gene and genome-wide association studies showed numerous susceptibility genes of SLE, and the association of some of these genes has been confirmed in distinct populations [3].

The immune cells from patients with SLE show many abnormalities, including reduced T-cell cytotoxicity, abnormal function of CD4 T cells, abnormal activation of B cells, and alterations in cytokine biosynthesis [5,6].

The *STAT4* (signal transducer and activator of transcription) gene is expressed in T and B cells, monocytes, macrophages, natural killer cells, and dendritic cells. *STAT4* is a transcription factor and a member of the STAT family. Its expression may support the differentiation of immune cells to inflammatory subsets, production of inflammatory cytokines and autoantibodies, prevention of apoptosis, and presentation of autoantigens, which may promote the development of autoimmune diseases [7].

Participants and methods

This study was carried out on 57 SLE patients, two males and 55 females, age range 11–37 years old. Fifty-four age-matched and sex-matched individuals were selected as a control group.

All patients were subjected to the following:

(1) Full assessment history and a thorough clinical examination.
(2) Routine laboratory investigations including complete blood picture, renal function tests, ESR, urine analysis, and anti-dsDNA titer.

(3) *STAT4* G>C (rs7582694) polymorphism by PCR-RFLP.

Detection of the *STAT4* G>C (rs7582694) polymorphism by PCR-RFLP

DNA extraction

Genomic DNA was extracted from whole blood cells using the GeneJET Whole Blood Genomic DNA Purification Mini Kit (Thermo Scientific, San Jose, California, USA).

PCR amplification of STAT 4 gene

The *STAT4* gene was amplified from the extracted DNA using DreamTaq Green PCR Master Mix (2×) (Promega, USA) and STAT primers (Thermo Scientific). Promega DreamTaq Green PCR Master Mix (2×) is a ready-to-use solution containing DreamTaq DNA polymerase, optimized DreamTaq Green buffer, $MgCl_2$, and dNTPs. The master mix is supplemented with two tracking dyes and a density reagent that allows for direct loading of the PCR product on a gel.

Primers

Two pairs of primers were utilized to evaluate the *STAT4* G>C (rs7582694) polymorphism by PCR-RFLP. The lyophilized primers were reconstituted by the addition of sterile-distilled water to a final concentration of 100 pmol/μl, distributed in aliquots, and stored at −20°C.

Forward primer sequence: 5′ ATCCAACTC TTCTCAGCCCTT 3′.

Reverse primer sequence: 5′ TCATAATCAG GAGAGAGGAGT 3′.

Determination of the STAT 4 genotype

The amplified PCR products were digested using the fast digest *TAA*I restriction enzyme.

Detection of the digested fragment

Fifteen microliter of the digested fragment was then resolved on a 3% agarose gel using TBE buffer. The gel was run at 80 V for 35 min. A 100 bp ladder was used as a reference marker. U/V was used for visualization of DNA bands and they were photographed. The *STAT4* C allele was cleaved into 258 and 80 bp, whereas the *STAT4* G allele remained uncut.

The clinical and immunological criteria used in the Systemic Lupus International Collaborating Clinics (SLICC) Classification criteria are presented in Table 1 [8].

Results

This study was carried out on 57 SLE patients, two males and 55 females, age range 11–37 years, attending the Rheumatology department, Ain Shams University Hospitals. Fifty-four age-matched and sex-matched individuals were selected as a control group. We evaluated the *STAT4* G>C (rs7582694) polymorphism distribution in SLE patients and its association with various clinical manifestation of SLE and anti-dsDNA titer.

STAT4 genotype distribution among patients and controls

Comparison of patients and controls in their *STAT4* genotypes considering the GG genotype as the reference indicated an insignificant difference between them ($P > 0.05$). Also, comparison between them in the prevalence of the C allele considering the G allele as the reference indicated an insignificant difference between them ($P > 0.05$) (Table 2).

Association of *STAT4* genotypes with various clinical manifestations of the patients included

Comparison of the distribution of polymorphisms among the patients in terms of different symptoms such as neurogenic manifestations, malar rash, arthritis, nephritis, photosensitivity, respiratory symptoms, and oropharyngeal ulcer indicated an insignificant difference between different types of polymorphisms ($P > 0.05$) (Table 3).

Distribution of the polymorphism among the patients in terms of hematological disorders

Comparison of the distribution of polymorphisms among the patients in terms of hematological disorders, with *P* value more than 0.05 indicating an insignificant difference between different types of polymorphisms, is shown in Table 4.

Distribution of the polymorphism among the patients in terms of the anti-dsDNA titer

Comparison of the distribution of polymorphism among the patients in terms of the anti-dsDNA titer, with *P* value more than 0.05 indicating an insignificant difference between different types of polymorphisms, is shown in Table 5.

Distribution of the polymorphism among the patients in terms of renal disorders

Comparison of the distribution of polymorphism among the patients in terms of renal disorders, with *P* value more than 0.05 indicating an insignificant difference between different types of polymorphisms, is shown in Table 6.

Table 1 Clinical and immunological criteria used in the SLICC classification criteria

Clinical criteria	
Acute cutaneous lupus	Including lupus malar rash (do not count if malar discoid); bullous lupus; toxic epidermal necrolysis variant of SLE; maculopapular lupus rash; photosensitive lupus rash in the absence of dermatomyositis; or subacute cutaneous lupus (nonindurated psoriasiform and/or annular polycyclic lesions that resolve without scarring, although occasionally with postinflammatory depigmentation or telangiectasia)
Chronic cutaneous lupus	Including classical discoid rash; localized (above the neck); generalized (above and below the neck); hypertrophic (verrucous) lupus; lupus panniculitis (profundus); mucosal lupus; lupus erythematosus tumidus; chilblains lupus; discoid lupus/lichen planus overlap
Oral ulcers	Palate, buccal, tongue, or nasal ulcers in the absence of other causes, such as vasculitis, Behçet's, infection (herpes), inflammatory bowel disease, reactive arthritis, and acidic foods
Nonscarring alopecia	Diffuse thinning or hair fragility with visible broken hairs in the absence of other causes such as alopecia areata, drugs, iron deficiency, and androgenic alopecia
Synovitis	Involving two or more joints, characterized by swelling, effusion, or tenderness in two or more joints, and 30 min or more of morning stiffness
Serositis	Typical pleurisy for more than 1 day or pleural effusions or pleural rub; typical pericardial pain (pain with recumbency improved by sitting forward) for more than 1 day or pericardial effusion or pericardial rub or pericarditis by ECG in the absence of other causes, such as infection, uremia, and Dressler's pericarditis
Renal	Urine protein/creatinine (or 24-h urine protein) representing 500 mg of protein/24 h or red blood cell casts
Neurologic	Seizures; psychosis; mononeuritis multiplex in the absence of other known causes such as primary vasculitis; myelitis; peripheral or cranial neuropathy in the absence of other known causes such as primary vasculitis and diabetes mellitus; acute confusional state in the absence of other causes, including toxic-metabolic, uremia, drugs
Hemolytic anemia	
Leukopenia	<4000/mm^3 at least once (in the absence of other known causes such as Felty's, drugs, and portal hypertension) or lymphopenia (<1000/mm^3 at least once) in the absence of other known causes such as corticosteroids, drugs, and infection
Thrombocytopenia	(<100 000/mm^3) at least once in the absence of other known causes such as drugs, portal hypertension, and thrombotic thrombocytopenic purpura
Immunologic criteria	
ANA	Above laboratory reference range
Anti-dsDNA	Above laboratory reference range, except ELISA: two times beyond the laboratory reference range
Anti-Sm	
Antiphospholipid antibody	Any of the following lupus anticoagulant false-positive RPR medium or high-titer anticardiolipin (IgA, IgG, or IgM) anti-β2 glycoprotein I (IgA, IgG, or IgM)
Low complement	Low C3, low C4, low CH50
Direct Coombs test	In the absence of hemolytic anemia

Ig, immunoglobulin; SLE, systemic lupus erythematosus; SLICC, Systemic Lupus International Collaborating Clinics.

Table 2 *STAT4* genotype distribution among patients and controls

variables	Groups [*n* (%)]		χ^2	*P* value	OR (95% CI)
	Patients (*n* = 57)	Controls (*n* = 54)			
Polymorphism					
GG	34 (59.6)	34 (63.0)	0.34	>0.05	Reference
CG	17 (29.8)	16 (29.6)			CG: 1.06 (0.46–2.44)
CC	6 (10.5)	4 (7.4)			CC: 1.20 (0.69–2.10)
					Both: 1.07 (0.74–1.54)
G	51 (70.8)	50 (72.5)	—	—	Reference
C	21 (29.2)	19 (27.5)	0.05	>0.05	1.08 (0.52–2.26)

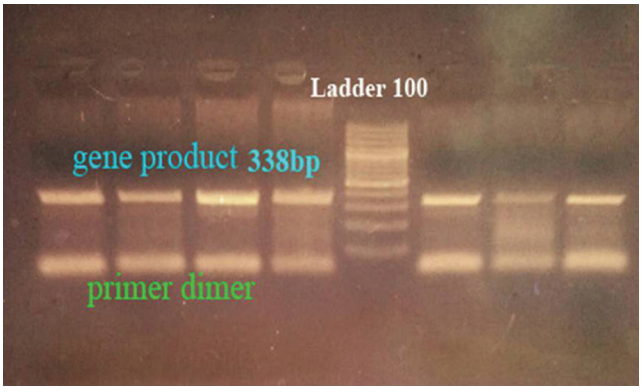
CI, confidence interval; OR, odd ratio.

Agarose gel electrophoresis: the PCR-amplified fragments of *STAT4 gene* were 338 bp in length

The PCR-amplified fragments of the *STAT4* gene were 338 bp in length and among PCR products after enzyme digestion, the *STAT4* C allele was cleaved into 258 and 80 bp fragments, whereas the *STAT4* G allele remained uncut (Fig. 1).

PCR product after enzyme digestion: The *STAT4* C allele was cleaved into 258 and 80 bp fragments, whereas the *STAT4* G allele remained uncut.

Figure 1



Agarose gel electrophoresis: the PCR-amplified fragments of the *STAT4* gene were 338 bp in length.

Discussion

SLE is a chronic autoimmune disorder characterized by the development of an immune response directed against any part of the host body. The course of SLE is unpredictable, with periods of remission and flare-ups. Moreover, this autoimmune disorder is highly heterogeneous, with various clinical manifestations

and biosynthesis of a broad array of autoantibodies. The occurrence of SLE is nine times more frequent in premenopausal women than in men [1].

It is known that environmental factors together with genetic components are involved in the abnormal immune responses and pathogenesis of SLE [2,3]. Flare-ups of SLE can be triggered by various environmental components, such as exposure to ultraviolet light, drugs, chemicals, and viral infections [4]. Candidate gene and genome-wide association studies reported numerous susceptibility genes of SLE, and the associations of some of these genes have been confirmed among distinct populations [3].

The immune cells from patients with SLE show many abnormalities, including reduced T-cell cytotoxicity, abnormal function of CD4 T cells, abnormal activation of B cells, and alterations in cytokine biosynthesis [5,6].

The *STAT4* gene is expressed in T and B cells, monocytes, macrophages, natural killer cells, and dendritic cells. *STAT4* is a transcription factor and a member of the STAT family. Its expression may support the differentiation of immune cells into inflammatory subsets, production of inflammatory cytokines and autoantibodies, prevention of apoptosis, and presentation of autoantigens, which may promote the development of autoimmune diseases [7].

STAT4 is essential for signal transduction by interleukin-12 (IL-12), IL-23, and type 1 interferon (IFN) in T cells and monocytes [7]. IL-12 induces the *STAT4*-dependent NK cell activation and differentiation of naive CD4 lymphocytes into Th1 effector cells and IFNγ production [9].

STAT4 also mediates the IL-23-dependent expansion of Th17 cells, contributing toward autoimmune diseases [10]. It has been reported that *STAT4*-deficient mice show reduced manifestation of T cell-linked experimental autoimmune diseases including encephalomyelitis, arthritis, myocarditis, colitis, and autoimmune diabetes [7]. Moreover, *STAT4* deficiency results in a reduction in IFNγ biosynthesis in immune cells [7]. Accordingly, an association between disease activity in SLE patients and activation of the type 1 IFN system has been observed [11].

This study was carried out to evaluate the distribution of the *STAT4* G>C (rs7582694) polymorphism in SLE patients and its association with various clinical manifestations of SLE and anti-dsDNA titer in an Egyptian population.

We found an insignificant difference between SLE patients and controls in their phenotypes ($P > 0.05$). Also, the distribution of polymorphisms among SLE the patients in terms of different symptoms and DNA showed an insignificant difference ($P > 0.05$).

The above-mentioned results are in agreement with the results of Zervou *et al.* [12], who reported the same findings in a Turkish population. Numerous studies have been carried out on the role of the *STAT4* in the genetic predisposition to SLE; the data presented here show that the distribution of the *STAT4* G>C (rs7582694) polymorphism is not associated with an increased susceptibility for SLE in the population

Table 3 Association of *STAT4* genotypes with various clinical manifestations of the patients included

Symptoms	Polymorphism [*n* (%)]			χ^2	*P* value
	GG (*n* = 34)	CG (*n* = 17)	CC (*n* = 6)		
Neurogenic					
Positive	3 (8.8)	4 (23.5)	1 (16.7)	2.07	>0.05
Negative	31 (91.2)	13 (76.5)	5 (83.3)		
Malar rash					
Yes	27 (79.4)	9 (52.9)	3 (50.0)	4.72	>0.05
No	7 (20.6)	8 (47.1)	3 (50.0)		
Arthritis					
Yes	15 (44.1)	8 (47.1)	5 (83.3)	3.18	>0.05
No	19 (55.9)	9 (52.9)	1 (16.7)		
Nephritis					
Yes	24 (70.6)	12 (70.6)	2 (33.3)	3.35	>0.05
No	10 (29.4)	5 (29.4)	4 (66.7)		
Photosensitivity					
Yes	23 (67.6)	12 (70.6)	4 (66.7)	0.05	>0.05
No	11 (32.4)	5 (29.4)	2 (33.3)		
Respiratory					
Yes	11 (32.4)	3 (17.6)	1 (16.7)	1.58	>0.05
No	23 (67.6)	14 (82.4)	5 (83.3)		
Oropharyngeal ulcer					
Yes	19 (55.9)	8 (47.1)	4 (66.7)	0.76	>0.05
No	15 (44.1)	9 (52.9)	2 (33.3)		

Table 4 Distribution of the polymorphism among the patients in terms of hematological disorders

Variable	Polymorphism (mean ± SD)			Kruskal–Wallis	*P* value
	GG (*n* = 34)	CG (*n* = 17)	CC (*n* = 6)		
WBCs×10^3/μl	7.86 ± 3.24	7.40 ± 2.25	5.93 ± 1.46	2.23	>0.05
Hb (g%)	10.21 ± 1.53	9.34 ± 1.90	11.10 ± 2.98	*F* = 2.39	>0.05
Platelets×10^3/μl	273.38 ± 71.11	241.94 ± 79.06	243.83 ± 169.90	4.31	>0.05
Lymphocytes	23.05 ± 6.65	21.64 ± 4.19	19.83 ± 2.40	*F* = 0.96	>0.05
ESR	70.91 ± 24.91	64.88 ± 26.06	59.33 ± 33.79	1.13	>0.05

Table 5 Distribution of the polymorphism among the patients in terms of the anti-dsDNA titer

Anti-dsDNA	Polymorphism (mean ± SD)			Kruskal–Wallis	*P* value
	GG (*n* = 34)	CG (*n* = 17)	CC (*n* = 6)		
Anti-dsDNA	1012.7 ± 454.96	911.94 ± 319.79	616.33 ± 350.44	5.75	>0.05

Table 6 Distribution of the polymorphism among the patients in terms of renal disorders

Renal laboratory results	Polymorphism (mean ± SD)			Kruskal–Wallis	*P* value
	GG (*n* = 34)	CG (*n* = 17)	CC (*n* = 6)		
Creatinine (mg/dl)	1.80 ± 1.01	1.93 ± 1.24	1.23 ± 0.50	2.60	>0.05
Protein/creatinine	1.02 ± 0.69	1.25 ± 1.13	0.85 ± 0.65	0.96	>0.05

of Egypt, indicating that the geographic location of both populations (Egypt and Turkey) may be implicated in the lack of susceptibility to the disease observed [13,14].

In addition to racial and ethnic differences that may affect the causes, expression, and prevalence of the disease, environmental factors together with the extensive differences in lifestyle (diet, alcohol, smoking, etc.) may play a major role in the development of some diseases to the same degree as genetic factors [9]. Thus, the same disease may have different contributing factors in one ethnic group compared with another as certain polymorphisms may exist only in one ethnic group.

Recent studies carried out by Luan *et al.* [15] reported a statistically significant contribution of *STAT4* G/C (rs7582694) toward the incidence of SLE in the Mainland Chinese female population. The contribution of the *STAT4* G>C (rs7582694) polymorphism toward the incidence of SLE was also observed in large groups of patients of European origin, among them a Finnish family cohort as well as Spanish, Swedish, and other populations [16–19].

The different effects of the *STAT4* G>C (rs7582694) on clinical manifestations in various ethnicities may result from different sizes of the groups studied, genetic heterogeneity, or patient interaction with several environmental factors [20]. *STAT4* gene variants have also been found to be risk factors for other autoimmune diseases including rheumatoid arthritis, Crohn's disease, asthma, systemic sclerosis, and Sjogren's syndrome [21].

In conclusion, here, we report that there was no association between the *STAT4* G>C (rs7582694) polymorphism and susceptibility for SLE in a population of Egypt that is different from other populations in terms of the geographic location, together with the racial and ethnic differences, and also environmental factors with differences in lifestyle.

Conclusion

Here, we found that there was an insignificance difference between SLE patients and controls in the *STAT4* G>C (rs7582694) polymorphism in a population of Egypt. In addition, our study showed that there was an insignificance difference between SLE patients in the *STAT4* G>C (rs7582694) polymorphism and its association with various clinical manifestations of SLE and anti-dsDNA titer.

Conflicts of interest

None declared.

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