The Egyptian Rheumatologist (2014) xxx, xxx–xxx

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The Egyptian Rheumatologist

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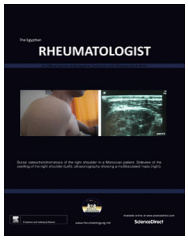

ORIGINAL ARTICLE

Signal transducer and activator of transcription 4 (STAT4) G/T gene polymorphism in Egyptian systemic lupus erythematosus female patients

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Received 5 July 2014; accepted 13 July 2014

KEYWORDS

STAT4;
G/T rs7574865 polymorphism;
SLE;
Egypt

Abstract *Background:* Systemic lupus erythematosus (SLE) is a systemic autoimmune disease with complex genetic inheritance. Many reports have provided evidence that signal transducer and activator of transcription-4 (STAT4) may participate in the pathogenesis of SLE.

Aim of the work: The aim was to investigate the clinical significance and possible association of STAT4 (G/T) genetic polymorphism and the susceptibility to SLE in a cohort of Egyptian female patients.

Patients and methods: Sixty-five Egyptian SLE female patients and 100 age and sex-matched unrelated female healthy blood donors who served as controls, were included in the study. STAT4 genotyping was performed by real time PCR-allelic discrimination technique.

Results: STAT4 genotyping in patients revealed that 63.1% had GG, 32.3% GT and 6.15% wild (TT) genotype. There was a non-significant difference in the distribution of STAT4 genotypes between patients and controls. Vasculitis, photosensitivity and lupus nephritis were significantly increased in patients with the homomutant (GG and TT) compared to heteromutant (GT) genotype ($p = 0.01$, $p = 0.04$ and $p < 0.01$ respectively). Patients with a TT genotype had a significantly consumed C3 and C4 levels and higher anti-dsDNA positivity compared to those with GG and GT genotypes. Promoter polymorphism tended to be higher in juvenile-onset SLE cases.

Conclusions: STAT4 (G/T) polymorphism was not associated with an increased risk of SLE in Egyptian females. However, vasculitis, photosensitivity and renal involvement were significantly higher among patients harboring the homomutant genotypes. Genetic polymorphism may be an important determinant affecting disease progression and is associated with DNA positivity and younger age of onset.

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Peer review under responsibility of Egyptian Society for Joint Diseases and Arthritis.

http://dx.doi.org/10.1016/j.ejr.2014.07.004

1. Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease with involvement of different body systems and the development of an immune response against self-antigens, leading to tissue inflammation, destruction and even end-organ damage [1]. SLE is more prevalent in females compared to males (9:1) and in African-American (AA), Asian (AS) and Hispanic (HI) populations compared to European (EA) [2,3]. There are also marked disparities in SLE incidence and prevalence worldwide; varying among different ethnic and geographical populations [4]. SLE is four times more common in people of African-American ancestry than those of European ancestry [5]. In addition, African-Americans have a markedly increased risk for developing lupus nephritis relative to European-Americans [6].

The etiology of SLE is not clearly understood, but genetic factors likely influence the pathogenesis, disease expression, and production of auto antibodies [7]. Environmental factors together with genetic components are involved in the abnormal immune responses and in the pathogenesis of SLE. SLE flare-ups in response to various environmental components, such as exposure to ultraviolet light, certain drugs, chemicals, and viral infections are documented [8]. In SLE, there is loss of tolerance to self-antigens with polyclonal activation of B lymphocytes, production of different auto-antibodies, and an altered function of T cells with alterations in cytokine biosynthesis [9]. Numerous susceptibility genes for SLE have been reported in candidate gene and genome wide association (GWA) studies; moreover, some of these genes have been confirmed to have some association among distinct populations [10]. The complexity of etiology and pathogenesis in SLE, enclosing genetic and environmental factors, apparently is one of the greatest challenges for both researchers and clinicians [11]. By participating in transcription complex with other co-factors, STAT4 harbors the potential of regulating a large number of target genes, which may contribute to their strong association with SLE [12].

The discovery of the link between the INF-α activation pathway and SLE has greatly contributed to a better understanding of pathogenesis of SLE [13] variants of certain INF-α pathway genes, including IRF5, IRF7, signal transducer and activator of transcription (STAT4), and tyrosine kinase2 have been associated with SLE susceptibility in many ethnic groups, but the complete impact of genetic variation on pathway activation is not fully understood [14,15]. STAT4 is a transcription factor that is expressed in the activated peripheral blood monocytes, dendritic cells and macrophages at the sites of inflammation in humans [16]. It encodes transcription factor that mediates the effect of several cytokines, including interleukin-12 (IL-12), INF-α, and IL-23, in T-cell and monocytes, TH1 and TH17 differentiation, monocyte activation and interferon gamma production [17,18], all playing a role in chronic inflammatory disorders, which means a possible crucial role of STAT4 in the development of autoimmune diseases such as SLE [19].

Previous studies have provided evidence that STAT4 may participate in the pathogenesis of SLE, while studies of relation between this gene and genetic susceptibility to subphenotypes of SLE were seldom observed [15]. The aim of current work was to investigate the possible role of STAT4 (rs7576485) G/T gene polymorphism as a molecular risk factor for the development of SLE and its possible association with certain clinical or laboratory characteristics of the disease in a cohort of Egyptian females.

2. Patients and methods

2.1. Study population

The current case–control study was conducted on sixty-five Egyptian SLE female patients diagnosed according to Systemic Lupus International Collaborating Clinics classification criteria for SLE [20]. All patients were attending the Rheumatology and Rehabilitation department, Faculty of medicine, Cairo University. Also, one hundred age and sex-matched unrelated female healthy blood donors were included in the study as a control group. Patients were subjected to a thorough clinical examination and information with regard to clinical manifestations of patients was obtained (age, malar rash, discoid lesions, photosensitivity, oral ulcers, arthritis, and renal, neurological and hematological disorder). On the day of sampling, laboratory assessment was done in the form of complete hemogram, erythrocyte sedimentation rate (ESR), serum complement level (C3 and C4), serum antinuclear antibodies (ANA) and anti-double stranded deoxyribonucleic acid (ds-DNA) antibodies by indirect immune-fluorescence, complete urine analysis and kidney function tests. LN was defined as clinical and laboratory manifestations that meet ACR criteria (persistent proteinuria > 0.5 g per day or greater than + + + by dipstick, and/or cellular casts including red blood cells [RBCs], hemoglobin, granular, tubular, or mixed) and/or a renal biopsy sample demonstrating immune complex–mediated glomerulonephritis compatible with LN [20]. Lupus disease activities were assessed using SLE disease activity index (SLEDAI) score [21]. The results of renal biopsy grading from patients with nephritis were retrospectively obtained from patients' medical records.

The protocol for this research conforms to the provisions of the World Medical Association's Declaration of Helsinki. Informed Consent was obtained from all participants prior to the study. Approval from our institutional scientific and ethics committee was also obtained.

2.2. Genotyping of STAT4 (rs7574865) by real time PCR based allelic discrimination assay

For all participants, genomic DNA was extracted from peripheral blood leukocytes using a QIA Amp DNA Minikit (Qiagen, Germany) according to the manufacturer's instructions. Identification of the *STAT4* G/T (rs7574865) polymorphic variant was performed by real time polymerase chain reaction using allelic discrimination (AD) assay; a multiplexed end point assay that detects variants of a single nucleic acid sequence using two primer/probe pairs in each reaction allowing genotyping of the two possible variants at the single-nucleic polymorphism (SNP) site in a target template sequence using a specific, fluorescent, dye-labeled probe for each allele. Probes contained different fluorescent reporter dyes (FAM and VIC). Primers and probes were purchased from Applied Biosystems (PN: 4351379, USA) as a commercially complete kit prepared by the company. Allelic discrimination assay

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was performed by Applied Biosystems (ABI) 7500 Real-Time PCR System. For quality control, genotyping was repeated blindedly with respect to case/control status to confirm our results for 30 samples and were 100% concordant.

Statistical analysis: Data were analyzed using SPSS statistical package version 17. For numerical data, parametric data were expressed as mean, standard deviation and range, while non-parametric data were expressed as median and interquartile range. Qualitative data were expressed as frequency and percentage. Chi-square test or Fisher's exact test was used to examine the relation between qualitative variables. Non-parametric numerical data were analyzed using Mann–Whitney test. Correlation analysis was performed by Spearman's rank correlation. Unconditional logistic regression analysis was used to calculate odds ratios (OR) and 95% confidence intervals (CI) for risk estimation. *P*-value less than 0.05 was considered significant. Chi-square (χ^2) test was performed to assess deviation from Hardy–Weinberg equilibrium in controls.

3. Results

The study included 65 female SLE patients with a mean age of 29.22 ± 9.01 years (10–50 years). There were 8 patients with juvenile-onset and 57 adult-onset SLE cases. One hundred age-matched unrelated female healthy blood donors were included in the study as a control group, their mean age was 30.11 ± 9.41 years. The frequencies of STAT4 genotypes in SLE patients and controls are presented in Table 1. Also, the age, clinical manifestations, laboratory investigations, renal biopsy class and disease activity grades among the three STAT4 genotypes in SLE patients are shown in Table 2.

Vasculitis and photosensitivity were significantly higher in patients with the homomutant STAT4 genotype (GG and TT) compared to those with the heteromutant genotype (GT) ($p = 0.01$ and $p = 0.04$ respectively). Regarding renal involvement, the incidence of lupus nephritis was significantly higher in patients with the homomutant genotype (75%) than those with the heteromutant genotype (25%) ($p < 0.01$).

The TLC was significantly lower in SLE patients with the GT genotype compared to the count in those with GG genotype ($p = 0.02$). The C3 and C4 levels in SLE patients with the TT genotype were significantly consumed compared to those with GG ($p < 0.0001$ and $p = 0.009$) and GT ($p = 0.001$ and 0.03) genotypes. Those with the TT genotype had a significantly higher anti-ds DNA positivity compared to those with GG ($p = 0.001$) and GT ($p = 0.04$) genotypes.

The STAT4 genotype in the juvenile onset SLE cases ($n = 8$) was GG in 3 (37.5%), GT in 4(50%) and TT in 1 (12.5%). Fifty-seven were adult onset SLE cases with a STAT4 genotype of GG in 37 (64.9%), GT in 17 (29.8%) and TT in 3 (5.3%). There were no significant differences between patients according to disease onset as regards the clinical and laboratory manifestations or SLEDAI. Only 2 patients with a GG genotype had a positive family history of SLE.

Table 1 Frequencies of STAT4 genotypes in SLE patients and controls.

STAT4 *n* (%)	SLE patients ($n = 65$)	Controls ($n = 100$)
GG	40 (61.5)	68 (68)
GT	21 (32.3)	27 (27)
TT	4 (6.2)	5 (5)

STAT4: signal transducer and activator of transcription 4; SLE: systemic lupus erythematosus.

4. Discussion

Systemic lupus erythematosus is a complex autoimmune disease characterized by autoantibody production and organ damage in which environmental factors together with genetic components are involved in the abnormal immune responses and in its pathogenesis [7]. The ethnic and genetic heterogeneity of SLE may contribute to the diversity of its clinical characteristics. Genome-wide association (GWA) and candidate gene studies have identified more than 30 common SLE risk alleles in European and Asian derived populations [10,22]. The contribution of single nucleotide polymorphisms (SNPs) of STAT4 G/C (rs7582694) and G/T (rs7574865) to the incidence of SLE and its clinical manifestations has been demonstrated [15,23].

The current study is an age-gender-ethnic-matched case–control study, that aimed at investigating the impact of STAT4 G/T (rs7574865) genetic polymorphism on the susceptibility to SLE and its possible association with certain clinical or laboratory characteristics of the disease in Egyptian females. This study included 65 female SLE patients and 100 healthy female volunteers. STAT4 genotyping was performed by real time PCR-allelic discrimination technique.

Regarding STAT4 genotyping in SLE patients, 32.3% were heterozygous harboring the GT genotype and 6.2% were Homozygous having the TT genotype. The frequency of STAT4 polymorphic genotypes was nearly similar to that reported in Polish population being 37% and 11% for the GT and TT genotypes [24]. However, In Northern Han Chinese population [25] it was 48.1% and 17.0% for GT and TT genotypes, and in a Japanese cohort it was 55.5% and 18.5% for GT and TT genotypes respectively [26].

In our study, there was no statistically significant difference found on comparing the frequency of STAT4 genotypes in SLE patients and controls. Although our results are in accordance with those of Zervou et al. [27] in a Turkish population, they are contradictory to those reported in studies from Japanese [26] and Chinese populations [25,28]. Luan and colleagues [15] reported that STAT4 was a common and critical susceptibility gene involved in the SLE pathogenesis in female Chinese. STAT4 (rs7574865) has also been confirmed as a genetic risk factor for SLE in Colombians, Mexicans and Argentinean cohorts [14]. This could be attributed to the ethnic difference between the studied populations, in addition to the different sample size of these studies. As SLE has a complex genetic background, none of the genes is likely to be entirely responsible for triggering autoimmune response in SLE even if they present potentially novel molecular mechanisms in the pathogenesis of the disease [11].

Study of the influence of STAT4 (G/T) genetic polymorphism on the clinical and pathological characteristics of the disease revealed that vasculitis and photosensitivity were significantly higher in SLE patients with the homomutant STAT4 genotype (GG and TT) compared to heteromutant (GT) ones. Moreover, the incidence of lupus nephritis was significantly

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Table 2 Comparison of the age, clinical manifestations, laboratory investigations, renal biopsy class and disease activity grades among 3 STAT4 genotypes in SLE patients.

Feature		STAT4 genotype in SLE patients (n = 65)			p
		GG (n = 40)	GT (n = 21)	TT (n = 4)	
Age		30.85 ± 9.36	27.19 ± 8.29	23.5 ± 5.45	0.14
Clinical manifestations					
Fever		20 (50)	10 (47.62)	2 (50)	0.99
Myalgia		10 (25)	2 (9.52)	1 (25)	0.36
Muco cutaneous	Malar rash	36 (90)	17 (80.95)	2 (50)	0.09
	Photosensitivity	18 (45)	7 (33.33)	2 (50)	0.65
	Alopecia	25 (62.5)	15 (71.43)	2 (50)	0.59
	Oral ulcers	21 (52.5)	10 (47.62)	2 (50)	0.94
Arthritis		35 (87.5)	20 (95.24)	4 (100)	0.51
Vasculitis		7 (17.5)	1 (4.76)	1 (25)	0.32
Pleurisy		21 (52.5)	10 (47.62)	2 (50)	0.94
Lupus cerebritis	Lupus headache	5 (12.5)	0 (0)	0 (0)	0.19
	Seizures	3 (7.5)	0 (0)	1 (25)	0.14
	Psychosis	5 (12.5)	1 (4.76)	0 (0)	0.51
Laboratory investigations					
ESR (mm/1st hr)		79.58 ± 39	75.76 ± 44.95	66.25 ± 34.97	0.8
Hb (g/dl)		9.82 ± 1.99	9.59 ± 2.73	10.78 ± 2.36	0.64
TLC ($\times 10^3/cm^3$)		8.18 ± 5.24	5.67 ± 2.78	8.78 ± 3.42	0.11
Platelets ($\times 10^3/cm^3$)		324.1 ± 142.8	278.8 ± 118.2	199.75 ± 69.7	0.14
Creatinine (mg/dl)		0.99 ± 0.63	0.96 ± 1.03	0.8 ± 0.34	0.89
Urea (mg/dl)		43.18 ± 28.8	55.05 ± 49.02	49.75 ± 38.9	0.49
C3 (mg/dl)		53.19 ± 36.6	49.08 ± 40.07	0.56 ± 0.2	0.17
C4 (mg/dl)		14.83 ± 25.9	8.7 ± 12.2	0.17 ± 0.04	0.53
Albuminuria N (%)		29 (72.5)	15 (71.43)	4 (100)	0.12
Grade	3+	13 (32.5)	0 (0)	2 (50)	–
	2+	7 (17.5)	9 (42.86)	1 (25)	–
	1+	9 (22.5)	6 (28.57)	1 (25)	–
	Nil	11(27.5)	6 (28.57)	0 (0)	–
ANA positivity		39 (97.5)	20 (95.24)	4 (100)	0.84
Anti-ds DNA		30 (75)	17 (80.95)	4 (100)	0.5
Renal biopsy and LN (N = 20)					0.63
Class	II	3 (7.5)	2 (9.5)	1 (25)	
	III	5 (12.5)	2 (9.5)	–	
	IV	3 (7.5)	1 (4.8)	1 (25)	
	V	2 (5)	–	–	
SLEDAI grade					0.63
Grade	Mild (< 8)	12 (30)	5 (23.8)	1 (25)	
	Moderate (8–18)	14 (35)	14 (66.7)	2 (50)	
	Severe (> 18)	14 (35)	2 (9.5)	1 (25)	

Results are presented as mean ± SD or N (%). STAT4: signal transducer and activator of transcription 4; ESR: erythrocyte sedimentation rate; Hb: hemoglobin; TLC: total leukocytic count; C: complement; DNA: deoxyribonucleic acid; LN: lupus nephritis; SLEDAI: systemic lupus erythematosus disease activity index.

higher in patients with the homomutant than those with a heteromutant genotype. Lupus nephritis (LN) is one of the most serious SLE complications since it is a major predictor of poor prognosis. It is a major concern as it affects approximately 50% of the patients and accounts for significant morbidity and mortality [29]. Our results go with those previously reported by Kawasaki et al. [26] as lupus nephritis was higher in SLE patients with STAT4 polymorphic alleles. Genetic variations in STAT4 predispose to lupus nephritis and a worse outcome with severe renal insufficiency [30]. The clinical manifestations and disease severity vary greatly among patients, thus several studies try to associate clinical heterogeneity and prognosis with specific genetic polymorphisms in SLE associated genes. The continuous effort to describe new predisposing or modulating genes in SLE is justified by the limited knowledge about pathogenesis, assorted clinical manifestation and possible prevention strategies [11].

The TLC was significantly lower in SLE patients with the GT genotype compared to the count in those with GG genotypes. The C3 and C4 levels in SLE patients with the TT genotype were significantly consumed compared to those with GG and GT genotypes. It was reported that complement activation and sublytic C5b-9 assembly on the plasma membrane were able to activate STAT4 in endothelial cells [31].

In our study, those with the TT genotype had a significantly higher anti-ds DNA positivity compared to those with GG and

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GT genotypes. Anti-dsDNA antibodies are significant predictors of LN [32]. Kawasaki et al. [26] reported that anti-DNA antibodies were higher in SLE patients with STAT4 polymorphism. It has been reported that STAT4 polymorphisms are associated with autoimmune diseases which are characterized by a systemic pathology and anti-dsDNA antibody [33]. The *rs7574865* SNP in *STAT4* has been associated with a severe SLE phenotype defined not only with the immunologic disorder (specifically, ds-DNA autoantibodies) but also with nephritis and age at diagnosis < 30 years old [23].

The STAT4 genotype in the juvenile onset SLE cases tended to show a higher GT (50%) and TT (12.5%) polymorphism compared to that in the adult onset cases as GT was present in 29.8% and TT in 5.3%. It was reported that the prevalence of damage was increased in juvenile-onset SLE Egyptian patients compared to adult-onset ones [34] .Kawasaki and Colleagues [26] reported that STAT4 polymorphism was higher in SLE Japanese patients with an age of onset of less than 20 years. Associations of polymorphisms in STAT4 with childhood-onset SLE were confirmed in another Japanese population. The cumulative number of risk alleles was significantly increased in childhood-onset cases [35].

In conclusion, in the present study STAT4 (G/T rs7574865) polymorphism was not associated with an increased risk of SLE in Egyptian females. An increased frequency of vasculitis, photosensitivity and renal involvement was present among SLE patients harboring homomutant genotypes, raising the possibility that genetic polymorphism may be an important determinant affecting disease progression. Genetic polymorphism of STAT4 (TT) was associated with an increased complement consumption and anti-DNA positivity compared to other genotypic alleles. Larger studies are required to elucidate the relevance of STAT4 polymorphism in the susceptibility to systemic lupus and its impact on the clinical and laboratory features of the disease.

Conflict of interest

All authors declare that they have no conflict of interest.

Disclosures

None.

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