Human Immunology 72 (2011) 1210-1213

Contents lists available at SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/humimm

TRAF1/C5, *eNOS*, *C1q*, but not *STAT4* and *PTPN22* gene polymorphisms are associated with genetic susceptibility to systemic lupus erythematosus in Turkey

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ARTICLE INFO

Article history:
Received 5 July 2011
Accepted 19 September 2011
Available online 24 September 2011

Keywords:
Gene polymorphisms
Systemic lupus erythematosus
Association study

ABSTRACT

A significant source of variability in the literature on systemic lupus erythematosus (SLE) susceptibility genes has been the inability to replicate genetic findings across different racial or ethnic groups. We investigated whether a single nucleotide polymorphism (SNP) of the *STAT4* (rs7574865), *PTPN22* (rs2476601), *TRAF1/C5* (rs10818488), and *C1q* (rs292001) genes as well as the 27-bp VNTR polymorphism on intron 4 of *eNOS*, previously associated with SLE in other populations, are also associated with SLE risk in Turkey. A group of 158 SLE patients and 155 healthy controls were included in this study. A genetic association of the *TRAF1/C5*, *C1q*, and *eNOS* gene polymorphism, but not of *STAT4* and *PTPN22*, was found to confer a degree of risk for SLE. These data highlight the importance of comparative studies in different populations to confirm the previously detected genetic associations.

1. Introduction

Systemic lupus erythematosous (SLE) is the prototype of complex autoimmune diseases, whereby multiple susceptibility genes are thought to interact with a variety of potential environmental exposures. It is a systemic disease characterized by the production of autoantibodies against various nuclear antigens owing to a breakdown in self-tolerance that can affect several organs or organ systems, resulting in a broad spectrum of clinical and immunologic manifestations.

Although the etiology of SLE is unknown, both genetic and environmental factors play a role in the pathogenesis of the diseases. The strong genetic component of the disease is supported by high familial aggregation and concordance in twins [1]. Genome-wide association studies (GWAS) have led to the association of a number of new susceptibility genes or confirmed previous findings from gene association studies and/or candidate gene approaches. These studies, like in most autoimmune diseases, have shown that *HLA* has an important contribution in SLE development [2], whereas several non–human leukocyte antigen (HLA) SLE susceptibility loci that mainly confer a modest level of risk have been mapped as well [3–5]. For these loci to be confirmed as risk factors for SLE, comparative studies are needed to replicate the association findings across other ethnic or racial groups, thus verifying possible locus heterogeneity for various autoimmune diseases.

Among the SLE-correlated genes that have been analyzed so far are included endothelial nitric oxide synthase (eNOS), tumor necrosis factor (TNF)–receptor–associated factor 1 and complement component 5 region (*TRAF1/C5*), signal transducer and activator of transcription 4 (*STAT4*), complement component 1q (*C1q*) and protein-tyrosine-phosphatase nonreceptor type 22 (*PTPN22*). To date, no comprehensive gene association study has been performed in the Turkish population so far. The aim of this study is, to validate polymorphisms from each of the aforementioned genes in this population.

2. Subjects and methods

2.1. Study population

The study group consisted of 155 healthy subjects and 158 SLE patients from unrelated families living in Istanbul, Turkey. All SLE patients met the 1982 American College of Rheumatology (ACR) revised criteria for the classification of SLE [6], whereas all patients and control healthy volunteers (age and gender matched) included if thay had no other autoimmune diseases. The study was performed after approval of the research committee and signed informed consent by the patients and the healthy controls had been provided.

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0198-8859/$36.00
doi:10.1016/j.humimm.2011.09.003

Table 1
TRAF1/C5 rs10818488 polymorphism in Turkish SLE patients and healthy controls

	rs10818488 TRAF1/C5				
	Genotype			Allele	
	GG	GA	AA	G	A
Turkish cohort					
SLE patients ($N = 158$)	43 (27.2%)	86 (54.4%)	29 (18.4%)	172 (54.4%)	144 (45.6%)
Healthy controls ($N = 155$)	61 (39.4%)	75 (48.4%)	19 (12.2%)	197 (63.5%)	113 (36.5%)
OR (95% CI)[a]	1.00	1.63 (0.99–2.68)	2.17 (1.08–4.35)	1.00	1.46 (1.06–2.01)
p Value (df = 1)	–	5.5×10^{-2}	2.9×10^{-2}	–	2.0×10^{-2}

CI, confidence interval; df, degrees of freedom; OR, odds ratio; SLE, systemic lupus erythematosus.
[a] p Values with df = 1 and OR (95% CI) were calculated taking as reference the G/G genotype or the major (G) allele.

2.2. Analysis of the gene polymorphisms

Whole blood was collected in ethylenediaminetetraacetic acid (EDTA)– containing tubes. Genomic DNA was isolated from peripheral blood leukocytes by using the commercial kit PUREGENE (Gentra Systems, Minneapolis, MN). The extracted DNA was stored at −20°C until analyzed. Amplification and genotyping for the 27-bp repeat polymorphism on intron 4 of *eNOS*, *TRAF1/C5* rs10818488, *STAT4* rs7574865, and *PTPN22* rs2476601was performed by restriction fragments lengths polymorphism (RFLPs) as published previously [7–9]. The amplification of the genomic fragments harboring the polymorphic sites was carried out using the GoTaq polymerase provided by Promega. Moreover, to analyze the *C1q* rs292001 single nucleotide polymorphism (SNP), the upstream 5′- GTC CAA AGC AGA CCA GAA GGA TCA CAT AGA CAT TTA -3′ and the downstream 5′- GGC ACT TGG GAA AGT GTC AG -3′ primers were used to generate a 197-bp polymerase chain reaction (PCR) fragment of the intron 2 of *C1q* gene (located on chromosome 1). A hot start was used with initial heating at 94°C for 5 minutes and then 35 cycles of denaturing (at 94°C for 30 seconds), annealing (at 63°C for 30 seconds), and chain extension (at 72°C for 30 seconds), followed with a final extension step at 72°C for 5 minutes. Genotyping for the *C1q* rs292001 SNP was performed by restriction enzyme-analysis; thus, the 197-bp PCR product was digested with *HpyCH4*III (New England Biolabs, Ipswich, MA), which digests specifically DNA amplified from the allele G into 159-bp and 38-bp fragments. PCR products were analyzed through electrophoresis on 2.3% agarose gel and ethidium bromide fluorescence in reference to a molecular weight marker. Genotypes were scored blindly, and analysis of all ambiguous samples was repeated. Moreover, 10% of the samples was amplified twice for checking the accuracy of the results.

2.3. Statistical analysis and power calculation

Statistical analysis was performed with GraphPad Prism statistical program (GraphPad Software, San Diego, CA). In case-control comparisons, only unrelated individuals were used. The genes variants under investigation were evaluated for deviation from Hardy–Weinberg equilibrium by comparing observed and expected genotype frequencies by means of the χ^2 test both in patients and control groups. The χ^2 test, with 1 or 2 degrees of freedom (df) or Fisher's exact test was used to examine differences of genotype and allele frequencies between patients and controls. Odds ratios (OR) and their 95% confidence intervals (CI) were calculated according to the Rothman test. A two-tailed p value of less than 0.05 was defined as statistically significant. Power was calculated by using the Quanto software at a two-sided level of $p < 0.05$ based on previously published effect sizes, assuming a recessive model [10].

3. Results

The SLE study group ($n = 158$) consisted of 153 women (96.8%) and 5 men (3.2%), whereas unrelated healthy controls ($n = 155$) consisted of 150 women (96.7%) and 5 (3.3%) of similar age. The mean (± SD) age of patients was 41.1 ± 11 years and that of controls was 38.7 ± 6.6 years. Allele and genotype frequencies of the *TRAF1/C5 rs10818488* **G/**SNP and *eNOS* gene intron 4 a/b VNTR polymorphism (a 27-bp tandem repeat based polymorphism) are depicted in Tables 1 and 2, respectively. With regard to the *TRAF1/C5* polymorphism, we found that the **A/A** genotype and the minor allele **A** were more common in SLE patients (18.35% and 47.57%, respectively) than in control individuals (12.26% and 36.45%, respectively). The observed difference was statistically significant. In the case of *eNOS* gene intron 4 a/b VNTR polymorphism (a 27-bp tandem repeat based polymorphism), our results show that the a/a genotype and the **a** allele were more frequent in cases (8.86% and 24.68%, respectively) than in controls (3.23% and 17.42%, respectively). Allele and genotype frequencies of the *C1q* rs292001 G/A polymorphism analyzed in patients and controls are summarized in Table 3. Patients with SLE presented more commonly with the A allele (47.15%) than controls (35.81%) ($p = 4.0\times10^{-3}$, OR = 1.60, 95% CI = 1.16–2.20). Moreover, the genotype **A/A** was observed in 21.52% of SLE patients and 6.45% of controls ($p = 3.0\times10^{-4}$, OR = 4.27, 95% CI = 1.90–9.61). Both allelic and genotypic frequency differences were statistical significant. The distribution of genotypes showed no deviation from Hardy–Weinberg equilibrium for controls (at the 5% significance threshold), with an exception in the case of *C1q* SNP ($p = 0.0006$, $\chi^2 = 11.9$, df = 1). Altogether, these findings support clearly the implication of the

Table 2
eNOS intron 4 VNTR polymorphism in Turkish SLE patients and healthy controls

	Intron 4 VNTR eNOS				
	Genotype			Allele	
	bb	ba	a/a	b	a
Turkish cohort					
SLE patients ($N = 158$)	94 (59.5%)	50 (31.7%)	14 (8.8%)	238 (75.3%)	78 (24.7%)
Healthy controls ($N = 155$)	106 (68.4%)	44 (28.4%)	5 (3.2%)	256 (82.3%)	54 (17.7%)
OR (95% CI)[a]	1.00	1.27 (0.78–2.07)	3.12 (1.08–9.00)	1.00	1.55 (1.05–2.30)
p Value (df = 1)[a]	–	3.4×10^{-1}	2.7×10^{-2}	–	2.6×10^{-2}

CI, confidence interval; df, degrees of freedom; OR, odds ratio; SLE, systemic lupus erythematosus.
[a] p Values with df = 1 and OR (95% CI) were calculated taking as reference the bb genotype or the major (b) allele.