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Association of genetic variations in the STAT4 and IRF7/KIAA1542 regions with systemic lupus erythematosus in a Northern Han Chinese population

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ABSTRACT

Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease with complex genetic inheritance. Genome-wide association studies have identified SLE susceptibility variations at the IRF7/KIAA1542 locus and with STAT4 gene in European populations. We decided to investigate the association of single-nucleotide polymorphisms (SNPs) in the IRF7/KIAA1542 region (rs4963128, rs2246614, and rs702966) and in STAT4 (rs7574865 and rs7582694) with SLE disease in a Northern Han Chinese population of 748 patients and 750 healthy controls. Our study indicated a strong association between rs7574865 (odds ratio = 0.68; 95% confidence interval 0.59–0.79; $p = 1.57 \times 10^{-6}$) and SLE and between rs7574865 and the production of anti-Sm antibodies. Additionally, rs4963128 and rs2246614 were correlated with a variety of clinical subphenotypes, such as lupus nephritis, arthritis, and the production of anti-SSA/B autoantibodies, despite a lack of significant association between these two SNPs and SLE disease susceptibility in general.

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic, inflammatory autoimmune disease that can result in multiple organ damage and is characterized by production of autoantibodies to nuclear antigens and immune complex formation [1]. The etiology of SLE is not clearly understood, but genetic factors likely influence the pathogenesis, disease expression, and production of autoantibodies [2]. Population differences in disease prevalence and clinical manifestations exist [3]. The prevalence of SLE ranges from 31 to 70 cases per 100,000 persons among Chinese populations and from 7 to 71 cases per 100,000 persons in European populations [3,4]. Clinically, Asians have more renal involvement than Caucasians [5]. Studies in both mice and humans have demonstrated several genetic susceptibility loci that have roles in immune activation and regulation, as well as clearance of apoptotic cells. A major breakthrough in the understanding of pathogenesis of SLE was the discovery of the link between the interferon-α (IFN-α) activation pathway and SLE [6,7]. IFN-α is part of the innate immune response, and abnormally high levels of IFN-α have been observed in patients with SLE [8]. This state can promote dendritic cell maturation and proinflammatory cytokine production, which can affect stimulation of T-helper 1 pathways, promotion of B-cell activation, and regulation of apoptosis. Variants of certain IFN-α pathway genes, including IRF5, IRF7, signal transducer and activator of transcription 4 (STAT4), and tyrosine kinase 2, have been associated with SLE susceptibility in multiple ethnic groups, but the complete impact of genetic variation on pathway activation is not fully understood [9–11].

STAT4, the signal transducer and activator of transcription 4 gene, lies adjacent to STAT1 at 2q32.2–2q32.3, contains 24 exons, and spans 122 kb. The STAT4 gene encodes a transcription factor that mediates the effect of several cytokines, including interleukin (IL)-12, the type I interferons, and IL-23 in T cells and monocytes. Thus, STAT4 has a role in T-helper type 1 and type 17 differentiation, monocyte activation, and IFN-γ production. In 2003, Jacob et al. [12] confirmed STAT4 deficiency was associated with accelerated renal disease and increased mortality in a murine lupus model [13]. Associations between variants of the STAT4 single-nucleotide polymorphism (SNP) rs7574865 with other autoimmune diseases, such as rheumatoid arthritis [14,15], Sjögren's syndrome [16], inflammatory bowel disease, and type I diabetes mellitus [17], have been demonstrated. In addition to the link between STAT4 SNP rs7574865 and SLE, genome-wide association studies (GWAS) have demonstrated a link among rs3821236, rs7601754, and rs7582694 SNPs and SLE

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in Caucasian populations [18–20] and between rs10168266 and SLE in a Chinese population [21].

IRF7, a member of the IRF family of transcription factors that control inflammatory and immune responses, is the master regulator of type I IFN-dependent immune responses and is required for type I IFN production [22]. A GWAS of a European population with SLE showed an association between disease susceptibility and the SNP rs4963128, located 23 kb telomeric to IRF7 in KIAA1542 at 11p15.5 [20]. KIAA1542 is also known as PHRF1 or carboxyl-terminal domain-binding SR-like protein rA9 and is homologous to a gene encoding an elongation factor. This SNP was also in high linkage disequilibrium ($r^2 = 0.94$) with the rs702966 SNP in IRF7 [20]. Another SNP, rs2246614, located in the IRF7/KIAA1542 region, demonstrated an association with IFN-α activity in SLE patients [23].

Until this study, there have been no reports of associations between SNPs in the IRF7/KIAA1542 region with SLE susceptibility in Chinese populations. Hence, we examined the association of 3 SNPs (rs4963128, rs2246614, and rs702966) in the IRF7/KIAA1542 region, as well as 2 SNPs (rs7574865, rs7582694) in the STAT4 gene with SLE susceptibility in a northern Han Chinese population of 748 patients and 750 healthy controls. Our goal was to detect population differences in genetic variations involved in the pathogenesis of SLE to improve clinical intervention of this disease.

2. Subjects and methods

2.1. Patients and controls

The study population included 748 patients with SLE recruited from the Rheumatology Department of Peking Union Medical College Hospital between October 2008 and December 2009. Eligible patients fulfilled at least 4 of the American College of Rheumatology 1982 revised criteria for SLE diagnosis [24]. The SLE group had a mean age of 34.79 ± 12.75 years and consisted of 675 females and 73 males. The 750 control samples had a mean age of 35.14 ± 10.94 years (678 females and 72 males) with no family history of SLE or other autoimmune disease, such as rheumatoid arthritis, Sjögren's syndrome, ankylosing spondylitis, or diabetes. Patients and controls were ascertained from the same geographic location and the sex ratio and mean age were matched between groups. All patients and controls were unrelated individuals of self-reported Han Chinese ethnicity. In SLE patients, autoantibodies, including anti-Sm, anti-SSA/B, anti-RNP, and anti-dsDNA, were determined either by indirect immunofluorescence or by double immunodiffusion methods. Clinical manifestations, such as lupus nephritis, arthritis, neuropsychiatric disorder, hematologic disorder, malar rash, and complement, were also recorded for each patient (Table 1). The study was approved by the ethics committee of the Peking Union Medical College hospital and all subjects gave informed consent.

Table 1
Clinical symptoms of SLE[a] patients: stratified information

Characteristics	Positive (%)	Negative (%)
Nephritis	456 (61.0)	292 (39.0)
Anti-Sm antibodies	124 (16.6)	624 (83.4)
Anti-SSA antibodies	355 (47.5)	393 (52.5)
Anti-SSB antibodies	72 (9.6)	676 (90.4)
Anti-RNP antibodies	211 (28.2)	537 (71.8)
Anti-dsDNA antibodies	324 (43.3)	424 (56.7)
Low complement	414 (55.3)	334 (44.7)
Arthritis	465 (62.2)	283 (37.8)
Neuropsychiatric disorder	122 (16.3)	626 (83.7)
Hematologic disorder	324 (43.3)	424 (56.7)
Malar rash	372 (49.7)	376 (50.3)

[a]SLE, systemic lupus erythematosus.

Table 2
Primer sequences of all SNPs

SNPs	Primer[a]	Primers sequences (5′–3′)
rs7574865 (STAT4)	F	ACGTTGGATGAGTATGAAAAGTTGGTGAC
	R	ACGTTGGATGAATCCCCTGAAATTCCACTG
	E	GTTGGTGACCAAAATGT
rs7582694 (STAT4)	F	ACGTTGGATGGAACAAGCAAACATGCATAGG
	R	ACGTTGGATGGCCTTTACATTGTTCTACCC
	E	TGCATAGGTTGCATACT
rs4963128 (IRF7/ KIAA1542)	F	ACGTTGGATGGGTTTTGCAGGGGCTTAGAA
	R	ACGTTGGATGTGTGAGCAGCCCCCAGCTGA
	E	AGGGGCTTAGAAGAGGCC
rs2246614 (IRF7/ KIAA1542)	F	ACGTTGGATGACGCGCCACGGTGCCACG
	R	ACGTTGGATGAGGTCACCGTGGAGGCTGT
	E	CCACGGTGCCACGATACAG
rs702966 (IRF7/ KIAA1542)	F	ACGTTGGATGTGCACAGACCAGGTGTCAG
	R	ACGTTGGATGTGTCGGGAGTGGCGGGAAAT
	E	GGAACCCGACGGCAGGCATG

SNP, single-nucleotide polymorphism; STAT4, signal transducer and activator of transcription 4.
[a]F, forward primer; R, reverse primer; E, extend primer.

2.2. Genotyping

Each study participant provided a 2-mL peripheral blood sample collected in EDTA tubes. Genomic DNA was extracted using a Bioteke DNA isolation kit (Beijing, China), and SNP genotyping was performed at the Beijing Institute of Genomics, Chinese Academy of Sciences (Beijing, China), using the Sequenom MassArray system (Sequenom iPLEX assay, San Diego, CA). All procedures were carried out according to the manufacturer's instructions. Approximately 15 ng of genomic DNA was amplified by multiplex polymerase chain reaction and the products were then used in locus-specific single-base extension reactions. Then, the resulting products were desalted and transferred to a 384-element SpectroCHIP array. Allele detection was accomplished using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry, and the mass spectrograms were analyzed by the MassARRAY TYPER software (Sequenom). Primers were designed using the MassARRAY Design 3.0 software (Sequenom; Table 2).

2.3. Statistical analysis

The 5 SNPs were tested for Hardy–Weinberg equilibrium (HWE) in the patient and control populations, and any SNPs that deviated from HWE ($p < 0.05$ in the control group) were excluded from subsequent analyses. Genotype and allele frequencies of cases and controls were assessed by χ^2 test based on 2×3 and 2×2 contingency tables. Since the incidence of SLE is both gender and age-related, we took gender and age as covariates in our analysis. For genetic model testing (additive, dominant, and recessive models), genotype frequencies were analyzed using a logistic regression model that adjusted for gender and age. Subphenotype stratification analysis was performed using 3 comparisons: patients having a certain subphenotype with all controls, patients without the subphenotype with all controls, and patients with and without the subphenotype. All data were analyzed using the PLINK toolset [25]. The odds ratio (OR) and 95% confidence interval (95% CI) were calculated, and p values (corrected for multiple testing by Bonferroni adjustment) less than 0.05 were considered statistically significant. Linkage disequlibrium (LD) patterns in our analysis were generated using Haploview [26]. Genetic Power Calculator was applied to compute statistical power, assuming a genetic model as follows: the risk allele frequency is 30% (similar to the average minor allele frequency of the 3 SNPs genotyped in our study) and the prevalence of SLE in the Chinese population is 0.07% [27].