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A New Possible Single-Nucleotide Polymorphism Locus Associated with Systemic Sclerosis Susceptibility: A Genetic Association Study in a Chinese Han Population

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Abstract:	Objective The aim of this study was to confirm the association of RHOB and FAM167A-BLK gene polymorphisms with susceptibility to systemic sclerosis (SSc) in a Chinese Han population. Methods A total of 248 SSc patients and 251 healthy controls of Chinese Han ethnicity, who visited the department of dermatology of Peking Union Medical College Hospital, were included in the study. A few selected single nucleotide polymorphisms (SNPs) in the RHOB and FAM167A-BLK genes were selected as genetic markers and were genotyped using a MassARRAY system, which is based on the matrix-assisted laser desorption/ionization time of flight mass spectrometry technique. Results Three SNPs in the coding regions of the RHOB and FAM167A-BLK genes displayed an association with SSc: (1) rs1062292G, which is a newly discovered SNP in the RHOB gene (P = 0.02948, odds ratio [OR] = 0.618, 95% confidence interval (CI) = 0.400-0.956), (2) rs2736340C (P = 0.02583, OR = 0.722, 95%CI = 0.539-0.967), and (3) rs13277113G (P = 0.03980, OR = 1.335, 95%CI = 1.013-1.760), both in the FAM167A-BLK gene. Our results support previous findings that polymorphisms in the RHOB and FAM167A-BLK genes may be associated with SSc. However, the loci of the SNPs that displayed an association with SSc are quite different from the loci identified in studies of Caucasian populations. Conclusion This is the first large-scale genetic association study of polymorphisms in non-human leukocyte antigen genes associated with SSc in a Chinese Han population. Our results confirm that RHOB and FAM167A-BLK polymorphisms exist in Chinese Han SSc patients. Therefore, variants of the RHOB and FAM167A-BLK genes are promising genetic markers for SSc.
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Ethics Statement All research involving human participants must have been approved by the authors' institutional review board or equivalent committee(s) and that board must be named by the authors in the manuscript. For research involving human participants, informed consent must have been obtained (or the reason for lack of consent explained, e.g. the data were analyzed anonymously) and all clinical investigation must have been conducted according to the principles expressed in	The study was approved by the Ethics Committee of Peking Union Medical College Hospital. Both the patients and controls were included in the study after providing written informed consent. The DNA from patients and controls was obtained using standard methods.

the Declaration of Helsinki. Authors should submit a statement from their ethics committee or institutional review board indicating the approval of the research. We also encourage authors to submit a sample of a patient consent form and may require submission of completed forms on particular occasions. All animal work must have been conducted according to relevant national and international guidelines. In accordance with the recommendations of the Weatherall report, "The use of non-human primates in research" we specifically require authors to include details of animal welfare and steps taken to ameliorate suffering in all work involving non-human primates. The relevant guidelines followed and the committee that approved the study should be identified in the ethics statement. Please enter your ethics statement below and place the same text at the beginning of the Methods section of your manuscript (with the subheading Ethics Statement). Enter "N/A" if you do not require an ethics statement.	

A New Possible Single-Nucleotide Polymorphism Locus Associated with Systemic Sclerosis Susceptibility: A Genetic Association Study in a Chinese Han Population

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Abstract

Objective The aim of this study was to confirm the association of RHOB and FAM167A-BLK gene polymorphisms with susceptibility to systemic sclerosis (SSc) in a Chinese Han population.

Methods A total of 248 SSc patients and 251 healthy controls of Chinese Han ethnicity, who visited the department of dermatology of Peking Union Medical College Hospital, were included in the study. A few selected single nucleotide polymorphisms (SNPs) in the RHOB and FAM167A-BLK genes were selected as genetic markers and were genotyped using a MassARRAY system, which is based on the matrix-assisted laser desorption/ionization time of flight mass spectrometry technique.

Results Three SNPs in the coding regions of the RHOB and FAM167A-BLK genes displayed an association with SSc: (1) rs1062292G, which is a newly discovered SNP in the RHOB gene (P = 0.02948, odds ratio [OR] = 0.618, 95% confidence interval (CI) = 0.400–0.956), (2) rs2736340C (P = 0.02583, OR = 0.722, 95%CI = 0.539–0.967), and (3) rs13277113G (P = 0.03980, OR = 1.335, 95%CI = 1.013–1.760), both in the FAM167A-BLK gene. Our results support previous findings that polymorphisms in the RHOB and FAM167A-BLK genes may be associated with SSc. However, the loci of the SNPs that displayed an association with SSc are quite different from the loci identified in studies of Caucasian populations.

Conclusion This is the first large-scale genetic association study of polymorphisms in non-human leukocyte antigen genes associated with SSc in a Chinese Han population.

Our results confirm that RHOB and FAM167A-BLK polymorphisms exist in Chinese Han SSc patients. Therefore, variants of the RHOB and FAM167A-BLK genes are promising genetic markers for SSc.

Keywords: systemic sclerosis, Chinese Han population, single nucleotide polymorphism

Introduction

Scleroderma or systemic sclerosis (SSc) is a chronic, connective tissue disease characterized by widespread fibrosis of the skin and internal organs, small-vessel vasculopathy, and immune dysregulation with or without production of autoantibodies. SSc patients have markedly lower survival rates than that of the age- and sex-matched general population. In a recently published meta-analysis, the overall pooled standardized mortality ratio of patients with SSc was 3.53[1]. During the past few years, knowledge of the genetic basis of SSc has increased rapidly because of large and well-powered candidate gene association studies as well as genome-wide association studies (GWASs)[2]. Currently, it is widely accepted that different genetic factors contribute to the development and prognosis of SSc. Further, GWASs have been a useful tool for studying the genetic basis of autoimmune and other complex diseases. Radstake *et al.*[3] performed the first SSc GWAS in a Caucasian population, which also represented the first large-scale GWAS in an SSc cohort. In a recent GWAS in a French Caucasian SSc discovery cohort[4] 17 single-nucleotide polymorphisms (SNPs) displaying tier two associations were selected for follow-up in independent cohorts. Three of the selected SNPs were located within the human leukocyte antigen (HLA) region corresponding to the HLA-DQB1 and PSORS1C1 genes. The remaining SNPs were located in six independent non-HLA loci. After the replication step, six SNPs located in three loci (TNIP1, RHOB, and PSORS1C1) were proposed as novel SSc risk factors. However, later, in a large independent replication study by a Spanish group, TNIP1, but not RHOB and PSORS1C1, was confirmed to

be associated with SSc.

The associations identified in a single GWAS, despite crossing established statistical significance thresholds, tend to display inflated effect sizes. This effect size is called the winner's curse, and it affects the predictive ability of the discovered associations and the estimation of the risk variance based on the associations. Thus, it is essential to replicate these studies in independent comparable populations for firmly establishing a genotype-phenotype association. On the other hand, China has a large SSc patient population, and genotyping data is not available for this population. Peking Union Medical College Hospital (PUMCH) is believed to have the largest SSc patient group in China, and a large portion of this group regularly visits the dermatology department. We collected SSc data for more than three years, including data on two major subgroups: lcSSc and dcSSc, the latter of which has the worse prognosis[5]. Therefore, we performed a replication study in a previously unexamined SSc population to confirm the results of previous GWAS and candidate gene association studies.

Patients and methods

Subjects

This study was approved by the Ethics Committee of Peking Union Medical College Hospital. Both patients and controls were included in the study after providing written informed consent. DNA was obtained from patients and controls using standard methods.

We performed a case-control association study in 248 SSc patients from the outpatient dermatology and rheumatology departments at the PUMCH. Two hundred fifty-two controls were recruited from the physical examination center of PUMCH. All subjects were of Chinese Han ethnicity and from the mainland of China. All the patients fulfilled the 1980 American College of Rheumatology classification criteria for SSc. The control population consisted of unrelated healthy individuals who were from the same geographical regions as the SSc patients and who were matched by age and sex with the SSc patients.

Genotyping

The rs1062292, rs13021401, rs342054, and rs342070 alleles were selected as genetic markers of RHOB. rs342070 and rs13021401 were previously identified in a GWAS, and the other two were selected via tagger SNP analysis implemented in HaploView 4.2 [6] at an r^2 threshold of 0.80 and an LOD threshold for multi-marker tests of 3.0. rs2736340 and rs13277113 in FAM167A-BLK have been previously reported to be associated with SSc in a Japanese population; therefore, we decided to confirm whether the same association exists in a Chinese population.

Genomic DNA was extracted from the peripheral blood of each subject. The six SNPs were genotyped using a matrix-assisted laser desorption/ionization time of flight mass spectrometry genotyping assay using the MassARRAY platform from Sequenome following the manufacturer's suggestions (Foster City, CA, USA). The genotyping call rate was larger than 95% for all six SNPs.

Statistical analysis

SHEsis [7] was used for the individual population association tests (significance was calculated using 2×2 contingency tables and Fisher's exact test or a χ^2-test when necessary).SHEsis is a web-based platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphic loci designed by YongYong Shi from Shanghai Jiao Tong University. The software calculations are based on an expectation-maximization algorithm[8]. The odds ratios (OR) and their 95% confidence intervals (CI) are also reported by the software. All cohorts were in Hardy–Weinberg equilibrium (HWE) at a significance level of 0.05 for all the included SNPs. Power was calculated using the software Power Calculator for Genetic Studies 2006 and assuming an additive model at the 5% significance level.

Results

Frequencies of Alleles and Genotypes in Patients and Controls

The genotype distribution of the six SNPs was in HWE in the controls ($P > 0.05$). The results of the association are shown in Table 2. As the data indicate, the rs1062292G allele showed a lower prevalence in SSc patients (8.1%) than in controls (12.4%), thereby indicating a statistically significant association between the rs1062292G allele and SSc risk ($P = 0.02948$, OR = 0.618, 95%CI = 0.400–0.956) in the $\chi 2$ test performed on SHEsis. The rs2736340C allele was associated with lower risk of SSc in patients (25.1%) than in controls (31.7%, $P = 0.02583$, OR = 0.722, 95%CI = 0.539–0.967). The rs13277113G allele was associated with higher risk of SSc in patients (27.4%) than in controls (33.5%, $P = 0.03980$, OR = 1.335, 95%CI =

1.013–1.760). No significant differences were observed between the SSc patients and controls for the other SNPs and genotypes.

Discussion

Allanore *et al.* identified RHOB to be associated with SSc susceptibility in a GWAS aimed at identifying loci associated with SSc risk. [4] We confirmed the association of RHOB with SSc, but the loci identified are slightly different from the ones identified by Allanore *et al*. However, in a replication study of the same GWAS in Caucasians [9], association between RHOB and SSc was not observed. This interesting contradiction may be explained by the genetic heterogeneity among human races. rs1062292, which displayed an association with SSc in our study, is an SNP located in the 3'-untranslated region(3'-UTR) of RHOB. This region is commonly involved in post-transcriptional regulation. The exact roles that this SNP plays require further exploration. The RHOB gene mediates apoptosis in neoplastically transformed cells after DNA damage[10]. RHOB is not essential for development but affects cell adhesion and growth factor signaling in transformed cells[11].This gene is also required for stability and nuclear trafficking of AKT1/AKT, which promote endothelial cell survival during vascular development and may play a role in SSc pathogenesis[12]. The SNP identified in this study has not been reported previously. It appears that the polymorphism at this locus is distinctive in Chinese SSc patients.

FAM167A (previously referred to as C8orf13)-BLK is a region in chromosome 8. BLK encodes a nonreceptor tyrosine kinase of the src family of proto-oncogenes,

which are typically involved in cell proliferation and differentiation. The protein plays a role in B-cell receptor signaling and B-cell development[13].

The association of SNPs in the FAM167A-BLK region with systemic lupus erythematosus (SLE) has been demonstrated in Caucasians and Asians. The SNP in this region was first found to be associated with SLE through a GWAS. Previous findings indicate that the rs2736340C and rs13277113A alleles are associated not only with SLE but also with SSc in European and Japanese populations[13,14] and that the FAM167A-BLK region is a common genetic risk factor for both SLE and SSc. The association of these two SNPs with SSc was also confirmed in our study. In addition, polymorphisms in this region has been determined to be associated with other immune diseases such as Kawasaki disease and Sjögren's syndrome[15,16]. These results suggest that the B-cell receptor signaling pathway may play an important role in the pathogenesis of multiple immune diseases.

Conclusion

This is the first study to determine an association between non-HLA genes and SSc in a large Chinese population. Our results confirm that RHOB and FAM167A-BLK polymorphisms exist in Chinese Han SSc patients, which indicates that RHOB and FAM167A-BLK may be associated with susceptibility to SSc. However, the SNPs that displayed an association with SSc are quite different from those in Caucasian populations. Moreover, we observed a new SNP, rs1062292G, which was significantly associated with the risk of SSc in Chinese Han populations. Thus, RHOB

and FAM167A-BLK are promising genetic markers of SSc.

Conflict of interest

The authors have declared that no competing interests exist.

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234 **Table 1.**

Features	SSc patients	Healthy controls
Patients	248	252
Average age (year)	42.7	42.7
Age range (year)	14-81	20-77
Male (%)	11.3	11.1

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Table 2. Frequencies of Alleles in Patients and Controls

Gene region	SNP	Minor Allele	Cases n = 238	Controls n = 245	*P* value	OR (95%CI)
RHOB	rs1062292	G	37(0.081)	57(0.124)	**0.02948**	**0.618286 (0.399871–0.956000)**
RHOB	rs13021401	C	162(0.352)	174(0.378)	0.4113	
RHOB	rs342054	T	23(0.049)	25(0.052)	0.8601	
RHOB	rs342070	A	178(0.376)	187(0.383)	0.8064	
FAM167A-BLK	rs2736340	C	111(0.251)	144(0.317)	**0.02853**	**0.721928 (0.539094–0.966771)**
FAM167A-BLK	rs13277113	G	130(0.274)	163(0.335)	**0.03980**	**1.335366 (1.013286–1.759821)**