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The Open Rheumatology Journal, **2014**, *8*, 43-45 **43**

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Lack of Association of the CD247 SNP rs2056626 with Systemic Sclerosis in Han Chinese

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Abstract: Systemic sclerosis (SSc) is a complex disease involving multiple genetic factors. A recent genome-wide association study (GWAS) indicated that *CD247* was strongly associated with SSc, which was subsequently confirmed in a SSc cohort of European population. However, genetic heterogeneity in different ethnic populations may significantly impact the complex trait of SSc. The studies herein aimed to examine whether the SSc-associated SNP rs2056626 of *CD247* identified in Caucasian is also associated with Han Chinese SSc. A Han Chinese cohort consisting of 387 SSc patients and 523 healthy controls were examined in the studies. TaqMan assays were performed to examine the SNP. Exact *p*-values were obtained (Fisher's test) from 2x2 tables of allele counts and disease status. The results showed that there was no association between rs2056626 of *CD247* and SSc or any SSc subtypes of Han Chinese. The negative results are important in understanding genetics of SSc in different ethnic populations, which further suggest complex nature of genetics of SSc.

Keywords: CD247, Chinese population, genetics, polymorphism/SNP, scleroderma systemic sclerosis/SSc.

INTRODUCTION

T-cell surface glycoprotein CD3 zeta chain (CD247), a component of T cell receptor (TCR)/CD3 complex, plays an important role in assembly and transport of the TCR/CD3 complex to the cell surface and in receptor signaling function [1,2]. Somatic CD3-zeta mutations have been shown to impair immune function [3]. Recently, an intronic single nucleotide polymorphism (SNP) rs2056626 of the CD247gene was reported to be associated with systemic sclerosis (SSc) in a genome-wide association study (GWAS) of European and US Caucasians [4]. An independent study with a French Caucasian cohort (1031 patients/1014 controls) confirmed the association between rs2056626 and SSc, and further indicated that the rs2056626G minor allele was associated in a dominant pattern with a protective effect to SSc [5]. However, genetic heterogeneity in different ethnic populations may significantly impact the complex trait of SSc and SSc clinical features. Chinese SSc patients have some unique serological and clinical features with high

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1874-3129/14 2014 Bentham Open

frequency of ATA and pulmonary fibrosis [6]. Association between *CD247* and SSc has not been reported in Chinese SSc. Recently, we established a SSc cohort of Han Chinese through multicenter SSc consortium in China under the International Network of Scleroderma Clinical Care and Research (InSCAR) (http://www.inscar-global.org). This cohort has been extensively examined in multiple genetic association studies of SSc [7-10]. We undertook the current study to examine whether the genotype of the *CD247* rs2056626 confer susceptibility to SSc and clinical features of SSc in Han Chinese population.

MATERIALS AND METHODS

Study Subjects

A case-control study including 387 SSc patients and 523 healthy controls of Han Chinese was performed. SSc patients were recruited from a multicenter study including hospitals and outpatient clinics in Shanghai, Hebei province, Sichuan province, and Hunan province in China [6-9]. All patients met the American College of Rheumatology (ACR) classification criteria for SSc [10]. None of the controls had autoimmune diseases. The studies were approved by the institutional review board, and written informed consents were obtained from all subjects.

Tests for Autoantibodies and Pulmonary Fibrosis

Patient's sera were tested for antinuclear antibodies (ANA) by indirect immunofluorescence using HEp-2 cells as antigen substrate (Antibodies, Davis, CA). Anti-topoisomerase I (ATA) was detected by passive immunodiffusion against calf thymus extracts (INOVA, Diagnostics). Anti-centromere autoantibody (ACA) was determined by indirect immunofluorescence using HEp-2 cells. Diagnosis of pulmonary fibrosis was confirmed with either chest X-ray (41.9 %) or thorax CT (58.1 %) in different hospitals.

Genotyping Assays

The SNP genotyping for rs2056626 of *CD247* was performed with TaqMan assays as we described previously [9]. A standard control DNA (Life Technology, Forster city, CA, USA) was used for quality control. The probe was purchased from the pre-designed SNP Assays of Life Technologies. The SDS2.4 was used for reading genotyping (Life Technologies).

Statistical Analysis

Exact *p*-values were obtained (Fisher's test) from 2x2 tables of allele counts and disease status. The disease status includes SSc in general, SSc subtypes, and SSc with pulmonary fibrosis (PF). SSc subtypes include diffuse cutaneous SSc (dcSSc) and limited cutaneous SSc (lcSSc) of clinical subsets, and SSc patients with autoantibodies to DNA topoisomerase I (ATA) or autoantibodies to centromere protein (ACA). The *p* values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

SSc patients of the Han Chinese cohort were 93% positive for ANA. There were 137 limited cutaneous SSc (lcSSc) (42.4%) and 186 diffused cutaneous SSc (dcSSc) (57.6%), other were undefined. There were 349 patients examined for ATA with 167 positive (47.9%), 321 were examined for ACA with 45 positive (14.0%). In addition, there were 287 patients examined with chest X-ray and/or CT for diagnosis of pulmonary fibrosis (PF), and 210 were positive (73.2%). The high rate of PF correlates with the incidence of dcSS, and which was consistent with SSc features in Han Chinese [6].

In contrast to the previous reports in other ethnic populations, SSc patients of Han Chinese showed no association between rs2056626 and SSc or any subtypes of SSc including lcSSc, dcSSc, ATA, ACA and PF (Table **1**). Each genotype of the rs2056626 showed similar frequency between cases and controls. The rs2056626G minor allele did not show protective effect from SSc in this cohort that was reported in the French SSc studies [5]. It is worth noting that this Han Chinese cohort has been examined in multiple genetic studies of SSc [7-9]. In the studies of *STAT4*, another SSc-associated gene, the corresponding polymorphism was found to be strongly associated with Chinese SSc in this

Table 1. Association studies of the rs2056626 of the CD247 gene with SSc of Han Chinese population.

Number (%)				Allelic Association			
Genotype	**GG**	**GT**	**TT**	**Total**	**G Allele**	***p*-Value**	**OR (95% CI)**
control	7 (1.3)	119 (22.8)	397 (75.9)	523	133 (12.7)	-	-
SSc	10 (2.7)	82 (22.5)	273 (74.8)	365	102 (14.0)	0.442	1.12 (0.85-1.47)
dcSSc	4 (2.3)	36 (20.6)	135 (77.1)	175	44 (12.6)	0.944	0.99 (0.69-1.42)
lcSSc	4 (3.1)	30 (23.1)	96 (73.8)	130	38 (14.6)	0.417	1.18 (0.80-1.73)
ATA+	4 (2.5)	37 (23.6)	116 (73.9)	157	45 (14.3)	0.457	1.15 (0.80-1.65)
ACA+	1 (2.3)	7 (16.3)	35 (81.4)	43	9 (10.5)	0.546	0.80 (0.39-1.64)
PF	6 (3.0)	40 (20.2)	152 (76.8)	198	52 (13.1)	0.833	1.04 (0.74-1.46)

ATA = anti-topoisomerase I autoantibodies; ACA = anti-centromere autoantibodies; PF = pulmonary fibrosis; *p* = *p* value; OR = odds ratio; CI = confidence interval.

cohort [9], which was consistent with the reports in Caucasian population [4, 11, 12]. In the studies of *HLA-DPB1* and *–DQB1*, the specific HLA alleles in this Chinese cohort were associated with SSc, and that was also consistent with Caucasian population [13]. For instance, *HLA-DPB1**13:01 was strongly associated with ATA positive SSc [7,13], and *HLA-DQB1**05:01 with ACA positive SSc in both Han Chinese and US Caucasian cohorts [8,13]. On the other hand, *DQB1**06:01 appeared more common in ATA positive Chinese SSc, which was not reported in US SSc, but was consistent with a report of Japanese SSc cohort [14]. Moreover, two previously reported SSc-protective alleles *DQB1**02:02 and *06:02 in US Caucasian [13] did not show association with Han Chinese SSc, but which appeared in consistent with SSc of US Hispanics and Africa Americans [13]. Therefore, one possible explanation of the discrepancies may be genetic heterogeneity between Han Chinese and other ethnic populations, especially Caucasian population, which may significantly impact the complex trait of SSc.

This is the first report of studying *CD247* in Han Chinese SSc, and the first time to demonstrate a discrepancy in genetic association between the SNP of *CD247* and SSc. It revealed different genetic aspects of SSc, and suggested that previously reported association of the *CD247* polymorphism may be ethnic specific, and further verification in different ethnic populations may be necessary.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

The studies were supported by research grants from the US NIH NIAID UO1, 1U01AI09090 and the Science and Technology Committee of Shanghai Municipality (114107 01800, 11DJ1400102), International S&T Cooperation Program of China (2013DFA30870), the National Basic Research Program (2012CB944600), Ministry of Science and Technology (2011BAI09B00), Ministry of Health (201002007). Key projects of Shanghai Municipal Health Bureau (2011027), The National Natural Science Funds (81273979), National Science Foundation of China (NSFC): Oversea Collaboration Project 81328001.

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Received: July 14, 2014 Revised: September 5, 2014 Accepted: September 11, 2014