



# Lack of association between rare mutations of the *SIAE* gene and rheumatoid arthritis in a Han Chinese population

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**ABSTRACT.** The function of rare genotypes encoding defective variants of sialic acid acetyltransferase (*SIAE*) in some autoimmune diseases, including rheumatoid arthritis (RA), is ambiguous. We determined whether mutations in the *SIAE* gene are responsible for RA in a Han Chinese population. DNA was prepared from the venous leukocytes of 444 RA patients and 647 normal controls. The coding regions and adjacent intron sequences of *SIAE* were amplified by polymerase chain reaction. The products were

then subjected to sequencing analysis. The detected variations were further evaluated in the normal controls and available family members by sequencing. Seven variants of RA were identified in this study, including four known single nucleotide polymorphisms SNPs (rs7941327, rs7941523, rs1942663, and rs12282107) and three novel SNPs. The genomic positions of the three novel SNPs are chr11:124013712, chr11:124023268, and chr11:124044505. No significant differences in the seven SNPs of *SIAE* were observed between patients with RA and controls in this cohort ( $P > 0.05$ ). Three novel variations and four known SNPs in *SIAE* were detected in the Chinese RA patients and normal controls. Our results imply that *SIAE* does not play a major role in RA in this population.

**Key words:** Rheumatoid arthritis; Sialic acid acetyltransferase; Sequencing; Single nucleotide polymorphism; Han Chinese population

## INTRODUCTION

Rheumatoid arthritis (RA) is considered a systemic autoimmune disease. It causes chronic inflammation of the joints and may affect many different tissues and organs. The prevalence of RA is roughly 0.2-0.3% in China and nearly 0.8% in the Western world (Silman and Hochberg, 2001). Although the cause of RA has not been fully elucidated, it is believed that the combined effect of genetic and environmental factors, as well as exposure to certain infections, may cause the errant activation of the immune system in susceptible individuals. Several studies have shown that a group of genes are implicated in susceptibility to RA and its severity, including class II major histocompatibility complex genes, *PTPN22* (protein tyrosine phosphatase, non-receptor type 22), and *PADI4* (peptidyl arginine deiminase) (Chung and Criswell, 2007; Lee et al., 2007; Taneja and David, 2010).

The sialic acid acetyltransferase (*SIAE*) gene encodes an enzyme that removes 9-O-acetylation modifications from sialic acid. It negatively regulates B lymphocyte antigen receptor signaling and is required for the maintenance of immunological tolerance in mice. Compelling evidence suggests that *SIAE* may play a critical role in the pathogenesis of autoimmune diseases (Cariappa et al., 2009; Pillai et al., 2009).

Recently, studies on *SIAE* in European populations have attracted particular interest owing to the identification of rare loss-of-function genetic variants in the *SIAE* gene that appear to predispose individuals to multiple autoimmune diseases. Surolia et al. (2010) identified distinct nonsynonymous *SIAE* risk variant genotypes of autoimmune diseases in individuals of European origin. Hirschfield et al. (2012) revealed the potential role of functionally defective *SIAE* variants in the risk of primary biliary cirrhosis (PBC). These studies showed that defective *SIAE* variants have a strong effect on susceptibility to autoimmunity. Furthermore, studies by other investigators have confirmed the role of *SLAE* mutation in the development of other chronic diseases such as diabetes. Szymański et al. (2011) tested the homozygous Met89Val substitution of *SIAE* in patients with Graves' disease (GD) and type 1 diabetes mellitus (T1DM) patients in a Polish population. Hunt et al. (2011) replicated and extended the *SIAE* findings of Surolia et al. (2010) in a much larger independent study of autoimmune and chronic immune diseases. However, Hunt et al.'s results illustrated that rare and functional *SIAE* variants are not associated with autoimmune disease risk. In the present study, we sought to determine whether the variations of *SIAE* are associated with RA in a Han Chinese population.

## MATERIAL AND METHODS

### Study population

In total, 444 patients with RA and 647 normal age-matched controls were recruited. All the patients enrolled in this study had received a clinical diagnosis of RA at least two years before, and the mean duration of the disease was five years. The normal age-matched controls and the RA patients, including individuals who had normal joints upon examination, were recruited at the Rheumatology and Immunology Clinic of the Sichuan Provincial People's Hospital and North Sichuan Medical College, China. All the participants underwent a standard examination protocol according to the guidelines of the American Rheumatism Association, as noted previously: (<http://medcalc3000.com/RheumatoidArthritis.htm>). In the normal controls, no sign of joint or other autoimmune diseases were detected. Clinical information about the cases and controls is provided in Table 1. All the participants signed an informed consent form.

**Table 1.** Characteristics of rheumatoid arthritis (RA) cases and controls matched for age and ethnicity.

Subject	Total number	anti-CCP-positive	RF-positive	Male	Female	Average age
Cases	444	297	411	176	268	62 ± 6.3
Controls	647	0	0	312	335	60 ± 5.8

CCP = cyclic citrullinated peptide; RF = rheumatoid factor; ± represents standard deviation.

### Mutation analysis

Genomic DNA was prepared from peripheral leukocytes as described previously (Hunt et al., 2011). Table 2 lists the primers used to amplify the coding exons and adjacent introns of *SIAE* (NCBI human genome build 36.3, NC\_000011.9 for genomic DNA, NM\_032811.2 for mRNA, and NP\_001186851.1 for proteins). The nucleotide sequences of the *SIAE* exons were determined using an Applied Biosystems 3710 DNA Analyzer (Carlsbad, CA, USA). The sequencing results from the cases and the controls, as well as the *SIAE* consensus sequences, were compared to identify variations. The variations were confirmed by bidirectional sequencing.

### Statistical analysis

The  $\chi^2$  test was used to analyze the Hardy-Weinberg equilibrium (HWE) of the SNPs. Allele and genotype frequencies between the cases and the controls were compared by  $\chi^2$  analysis. Statistical significance was defined as  $P < 0.05$ . All statistical analyses were performed using SPSS version 10.0 (SPSS Inc., Chicago, IL, USA).

## RESULTS

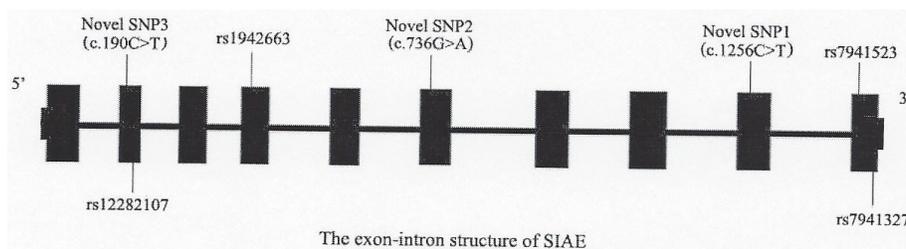
Among the 444 patients with RA, 297 were anti-cyclic citrullinated peptide (CCP) antibody-positive, while 411 were rheumatoid factor (RF)-positive. The frequency of females was 268/444 (60.4%). The patients diagnosed with RA had an average age of 62 years (range 55.7-68.3 years), and the average age of the normal controls was 60 years (range 54.2-65.8 years) (Table 1).

**Table 2.** Primers used for the amplification and sequencing of sialic acid acetyltransferase gene (*SIAE*).

Exon number	Direction	Primer sequence	Size of product (bp)	Annealing temperature (°C)
1	F	5'-AAGACCCCTTCTGACCTAA-3'	351	52
	R	5'-TAAATCTGAAGACCCA-3'		
2	F	5'-AGCTGACTAGCCACTGATCACAGA-3'	460	58
	R	5'-GCTCAGCCCCCAACCAACCA-3'		
3	F	5'-CCAGTAAAAAGACTCCAGTGTGGG-3'	254	58
	R	5'-TGCAGCACACCCAGCATAACG-3'		
4	F	5'-TTGCTGATGCCCACTGGCTG-3'	374	60
	R	5'-GCACATCCCCACCAATGCC-3'		
5	F	5'-CATAGAAAGCTGACCGATAC-3'	471	55
	R	5'-ACTACCCTGCTCACCCAC-3'		
6	F	5'-CCCCACAGAGGACGATGACCCT-3'	338	60
	R	5'-CCCCACAGAGGACGATGACCCT-3'		
7	F	5'-CAGTGACAGCCATTGGTTTTGGT-3'	337	58
	R	5'-TCCACTTAGGGCGTAACCCA-3'		
8	F	5'-ACAGAGGCTGGGAATAGCCACA-3'	361	58
	R	5'-GCCCTGCTTCTCCTTGTGCC-3'		
9	F	5'-CTCTCGATGATGCTTCCCTCAGT-3'	234	58
	R	5'-AGCAGGTGGCAGAAGAGGGCAA-3'		
10	F	5'-CCCTGCCGGGAACCCACTTTC-3'	179	60
	R	5'-GGTCTCGAGCCCGCACAGG-3'		

F = forward; R = reverse.

Complete sequencing of the coding regions and the adjacent intron regions of *SIAE* in 1091 DNA samples identified three novel mutations and four known SNPs (Figure 1). The results of this analysis revealed two synonymous variations (c.1452C>T and c.736G>A) and five missense variations (Table 3). Among the five missense variations, the c.1400G>A mutation (p.A467V), a known polymorphism, was detected in 11 of the 444 patients with RA (10 heterozygous and 1 homozygous), as well as in 12 of the 647 controls (12 heterozygous). The c.1316C>T mutation, a novel heterozygous variation (p.G419E) named novel SNP1, was detected in 9 of the 444 patients with RA (9 heterozygous), as well as in 23 of the 647 controls (1 heterozygous and 24 homozygous). The c.1256G>A mutation, another novel heterozygous variation (p.D246N) named novel SNP2, was detected in 15 of the 444 patients with RA (1 heterozygous and 14 homozygous), as well as in 15 of the 647 controls (15 heterozygous). The c.212T>C mutation (p.K71A), a known polymorphism, was detected in 86 of the 444 patients with RA (78 heterozygous and 8 homozygous), as well as in 148 of the 647 controls (138 heterozygous and 10 homozygous). The c.190C>T mutation (p.G64S), a novel polymorphism named novel SNP3, was detected in 93 of the 444 patients with RA (89 heterozygous and 4 homozygous) as well as in 119 of the 647 controls (112 heterozygous and 7 homozygous).



**Figure 1.** Schematic diagram of the exon-intron structures and variants identified in the study of the sialic acid acetyltransferase gene (*SIAE*). Black boxes represent exons. The location and ID of single-nucleotide polymorphisms (SNPs) genotyped in the *SIAE* panel are shown.

**Table 3.** Sialic acid acetyltransferase gene (*SIAE*) variants detected in rheumatoid arthritis patients and healthy controls in a Han Chinese population by direct sequencing of all the exons.

SNP ID	Exon number	Genomic position	Allele*	Residue change	Genotype counts†		P value	OR (95%CI)
					Cases	Controls		
rs7941327	1	chr11:124012177	T/C	T484T	9/90/345	10/131/506	0.731	1.05 (0.81-1.36)
rs7941523	1	chr11:124012229	A/G	A467V	1/10/433	0/12/635	0.368	1.46 (0.65-3.27)
Novel SNP1	2	chr11:124013712	T/C	G419E	0/9/434	1/24/622	0.076	0.50 (0.23-1.07)
Novel SNP2	5	chr11:124023268	A/G	D246N	1/14/430	0/15/632	0.227	1.56 (0.77-3.17)
rs1942663	7	chr11:124029837	G/A	S156S	5/82/357	8/127/512	0.610	0.19 (0.12-0.30)
rs12282107	9	chr11:124035880	C/T	D87Y	8/78/358	10/138/499	0.251	0.17 (0.10-0.27)
Novel SNP3	9	chr11:124044505	T/C	G64S	4/89/351	7/112/528	0.378	0.21 (0.13-0.35)

SNP = single-nucleotide polymorphism. \*Minor allele/major allele. †The genotype counts are presented as homozygote/heterozygote/wild type.

There was no significant association ( $P > 0.05$ ) between the RA patients and the normal controls in the Chinese population with any of the *SIAE* variations.

## DISCUSSION

*SIAE* plays an important role in the maintenance of B-cell tolerance. It influences B-cell signaling and development, and participates in inhibitory signaling mechanisms (Pillai, 2013; Yamamoto et al., 2014). *SIAE* represses B-cell receptor (BCR) signaling via the *SIAE*-Siglec-Lyn-SHP-1 pathway. Siglec-2 is a member of the Siglec family that binds to N-glycans containing sialic acid via an  $\alpha$ -2,6 linkage to an N-acetyllactosamine moiety of the BCR (Siglec is an abbreviation for sialic acid-binding immunoglobulin-type lectin). Siglec-2 is rapidly phosphorylated on its cytoplasmic inhibitory tyrosines by Lyn, an Src family tyrosine kinase. Following the phosphorylation of Siglec-2, Src homology region 2 domain-containing phosphatase-1 (SHP-1) is recruited to the cytoplasmic tail of CD22, thereby inhibiting BCR signaling. *SIAE* can also remove O-acetyl moieties from 9-O-acetylated sialic acid (Stoddart et al., 1996; Takematsu et al., 1999). Thus, this pathway impairs BCR signaling. However, mutations in *SIAE* presumably lead to autoimmunity by enhancing the signal strength of BCR.

Recently, Surolija et al. (2010) investigated *SIAE* risk variant genotypes in 923 European patients with 10 common autoimmune diseases and 648 controls. All the heterozygous loss-of-function *SIAE* mutations showed a dominant negative function; whereas a homozygous secretion-defective polymorphic variant of *SIAE* was catalytically active and lacked the dominant negative function. This was seen in eight autoimmune subjects but not in any of the control subjects. Therefore, the researchers concluded that rare and polymorphic variants of *SIAE* give rise to defects in function, which points to an association between *SIAE* and autoimmune disorders. Hirschfield et al. (2012) sequenced the *SIAE* gene in 1450 PBC cases and 2957 healthy controls, and identified six cases with functional nonsynonymous *SIAE* mutations and functional *SIAE* variants that were associated with PBC risk.

However, there is still some controversy regarding the association of *SIAE* with autoimmune diseases. A study by Szymański et al. (2011) comprising 561 GD patients, 379 T1DM patients, and 1822 controls found that the prevalence of Met89Val homozygosity was similar among patients and controls, yielding ORs of 0.93 and 0.69 for GD and T1DM, respectively. This study highlighted the fact that the strong effect of defective *SIAE* variants on susceptibility to autoimmunity needs to be investigated further. Moreover, Hunt et al. (2011) tested for the presence of the *SIAE* variant

in 66,924 samples from five ethnic groups with autoimmune diseases and found homozygotes of the variant in all cases and controls. The variant encoding *SIAE* p.Met89Val was genotyped in 4805 offspring-parent trios of European origin in five autoimmune diseases. The study found no evidence indicating an effect of the p.Met89Val alteration on autoimmune disease risk. An analysis of eight additional rare *SIAE* variants in 43,378 subjects identified no functional *SIAE* variants as potential factors for autoimmune disease risk (Hunt et al., 2011). Finally, Gan et al. (2012) analyzed nine *SIAE* gene variants in a UK cohort of 378 subjects with autoimmune Addison's disease (AAD) and 387 healthy controls. Their analysis did not support a role for rare variants in *SIAE* in the pathogenesis of AAD. Thus, further studies are needed to validate the relationship between the *SIAE* gene and autoimmune diseases.

In this study, we resequenced the 10 exons of *SIAE* in the 444 RA patients and the 647 normal controls of Han origin. We then attempted to identify novel, rare, and functional mutations in *SIAE* and investigate the association between these SNPs in *SIAE* and RA. We detected seven SNPs, including three novel SNPs and four known SNPs. Among them, two variations cause synonymous-coding, while five were missense. None of the seven SNPs showed a significant association with RA ( $P > 0.05$ ) (Table 3). Consistent with the results obtained in Dutch populations (Hunt et al., 2011), our study did not identify *SIAE* as a significant risk factor for RA in a Han Chinese population. This suggests that possible heterogeneity among different ethnicities or genetic variants in the *SIAE* gene is not associated with RA.

In summary, we established a replication cohort of 444 RA patients and 647 controls to determine the potential presence of *SIAE* variants in a Han Chinese population. Our results suggest that *SIAE* is unlikely to play a major role in RA. Additional studies will be necessary to clarify these results.

## Conflicts of interest

The authors declare no conflict of interest.

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