Polymorphisms of *STAT4* and the risk of inflammatory bowel disease: A case-control study in Chinese Han population

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Abstract. Signal transducer and activator of transcription 4 (STAT4) is a transcription factor involved in the signaling pathways of several cytokines, playing an essential role in the development of inflammation in various immune-mediated diseases. Genetic association studies have shown that the *STAT4* gene was significantly associated with inflammatory bowel disease (IBD) in Spanish and Caucasian populations. However, these associations in other ethnic populations remain unknown. In the present study, we evaluated the role of the *STAT4* rs7574865 and rs7582694 polymorphisms on IBD in 562 unrelated Chinese Han subjects by assessing distributions of genotypes and allele frequencies. Results showed that neither rs7574865 [Crohn's disease (CD): P=0.66, odds ratio (OR) = 0.95, 95% confidence interval (CI) 0.74-1.21; ulcerative colitis (UC): P=0.43, OR=0.85, 95% CI 0.56-1.28; IBD: P=0.52, OR=0.93, 95% CI 0.73-1.17] nor rs7582694 (CD: P=0.40, OR=1.12, 95% CI 0.86-1.44; UC: P=0.50, OR=0.86, 95% CI 0.56-1.33; IBD: P=0.62, OR=1.06, 95% CI 0.83-1.36) was significantly associated with IBD, although the genotype frequency of rs7574865 varied in patients and the controls. In conclusion, our data did not support that *STAT4* variants contribute to IBD susceptibility in the Chinese Han population.

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Introduction

Inflammatory bowel disease (IBD) is a series of inflammatory conditions of the colon and small intestine comprising Crohn's disease (CD) and ulcerative colitis (UC) (1-4). IBD, with a complex pathogenesis influenced by a combination of genetic risk factors and environmental events, is highly heritable, thus investigations into the genetic susceptibility of this disease are ongoing (5,6). Numerous loci, such as NOD2, IL23R, ATG16L1 and IRGM, are associated with IBD susceptibility (7-14). However, the genetic polymorphisms located within these genes are not sufficient to adequately explain the pathogenesis and development of IBD and its various phenotypes.

Signal transducer and activator of transcription 4 (STAT4), a transcription factor whose gene is located on chromosome 2q33, is important in the development of inflammation of various immune-mediated diseases (15). *STAT4* variation has been reported to be significantly associated with rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) (16). The association of *STAT4* haplotype characterized by the rs7574865 polymorphism with IBD was originally reported by Martinez *et al* (17) in a Spanish population, a study that was replicated by Diaz-Gallo *et al* (18). Glas *et al* (19) reported that rs7574865 polymorphism was associated with colonic CD and early disease onset in Caucasians.

These associations need to be confirmed by replication studies, particularly in other ethnic populations. Additionally, the differences in risk allele frequencies and linkage disequilibrium structure in ethnicities may provide insights to refine the association signal and identify the true risk variant. Therefore, the aim of the present study was to evaluate whether the previously identified *STAT4* single-nucleotide polymorphisms (SNPs) are associated with IBD susceptibility in a population-based Chinese Han cohort including 562 unrelated individuals (232 CD, 56 UC and 274 healthy controls).

Patients and methods

Human subjects. A total of 288 unrelated Chinese patients with IBD (232 with CD and 56 with UC) and 274 matched healthy controls were included in this study. The patients were recruited from the Department of Gastroenterology of the Ruijin Hospital Affiliated to the Shanghai Jiaotong University School of Medicine. The patients were diagnosed by senior physicians according to standard clinical, endoscopic, radiologic and histological criteria (20,21). Healthy individuals with no history of digestive system disease, unrelated to each other or to the patients were randomly selected under routine health screening as controls (Table I). The study was approved by the Research Ethics Committee of the Ruijin Hospital and the enrolled patients provided informed consent to participate in the study.

STAT4 genotyping. Genomic DNA was extracted from whole blood samples using QIAamp blood extraction kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. The DNA samples were genotyped for the rs7574865 and rs7582694 polymorphisms via polymerase chain reaction (PCR) with sequence-specific primers. The primer sequences, designed using the genomic sequences in the GenBank (http://www.ncbi.nlm.nih.gov) are listed in Table II. Genotyping of the amplified products was assessed for the presence/absence of PCR amplicons specific to the particular alleles by using a standard 2% agarose gel electrophoresis with ethidium bromide staining. Then, ~10% samples were confirmed using Sanger sequencing.

Statistical analysis. Hardy-Weinberg equilibrium testing (HWE), P-value computations (P>0.05), in the healthy control and patient groups, calculations of allelic and genotypic associations of SNPs with susceptibility to IBD were performed using the SHEsis software (http://analysis.bio-x.cn) (22,23). The tests were two-tailed and P<0.05 was considered to indicate a statistically significant difference.

Results

Patients. To evaluate the association of the *STAT4* polymorphisms with IBD in the Chinese Han population, we genotyped SNP rs7574865 and rs7582694 among 232 CD and 56 UC patients, as well as 274 healthy controls. Characteristics of the study subjects are shown in Table I.

Table I. Characteristics of the study samples.

Characteristics	Patients		Control subjects
	UC	CD	
Number	56	232	274
Age (years)			
Mean ± SD	42.5±16.7	33.6±13.5	62.1±10.1
Range	3-77	1-76	37-88
Male/female	33/23	149/83	119/155

UC, ulcerative colitis; CD, Crohn's disease; SD, standard deviation.

Genotypic and allele frequencies. Distributions of the genotype and allele frequencies of the two SNPs between the cases and healthy controls are shown in Table III. Results revealed that the *STAT4* rs7574865 genotype was significantly different between CD/IBD patients and the controls. However, the association analysis showed that neither of the two SNPs was significantly associated with IBD susceptibility (rs7574865 CD: P=0.66, OR=0.95, 95% CI 0.74-1.21; UC: P=0.43, OR=0.85, 95% CI 0.56-1.28; IBD: P=0.52, OR=0.93, 95% CI 0.73-1.17; rs7582694 CD: P=0.40, OR=1.12, 95% CI 0.86-1.44; UC: P=0.50, OR=0.86, 95% CI 0.56-1.33; IBD: P=0.62, OR=1.06, 95% CI 0.83-1.36) (Table III).

Discussion

IBD is a series of inflammatory conditions of the colon and small intestine, characterized by a combination of genetic risk factors and environmental events. Numerous gene variants are reportedly associated with IBD or IBD-related phenotypes (7-14). However, these variants are not sufficient to adequately explain the pathogenesis and development of IBD and its various phenotypes. The *STAT4* gene has been previously reported to be significantly associated with certain autoim-

Table II. Primer sequences used for STAT4 genotyping.

Polymorphism	Description	Primer sequences
rs7574865	Internal control forward primer	CTGTTAATACGGATGTCT
	Common reverse primer	ACTTCTTGCTTTAGGAGT
	Specific primer G	AAGTTGGTGACCAAAATGTG
	Specific primer T	AAGTTGGTGACCAAAATGTT
rs7582694	Internal control forward primer	TGGAATCCAACTCTTCTCAGCC
	Common reverse primer	AAAATGTTACCAATGCTTATCT
	Specific primer C	TTCATGAAGGGATGACACATAC
	Specific primer G	TTCATGAAGGGATGACACATAG

STAT4, signal transducer and activator of transcription 4.

Table III. Allele and genotype frequency of the two loci in IBD (CD and UC).

Cases	SNP ID	Alleles		OR (95%CI)	P-value	Genotypes			HWE P[a]	P-value
IBD										
	rs7574865	G (freq)	T (freq)			G/G (freq)	G/T (freq)	T/T (freq)		
	Case	271 (0.477)	297 (0.523)	0.93 (0.73-1.17)	0.52	23 (0.081)	225 (0.792)	36 (0.127)	0.42	**0.031**
	Control	269 (0.496)	273 (0.504)			39 (0.144)	191 (0.705)	41 (0.151)	0.16	
	rs7582694	C (freq)	G (freq)			C/C (freq)	C/G (freq)	G/G (freq)		
	Case	211 (0.369)	361 (0.631)	1.06 (0.83-1.36)	0.62	44 (0.154)	123 (0.430)	119 (0.416)	0.20	0.89
	Control	193 (0.355)	351 (0.645)			39 (0.143)	115 (0.423)	118 (0.434)	0.21	
CD										
	rs7574865	G (freq)	T (freq)			G/G (freq)	G/T (freq)	T/T (freq)		
	Case	220 (0.482)	236 (0.518)	0.95 (0.74-1.21)	0.66	19 (0.083)	182 (0.798)	27 (0.118)	0.17	**0.042**
	Control	269 (0.496)	273 (0.504)			39 (0.144)	191 (0.705)	41 (0.151)	0.16	
	rs7582694	C (freq)	G (freq)			C/C (freq)	C/G (freq)	G/G (freq)		
	Case	175 (0.380)	285 (0.620)	1.12 (0.86-1.44)	0.40	36 (0.157)	103 (0.448)	91 (0.396)	0.45	0.68
	Control	193 (0.355)	351 (0.645)			39 (0.143)	115 (0.423)	118 (0.434)	0.21	
UC										
	rs7574865	G (freq)	T (freq)			G/G (freq)	G/T (freq)	T/T (freq)		
	Case	51 (0.455)	61 (0.545)	0.85 (0.56-1.28)	0.43	4 (0.071)	43 (0.768)	9 (0.161)	0.42	0.34
	Control	269 (0.496)	273 (0.504)			39 (0.144)	191 (0.705)	41 (0.151)	0.16	
	rs7582694	C (freq)	G (freq)			C/C (freq)	C/G (freq)	G/G (freq)		
	Case	36 (0.321)	76 (0.679)	0.86 (0.56-1.33)	0.50	8 (0.143)	20 (0.357)	28 (0.500)	0.17	0.62
	Control	193 (0.355)	351 (0.645)			39 (0.143)	115 (0.423)	118 (0.434)	0.21	

[a]Hardy-Weinberg equilibrium testing (HWE) P-value. IBD, inflammatory bowel disease; CD, Crohn's disease; UC, ulcerative colitis; SNP; single-nucleotide polymorphism; OR; odds ratio; CI, confidence interval; HWE, Hardy-Weinberg equilibrium testing; freq, frequency. Bold, statistically significant.

mune diseases such as SLE, RA (16,24,25) and psoriasis (24,26).

The Chinese Han is one of the largest populations in the world. Evaluation of the association of risk factors with specific diseases is crucial. In this study, we present a genotype-phenotype analysis of *STAT4* gene polymorphisms in an IBD cohort. This study did not reproduce the association of *STAT4* variants with susceptibility to IBD in the Chinese population. However, we found the genotype frequency of rs7574865 to vary significantly in CD/IBD patients and the healthy controls. Previous studies have shown that the *STAT4* SNP rs7574865 was significantly associated with RA (16), IBD and type I diabetes (17). Additionally, the association analysis of rs7574865 with IBD was carried out independently by several groups based on various ethnic populations. Unlike the original findings of Martinez *et al* (17), Glas *et al* (19), who analyzed 2,704 individuals of Caucasian origin, hypothesized that rs7574865 was associated with CD and early disease onset, but not with UC. Nevertheless, in another independent Spanish cohort Diaz-Gallo *et al* (18) observed that rs7574865 was associated with UC but not CD. A further discrepancy was noted by Moon *et al* (27) who analyzed eight *STAT4* SNPs in 657 unrelated Korean participants, and did not detect any statistically significant association between rs7574865 and CD or UC. These varying results may be due to the variation of complex circumstances and extended genetic background.

Other SNPs of *STAT4*, such as rs7582694, also analyzed in our study, may affect several autoimmune diseases in various ethic populations (28-30). However, rs7582694 was not found to be associated with IBD. Although numerous gene variants are reportedly associated with IBD susceptibility (7,10-14), a re-evaluation of the association is required in additional ethic populations. Lack of replication has long been a challenge in genetic association studies of IBD.

Taken together, although our study was sufficiently powered, there was no evidence to show that the previously reported common variants rs7574865 and rs7582694 in the *STAT4* gene increased the risk of IBD in a Chinese Han population. However, this study has a limitation. Although the two SNPs were not associated with IBD susceptibility and phenotypes, the relatively small study population caused the weak diversity of the genotype frequency of rs7574865 in the CD patients and controls. The association analysis in our study should be replicated in a larger sample-sized study of Chinese patients, as well as in other ethnic populations.

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