GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Association between *STAT3* gene polymorphisms and ulcerative colitis susceptibility: a case-control study in the Chinese Han population

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Genet. Mol. Res. 13 (2): 2343-2348 (2014)
Received March 30, 2013
Accepted June 7, 2013
Published April 3, 2014
DOI http://dx.doi.org/10.4238/2014.April.3.6

ABSTRACT. Ulcerative colitis (UC) is a chronic inflammation of the large intestine. The aim of this study was to investigate the association of two polymorphisms in *STAT3* with the risk of UC development in the Chinese Han population. This is a hospital-based case-control study involving 56 UC patients and 274 controls. Genotyping was performed using the polymerase chain reaction with sequence-specific primers (PCR-

SSP) method. Statistical analyses were conducted using logistic regression and genotype risk score. Overall, there was a significant difference between patients and controls in the genotype distribution of rs2293152 (P = 0.044). The risk for UC associated with the rs2293152-G mutant allele was increased (odds ratio = 2.76; 95% confidence interval = 1.06-7.24) under the dominant model. However, we failed to find any obvious differences in the rs4796793 genotype or allele distributions between the UC patients and controls, and did not detect any significant association of the rs4796793 polymorphism with UC across different genetic models of inheritance. Our study implies that the *STAT3* rs2293152 polymorphism may be associated with the occurrence of UC and might be used as a predictive factor for UC in the Chinese Han population.

Key words: *STAT3* gene; Ulcerative colitis; Polymorphism

INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammation of the large intestine that, together with Crohn's disease (CD), makes up the majority of what are called inflammatory bowel diseases (IBD). The etiology and pathogenesis of UC are currently not fully understood. However, familial aggregation and twin studies reported that patients with UC carry a strong genetic predisposition (Zheng et al., 2003). Moreover, dozens of studies strongly suggest that UC results from a combination of factors, such as commensal bacteria, food antigens, and immunologic factors, as well as multiple genetic factors (Molodecky and Kaplan, 2010; Cho, 2008).

The signal transducer and activator of transcription 3 (STAT3) is a member of a family of seven proteins (STATs 1, 2, 3, 4, 5a, 5b, and 6), which plays an important role in the development of the human immune system and hematopoiesis. It has been implicated in the signal transduction pathways of multiple cytokines, including the IL-2/γc, IL-6/gp130, IFN, and IL-10 families of cytokines, as well as IL-12, IL- 23, Flt3 ligand, M-CSF, G-CSF, leptin, and growth hormone (Takeda et al., 1999; Akira, 2000; Laouar et al., 2003; Murray, 2007; Stumhofer et al., 2007; O'Shea and Murray, 2008). Several studies have highlighted that the STAT3 signaling pathway is important in the occurrence and development of IBD in both patient and animal models (Suzuki et al., 2001; Lovato et al., 2003; Musso et al., 2005; Li et al., 2012). In a genome-wide association study, Barrett et al. (2008) reported that the *STAT3* locus is significantly associated with susceptibility to CD. Since then, a number of studies demonstrated that *STAT3* polymorphisms are associated with UC as well as CD, but the results were not consistent in different population cohorts (Franke et al., 2008; Sato et al., 2009; Cenit et al., 2010; Ferguson et al., 2010; Peter et al., 2011; Polgar et al., 2012). Therefore, in this study, we analyzed the association between UC and two *STAT3* polymorphisms (rs2293152 and rs4796793) in the Chinese Han population.

MATERIAL AND METHODS

Patient and control subjects

This was a hospital-based case-control study of the Chinese Han population performed

between January 2009 and December 2010. It involved 56 UC patients and 274 healthy controls recruited from the Gastroenterology Department of Ruijin Hospital appended to Shanghai Jiaotong University School of Medicine. All patient diagnoses were made by senior physicians and were based on clinical, endoscopic, radiological, and histopathological findings, in accordance with previously established international criteria (Ooi et al., 2010). All patients were registered with an integrated clinical and epidemiological registry and were followed up for at least one year. Controls were randomly selected from healthy individuals receiving routine health screening. The study was approved by the Research Ethics Committee of Ruijin Hospital, Shanghai, China. Informed consent was obtained from all subjects before blood sampling.

Genotyping

Genomic DNA was isolated from EDTA peripheral blood using QIAamp blood extraction kit (Qiagen, Hilden, Germany) following manufacturer instructions. All DNA samples were genotyped for single nucleotide polymorphisms (SNPs) by polymerase chain reaction with sequence-specific primers (PCR-SSP). All primers for PCR-SSP were designed using genomic sequences in GenBank (http://www.ncbi.nlm.nih.gov). The primer sequences are listed in Table 1. The amplified products were assessed for the presence/absence of PCR amplicons specific to the particular alleles using standard 2% agarose gel electrophoresis followed by ethidium-bromide staining. About 10% of the samples were then confirmed by DNA sequencing.

Table 1. Primer sequences used for genotyping.

Polymorphisms	Primer	Sequence
rs2293152	Internal control forward primer	CCGTTTAACCTAACTTCAT
	Common reverse primer	CCAGTTGTCTTTCATCCC
	Specific primer C	ACAAAGGGCCTCTGGCTGCC
	Specific primer G	ACAAAGGGCCTCTGGCTGCG
rs4796793	Internal control forward primer	TCTGGTAGACACAGCTCAGTATGG
	Common reverse primer	CCATAGTCGCAGAGGTAGATTTTA
	Specific primer C	TGTTTAGTGATTTACTGCTTACAAAGG
	Specific primer G	TGTTTAGTGATTTACTGCTTACAAAGC

Statistical analysis

Comparisons between UC patients and controls were conducted by the unpaired *t*-test for continuous variables, and by the χ^2 test for categorical variables. To avoid gross genotyping error, all polymorphisms were checked for consistency with Hardy-Weinberg equilibrium on a contingency table of observed-versus-predicted genotype frequencies, using Pearson's χ^2 test or Fisher's exact test. Genotypes were compared by logistic regression analysis, assuming additive, dominant, and recessive models of inheritance, respectively. Statistical significance was defined as $P < 0.05$.

RESULTS

Detailed information on patients and controls is shown in Table 2. Cases and controls were well matched by age and gender distribution. The frequency and distribution of alleles and genotypes of the rs2293152 and rs4796793 polymorphisms in *STAT3* were identified and

compared between UC patients and controls. The genotype distributions of the two polymorphisms were in Hardy-Weinberg equilibrium in the control groups ($P > 0.05$).

Table 2. Characteristics of ulcerative colitis (UC) patients and healthy controls in the Chinese Han population.

Characteristics	UC patients	Control subjects
Number	56	274
Age (mean ± SD, years)	42.5 ± 16.7	50.3 ± 12.5
Age range (years)	3-77	18-70
Male/female	33/23	155/119

As shown in Table 3, a significant difference between UC patients and controls was observed in genotype distribution for rs2293152, but not in allele distribution ($P^{allele} = 0.399$, and $P^{genotype} = 0.044$). A significant difference was identified for rs2293152 in association with UC under the dominant model (OR = 2.76; 95%CI = 1.06-7.24), while no significant association was detected under the additive (OR = 1.21; 95%CI = 0.79-1.85) or recessive (OR = 0.83; 95%CI = 0.43-1.60) models.

Table 3. Genotype distributions and allele frequencies of the polymorphisms studied between patients and controls, and their risk prediction for UC under three genetic models of inheritance.

Polymorphism	Patients (N = 56)	Controls (N = 274)	P χ^2	Genetic models	OR (95%CI)
rs2293152					
CC	5	58		Additive	1.21 (0.79-1.85)
CG	37	136	0.044	Dominant	2.76 (1.06-7.24)
GG	14	78		Recessive	0.83 (0.43-1.60)
C (%)	42.0	46.3	0.399		
rs4796793					
CC	23	112		Additive	1.08 (0.72-1.62)
CG	23	121	0.776	Dominant	1.00 (0.56-1.80)
GG	10	39		Recessive	1.30 (0.61-2.79)
C (%)	61.6	63.4	0.717		

With regard to rs4796793, there was no significant difference in genotype or allele distributions between UC patients and controls, and this non-significance was mirrored under the assumptions of the additive (OR = 1.08; 95%CI = 0.72-1.62), dominant (OR = 1.00; 95%CI = 0.56-1.80), and recessive (OR = 1.30; 95%CI = 0.61-2.79) models.

DISCUSSION

The *STAT3* gene is located on chromosome 17q21. Its protein product is a member of the STAT protein family that carries out a dual function: signal transduction and activation of transcription. STAT3 is a widely expressed latent cytoplasmic transcription factor that relays signals from the cell membrane directly to the nucleus. STAT3 becomes activated as a DNA binding protein through phosphorylation of tyrosine in response to a variety of stimuli and mediates the expression of a variety of genes. Thus, it plays a key role in many biological pathways crucial to cell function, including proliferation, migration, survival, and differentiation (Levy and Darnell, 2002). Several studies indicate that STAT3 activation plays distinctly different roles in innate and acquired immune responses in colitis: activation of STAT3 in innate immune cells enhances mucosal barrier function, whereas STAT3 activation in T-cells exacerbates colitis (Lovato et al., 2003; Musso et al., 2005). A number of studies also suggest

that polymorphisms of *STAT3* are associated with susceptibility to CD or UC in some population cohorts (Franke et al., 2008; Sato et al., 2009; Cenit et al., 2010; Ferguson et al., 2010; Peter et al., 2011; Polgar et al., 2012).

In this study, we examined the rs2293152 and rs4796793 polymorphisms of *STAT3* in 56 UC patients and 274 normal controls from the Chinese Han population. rs2293152, which has been reported to be significantly associated with CD in the Japanese population (Sato et al., 2009), was associated with susceptibility to UC in the Chinese Han population in this study. rs4796793, which has been reported to be associated with dilated cardiomyopathy in the Chinese Han population (Peng et al., 2012), did not show significant association with UC when studied in the UC patient and normal control groups.

A general problem with multifactorial disorders like UC is that the association of one gene with the disease in one population cannot be exactly replicated in another population. Since the incidence, epidemiology and phenotype are different between patients from the Chinese Han population and western countries, the genetic susceptibility may be different too. Other reasons, such as sample size and different endophenotypes, may also lead to inconsistency. In this study, our sample size was not large, so more SNP sites for pair-loci D'/r^2 value and haplotype analyses are necessary on a larger number of Chinese subjects and on other ethnicities to confirm the association. Nevertheless, our study is the first demonstration of the single-marker association of *STAT3* with UC susceptibility in the Chinese Han population. Our results provide a reference for further studies on the *STAT3* gene in other populations.

ACKNOWLEDGMENTS

Research supported by a grant from the National Natural Science Foundation of China (#31000408).

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