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

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ABSTRACT. Ulcerative colitis (UC) is an immune-related disease with genetic predisposition. The aim of this study was to investigate the association of three polymorphisms in the receptor for advanced glycation end-products (*RAGE*) gene with UC risk in a Chinese population. This case-control study involved 72 UC patients and 479 age- and gender-matched healthy controls. Genotyping was performed using the polymerase chain reaction-ligase detection reaction method. Data were analyzed using the Haplo.stats program. There were no significant differences between patients and controls in the allele/genotype distributions of rs1800624 (P_{allele} = 0.11; $P_{genotype}$ = 0.20), rs1800625 (P_{allele} = 0.16; $P_{genotype}$ = 0.11), or rs2070600 (P_{allele} = 0.37; $P_{genotype}$ = 0.65). In addition, no positive haplotypes were identified. To the best of our knowledge, the current study describes polymorphisms of *RAGE* in Chinese UC for the first time. We found no

association between *RAGE* polymorphisms and the development of UC in the Chinese population.

Key words: RAGE; Polymorphism; Ulcerative colitis; Susceptibility; Association study

INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammation disorder of the large intestine and, together with Crohn's disease (CD), is referred to as inflammatory bowel disease (IBD). The etiology and pathogenesis of UC are not fully understood currently. However, familial aggregation and twin studies have reported that patients with UC carry 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, immunologic factors, and multiple genetic factors (Cho, 2008; Molodecky and Kaplan, 2010). In view of the importance of immunity in IBD, IBD-susceptibility genes involved in immunity are attracting increasing attention from researchers (Wang et al., 2014a,b).

The receptor for advanced glycation end-products (RAGE) is a member of the immunoglobulin protein family of cell-surface molecules (Basta, 2008). It binds multiple structurally diverse ligands, leading to the activation of several proinflammatory signaling pathways (Han et al., 2011). Nowadays, RAGE is recognized as a pattern recognition receptor that is involved in several pathophysiological processes associated with inflammation, such as diabetes complications (Yan et al., 2008), arthritis (Foell et al., 2007), and CD (Däbritz et al., 2011). In addition, animal model studies have suggested that RAGE plays an important role in innate defense mechanisms (Liliensiek et al., 2004). A subsequent study reported convincing statistical evidence for a novel functional single nucleotide *RAGE* polymorphism, -374T/A, which was the CD-susceptibility locus in a German population (Däbritz et al., 2011). However, the role of *RAGE* polymorphisms in UC susceptibility remains unclear. Therefore, in this study, we performed an analysis on three widely evaluated polymorphisms (rs1800624, rs1800625, and rs2070600) of the *RAGE* gene and UC in a Chinese Han population.

MATERIAL AND METHODS

Patients and control subjects

This was a hospital-based case-control study involving 72 sporadic UC patients and 479 healthy controls from the Chinese Han population recruited from the Department of Gastroenterology of Ruijin Hospital, School of Medicine, Shanghai Jiaotong University, between January 2009 and December 2011. All patients were diagnosed by senior physicians based on clinical, endoscopic, radiological, and histopathological findings in accordance with previously established international criteria (Ooi et al., 2010). All patients were followed-up for at least 1 year and registered with an integrated clinical and epidemiological registry. Controls were randomly selected from healthy people under routine health screening. The study was approved by the Research Ethics Committee of Ruijin Hospital, Shanghai, China, and informed consent was obtained from each subject before blood sampling.

Genotyping

Blood samples (1 mL) were collected, and genomic DNA was extracted from white blood cells using the TIANamp Blood DNA Kit (TianGen Biotech (Beijing) Co., Ltd., China). Genotyping was conducted by the polymerase chain reaction-ligase detection reactions (PCR-LDR) method using the ABI 9600 system (Applied Biosystems, Foster City, CA, USA) (Hu et al., 2014; Wang et al., 2014c). Cycling parameters were as follows: 94°C for 2 min; 35 cycles of 94°C for 20 s; 56°C for 20 s; 72°C for 40 s; and a final extension step at 72°C for 3 min. Two probes to discriminate the specific bases and one common probe were synthesized. The common probe was labeled at the 3'-end with 6-carboxy-fluorescein, and phosphorylated at the 5'-end. The reaction conditions for LDR were: 94°C for 2 min; 30 cycles of 94°C for 30 s; and 56°C for 3 min. After the reaction, 1 mL LDR products was mixed with 1 mL carboxy-X-rhodamine (ROX) passive reference and 1 mL loading buffer, denatured at 95°C for 3 min, and chilled rapidly in ice water. The fluorescent products of LDR were differentiated using ABI sequencer 377 (Applied Biosystems).

Statistical analysis

Comparisons between UC patients and controls were conducted by unpaired Student *t*-tests for continuous variables and by the χ^2 test for categorical variables. To avoid gross genotyping errors, all polymorphisms were checked for consistency with the Hardy-Weinberg equilibrium on a contingency table of observed-versus-predicted genotype frequencies using the Pearson χ^2 test or the Fisher exact test. Genotypes were compared by logistic regression analysis under assumptions of additive, dominant, and recessive models of inheritance. Statistical significance was defined as $P < 0.05$.

Haplotype frequencies were estimated using the haplo.em program, and odds ratios (ORs) and 95% confidence intervals (CIs) were estimated by the haplo.cc and haplo.glm programs according to a generalized linear model (Stram et al., 2003). Furthermore, haplo.score was used to model an individual's phenotype as a function of each inferred haplotype, which was weighted by their estimated probability to account for haplotype ambiguity. The haplo.em, haplo.glm, and haplo.score programs were implemented using the Haplo.stats software (version 1.4.0) developed in the R language (http://www.r-project.org/).

RESULTS

rs1800624 genotypes and alleles

The frequency of the rs1800624 A allele in patients and controls was 20.8 and 15.6% ($\chi^2 = 2.57$, $P = 0.11$), respectively (Table 1).

The distribution of rs1800624 genotypes in patients did not differ significantly from that of controls ($\chi^2 = 2.73$, $P = 0.20$). We failed to find significant associations between rs1800624 and UC risk in the three genetic models (Table 1). The observed frequencies of the rs1800624 genotypes and alleles were in Hardy-Weinberg equilibrium in both patients ($\chi^2 = 0.18$, $P > 0.1$) and controls ($\chi^2 = 0.94$, $P > 0.1$).

Table 1. 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 = 72)	Controls (N = 479)	P χ^2	Genetic models	OR (95%CI)
rs1800624					
TT	46.0	343.0		Additive	1.41 (0.92-2.18)
AT	22.0	123.0	0.20	Dominant	1.43 (0.85-2.40)
AA	4.0	13.0		Recessive	2.11 (0.67-6.65)
A (%)	20.8	15.6	0.11		
rs1800625					
TT	52.0	353.0		Additive	1.43 (0.86-2.38)
CT	20.0	118.0	0.11	Dominant	1.63 (0.94-2.83)
CC	0.0	8.0		Recessive	
C (%)	14.3	14.0	0.16		
rs2070600					
GG	48.0	303.0		Additive	0.82 (0.53-1.28)
AG	22.0	148.0	0.65	Dominant	0.86 (0.51-1.45)
AA	2.0	28.0		Recessive	0.46 (0.11-1.97)
A (%)	18.1	21.3	0.37		

OR = odds ratio; 95%CI = 95% confidence interval.

rs1800625 genotypes and alleles

The frequency of the rs1800625 C allele in patients and controls was 14.3 and 14.0% (χ^2 = 2.57, P = 0.11), respectively (Table 1). The frequency of the CC + CT genotype in patients was slightly higher (27.8%) compared with controls (26.3%) (OR = 1.63; 95%CI = 0.94-2.83; Table 1). The presence of the rs1800625 C allele was not associated with susceptibility to UC (OR = 0.99; 95%CI = 0.60-1.65; Table 1). The observed frequencies of the rs1800625 genotypes and alleles were in Hardy-Weinberg equilibrium in both patients (χ^2 = 1.6, P > 0.1) and controls (χ^2 = 0.14, P > 0.1).

rs2070600 genotypes and alleles

The allele and genotype distributions of rs2070600 are depicted in Table 1. The frequency of the A allele in patients and controls was 18.1 and 21.3%, respectively. The distribution of rs2070600 A genotypes in patients did not differ significantly from that of controls (χ^2 = 0.80, P = 0.37). Moreover, the rs2070600 A allele did not decrease the risk of UC (OR = 0.81; 95%CI = 0.52-1.28; Table 1). The observed frequencies of the rs2070600 genotypes and alleles were in Hardy-Weinberg equilibrium in both patients (χ^2 = 0.04, P > 0.1) and controls (χ^2 = 1.37, P > 0.1).

Haplotype analysis

Haplotype frequencies of the three polymorphisms examined were estimated and compared between cases and controls (Table 2).

The frequency of haplotype T-A-T (in the order rs1800625, rs1800624, and rs2070600) was lower in patients than in controls (18.1 *vs* 21.3%), whereas the frequency of haplotype T-G-A was higher (20.8 *vs* 15.6%) in patients. After assigning the commonest haplotype T-G-T as the reference, no haplotypes were identified as being associated with the risk of UC.

Table 2. Haplotype frequencies of the polymorphisms studied between patients and controls, and their risk prediction for UC.

Haplotype*	Case (%)	Control (%)	Hapscore	P value	P_{sim}	OR	95%CI	P value
T-G-T	47.2	49.2	-0.44	0.66	0.70	Reference		
T-A-T	18.1	21.3	-0.86	0.39	0.35	0.90	(0.56, 1.44)	0.66
C-G-T	13.9	14.0	-0.03	0.97	0.91	1.05	(0.60, 1.82)	0.88
T-G-A	20.8	15.6	1.58	0.12	0.08	1.38	(0.87, 2.21)	0.17

*Alleles in haplotype are reported in order of polymorphisms rs1800625, rs1800624, and rs2070600. P_{sim} = simulated P; OR = odds ratio; CI = confidence interval.

DISCUSSION

RAGE is recognized as a pattern recognition receptor and is capable of binding to numerous proinflammatory molecules, including S100 proteins (Foell et al., 2003). Previous data have shown that RAGE and its ligands are highly hyperexpressed in the intestinal mucosa of patients with IBD (Andrassy et al., 2006; Cirillo et al., 2009), indicating the role of RAGE and its ligands in the augmentation of intestinal injury. Further studies have found that serum levels of soluble RAGE (sRAGE) are higher in UC patients and correlate with disease activity (Yilmaz et al., 2011). Moreover, the -374T/A *RAGE* polymorphism was negatively associated with CD in a German population (Däbritz et al., 2011). Therefore, the *RAGE* gene is a logical candidate for potential causative factors of IBD.

To the authors' knowledge, this is the first study exploring the association between the *RAGE* gene and genetic susceptibility to UC risk. In this study, we performed an analysis on three widely evaluated polymorphisms (rs1800624, rs1800625, and rs2070600) of the *RAGE* gene and UC in a Chinese Han population. We found a higher prevalence of the rs1800624 A allele in patients compared with controls, and an elevated risk of UC in its presence. In contrast, there was a lower prevalence of the rs2070600 A allele in patients compared with controls, and a reduction in the risk of UC in its presence. However, since these differences were not statistically significant, we concluded that these polymorphisms are not associated with the risk of UC in the Chinese Han population. The three polymorphisms of *RAGE* have been widely studied in inflammatory and autoimmune-related diseases. The rs1800624 and rs2070600 polymorphisms, located at positions -374 and -429 of the promoter region, respectively, have been reported to increase the transcriptional activity and protein expression of the *RAGE* gene *in vitro*. In addition, the -374A allele also influences the binding affinity of the transcription factor (Hudson et al., 2001). Recently, Däbritz et al. (2011) found that the -374T/A *RAGE* polymorphism was negatively associated with CD in a German population, raising the hypothesis that the -374T/A *RAGE* polymorphism might increase the serum levels of sRAGE to neutralize proinflammatory mediators. Another polymorphism (rs2070600, also known as G82S) causes a glycine-to-serine substitution at position 82 within the V-domain, and is located in exon 3, a region that plays a pivotal role in ligand binding. The G82S variant has been shown to increase the ligand-binding affinity of the receptor (Hofmann et al., 2002; Osawa et al., 2007), and consequently to increased nuclear factor-κB activation and inflammatory gene expression. In addition, the G82S polymorphism is associated with reduced levels of sRAGE that in a number of diseases increases the contribution made by RAGE to inflammation (Jang et al., 2007). The G82S RAGE polymorphism is associated with arthritis (Hofmann et al., 2002). However, we failed to find a significant association between the three polymorphisms and UC risk in the Chinese Han

population. Given the relatively small sample size in this study, we acknowledge that confirmation by large, well-designed studies is critical.

Moreover, it is widely believed that genetic markers of predisposition to IBD vary across geographical and racial groups. As evidenced in our previous meta-analyses, the *CD14* gene C-260T polymorphism exhibited remarkable heterogeneity in terms of association with UC across ethnic groups, with significance attained in Asians but not in Caucasians (Wang et al., 2012). Therefore, further studies and biological research utilizing large sample sizes from different ethnic origins should be carried out to verify this association.

Finally, interpretation of our results should be viewed in the light of several limitations. First, the sample size in our study was relatively small, and may not have had sufficient statistical power to detect a small genetic effect, resulting in a fluctuating estimation. Second, we only revealed limited polymorphisms of the *RAGE* gene associated with susceptibility to UC; there might be other unidentified polymorphisms that influence the development of UC. Furthermore, we only centered on the *RAGE* polymorphisms and did not evaluate other genes. The question of whether these polymorphisms when integrated with other risk factors enhance the predictive power requires additional research. Thus, we must refrain from drawing a firm conclusion until large, well-performed studies confirm or refute our results.

In summary, our results indicate that the *RAGE* polymorphisms are not significantly associated with the risk of UC. Future studies on these polymorphisms in larger UC samples with defined ethnicity could help to elucidate the role of the *RAGE* gene in UC susceptibility.

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