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ORIGINAL RESEARCH

STAT6 rs324015 Gene Polymorphism Increases Ulcerative Colitis Risk: A Case–Control Study

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Introduction: Phosphorylation of signal transducer and activator of transcription 6 (STAT6) in the colonic epithelium is elevated in ulcerative colitis (UC) patients, and its inhibition prevents IL-13-associated apoptosis and barrier disruption. Recently, the *STAT6* rs324015 polymorphism was reported to be related to genetic susceptibility to UC.

Methods: We examined *STAT6* rs324015 using the PCR–RFLP method in 268 UC cases and 357 controls. *STAT6* expression was determined by quantitative reverse-transcription PCR. The gene–environment interactions were addressed by cross-over analysis.

Results: We found that the *STAT6* rs324015 polymorphism enhanced the risk of UC under the homozygous, dominant, and allelic models. Further subgroup analyses indicated that this relationship was more evident in alcohol users, smokers, and those younger than 40 years. Cross-over analysis showed strong interactions of *STAT6* rs324015 with smoking/alcohol use. In addition, this polymorphism was associated with the severity, and location of UC. The GG genotype was significantly associated with increased *STAT6* gene levels.

Conclusion: In summary, the *STAT6* rs324015 polymorphism is related with predisposition to UC in a Chinese Han population.

Keywords: *STAT6*, ulcerative colitis, case–control study, rs324015 polymorphism

Introduction

Inflammatory bowel diseases (IBDs) are heterogeneous intestinal disorders, which affects multiple gastrointestinal tract organs.[1] IBD reflects an imbalance between an uncontrolled inflammatory response and the intestinal microbiota.[2,3] An estimated 1.5 million Americans and 2.2 million Europeans have IBD.[4] There are two main types of IBD: Crohn's disease (CD) and ulcerative colitis (UC). UC is a chronic IBD; it is characterized by chronic, relapsing inflammation process in the colorectal mucosa from the rectum to cecum. The etiology of UC is complex, and interactions among immune responses, genetic predisposition, and environmental factors contribute to its occurrence.[5,6] UC causes a loss of intestinal immune tolerance due to genetic and environmental factors.[7] Novel UC susceptibility loci were reported before.[8,9]

The signal transducer and activator of transcription 6 (STAT6) regulates Th1- and Th2-mediated immune responses.[10] The intestinal mucosa showed imbalanced activation of Th2 and Th1 lymphocytes in IBD.[11] STAT6 participated in IL-4- and IL-13-regulated Th2 responses.[12] Li et al suggested that downregulating miR-214-3p contributed to the development of UC via targeting STAT6.[13] Moreover, Rosen et al found that STAT6 deficiency ameliorated the severity of oxazolone-induced colitis by decreasing Th2-inducing cytokines.[14] They also suggested that UC was

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associated with elevated phosphorylation of STAT6 in the colonic epithelium, and that inhibition of STAT6 prevented IL-13-induced apoptosis and barrier disruption.[15] Based on the above evidence, STAT6 may play an indispensable role in the development and pathogenesis of UC. Therefore, we postulated that *STAT6* may be a candidate gene for determining UC predisposition because of immunoregulatory function. The chromosomal location of *STAT6* is at 12q13.3-14.1. Three studies have explored the connection between the *STAT6* rs324015 polymorphism and UC predisposition, with conflicting findings.[16–18] New case–control studies are required to verify previous findings; in that case, this study was designed to interpret the potential connection between *STAT6* rs324015 and UC susceptibility in this Chinese Han population.

Methods

Subjects

We recruited 268 UC patients from the Affiliated Hospital of the Nanjing University of Chinese Medicine. The UC cases were diagnosed according to clinical, endoscopic, radiographic, and histological criteria.[19] The severity of UC was evaluated using Truelove and Witts' severity index, and the disease location was classified using the Montreal classification.[20] The exclusion criteria were the presence of an (1) autoimmune disease, (2) malignant tumor, or (3) other known chronic inflammatory disease. The following clinical information was extracted from the medical records of the UC patients: family history, cigarette and alcohol use, and body mass index (BMI). During the same period, 357 age- and sex-matched healthy controls were recruited from healthy individuals undergoing physical examinations. Informed consents were got from all subjects.

The hospital ethics committee approved the conduction of this study; this study followed the guidelines of the Declaration of Helsinki.

Blood Sampling and Genotyping

First, blood samples (2 mL) were collected from all subjects in EDTA tubes and stored at −80°C. DNA was isolated by use of the DNA Purification Kit (Tiangen Biotech, Beijing, China) following the manufacturer's instructions. Genotyping was conducted by PCR–RFLP technique. The primers used were 5′-GAAGTTCAGGCTCTGAGAGAC-3′ (forward) and 5′-CCATCACCCTCAGAGAGC-3′ (reverse). To ensure the accuracy of genotyping, we directly sequenced 10% of the samples. The results were 100% concordant.

Quantitative Real-Time PCR (qRT-PCR)

Total RNA was isolated from PBMCs by use of the TRIzol reagent (Invitrogen, Carlsbad, CA, USA). By use of SYBR Green I Real-Time PCR kit (GenePharma, Shanghai, China), qRT-PCR was conducted on the CFX96 Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). GAPDH was regarded as the internal control to normalize the expression of *STAT6*. The respective forward and reverse primers for PCR were 5′-ATGGA CAATGCCTTCTCTGA-3′ and 5′-AACCACTGCCAAA ATGTGAAC-3′ for *STAT6* and 5′-AGGTCGGTGTGAA CGGATTTG-3′ and 5′-TGTAGACCATGTAGTTGAG GTCA-3′ for GAPDH. The experiment was repeated three times, and the relative expression levels were determined using the $2^{-\Delta\Delta CT}$ method.

Statistical Analysis

Continuous variables were determined by utilization of the One-Way ANOVA or unpaired Student's *t*-test, and the χ^2 test was utilized for analyzing categorical variables. If variables did not conform to normality, Nonparametric test was employed. Hardy–Weinberg equilibrium was assessed for this polymorphism in controls using a goodness-of-fit χ^2 test. Using logistic regression analyses, ORs and 95% CIs were analyzed to evaluate the connection between genetic variants and UC risk. Gene × environment interactions (including gene × smoking and gene × alcohol use) were addressed by the Cross-over analysis. Regression models were adjusted for sex and age. The power of the study was calculated at a significance level of 0.05.[21] All relevant statistical analyses were conducted by use of SPSS 22.0 (SPSS, Chicago, IL, USA). $P < 0.05$ was considered significant.

Results

Participant Characteristics

Table 1 summarizes the demographic and risk factors of all subjects. No significant differences were shown between two groups regarding age, sex, BMI, or alcohol use. There were more smokers among the UC patients than the controls ($P < 0.001$). Of the UC cases, 168 had distal colitis and 100 extensive colitis. There were 111 (41.4%) mild, 125 (46.6%) intermediate, and 32 (11.9%) severe UC cases, respectively.

Table 1 Demographics of Study Participants

Characteristics	Case (N=268)	Control (N=357)	*P*
Age	35.57±8.60	35.80±9.61	0.759
BMI	24.40±1.55	24.27±1.47	0.290
Sex			0.234
Male	132(49.3%)	193(54.1%)	
Female	136(50.7%)	164(45.9%)	
Smoking			**<0.001**
Yes	144(53.7%)	138(38.7%)	
No	124(46.3%)	219(61.3%)	
Alcohol			0.154
Yes	101(37.7%)	115(32.2%)	
No	167(62.3%)	242(67.8%)	
Family history			
Yes	25(9.3%)		
No	243(90.7%)		
Lesion location of UC			
Distal colitis(E1+E2)	168(62.7%)		
Extensive colitis(E3)	100(37.3%)		
Severity of UC			
Mild	111(41.4%)		
Intermediate	125(46.6%)		
Severe	32(11.9%)		

Note: Bold values are stitistically significant (P < 0.05).
Abbreviations: BMI, body mass index; UC, ulcerative colitis.

Relationship Between the STAT6 rs324015 Polymorphism and UC Risk

The genotype distribution of the rs324015 polymorphism in the controls was in line with Hardy–Weinberg equilibrium (Table 2). The GG genotype of this polymorphism was correlated with UC susceptibility (GG vs AA: OR, 1.88; 95% CI, 1.02–3.45; $P = 0.043$). The GG and AG genotypes or G allele significantly increased the risk of UC. These findings remained significant even after adjusting for age and sex. The power analysis revealed that this study had a power of 69.6% to detect the effect of the rs324015 polymorphism on UC susceptibility, assuming an OR of 1.37.

We conducted stratified analyses according to sex, age, and alcohol and smoking statuses to evaluate the effect of *STAT6* rs324015 on the risk of UC (Table 3). A significant association between *STAT6* rs324015 and UC risk was seen in smokers, alcohol users, and subjects <40 years. However, there was no positive finding according to sex. Due to the potential interactions of smoking/alcohol use with the *STAT6* rs324015 polymorphism, we used cross-over analysis to validate the results (Table 4). The GG or AG genotype was not related to the risk of UC compared with the AA genotype. Furthermore, no positive association was yielded between alcohol use and UC risk. However, we found that smokers with the GG or AG genotype presented a higher risk of UC than non-smokers with the AA genotype (GG + smoking vs AA + non-smoking: OR, 3.11, 95% CI, 1.35–7.16; $P = 0.006$; AG + smoking vs AA+ non-smoking: OR, 2.40, 95% CI, 1.52–3.80; $P < 0.001$). Alcohol drinkers with the AG genotype also showed a higher risk of UC than non-drinkers with the AA genotype. In total, this study observed interactions between environmental (alcohol and smoking) and genetic (GG or AG genotype of *STAT6* rs324015) factors.

Subsequently, we evaluated the relationships between this polymorphism and the clinical manifestations of the UC cases (Table 5). We found some genotypes of rs324015 polymorphism were related with the severity, and lesion location of UC.

Table 2 The Association of Genotype and Allele of the STAT6 rs324015 Polymorphism with Ulcerative Colitis Risk

Models	Genotype	Case (n, %)	Control (n, %)	OR (95% CI)	*P*-value	*OR (95% CI)	**P*-value
Co-dominant	AA	126(47.2%)	200(56.3%)	1.00(reference)	-	1.00(reference)	-
Heterozygote	AG	115(43.1%)	133(37.5%)	1.37(0.98-1.92)	0.064	1.40(1.00-1.96)	0.050
Homozygote	GG	26(9.7%)	22(6.2%)	**1.88(1.02-3.45)**	**0.043**	**1.92(1.04-3.54)**	**0.037**
Dominant	AA	126(47.2%)	200(56.3%)	1.00(reference)	-	1.00(reference)	-
	GG+AG	141(52.8%)	155(43.7%)	**1.44(1.05-1.99)**	**0.024**	**1.48(1.07-2.04)**	**0.018**
Recessive	AG+AA	241(90.3%)	333(93.8%)	1.00(reference)	-	1.00(reference)	-
	GG	26(9.7%)	22(6.2%)	1.63(0.90-2.95)	0.104	1.66(0.92-3.00)	0.095
Allele	A	367(68.7%)	533(75.1%)	1.00(reference)	-	1.00(reference)	-
	G	167(31.3%)	177(24.9%)	**1.37(1.07-1.76)**	**0.013**	-	-

Notes: The genotyping was successful in 267 cases and 355 controls for rs324015 polymorphism; Bold values are statistically significant ($P < 0.05$); *Adjust age and sex.

Table 3 Stratified Analyses Between the STAT6 rs324015 Polymorphism and the Risk of Ulcerative Colitis

Variables	(Case/Control)			AG vs AA	GG vs AA	GG vs AG+AA	GG+AG vs AA
	AA	AG	GG				
Sex							
Male	55/99	63/78	13/14	1.45(0.91-2.32); 0.116	1.67(0.73-3.81); 0.218	1.39(0.63-3.07); 0.409	1.49(0.95-2.33); 0.082
Female	71/101	52/55	13/8	1.35(0.83-2.19); 0.231	2.31(0.91-5.87); 0.072	2.06(0.83-5.13); 0.114	1.47(0.93-2.33); 0.102
Smoking							
Yes	56/72	71/54	17/10	**1.69(1.03-2.78); 0.038**	2.19(0.93-5.14); 0.073	1.69(0.74-3.83); 0.207	**1.77(1.10-2.84); 0.018**
No	70/128	44/79	9/12	1.02(0.64-1.63); 0.939	1.37(0.55-3.41); 0.496	1.36(0.56-3.33); 0.497	1.07(0.68-1.67); 0.782
Alcohol							
Yes	29/66	60/42	12/7	**3.25(1.81-5.86); <0.001**	**3.90(1.39-10.92); 0.007**	2.08(0.79-5.51); 0.134	**3.34(1.90-5.90); <0.001**
No	97/134	55/91	14/15	0.84(0.55-1.28); 0.405	1.29(0.60-2.80); 0.519	1.38(0.65-2.95); 0.401	0.90(0.60-1.34); 0.603
Age (years)							
<40	84/96	101/66	21/10	**1.75(1.14-2.68); 0.010**	**2.40(1.07-5.38); 0.030**	1.84(0.84-4.02); 0.122	**1.84(1.22-2.76); 0.004**
≥40	42/104	14/67	5/12	0.52(0.26-1.02); 0.055	1.03(0.34-3.11); 1.000	1.27(0.43-3.77); 0.885	0.60(0.32-1.10); 0.097

Note: Bold values are statistically significant ($P < 0.05$).

Table 4 Genetic (G) and Environmental (E) Factors 2×4 Fork Analysis

G[a]	E[b]	Case	Control	OR (95%CI); *P* value	Reflecting Information
GG vs AA	Smoking				
+	+	17	10	**3.11(1.35,7.16); 0.006**	G, E combined effect
+	-	9	12	1.37(0.55,3.41); 0.496	G alone effect
-	+	56	72	1.42(0.90,2.24); 0.128	E alone effect
-	-	70	128	1.0	Common control
AG vs AA	Smoking				
+	+	71	54	**2.40(1.52,3.80); <0.001**	G, E combined effect
+	-	44	79	1.02(0.64,1.63); 0.939	G alone effect
-	+	56	72	1.42(0.90,2.24); 0.128	E alone effect
-	-	70	128	1.0	Common control
GG vs AA	Drinking				
+	+	12	7	2.37(0.90,6.24); 0.074	G, E combined effect
+	-	14	15	1.29(0.60,2.80); 0.519	G alone effect
-	+	29	66	0.61(0.37,1.01); 0.053	E alone effect
-	-	97	134	1.0	Common control
AG vs AA	Drinking				
+	+	60	42	**1.97(1.23,3.17); 0.005**	G, E combined effect
+	-	55	91	0.84(0.55,1.28); 0.405	G alone effect
-	+	29	66	0.61(0.37,1.01); 0.053	E alone effect
-	-	97	134	1.0	Common control

Notes: [a]G (+): STAT6 gene rs324015 variants (Heterozygous or homozygous); G (-): Wild type. [b]E (+): Smoking/drinking; E (-): Non-smoking/non-drinking. Bold values are statistically significant ($P < 0.05$).

Last, we evaluated the impact of the *STAT6* rs324015 polymorphism on *STAT6* gene expression. Compared with the AA genotype, the GG genotype was significantly associated with increased *STAT6* expression ($P = 0.034$; Figure 1).

Discussion

In this present case-control study, we observed that the *STAT6* rs324015 polymorphism enhanced the risk of UC in Chinese individuals. Subgroup analyses indicated that the

Table 5 The Association Between the STAT6 rs324015 Polymorphism and Clinical Characteristics of Ulcerative Colitis

Characteristics	Genotype Distributions			
	AA	**AG**	**GG**	**AG+GG**
Family history				
Yes/No	11/115	9/106	5/21	14/127
OR (95%CI); *P*-value	1.0 (reference)	0.89(0.35-2.23); 0.799	2.49(0.78-7.90); 0.112	1.15(0.50-2.64); 0.737
Lesion location of UC				
E3/E1+E2	37/89	50/65	13/13	63/78
OR (95%CI); *P*-value	1.0 (reference)	**1.85(1.09-3.15); 0.023**	**2.41(1.02-5.68); 0.041**	**1.94(1.17-3.23); 0.010**
Severity of UC				
Severe/mild	7/58	20/43	5/10	25/53
OR (95%CI); *P*-value	1.0 (reference)	**3.85(1.50-9.93); 0.004**	4.14(1.10-15.66); 0.071	**3.91(1.56-9.78); 0.002**
Severity of UC				
Severe/intermediate	7/61	20/52	5/11	25/63
OR (95%CI); *P*-value	1.0 (reference)	**3.35(1.31-8.55); 0.009**	3.96(1.06-14.75); 0.079	**3.46(1.39-8.58); 0.005**

Note: Bold values are statistically significant ($P < 0.05$).

STAT6 rs324015 was related to an increased risk of UC in smokers, alcohol users, and those individuals under 40 years. *STAT6* rs324015 was linked with the location, and severity of UC, and the GG genotype was significantly associated with increased *STAT6* expression.

Xia et al first explored the relationship between the rs324015 polymorphism of *STAT6* gene and IBD risk in a Dutch population.[16] They showed that UC did not differ according to the genetic distribution when it was classified by disease extent, age of onset, and colectomy, indicating that *STAT6* rs324015 polymorphism did not confer susceptibility to UC.[16] A German study by Klein et al revealed that the G allele and GG genotype were significantly related with an elevated risk of CD only, but not of UC.[17] A subsequent Chinese study suggested that *STAT6* rs324015 was not associated with genetic susceptibility to UC in Chinese patients.[18] However, unlike the abovementioned studies,[16–18] we uncovered that this polymorphism elevated the risk of UC. We postulate the following reasons for these inconsistent findings. First, the allele and genotype distributions of *STAT6* rs324015 differed significantly between our cohort and the other study populations. Second, the exposure factors for UC were diverse among the studies. Third, UC is clinically heterogeneous; for example, the severities and lesion locations of UC are distinct. Fourth, the sample sizes of these studies varied.

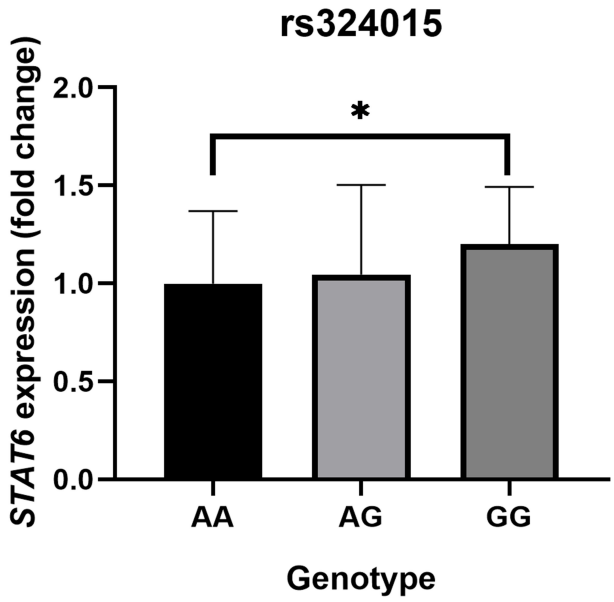

Figure 1 The relative *STAT6* mRNA expression among three genotypes of rs324015 polymorphism (*$P < 0.05$, GG genotype vs AA genotype).

In further analyses, we evaluated the relationships between *STAT6* rs324015 and environmental factors. This polymorphism was linked with an enhanced risk of UC in smokers, alcohol users, and those aged lower than 40 years, suggesting that interactions of smoking, alcohol use, and *STAT6* rs324015 may account for an increased risk of UC in this population. However, no significant association between the rs324015 polymorphism and sex was seen. Due to the potential interactions of smoking/alcohol use with the *STAT6* rs324015 polymorphism, cross-over analysis was conducted. Significant interactions between genetic factors (GG or AG genotype of *STAT6* rs324015) and

alcohol use/smoking were detected. Next, we investigated the connection between the *STAT6* rs324015 polymorphism and the clinical manifestations of the UC patients. This polymorphism was related to the severity, and location of UC. The data suggest that the rs324015 polymorphism confers increased susceptibility to UC in those with extensive colitis or severe UC.

The following study limitations should be noted. First, the sample size was small, which may reduce the reliability of the results. Second, selection bias existed in this hospital-based case-control study; however, we could not predict the impact of selection bias on the results. Third, the limited data restricted further analyses. Fourth, evaluation of only one polymorphism is insufficient because rs324015 may be in linkage disequilibrium with other polymorphisms. Fifth, immunological indicators such as IL-4, IL-13, Th1 and Th2 lymphocytes should be tested. Finally, other genetic–environmental interactions that were not assessed may exist.

Conclusion

The *STAT6* rs324015 polymorphism is related with an enhanced risk of UC in Chinese Han individuals and is also associated with *STAT6* expression. Further studies of this polymorphism in larger sample sizes are needed.

Author Contributions

All authors contributed to conception and design, acquisition of data, or analysis and interpretation of data substantially; were involved in drafting this paper or revising it critically; were in agreement with submitting to the current journal; approved the version to be published; and agree to be responsible for all aspects of this study.

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Disclosure

The authors report no conflict of interest.

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