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RESEARCH ARTICLE

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Interleukin-12B gene rs6887695 and rs2288831 polymorphisms are associated with an increased risk of ulcerative colitis development in Chinese Han population: A case-control study

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Abstract

Background: The association of the *interleukin* (*IL*)*-12B* gene rs6887695 and rs2288831 polymorphisms with ulcerative colitis (UC) risk has been extensively investigated, but results are conflicting. In this study, we investigated potential link between the *IL-12B* gene rs6887695 and rs2288831 polymorphisms and UC development in Chinese Han population.

Material and Methods: Genotyping was performed in 367 patients and 456 controls through polymerase chain reaction-restriction fragment length polymorphism analysis. Plasma levels of *IL-12B* were tested using an enzyme-linked immunosorbent assay.

Results: We found that the *IL-12B* gene rs6887695 and rs2288831 polymorphisms were related to a significantly increased risk of UC. Subgroup analyses revealed significant associations of the *IL-12B* gene rs6887695 and rs2288831 polymorphisms with UC risk among females, consumers of alcohol, and those aged <40 years. Additionally, the rs6887695 and rs2288831 polymorphisms were associated with lesion location and UC treatment. Last, we found that these two polymorphisms were associated with IL-12B levels.

Conclusions: The *IL-12B* gene rs6887695 and rs2288831 polymorphisms were associated with a higher risk, and the clinical characteristics, of UC.

KEYWORDS

IL-12B, rs2288831, rs6887695, ulcerative colitis

1 | INTRODUCTION

Inflammatory bowel disease (IBD), a prevalent chronic inflammatory disorder, occurs due to an abnormal immune reaction to intraluminal antigens in genetically predisposed individuals.[1] Distinct subtypes of IBD include Crohn's disease (CD) and ulcerative colitis (UC). The incidence of UC has been increasing worldwide for decades, particularly in developed countries.[2] UC has a prevalence rate of 0.02%-0.5% in the general population.[1] UC can be induced by many factors, including an abnormal immune reaction epithelial barrier disruption, genetic predisposition, and environmental causes.[3] Genome-wide association assays have revealed hundreds of genetic variants in UC,[4-6] which are regarded as susceptible sites for UC.

Interleukin (IL)-12, an important inflammatory factor, is involved in both innate and adaptive immunity.[7,8] The subunits of IL-12, P35 and p40, are encoded by IL-12A and IL-12B genes, respectively. IL-12 is mainly expressed due to activation of neutrophils, macrophages, dendritic cells, and microglia.[9,10] IL-12 expression levels are significantly higher in CD patients than in normal individuals.[11] IL-12 levels were shown by endoscopy to be related to the clinical activity of UC.[12] IL-12B is considered a potential biomarker for UC.[13] Furthermore, IL-12B mRNA expression in UC may be related to the changes in inflammatory and immune reactions.[14] Thus, *IL-12B* is a major candidate gene for UC susceptibility.

IL-12B is located on chromosome 5q31. *IL-12B* gene polymorphisms are heavily involved in many autoimmune disorders, such as psoriatic arthritis,[15] multiple sclerosis,[16] and rheumatoid arthritis.[17] An association between *IL-12B* gene polymorphisms (rs6887695 and rs2288831) and UC risk has been reported in various populations.[18-22] However, the results of these studies are conflicting, and no study has included a Chinese Han population. Thus, our study explored the association between *IL-12B* rs6887695 and rs2288831 polymorphisms and UC risk in a Chinese Han population.

2 | MATERIALS AND METHODS

2.1 | Subjects

We recruited 367 UC patients and 456 controls from Wuxi Third People's Hospital from May 2014 to December 2019. UC was diagnosed in accordance with histopathological, endoscopic, and radiological findings based on existing guidelines.[23] The controls all underwent health examinations at the same hospital and had no immune-associated disorders or disease history. The exclusion criteria were as follows: (a) history of cancer; (b) current autoimmune disease; and 3. history of drug use or abnormal immune status.

All subjects provided informed consent before enrollment. This study was approved by the Ethics Committee of our hospital and performed as per the tenets of the Helsinki Declaration.

2.2 | Blood sampling and genotyping

Blood was collected from all participants, and genomic DNA was extracted from surrounding blood leukocytes with a TIANamp Blood DNA Kit (Tiangen Biotech, Beijing, China) according to the manufacturer's instruction. The DNA was kept at −20°C before analysis. The *IL-12B* gene polymorphisms were genotyped by polymerase chain reaction (PCR) with sequence-specific primers. About 10% of the UC patients and controls were subjected to repeated genotyping; the genotypes were 100% concordant. The IL-12B levels in blood plasma were tested using an enzyme-linked immunosorbent assay kit (Boster Ltd). The levels of IL-12B were calculated by referring to a standard curve, according to the manufacturer's instructions.

2.3 | Statistical analysis

All data were analyzed using SPSS 22.0 (SPSS Inc). The observed number of genotypes was compared, via chi-square (χ^2) analysis, with that expected in accordance with Hardy-Weinberg equilibrium (HWE). The distributions of alleles and genotypes were compared between the cases and controls. Analyses stratified by alcohol consumption, smoking, sex, and age were conducted. $P < .05$ was taken to indicate significance. The correlations of the *IL-12B* gene polymorphisms with the risk of UC were assessed via logistic regression, and odds ratio (OR) and 95% confidence intervals (CIs) were calculated.

3 | RESULTS

3.1 | Characteristics of the study population

The participant characteristics are summarized in Table 1. The mean age of controls and patients was 35.98 and 36.77 years old

TABLE 1 Patient demographics and risk factors in ulcerative colitis

Characteristics	Case (N = 367)	Control (N = 456)	*P*
Age	35.98 ± 12.45	36.77 ± 9.94	.321
BMI	24.41 ± 1.60	24.28 ± 1.46	.235
Sex			
Male	200 (54.5%)	227 (49.8%)	.178
Female	167 (45.5%)	229 (50.2%)	
Smoking			
Yes	201 (54.8%)	176 (38.6%)	<.001
No	166 (45.2%)	280 (61.4%)	
Alcohol			
Yes	197 (53.7%)	149 (32.7%)	<.001
No	170 (46.3%)	307 (67.3%)	
Lesion location of UC			
Distal colitis (E1 + E2)	229 (62.4%)		
Extensive colitis (E3)	138 (37.6%)		
Severity of UC			
Mild	158 (43.1%)		
Intermediate	175 (47.7%)		
Severe	34 (9.3%)		
Treatment			
SASP/5-ASA	247 (67.3%)		
Prednisone	109 (29.7%)		
Antibiotics	105 (28.6%)		
Immunosuppressive	9 (2.5%)		
Infliximab	1 (0.3%)		
Colectomy	1 (0.3%)		

on average, respectively. The mean body mass index (BMI) was 24.41 and 24.28 kg/m^2, respectively. No significant between-group differences were identified in age, sex, or BMI, but differences in the proportions of smokers and alcohol consumers were seen ($P < .001$). We also investigated other clinical parameters of UC patients, such as lesion location, severity, and the treatment received.

3.2 | Associations of *IL-12B* gene rs6887695 and rs2288831 polymorphisms with UC risk

The genotype and allele distributions of the *IL-12B* gene polymorphisms both differed significantly between cases and controls (Table 2). The HWE test revealed no obvious difference in genotypic frequency between the rs6887695 and rs2288831 single nucleotide polymorphisms (SNPs) among the controls. Logistic regression analyses showed that the CC genotype of rs6887695 significantly increased the risk of UC (CC vs. GG: adjusted OR and 95% CI, 1.77 (1.09-2.84), $P = .002$; CC + GC vs GG: 1.40(1.08-1.88), $P = .016$) (Table 2). Allele genetic analysis revealed the C allele of rs6887695 was associated with a higher risk of UC (C vs. G: 1.32(1.07-1.63), $P = .009$) (Table 2). In addition, the CC genotype and C allele of rs2288831 polymorphism were associated with an increased risk of UC. Subgroup analyses showed a significantly higher risk of UC in females, alcohol drinkers, and those aged <40 years (Table 3).

3.3 | Correlations between *IL-12B* gene polymorphisms and clinical characteristics of UC

Next, we explored whether the *IL-12B* gene rs6887695 and rs2288831 polymorphisms were correlated with the clinical features of UC patients (Table 4). The GC, CC, and GC + CC genotypes of rs6887695 polymorphism were more common in UC patients with a lesion located in E3 (beyond the splenic flexure), and in those receiving non-salicyl azo sulfonamide pyridine (SASP) treatment. The rs2288831 polymorphism was associated with lesion location and SASP treatment for UC. In other words, *IL-12B* gene rs6887695 and rs2288831 polymorphisms were related to the clinical features of UC patients.

3.4 | Associations between *IL-12B* gene polymorphisms and IL-12B plasm levels

Finally, we explored the link between *IL-12B* gene polymorphisms and IL-12B plasm levels. Data showed that CC genotype carriers showed increased IL-12B plasma levels compared with GG genotype

TABLE 2 Genotype frequencies of IL12B gene polymorphisms in cases and controls

Models	Genotype	Case (n, %)	Control (n, %)	OR (95% CI)	*P*-value	[a]OR (95% CI)	[a]*P*-value
rs6887695							
Codominant	GG	156 (42.6%)	231 (50.9%)	1.00 (reference)	-	1.00 (reference)	–
Heterozygote	GC	166 (45.4%)	186 (41.0%)	1.33 (0.99-1.78)	.056	1.34 (0.95-1.75)	.058
Homozygote	CC	44 (12.0%)	37 (8.1%)	**1.76 (1.09-2.85)**	**.021**	**1.77 (1.09-2.84)**	**.020**
Dominant	GG	156 (42.6%)	231 (50.9%)	1.00 (reference)	-	1.00 (reference)	–
	CC + GC	210 (57.4%)	223 (49.1%)	**1.40 (1.06-1.85)**	**.017**	**1.40 (1.08-1.88)**	**.016**
Recessive	GC + GG	322 (88.0%)	417 (91.9%)	1.00 (reference)	-	1.00 (reference)	–
	CC	44 (12.0%)	37 (8.1%)	1.54 (0.97-2.44)	.068	1.58 (0.99-2.37)	.065
Allele	G	478 (65.3%)	648 (71.4%)	1.00 (reference)		1.00 (reference)	–
	C	254 (34.7%)	260 (28.6%)	**1.32 (1.07-1.63)**	**.009**	-	–
rs2288831							
Codominant	TT	109 (29.8%)	167 (36.8%)	1.00 (reference)		1.00 (reference)	
Heterozygote	TC	183 (50.0%)	216 (47.7%)	1.29 (0.95-1.76)	.109	1.29 (0.95-1.76)	.109
Homozygote	CC	74 (20.2%)	70 (15.4%)	**1.61 (1.07-2.42)**	**.022**	**1.60 (1.09-2.41)**	**.020**
Dominant	TT	109 (29.8%)	167 (36.8%)	1.00 (reference)		1.00 (reference)	
	CC + TC	257 (70.2%)	286 (63.2%)	**1.37 (1.02-1.84)**	**.037**	**1.35 (1.05-1.80)**	**.035**
Recessive	TT + TC	292 (79.8%)	383 (84.6%)	1.00 (reference)		1.00 (reference)	
	CC	74 (20.2%)	70 (15.4%)	1.38 (0.96-1.98)	.078	1.34 (0.90-1.93)	.074
Allele	T	401 (54.8%)	550 (60.7%)	1.00 (reference)		1.00 (reference)	
	C	331 (45.2%)	356 (39.3%)	**1.28 (1.05-1.55)**	**.018**		

Note: The genotyping was successful in 366 cases and 454 controls for rs6887695; the genotyping was successful in 366 cases and 453 controls for rs2288831; Bold values are statistically significant ($P < .05$).

[a]Adjust age and sex.

TABLE 3 Stratified analyses between rs6887695 and rs2288831 polymorphisms and the risk of ulcerative colitis

	(Case/control)						
Variable	**GG**	**GC**	**CC**	**GC vs. GG**	**CC vs. GG**	**CC vs. GC + GG**	**CC + GC vs. GG**
Sex							
Male	87/118	92/90	20/18	1.40 (0.94-2.10); 0.100	1.51 (0.75-3.02); 0.247	1.29 (0.66-2.51); 0.462	1.42 (0.97-2.08); 0.073
Female	69/113	74/96	24/19	1.26 (0.83-1.93); 0.284	**2.07 (1.06-4.05); 0.034**	1.85 (0.98-3.50); 0.060	1.40 (0.93-2.09); 0.105
Smoking							
Yes	93/86	104/74	34/14	**1.92 (1.23-2.98); 0.004**	**3.31 (1.64-6.67); 0.001**	**2.33 (1.20-4.50); 0.012**	**2.14 (1.41-3.26); 0.001**
No	63/145	62/112	10/23	0.87 (0.58-1.31); 0.504	0.68 (0.31-1.49); 0.333	0.72 (0.33-1.55); 0.399	0.84 (0.57-1.23); 0.370
Alcohol							
Yes	63/74	111/64	23/11	**2.04 (1.29-3.21); 0.002**	**2.46 (1.11-5.43); 0.026**	1.66 (0.78-3.52); 0.188	**2.10 (1.36-3.26); 0.001**
No	93/157	55/122	21/26	0.77 (0.51-1.16); 0.205	1.36 (0.73-2.56); 0.334	1.52 (0.83-2.79); 0.180	0.87 (0.60-1.27); 0.480
Age (years)							
<40	130/108	153/95	43/18	1.35 (0.94-1.94); 0.102	**1.98 (1.08-3.64); 0.027**	1.71 (0.96-3.04); 0.071	**1.45 (1.03-2.05); 0.034**
≥40	26/123	13/91	1/19	0.68 (0.33-1.39); 0.285	0.25 (0.03-1.94); 0.185	0.29 (0.04-2.22); 0.233	0.60 (0.30-1.21); 0.155
rs2288831	**TT**	**TC**	**CC**	**TC vs. TT**	**CC vs. TT**	**CC vs. TC + TT**	**CC + TC vs. TT**
Sex							
Male	65/85	100/106	34/35	1.23 (0.81-1.89);0.335	1.27 (0.72-2.25);0.466	1.13 (0.67-1.88);0.694	1.24 (0.83-1.85);0.310
Female	44/82	83/110	40/35	1.41 (0.88-2.24)0.162	**2.13 (1.19-3.82);0.012**	**1.73 (1.04-2.87);0.038**	**1.58 (1.02-2.45);0.049**
Smoking							
Yes	69/60	110/86	52/28	1.11 (0.71-1.59);0.650	1.62 (0.91-2.87);0.114	1.52 (0.91-2.52);0.130	1.24 (0.81-1.88);0.334
No	40/107	73/130	22/42	1.50 (0.95-2.39);0.105	1.40 (0.75-2.63);0.326	0.89 (0.51-1.57);0.776	1.48 (0.95-2.30);0.100
Alcohol							
Yes	39/56	110/79	25/14	**2.00 (1.21-3.30);0.008**	**2.56 (1.19-5.55);0.022**	1.62 (0.81-3.24);0.230	**2.08 (1.28-3.39);0.003**
No	70/111	73/137	49/56	0.84 (0.56-1.27);0.461	1.39 (0.85-2.26);0.214	1.52 (0.98-2.35);0.071	1.08 (0.74-1.57);0.706
Age (years)							
<40	88/75	173/119	65/29	1.24 (0.84-1.82);0.279	**1.91 (1.12-3.26);0.018**	**1.67 (1.04-2.68);0.038**	1.37 (0.95-1.98);0.106
≥40	21/92	10/97	9/41	0.45 (0.20-1.01);0.054	0.96 (0.41-2.28);0.559	1.34 (0.59-3.02);0.509	0.60 (0.31-1.18);0.165

Note: Bold values are statistically significant ($P < .05$).

for rs6887695 polymorphism. As for rs2288831 polymorphism, CC or TC genotype was related to elevated IL-12B levels compared with TT genotype (Table 5).

4 | DISCUSSION

We found that *IL-12B* gene rs6887695 and rs2288831 polymorphisms were associated with an increased risk of UC, as were female gender, alcohol consumption, and an age < 40 years. The rs6887695 and rs2288831 SNPs were also associated with lesion location (E3) and UC treatment.

The dynamic interplay between immune and non-immune cells may contribute to the intestinal tissue damage seen in UC, which is mediated by various cytokines.[24] Cytokines are involved in the onset of clinical manifestations and complications in UC.[25] Bunte et al found that the IL-23/IL-17 immune axis plays an important role in the pathogenesis of UC.[26] Suppression of IL-6 was useful for attenuating gut inflammation in colitis.[27] A clinical study showed that anti-IL-6 treatment could induce clinical remission in CD patients.[28] Previous studies showed that blocking IL-1R has beneficial effects on the IL-1 signaling pathway in IBD, and is a promising approach for the treatment of IBD.[29] IL-23 was found to be up-regulated in the inflamed intestine of UC patients.[30] IL-12 is regarded as a biomarker for assessing disease activity in UC patients.[12] Ustekinumab (anti-IL-12/23) is available for the treatment of UC.[31,32] Thus, treatments targeting cytokines may be useful for IBD.[31,33]

IL-12B, a potent immunomodulatory cytokine, participates in both conditioned and unconditioned immune reactions. In recent years, the association of IL-12B gene polymorphisms with autoimmune disorders, such as psoriasis, CD, type 1 diabetes, systemic lupus erythematosus, and autoimmune thyroid disease, has been widely explored. A recent meta-analysis showed that the *IL-12B* gene rs6887695 polymorphism was highly associated with susceptibility to autoimmune diseases, but not with CD.[34] However, the meta-analysis did not investigate the link between *IL-12B* gene rs6887695 and the risk of UC or IBD.[34]

TABLE 4 The associations between IL12B rs6887695 and rs2288831 polymorphisms and clinical characteristics of ulcerative colitis

Characteristics	Genotype distributions			
rs6887695	**GG**	**GC**	**CC**	**GC + CC**
Lesion location of UC				
E3/E1 + E2	46/110	71/95	21/23	92/118
OR (95% CI); *P*-value	1.0 (reference)	**1.79 (1.13-2.84); .014**	**2.18 (1.10-4.33); .025**	**1.86 (1.20-2.89); .005**
Severity of UC				
Severe/mild	18/63	14/74	2/21	16/95
OR (95% CI); *P*-value	1.0 (reference)	0.66 (0.31-1.44); .297	0.33 (0.07-1.56); .163	0.59 (0.28-1.24); .164
Severity of UC				
Severe/intermediate	18/75	14/78	2/21	16/99
OR (95% CI); *P*-value	1.0 (reference)	0.75 (0.35-1.61); .458	0.40 (0.09-1.85); .239	0.67 (0.32-1.41); .293
Treatment				
NOT/SASP	39/117	60/106	20/24	80/130
OR (95% CI); *P*-value	1.0 (reference)	**1.70 (1.05-2.75); .031**	**2.50 (1.25-5.01); .010**	**1.85 (1.17-2.92); .009**
rs2288831	**TT**	**TC**	**CC**	**TC + CC**
Lesion location of UC				
E3/E1 + E2	29/80	73/110	36/38	109/148
OR (95% CI); *P*-value	1.0 (reference)	**1.83 (1.09-3.07); .021**	**2.61 (1.40-4.87); .002**	**2.03 (1.24-3.32); .004**
Severity of UC				
Severe/mild	10/40	11/89	13/29	24/118
OR (95% CI); *P*-value	1.0 (reference)	0.49 (0.19-1.26); .134	1.79 (0.69-4.65); .227	0.81 (0.36-1.85); .622
Severity of UC				
Severe/intermediate	10/59	11/83	13/32	24/115
OR (95% CI); *P*-value	1.0 (reference)	0.78 (0.31-1.96); .599	2.40 (0.95-6.08); .061	1.23 (0.55-2.75); .611
Treatment				
NOT/SASP	18/91	66/117	35/39	101/156
OR (95% CI); *P*-value	1.0 (reference)	**2.85 (1.58-5.14); .000**	**4.54 (2.30-8.97); .000**	**3.27 (1.86-5.75); .000**

Note: Bold values are statistically significant ($P < .05$).

TABLE 5 Mean serum IL-12B values in pg/mL for IL-12B gene rs6887695 and rs2288831 polymorphisms

Genotypes	N (frequency)	Mean CRP (pg/mL)	*p*-value
rs6887695 G > C			
GG	156 (42.6%)	1.15	ref
GC	166 (45.4%)	1.37	.068
CC	44 (12.0%)	4.53	**.000**
rs2288831			
T > C			
TT	109 (29.8%)	1.43	ref
TC	183 (50.0%)	2.13	**.023**
CC	74 (20.2%)	4.78	**.000**

Note: Mean serum IL-12B levels for IL-12B gene rs6887695 and rs2288831 polymorphisms. Bold values are statistically significant ($P < .05$).

Several recent studies have explored the association between *IL-12B* gene rs6887695 polymorphism and UC risk. Márquez et al studied the role of this polymorphism in a Spanish population (344 CD patients, 363 UC patients, and 547 controls).[21] They observed that *IL-12B* rs6887695 was weakly associated with IBD and more strongly associated with UC; it was not a major risk factor for CD.[21] A Danish study including 624 CD patients, 411 UC patients, and 795 controls showed *IL-12B* gene rs6887695 was associated with a higher risk of CD, but not UC, indicating that this polymorphism may specifically affect CD susceptibility.[18] Another study of Caucasians performed in Germany revealed a weak association between the IL-12B rs6887695 polymorphism and the risk of IBD, but not with UC or CD.[19] In summary, the relationship between the IL-12B rs6887695 polymorphism and UC risk differed among Caucasian populations. The following factors may explain these inconsistencies. First, the genotype distributions of the IL-12B rs6887695 polymorphism differed among the populations. Second, both the severity and location of the lesions in

the UC patients were inconsistent among the studies. Third, there was clinical heterogeneity among the UC populations. Fourth, the sample sizes differed among the studies. Regarding Asian populations, a Japanese study found that the IL-12B rs6887695 polymorphism increased the risk of CD, but not UC.[20] Our study found that this SNP may increase the risk of UC in Chinese Han populations. Regarding the rs2288831 polymorphism, one Korean study investigated the relationship between this SNP and IBD risk, reporting that it was associated with CD, but not UC.[22] They observed that *IL12B* gene rs2288831 polymorphism was associated with the risk of CD, but not UC.[22] In this study, we found that *IL12B* gene rs2288831 polymorphism increased the risk of UC among this Chinese Han population. To the knowledge, this is the first study to show that *IL12B* gene rs2288831 polymorphism was related to increased risk for UC. Further studies in other populations should be performed to validate this finding in the future.

Next, we assessed the association of *IL-12B* gene polymorphisms with UC risk in subgroup analyses. The data implied that the rs6887695 and rs2288831 polymorphisms may increase the risk of UC in females, alcohol consumers, and people aged <40 years. Last, we explored the link between *IL-12B* gene polymorphisms and the clinical data of the UC patients, and found associations with lesion location (E3) and SASP therapy. Some carriers of the rs6887695 and rs2288831 polymorphisms showed a good response to SASP treatment. Individuals with certain genotypes receiving SASP treatment showed a decreased risk of UC in this study. N-acetyltransferase 2 (NAT2) is an important enzyme catalyzing the N-acetylation of SASP. Chen et al reported that the NAT2 slow acetylator genotype was associated with adverse effects of SASP treatment for IBD.[35] Thus, we assumed that IL-12B gene polymorphisms may affect the response to this treatment.

Some limitations of this case-control study should be mentioned. First, the relatively small sample size may have yielded false-positive results. Second, selection bias was inevitable. Third, we did not seek to determine why the rs6887695 and rs2288831 polymorphisms increased the risk of UC. Last, the interactive effects of environmental and genetic factors should be explored.

In conclusion, IL-12B gene rs6887695 and rs2288831 polymorphisms increased the risk of UC in our Chinese Han population. Further research involving individuals from other Asian populations is urgently needed.

ETHICAL APPROVAL

All experimental procedures involving humans were conducted as per ethical criteria of institutional and/or national research committee as well as the 1964 Helsinki Declaration and its later modifications or comparable ethical standards.

INFORMED CONSENT

Informed consent was obtained from all individuals in the study.

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