

Association of *MYO9B* gene polymorphisms with inflammatory bowel disease in Chinese Han population

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tion was found with ulcerative colitis in the comparison between the subgroups, the frequencies of rs962917 and rs1545620 were different in the Crohn's disease (CD) subgroup with ileocolitis (CC vs CT and TT, $P = 0.014$; and AA vs AC and CC, $P = 0.022$, respectively). rs1545620 variants appear to be the genetic susceptibility factor for perianal disease in CD patients (AA vs AC CC, $P = 0.029$). In addition, the L/M ratio was significantly higher in IBD patients than in controls (0.065 ± 0.013 vs 0.020 ± 0.002 , $P = 0.02$), but no association was found between the *MYO9B* gene and the L/M ratio in IBD patients.

CONCLUSION: *MYO9B* gene polymorphisms may influence the sub-phenotypic expression of CD in China. No association between these *MYO9B* polymorphisms and intestinal permeability in IBD patients was found.

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Abstract

AIM: To explore the association of *MYO9B* gene polymorphisms with clinical phenotypes and intestinal permeability of individuals with inflammatory bowel disease (IBD) in China.

METHODS: A total of 442 IBD patients and 402 healthy volunteers were genotyped for two single nucleotides (rs962917 and rs1545620) using the ligase detection reaction and polymerase chain reaction. Allelic and genotype frequency analyses were performed for the two groups. Intestinal permeability was evaluated using lactulose (L) and mannitol (M) excretion. The association of *MYO9B* gene polymorphisms with intestinal permeability between the normal and high intestinal permeability groups was analyzed.

RESULTS: Overall, there was no significant difference in the genotypic and allelic frequencies of *MYO9B* between IBD patients and controls. Although no associa-

Key words: Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; *MYO9B*; Genetic susceptibility; Intestinal permeability

Core tip: An association between *MYO9B* gene polymorphisms and inflammatory bowel disease (IBD) in the Chinese Han population has not yet been confirmed. The authors aimed to explore the association of *MYO9B* gene polymorphisms with the clinical phenotypes and intestinal permeability of IBD in China. The results suggested that *MYO9B* gene polymorphisms may influence the sub-phenotypic expression of Crohn's disease but failed to confirm an association between the *MYO9B* polymorphisms and intestinal permeability in Chinese Han IBD. These findings indicate that the *MYO9B* gene may differ among IBD patients of various races from various regions.

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INTRODUCTION

Inflammatory bowel disease (IBD), which includes Crohn's disease (CD) and ulcerative colitis (UC), is a chronic recurrent inflammation of the gastrointestinal tract of unknown origin^[1]. Environmental influences, immunological factors, and genetic background may play important roles in its etiopathogenesis^[2-4]. Since the initial identification of *CARD15* as a CD susceptibility gene in 2001, genetic studies have shown that there are numerous genetic susceptibility factors for IBD^[5-10]. The genetic background of IBD may be different in Asian individuals compared with Western populations. For example, variations in *OCTN* or *CARD15* are generally accepted to be associated with susceptibility to CD in Western populations^[11,12]. However, these associations have not been confirmed in Chinese individuals^[13].

Studies have provided evidence that IBD may result from a genetic predisposition that leads to defects in mucosal immune regulatory cells, barrier leakage, and susceptibility to environmental triggers, including luminal bacteria and specific antigens^[14,15]. The complex interaction of genetic, microbial, and environmental factors may result in continuous activation of the mucosal immune system, leading to IBD^[15]. *MYO9B* variants have been reported to potentially be involved in IBD pathogenesis^[16]. The *MYO9B* gene, encoding myosin IXB, was first identified as a susceptibility gene for celiac disease in a Dutch cohort study^[17]. This gene is a single motor protein with a Rho GTPase activating domain, and is involved in epithelial cell tight junction assembly and cytoskeletal remodeling^[18,19]. Cooney *et al.*^[16] recently genotyped 8 *MYO9B* single nucleotide polymorphisms (SNPs) in 652 CD patients, 650 UC patients, and 1190 controls and reported a significant association between genetic variants in *MYO9B* and IBD, which indicated that *MYO9B* variants may be involved in IBD pathogenesis. This involvement may be due to defects in *MYO9B*-dependent intestinal epithelial cells because IBD is often characterized by increased permeability of the intestinal epithelium^[20-22].

These findings have not been confirmed in China. Therefore, it is necessary to explore the association of *MYO9B* gene polymorphisms with IBD in the Chinese Han population and to assess the impact of *MYO9B* genetic variations on intestinal permeability in IBD. Most studies on the *MYO9B* gene polymorphisms associated with intestinal permeability mainly investigated rs962917 and rs1545620^[23,24]. Therefore, our study genotyped these two *MYO9B* SNPs to investigate the association of *MYO9B* gene polymorphisms with IBD clinical features and with the permeability of the intestinal mucosa in the Chinese Han population.

Table 1 Demographic and clinical characteristics of inflammatory bowel disease patients and controls, *n* (%)

	CD	UC	Controls
Total (<i>n</i>)	207	235	402
Sex (F/M)	91/116	106/129	196/206
Age at diagnosis			Mean age 40.21 ± 5.37
A1 (< 16 yr)	10 (4.8)	4 (1.7)	
A2 (17-40 yr)	133 (64.3)	127 (54.0)	
A3 (> 40 yr)	64 (30.9)	104 (44.3)	
Disease location, CD			
L1 (terminal ileum)	75 (36.2)	Proctitis 71 (30.2)	
L2 (colonic location)	42 (20.3)	Left-sided 103 (43.8)	
L3 (ileocolitis)	90 (43.5)	Extensive 61 (26.0)	
L4 ¹ (upper gastrointestinal tract)	18 (8.7)		
Disease behavior, CD			
B1 (inflammatory disease)	63 (30.4)		
B2 (structuring disease)	85 (41.1)		
B3 (penetrating disease)	59 (28.5)		
P ² (perianal disease)	47 (22.7)		

¹L4 is a modifier that can be added to L1-L3 when concomitant upper gastrointestinal disease is present; ²Perianal disease was categorized as P, that could be added to B1-B3 when concomitant perianal disease is present. CD: Crohn's disease; UC: Ulcerative colitis.

MATERIALS AND METHODS

Patients and controls

IBD patients were consecutively recruited from the Department of Gastroenterology, the First Affiliated Hospital of Anhui Medical University between February 2006 and May 2012. Diagnosis of IBD was based on established clinical, endoscopic, radiological, and histological criteria^[1]. The study cohort consisted of 235 UC patients (129 men; mean age: 42.14 ± 10.69 years) and 207 CD patients (116 men; mean age: 37.15 ± 9.25 years). The phenotype of these patients was classified based on age at diagnosis, location, and behavior of disease according to the Montreal classification of IBD^[25]. Demographic and clinical characteristic data are presented in Table 1. The control group (206 men; mean age: 40.21 ± 5.37 years) was recruited from healthy individuals from the medical examination center. There were no significant differences between the case and control groups with respect to age or sex. The Han ethnic group, with a population of 1225932641 (according to the 6th Population Survey of China in 2010), lived in most provinces of China. In this study, both patients and controls were of Han ancestry and were unrelated inhabitants in Anhui province. Approval of the protocol was obtained from the Ethics Committee of the First Affiliated Hospital of Anhui Medical University.

Measurements gene determination method

Venous blood (5 mL) was collected from each patient and control. DNA was extracted in accordance with the kit's instructions (Axygen Corp., CA, United States) and

preserved at -20 °C. Two mononucleotide polymorphic sites of the *MYO9B* gene, rs962917 and rs1545620, were detected by polymerase chain reaction (PCR)/ligase detection reaction (LDR). Primers and probes were synthesized by Shanghai Biotech Corp. (Shanghai, China). For rs962917, the sequence of the forward primer was 5'-CCTCCTGCCTCATACCGTAA-3', and the sequence of the reverse primer was 5'-AATC-CACGTCACGAGACGAC-3'; the LDR probe set included a fluorescent probe (P-CGTCACCTGTT-TATTGCTGCTTTTTTTTTT TTTTTTTTTT-FAM) and a pair of detection probes (5'-TTTTTTTTTTTTTTT TTGCAGGGCTCAGCGACTCCCTCCG-3' and 5'-TTTTTTTTTTTTTTTTTTTG CAGGGCTCAGC-GACTCCCTCCA-3'). For rs1545620, the sequence of the forward primer was 5'-GCGGATGATGCTCT-GTTTCT-3', and the sequence of the reverse primer was 5'-AAGTAGACGTCCTTCACGG-3'; the LDR probe set included a fluorescence probe (P-CGTCACCT-GTTTATGCTGCTTTTTT TTTTTTTTTT-FAM) and a pair of detection probes (5'-TTTTTTTTTTTTTT TTTTGGCTGCCGTGTACCTCCAGGCCT-3' and 5'-TTTTTTTTTTTTTTTTTT TTTGGCTGCCGTG-TACCTCCAGGCCG-3'). Then, 20 µL of the multiple PCR mixture was prepared for multiple PCR amplification, which included 2 µL of 1× buffer solution, 3.0 mmol/L MgCl₂, 2 mmol/L dNTPs, 0.4 µL each of the positive and negative primers, 0.4 µL of Tag polymerase 1U (Qiagen Corp., Hilden, Germany), 4 µL of 1× Q-solution, and 50 ng of genomic DNA. Double distilled water was added to the final volume. Then we performed initial denaturation at 95 °C for 2 min, 35 cycles of 94 °C for 30 s, 62 °C for 90 s, and 72 °C for 60 s, and final extension at 72 °C for 10 min. 3% agarose gel electrophoresis was used to detect the PCR products.

In addition, 10 µL of multiple LDR mixture was prepared for the multiple LDR, which included 1 µL of 1× buffer solution, 1 µL of the probe mix (0.05 pmol/L/each), 0.05 µL of Taq DNA ligase (NEB Corp., Beijing, China), and 2 µL of the PCR products (50 ng/µL). Double distilled water was added to the final volume. After sufficient mixing, the solution was centrifuged for the LDR, which consisted of the following steps: initial denaturation at 95 °C for 2 min, followed by 30 cycles of denaturation at 94 °C for 15 s and annealing at 50 °C for 25 s. The LDR product was sequenced with a 377 DNA Sequencer (ABI Corp., United States).

Evaluation of intestinal permeability

Intestinal permeability was evaluated using the lactulose (L) and mannitol (M) excretion test in all patients and healthy controls. After an overnight fast, the subjects drank 100 mL of the test solution containing 10 g of L and 5 g of M. No food or drink other than water was allowed until completion of the test. Urine samples over the following 6 h were collected in a plastic tube containing 2% thimerosal as a preservative. The total volume was recorded, and 20 mL of each sample was stored at -20 °C until analysis. The urinary concentrations of L

and M were measured by high-pressure liquid chromatography with pulsed electrochemical detection (HPLC-PED)^[26]. Intestinal permeability was evaluated based on the ratio of the concentrations of L and M (L/M) in the urine^[27]. As previously described, intestinal permeability was considered normal when the L/M ratio was less than 0.03^[23].

Statistical analysis

SPSS 13.0 (SPSS Inc., Chicago, IL, United States) statistical software was used for data analysis. Qualitative variables are expressed as percentages, and quantitative variables are calculated as the means. To compare groups, we used χ^2 tests. Haploview Software ver. 3.2 (<http://www.broad.mit.edu/mpg/haploview>) was used for testing the Hardy-Weinberg equilibrium, linkage disequilibrium, and transmission disequilibrium. Logistic regression was applied to model the association of SNPs with the sub-phenotypes. Odds ratios (OR) with 95% CIs were determined. For all allelic and genotype analyses, Bonferroni's correction for the number of SNPs tested was used to correct for multiple testing and a *P* value < 0.05 indicated statistical significance.

RESULTS

Demographics and clinical features

A total of 442 patients (245 male, 197 female) with IBD and 402 healthy controls, all of Han ancestry from China and none with a positive family history of IBD, were enrolled. There were no significant differences in age and sex between the two groups (Table 1). The age at diagnosis, disease location, and the behavior of IBD disease are shown in Table 1. According to the Montreal classification^[25], the most frequent disease location in our UC patients was the left side of the colon (43.8%), followed by the rectum (30.2%) and extensive colon (26.0%). In the CD patients, 43.5% exhibited ileocolitis, 36.2% exhibited pure ileitis, 20.3% had disease in the colon region, and only 8.7% had upper intestinal tract involvement. In addition, 41.1% of the CD patients had stricturing disease, 30.4% had nonstricturing nonpenetrating disease, and 28.5% had a penetrating phenotype, with concomitant perianal disease in 22.7% patients.

Association of *MYO9B* variants with IBD

A total of 442 IBD patients and 402 controls were genotyped for two *MYO9B* gene polymorphisms, rs962917 and rs1545620 (Tables 2 and 3). Both allelic and genotypic frequencies in the IBD and control groups were evaluated for Hardy-Weinberg equilibrium (*P* > 0.05). In the IBD patients, the rs962917 genotype frequencies of CC, CT, and TT were 8.6%, 36.9%, and 54.5%, respectively. For rs1545620, the genotype frequencies of AA, AC, and CC were 14.3%, 28.9%, and 56.8%, respectively. No significant differences in the allelic and genotypic frequencies were observed between the controls and IBD patients for either SNP. When the CD and UC patients were analyzed separately, no association was found

Table 2 Allele and genotype frequencies of the MYO9B SNPs rs962917 in inflammatory bowel disease patients *vs* controls, *n* (%)

Control (<i>n</i> = 402)		IBD (<i>n</i> = 442)			CD (<i>n</i> = 207)			UC (<i>n</i> = 235)		
		OR (95%CI)	<i>P</i> _{corr}		OR (95%CI)	<i>P</i> _{corr}		OR (95%CI)	<i>P</i> _{corr}	
GF										
CC	27 (6.6)	38 (8.6)	1.23 (0.79-1.65)	0.64	21 (10.1)	1.52 (0.63-2.17)	0.09	17 (7.2)	1.09 (0.82-1.40)	0.75
CT	173 (42.5)	163 (36.9)	0.87 (0.52-1.24)	0.26	79 (38.2)	0.81 (0.47-1.33)	0.24	84 (35.8)	0.83 (0.69-1.14)	0.31
TT	207 (50.9)	241 (54.5)	1.07 (0.82-1.41)	0.97	107 (51.7)	1.02 (0.85-1.24)	0.83	134 (57.0)	1.12 (0.67-1.46)	0.48
AF										
C	227 (27.9)	239 (27.0)	0.92 (0.66-1.71)	0.52	121 (29.2)	1.06 (0.75-1.41)	0.66	118 (25.1)	0.86 (0.67-1.35)	0.38
T	587 (72.1)	645 (73.0)	1.02 (0.81-1.30)	0.77	293 (70.8)	0.98 (0.61-1.59)	0.49	352 (74.9)	1.14 (0.82-1.33)	0.29

IBD: Inflammatory bowel disease; GF: Genotype frequencies; AF: Allele frequencies; OR: Odds ratio; CI: Confidence interval; SNP: Single nucleotide polymorphism; CD: Crohn's disease. Genotype and allele frequencies in patients and controls were compared using the χ^2 test; *P*_{corr}: Corrected *P* value.

Table 3 Allele and genotype frequencies of the MYO9B SNPs rs1545620 in IBD patients *vs* controls, *n* (%)

Controls (<i>n</i> = 402)		IBD (<i>n</i> = 442)			CD (<i>n</i> = 207)			UC (<i>n</i> = 235)		
		OR (95%CI)	<i>P</i> _{corr}		OR (95%CI)	<i>P</i> _{corr}		OR (95%CI)	<i>P</i> _{corr}	
GF										
AA	42 (10.4)	63 (14.3)	1.47 (0.56-3.85)	0.43	26 (12.6)	2.01 (0.71-5.66)	0.21	37 (15.7)	1.26 (0.31-3.25)	0.61
AC	135 (33.6)	128 (28.9)	0.86 (0.62-1.94)	0.29	63 (30.4)	0.91 (0.27-2.49)	0.07	65 (27.7)	0.82 (0.57-2.61)	0.37
CC	225 (56.0)	251 (56.8)	1.03 (0.37-2.19)	0.71	118 (57.0)	1.35 (0.33-2.19)	0.57	133 (56.6)	1.14 (0.61-3.77)	0.84
AF										
A	219 (27.2)	254 (25.9)	0.96 (0.44-2.91)	0.61	115 (27.8)	1.09 (0.39-2.46)	0.37	139 (29.6)	1.16 (0.57-3.14)	0.57
C	585 (72.8)	727 (74.1)	1.07 (0.56-2.28)	0.77	299 (72.2)	0.92 (0.49-3.17)	0.42	331 (70.4)	0.92 (0.77-2.51)	0.39

IBD: Inflammatory bowel disease; GF: Genotype frequencies; AF: Allele frequencies; CD: Crohn's disease. Genotype and allele frequencies in patients and controls were compared using the χ^2 test; *P*_{corr}: Corrected *P* value.

Table 4 Genotype frequency of rs962917 and rs1545620 SNPs in Crohn's disease phenotypes, *n* (%)

CD (<i>n</i> = 207)	rs962917			rs1545620		
	CC (21)	CT (79)	TT (107)	AA (26)	AC (63)	CC (118)
Age, CD						
A1 (< 16 yr)	1 (4.7)	4 (5.1)	5 (4.7)	1 (3.9)	3 (4.7)	6 (5.1)
A2 (17-40 yr)	14 (66.7)	51 (64.6)	68 (63.6)	16 (61.5)	41 (65.1)	76 (64.4)
A3 (> 40 yr)	6 (28.6)	24 (30.3)	34 (31.7)	9 (34.6)	19 (30.2)	36 (30.5)
Disease location, CD						
L1	9 (42.8)	28 (35.4)	38 (35.5)	11 (42.3)	22 (34.9)	42 (35.6)
L2	6 (28.6)	15 (19.0)	21 (19.6)	7 (26.9)	12 (19.0)	24 (20.3)
L3	6 (28.6) ¹	36 (45.6)	48 (44.9)	8 (30.8) ²	29 (46.0)	52 (44.1)
L4	2 (9.5)	7 (8.9)	9 (8.4)	2 (7.7)	6 (9.5)	10 (8.5)
Disease behavior, CD						
B1	6 (28.6)	24 (30.4)	33 (30.8)	8 (30.8)	19 (30.2)	36 (30.5)
B2	9 (42.8)	32 (40.5)	44 (41.1)	11 (42.3)	26 (41.2)	48 (40.7)
B3	6 (28.6)	23 (29.1)	30 (28.1)	7 (26.9)	18 (28.6)	34 (28.8)
<i>P</i>	5 (23.8)	18 (22.9)	24 (22.4)	3 (11.5) ³	16 (25.3)	28 (23.7)

Genotype frequencies in patients and controls were compared using the χ^2 test; ¹*P* = 0.014 CC *vs* CT TT; ²*P* = 0.022 AA *vs* AC CC; ³*P* = 0.029 AA *vs* AC CC. CD: Crohn's disease.

for the two IBD phenotypes.

Association of MYO9B variants with disease phenotype

The contribution of the different genotypes of the two SNPs in IBD patients was investigated to verify whether the MYO9B variants affected the clinical features. In the CD patients, the genotype frequencies of rs962917 and rs1545620 appeared to be differently distributed in ileocolitis (CC *vs* CT and TT, *P* = 0.014; and AA *vs* AC and CC, *P* = 0.022, respectively). Of note, an association was identified between the rs1545620 genotypes and the

subgroup of perianal disease (AA *vs* AC and CC, *P* = 0.029) in CD (Table 4). However, in the UC patients, no significant association with any specific sub-phenotype was observed.

Association of MYO9B variants with intestinal permeability in IBD

The L/M ratio was significantly higher in IBD patients than in controls (0.065 ± 0.013 *vs* 0.020 ± 0.002 , *P* = 0.006). The IBD patients were divided into two groups according to intestinal permeability (L/M ≥ 0.03 or

Table 5 Genotype frequencies of rs962917 and rs1545620 SNPs in ulcerative colitis phenotypes, *n* (%)

UC (<i>n</i> = 235)	rs962917			rs1545620		
	CC (17)	CT (84)	TT (134)	AA (37)	AC (65)	CC (133)
Age, UC						
A1 (< 16 yr)	0 (0)	1 (1.1)	3 (2.2)	1 (2.7)	1 (1.5)	2 (1.5)
A2 (17-40 yr)	9 (52.9)	49 (58.4)	69 (51.5)	17 (45.9)	37 (56.8)	73 (54.9)
A3 (> 40 yr)	8 (47.1)	34 (40.5)	62 (46.3)	19 (51.4)	27 (41.5)	58 (43.6)
Disease location, UC						
Proctitis	4 (23.5)	23 (27.4)	44 (32.8)	11 (29.7)	20 (30.8)	40 (30.1)
Left-sided	7 (41.2)	39 (46.4)	57 (42.6)	16 (43.2)	28 (43.1)	59 (44.4)
Extensive	6 (35.3)	22 (26.2)	33 (24.6)	10 (27.1)	17 (26.1)	34 (25.5)

χ^2 test was applied to compare the number of genotypes between patients and controls; All *P* values were > 0.05.

Table 6 Association of *MYO9B* variants with intestinal permeability in IBD, *n* (%)

IBD		rs962917			rs1545620		
		CC (17)	CT (84)	TT (134)	AA (37)	AC (65)	CC (133)
UC (<i>n</i> = 235)	L/M \geq 0.03	10 (58.8)	38 (45.2)	71 (53.0)	16 (43.2)	30 (46.2)	73 (54.9)
	L/M < 0.03	7 (41.2)	46 (54.8)	63 (47.0)	21 (56.8)	35 (53.8)	60 (45.1)
CD (<i>n</i> = 207)		CC (21)	CT (79)	TT (107)	AA (26)	AC (63)	CC (118)
	L/M \geq 0.03	9 (42.9)	42 (53.2)	59 (55.1)	12 (46.2)	33 (52.4)	65 (55.1)
	L/M < 0.03	12 (57.1)	37 (46.8)	48 (44.9)	14 (53.8)	30 (47.6)	53 (44.9)

χ^2 test was applied to compare the number of genotypes between patients and controls; All *P* values were > 0.05. IBD: Inflammatory bowel disease; CD: Crohn's disease.

L/M < 0.03), as previously described^[23]. The analysis showed no significant differences in the genotype frequency between the normal and high intestinal permeability groups.

DISCUSSION

Genetic susceptibility is a key factor in the pathogenesis of IBD^[28]. A focus on the genetic background of IBD in different geographic areas or races may provide insight into possible etiologic factors. Genetic variation in the *MYO9B* gene might produce an effect on epithelial cell tight junction assembly and cytoskeletal remodeling^[29,30]. Because IBD is often characterized by increased permeability of the intestinal epithelium^[21,22], *MYO9B* gene polymorphisms are a very valuable target for the study of IBD. Our research suggested that *MYO9B* gene polymorphisms influence the sub-phenotypic expression of CD. The frequencies of rs962917 and rs1545620 were significantly different in the CD subgroups according to disease location. In particular, when patients with CD were divided into perianal or non-perianal lesion groups, an association was identified between rs1545620 genotypes and the subgroup of perianal disease (*P* = 0.029) (Table 5). Although the L/M ratio was significantly higher in the IBD patients than in the controls, there was no association between the *MYO9B* gene and the L/M ratio in the IBD patients (Table 6).

The pathologic process of IBD involves many factors, including immune dysfunction of the intestinal mucosa, infection, heredity, and the environment^[2-4]. Intestinal mucosal barrier dysfunction is an essential component of IBD pathogenesis^[15]. The increase in intestinal

mucosal permeability may lead to the displacement of intestinal antigenic substances, induce and aggravate intestinal inflammation and immunoreactivity, further damage the intestinal mucosal barrier, and increase intestinal mucosal permeability^[31]. Büning *et al.*^[32] found that the permeability of the small intestine significantly increased in 89 UC cases in the remission stage. We used L and M to evaluate intestinal mucosal permeability and found that patients with CD or UC had markedly higher L/M ratios than controls, which indicates that intestinal permeability in IBD patients is increased.

The changes in intestinal permeability are associated with closely linked intestinal epithelial cells and myosin contraction^[33]. *MYO9B*, the gene encoding myosin, has been shown to be associated with diseases in the digestive system^[17]. van Bodegraven *et al.*^[34] studied eight SNPs in the *MYO9B* gene, including six loci associated with digestive diseases, and found that the *MYO9B* gene is closely related to IBD and that the rs1545620 locus has a marked association with UC. Nunez *et al.*^[35] also found that *MYO9B* gene polymorphisms were correlated with UC, but not with CD, in a study in a Spanish population. In a study in an Italian population, the *MYO9B* gene and IBD were closely associated, indicating that the rs1545620 and rs962917 genotypes may increase the susceptibility to IBD^[23]. Few studies of *MYO9B* gene polymorphisms in IBD patients are available in China. Shi *et al.*^[36] studied candidate genes for UC in a Chinese Han population and selected two polymorphic loci of the *MYO9B* gene. Their study observed an association of the TT genotype of rs1545620 in *MYO9B* with UC (*P* = 0.0169, OR = 0.29, 95%CI: 0.11-0.78). However, our study failed to find a significant difference in the

genotype frequency and allele frequency distributions of rs962917 and rs1545620 between IBD patients and normal controls. rs962917 and rs1545620 gene polymorphisms were not distributed differently in the UC clinical subgroup in our study. Furthermore, the study by Amundsen *et al.*^[37] failed to support the notion that *MYO9B* is a susceptibility gene in UC.

An association study of the *MYO9B* gene in Italian patients with IBD reported that the allele frequencies of *MYO9B* SNPs were different in CD subgroups according to disease location, with a trend towards an increased frequency of upper gastrointestinal involvement ($P = 0.057$) and perianal disease ($P = 0.042$)^[23]. Our research also found that the frequencies of rs962917 and rs1545620 were significantly different in the CD subgroups according to disease location.

Whether *MYO9B* gene polymorphisms affect intestinal permeability remains unknown. Latiano *et al.*^[23] reported that *MYO9B* gene polymorphisms were not significantly related to intestinal mucosal permeability. We used L and M to evaluate intestinal mucosal permeability. These sugars are not involved in metabolism and are urinated in prototype. Their excretion rate in the urine can reflect changes in intestinal mucosal permeability. HPLC-PED, which was adopted to detect the concentrations of L and M, is highly efficient and sensitive. This study confirmed that patients with IBD have markedly higher L/M ratios than controls, indicating increased intestinal permeability in IBD patients. However, we failed to find a correlation of *MYO9B* genotypes with intestinal permeability.

In summary, a significant association between genetic variants in *MYO9B* and IBD has been reported, which indicates that *MYO9B* variants may be involved in IBD pathogenesis in Western populations. However, our study suggested that *MYO9B* gene polymorphisms may influence the sub-phenotypic expression of CD but did not find an association between these *MYO9B* polymorphisms and intestinal permeability in IBD patients from a Han population in China. These findings indicate that IBD patients from different races and regions may express distinct clinical IBD characteristics and that the influence of *MYO9B* gene polymorphisms differs.

COMMENTS

Background

Genetic variation in the *MYO9B* gene might predispose individuals to inflammatory bowel disease (IBD) according to studies performed in Western populations. Furthermore, IBD is often characterized by increased permeability of the intestinal epithelium.

Research frontiers

The association of *MYO9B* gene polymorphisms with IBD has been studied in Western countries, but the conclusions from these studies have not been confirmed in China.

Innovations and breakthroughs

This study explored the association of *MYO9B* gene polymorphisms with the clinical phenotypes and intestinal permeability of IBD in China. This results suggested that *MYO9B* gene polymorphisms may influence the sub-phenotypic expression of Crohn's disease but did not find an association between *MYO9B* polymorphisms and intestinal permeability in IBD patients from a Han popula-

tion in China. These findings indicate that *MYO9B* gene polymorphisms may play a small role in changing the intestinal mucosal permeability in Chinese Han IBD patients.

Applications

The conclusions of this study involving *MYO9B* gene polymorphisms in IBD patients from a Han population in China were different from those of studies in Western populations. Studies of the genetic background of IBD in different geographic areas or races may provide insights into possible etiologic factors.

Peer review

This is a study from China aiming to explore the association of *MYO9B* gene polymorphisms with the clinical phenotypes and intestinal permeability of IBD. The study, which possesses a logical presentation of facts with regard to the description of the patient cohort, the performance of experiments, and the analysis of data, will provide more information about *MYO9B* gene polymorphisms in IBD to other authors.

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