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BRIEF ARTICLE

Association between *NOD2/CARD15* gene polymorphisms and Crohn's disease in Chinese Zhuang patients

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Abstract

AIM: To assess the relationship between the P268S, JW1 and N852S polymorphisms and Crohn's disease (CD) susceptibility in Zhuang patients in Guangxi, China.

METHODS: Intestinal tissues from 102 Zhuang [48 CD and 54 ulcerative colitis (UC)] and 100 Han (50 CD and 50 UC) unrelated patients with inflammatory bowel disease and 72 Zhuang and 78 Han unrelated healthy individuals were collected in the Guangxi Zhuang Autonomous Region from January 2009 to March 2013. Genomic DNA was extracted using the phenol chloroform method. The P268S, JW1 and N852S polymorphisms were amplified using polymerase chain reaction (PCR), detected by restriction fragment length polymorphism (RFLP), and verified by gene sequencing.

RESULTS: Heterozygous mutation of P268S in the *NOD2/CARD15* gene was detected in 10 CD cases (six Zhuang and four Han), two Han UC cases, and one Zhuang healthy control, and P268S was strongly associated with the Chinese Zhuang and Han CD populations (P = 0.016 and 0.022, respectively). No homozygous mutant P268S was detected in any of the groups. No significant difference was found in P268S genotype and allele frequencies between UC and control groups (P > 0.05). Patients with CD who carried P268S were likely to be ⩽ 40 years of age (P = 0.040), but were not significantly different with regard to race, lesion site, complications, and other clinical features (P > 0.05). Neither JW1 nor N852S polymorphisms of the *NOD2/CARD15* gene were found in any of the subjects (P > 0.05).

CONCLUSION: P268S polymorphism may be associated with CD susceptibility in the Zhuang population in the Guangxi Zhuang Autonomous Region, China. In contrast, JW1 and N852S polymorphisms may not be related to CD susceptibility in these patients.

Key words: Crohn's disease; *NOD2/CARD15*; Single nucleotide polymorphisms

Core tip: In this study, P268S, JW1 and N852S polymorphisms of the *NOD2/CARD15* gene were genotyped using the PCR-RFLP method and gene sequencing, and the presence of P268S in Guangxi Zhuang Crohn's disease patients was identified. However, no JW1 or N852S mutants were found in this cohort.

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INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic recurrent inflammatory disease of the gastrointestinal tract and includes ulcerative colitis (UC) and Crohn's disease (CD). In recent years, the incidence of IBD has increased in Western populations with an East-West gradient existing in Europe and is progressively increasing in Asia[1-3]. A recent systematic review revealed that the highest annual incidence of CD was 12.7 per 100000 person-years in Europe, 20.2 per 100000 person-years in North America, and 5.0 per 100000 person-years in Asia and the Middle East[4]. The etiology and pathogenesis of IBD are not completely clear, which involve a complex interaction of factors such as genetics, immunology, environment, and infection[5,6]. Several pathways may be crucial for intestinal homeostasis in IBD, for example barrier function, epithelial restitution, microbial defense, innate immune regulation, adaptive immunity regulation, reactive oxygen species (ROS) generation, and autophagy[7].

Genetic susceptibility to CD shows significant ethnic differences. A recent meta-analysis of multiple genome-wide association studies confirmed that 71 CD susceptibility loci were detected in a European population[8]. However, the majority of these genes could not be verified in the Asian region[9-11]. *NOD2/CARD15* was the first verified predisposing gene for CD. Multiple single nucleotide polymorphisms (SNPs) of *NOD2/CARD15* were shown to be significantly associated with CD in Caucasian populations[12-14]. Our previous studies confirmed that the R702W, G908R, and L1007fs SNPs of the *NOD2* gene were not associated with CD and UC in a Chinese Zhuang population from Guangxi Zhuang Autonomous Region, China[15]. In recent years, some gene mutation sites of *NOD2/CARD15* such as P268S, JW1, N852S, D113N, D357A, I363F, and L550V were shown to confer CD susceptibility[16-18]. The P268S SNP of the *NOD2/CARD15* gene was also associated with Chinese Han CD susceptibility and its clinical features[19]. The JW1 SNP of the *NOD2/CARD15* gene was shown to be associated with CD in Chinese Han, Malay, and Indians in Malaysia[17]. The N852S SNP of the *NOD2/CARD15* gene was found to be significantly associated with CD in Ashkenazi Jewish populations[18]. However, there are no data on the correlation between the P268S, JW1, and N852S SNPs of the *NOD2/CARD15* gene and the Chinese Zhuang CD population in the Guangxi Zhuang Autonomous Region.

In view of the differences in data regarding the correlation between key regulatory genes and IBD susceptibility, the purpose of the present study was to investigate whether the known gene SNPs (P268S, JW1 and N852S) of the *NOD2/CARD15* gene determine susceptibility to CD in the Guangxi Zhuang population from the Guangxi Zhuang Autonomous Region, China. Guangxi has a large Zhuang population in which genetic diseases and genetic SNPs are unique. Therefore, research on the correlation between the P268S, JW1, N852S SNPs of the *NOD2/CARD15* gene and CD in Chinese Zhuang patients from the Guangxi Zhuang Autonomous Region is needed.

MATERIALS AND METHODS

Specimen collection

Intestinal tissues from 102 Zhuang (48 CD and 54 UC) and 100 Han (50 CD and 50 UC) unrelated patients with IBD were collected at the Gastroenterology Department, First Affiliated Hospital of Guangxi Medical University, from January 2009 to March 2013. The control group included 72 Zhuang and 78 Han unrelated healthy individuals who did not have liver or gastrointestinal diseases. All patients had a well-established diagnosis of UC or CD based on the modified criteria framed by the World Gastroenterology Organization in 2010[20]. This study was approved by the hospital ethics committee and all the patients or their families provided written informed consent.

DNA extraction

Intestinal mucosa samples were digested using 450 μL of TES buffer (pH = 8.0) which consisted of Tris-HCl, ethylene diamine tetraacetic acid, and sodium chloride, 50 μL sodium dodecyl sulfate (10%), and 5 μL proteinase K (20 g/L) in a 56 ℃ water bath for 4-6 h. The supernatant was successively extracted by centrifugation at 12000 r/min for 10 min at 4 ℃ following the sequential addition of equal volumes of phenol, chloroform, and isoamyl alcohol (25:24:1), chloroform, and isoamyl alcohol (24:1). A white floc was precipitated from the final supernatant after the addition of 2.5 volumes of absolute ethanol and repeated aspiration. DNA was extracted from the white floc by centrifugation at 12000 r/min for 5 min at 4 ℃ after the addition of 75% ethanol. The DNA was dissolved by the addition of 50-120 μL of TE and stored at -20 ℃.

Genotyping of P268S, JW1 and N852S

The primer sequences were as published elsewhere[18] and were synthesized by SHENGGONG Biotechnology Co., Ltd., Shanghai, China. PCR reaction mixture contained 2 μL DNA template, 1 μL each of forward and reverse primers (10 μmol/L), 6 μL H_2O, and 10 μL of 2 × PCR Master Mix (TIANGEN Biotechnology Co., Ltd., Beijing, China). Reaction conditions consisted of an initial denaturation for 5 min at 94 ℃, followed by 35 cycles of denaturation at 94 ℃ for 45 s, annealing at different temperatures (Table 1) for 45 s, extension at 72 ℃ for 45 s, and a final extension at 72 ℃ for 10 min. All of the PCR

Table 1 Polymerase chain reaction primers, annealing temperatures and polymerase chain reaction fragment sizes

Mutation	Primer	Annealing temperature (℃)	PCR fragment size (bp)
P268S	F-TGCCTCTTCTTCTGCCTTCC R-AGTAGAGTCCGCACAGAGAG	60	422
JW1	F-TGCAGTTTTCTTGGGGAGAT R-TGTACCTGATCCAGCCCAAT	59	220
N852S	F-CTGTTTGCATGATGGGGGG R-CAGCCGTCAGTCAATTTGTAG	55	151

PCR: Polymerase chain reaction.

Table 2 Enzymes and gene polymorphism analysis

Mutation	Base change	Enzyme	Restriction fragment size (bp)	
			Wild-type	Mutant
P268S	C→T	*Bam*HI	422	Heterozygote 422 + 247 + 175 Homozygote 247 + 175
JW1	C→T	*Xho*I	125 + 95	Heterozygote 220 + 125 + 95 Homozygote 220
N852S	A→G	*Alu*I	151	Heterozygote 151 + 129 + 22 Homozygote 129 + 22

products were electrophoresed on a 1.5% agarose gel with 1 × Tris-borate-EDTA buffer at 100 V for 30 min and then observed under ultraviolet illumination (Bio-Rad Gel Doc-2000, Hercules, CA, United States).

The PCR products of P268S, JW1, and N852S SNPs of the *NOD2/CARD15* gene were digested at 37 ℃ for 11 h with *Bam*HI, *Xho*I, and *Alu*I restriction enzymes, respectively (Fermentas, Pittsburgh PA, United States). The digestion reaction contained 5 μL of the PCR product, 2 μL of 10 × buffer, 1 μL of restriction enzyme, and 9 μL of H_2O in a total of 17 μL. Following enzymatic digestion, the fragments were separated and visualized using gel electrophoresis (Yito Bio-Instrument Company Ltd., Shanghai, China) (Table 2).

The DNA mutative samples which were found by PCR-RFLP were reamplified. The products of each SNP were purified using a PCR purification kit (QIAGEN, Hilden, Germany) and sequenced using ABI 3730XL sequencer (Applied Biosystems, Foster, United States).

Statistical analysis

SPSS version 16.0 software was used for the statistical analysis, while comparisons of genotype and allelic frequencies among the different groups were performed using Fisher's exact test. The Hardy-Weinberg equilibrium test was used to test the distributions of each mutation genotype frequency. Values of $P < 0.05$ were considered statistically significant.

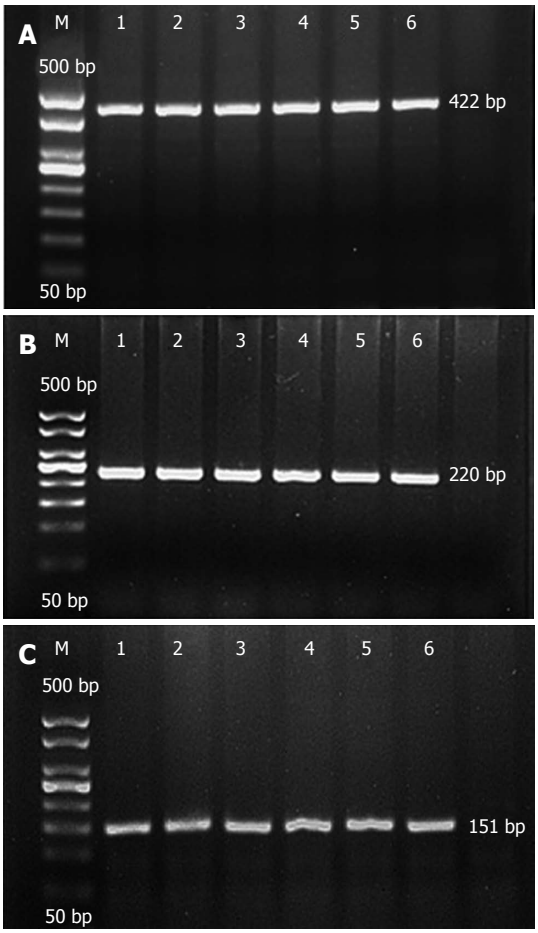

Figure 1 Electrophoresis of P268S, JW1, and N852S PCR products. A: P268S; B: JW1; C: N852S. M: Marker; 1, 2: Ulcerative colitis (UC), Crohn's disease (CD) of Han; 3, 4: UC, CD of Zhuang; 5, 6: healthy controls.

RESULTS

PCR

All three SNPs of the *NOD2/CARD15* gene were amplified by PCR, and the PCR products were then used for both RFLP analysis and gene sequencing. The target fragment sizes of the P268S, JW1, and N852S mutations were 422 bp (Figure 1A), 220 bp (Figure 1B), and 151 bp (Figure 1C), respectively.

PCR-RFLP

The PCR products of the P268S, JW1, and N852S mutations were digested using the *Bam*HI, *Xho*I, and *Alu*I enzymes, respectively. For P268S, a wild-type band of 422 bp was found in the majority of controls, CD patients, and UC patients, while heterozygous mutant bands of 422 bp, 247 bp, and 175 bp were found in six Zhuang CD cases, four Han CD cases, two Han UC cases, and one Zhuang healthy control, however, no homozygous mutants were detected (Figure 2A). For JW1, only wild-type bands of 125 bp and 95 bp were observed in all subjects (Figure 2B). Similarly, just one band of 151 bp was found in wild-type N852S in all subjects (Figure 2C), and no other mutant bands of JW1 or N852S were detected using PCR-RFLP fragment electrophoresis.

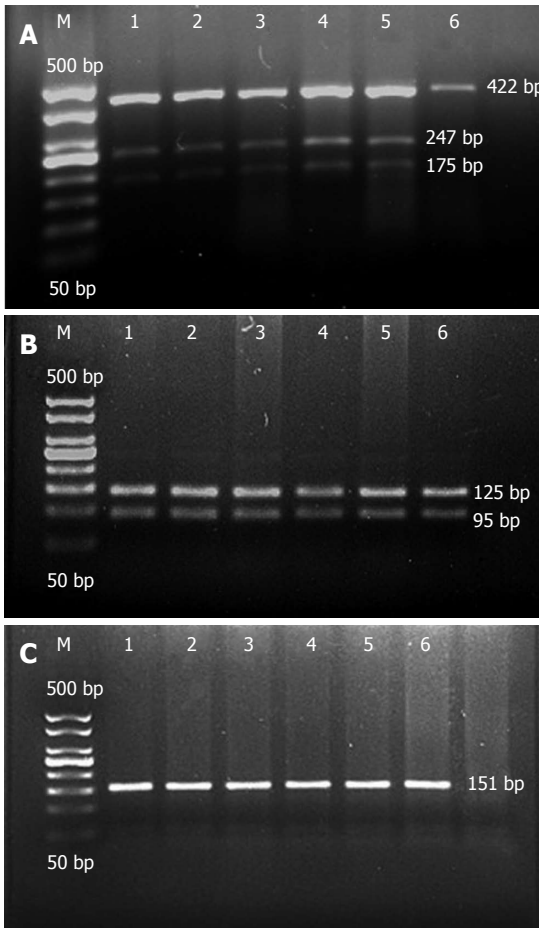

Figure 2 Electrophoresis of P268S, JW1, and N852S digestion products. A: M: Marker; 1-5: Heterozygote of P268S, 6: Wild-type of P268S. B: M: Marker; 1-6: Wild-type of JW1. C: M: Marker; 1-6: Wild-type of N852S.

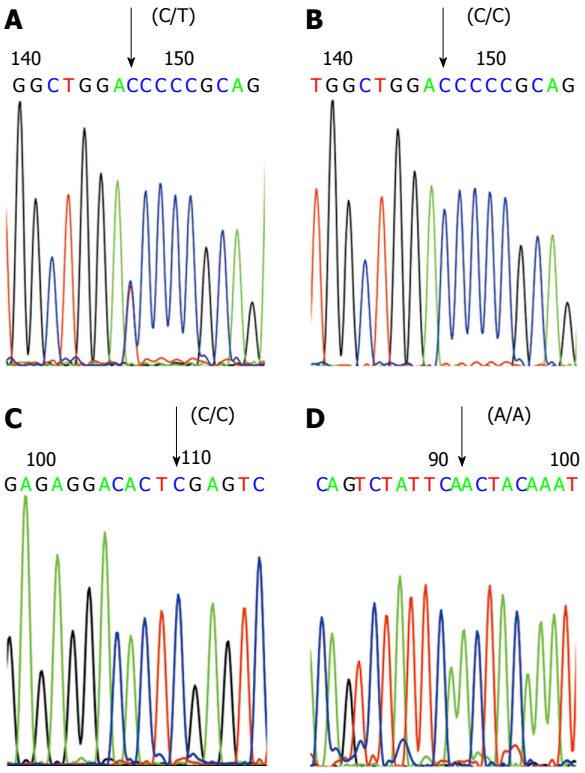

Figure 3 Gene sequencing analysis of P268S, JW1 and N852S polymerase chain reaction products. A: The forward sequencing map of the polymerase chain reaction (PCR) product of heterozygote P268S (C/T); B: The forward sequencing map of the PCR product of wild-type P268S (C/C); C: The forward sequencing map of the PCR product of wild-type JW1 (C/C); D: The forward sequencing map of the PCR product of wild-type N852S (A/A).

DNA sequencing

The gene sequencing results of the P268S, JW1 and N852S variants were consistent with those found on PCR-RFLP. For both mutant P268S and JW1, it is a C to T substitution mutation, and for mutant N852S, it is an A to G substitution mutation. In our study, heterozygous (C/T) (Figure 3A) and wild-type (C/C) (Figure 3B) P268S were detected in controls, CD patients, and UC patients, but no homozygous P268S (T/T) was detected. However, only wild-type JW1 (C/C) (Figure 3C) and wild-type N852S (A/A) (Figure 3D) were observed, and no other types (C/T, T/T, A/G, G/G).

Distribution of genotype and allelic frequencies

The distributions of P268S, JW1, and N852S genotypes were in accordance with the Hardy-Weinberg equilibrium test results ($P > 0.05$). In our cohort, only the P268S heterozygous mutation was found in six (12.5%) of 48 Zhuang CD cases, four (8.0%) of 50 Han CD cases, 0 (0.0%) of 54 Zhuang UC cases, two (4.0%) of 50 Han UC cases, one (1.4%) of 72 Zhuang controls, and zero (0.0%) of 78 Han controls. No P268S homozygous mutations were found. The genotype and allelic frequencies of P268S in the Zhuang and Han populations with CD were significantly higher than those in the control group ($^{a}P = 0.016$, $^{c}P = 0.022$ under the genotypic model, and $^{b}P = 0.017$, $^{d}P = 0.022$ under the allellic model, respectively); however, the differences between the control group and the UC group were not statistically significant ($P > 0.05$). The JW1 and N852S genotypes were homozygous wild-type in all three groups of Zhuang and Han. No differences in genotype and allelic frequencies of JW1 and N852S were detected among the groups ($P > 0.05$) (Tables 3 and 4).

P268S genotype and clinical features of CD

A comparison between CD patients in Guangxi including Zhuang and Han with and without P268S mutations was performed. Eight of the ten patients with CD who carried the P268S mutation were ≤ 40 years of age ($^{e}P = 0.040$), which suggested that the P268S mutation may be correlated with younger onset of CD in Guangxi patients. However, this mutation was not associated with lesion location, gender, ethnic groups, complications, or lesion severity ($P > 0.05$) (Table 5).

DISCUSSION

The *NOD2/CARD15* gene is located on chromosome 16q12. The protein that is encoded by the *NOD2/CARD15* gene is highly expressed in intestinal mucosal

Table 3 Distribution of genotype and allele frequencies of mutations in Crohn's disease and ulcerative colitis patients compared with healthy controls in the Guangxi Zhuang population *n* (%)

Mutant	Genotype	Allele	Control	CD		UC	
					P value		*P* value
P268S					[a]*P*[1]		NS[1]
	CC		71 (98.6)	42 (87.5)		54 (100.0)	
	CT		1 (1.4)	6 (12.5)		0 (0.0)	
	TT		0 (0.0)	0 (0.0)		0 (0.0)	
		T	1 (0.7)	6 (6.2)	[c]*P*[2]	0 (0.0)	NS[2]
JW1					NS[1]		NS[1]
	CC		72 (100.0)	48 (100.0)		54 (100.0)	
	CT		0 (0.0)	0 (0.0)		0 (0.0)	
	TT		0 (0.0)	0 (0.0)		0 (0.0)	
		T	0 (0.0)	0 (0.0)	NS[2]	0 (0.0)	NS[2]
N852S					NS[1]		NS[1]
	AA		72 (100.0)	48 (100.0)		54 (100.0)	
	AG		0 (0.0)	0 (0.0)		0 (0.0)	
	GG		0 (0.0)	0 (0.0)		0 (0.0)	
		G	0 (0.0)	0 (0.0)	NS[2]	0 (0.0)	NS[2]

[1]Comparisons of genotype frequencies; [2]Comparisons of allele frequencies. [a]$P < 0.05$ *vs* control using Fisher's exact test; [c]$P < 0.05$ *vs* control using Fisher's exact test. CD: Crohn's disease; UC: Ulcerative colitis; NS: No significance.

Table 4 Distribution of genotype and allele frequencies of mutations in Crohn's disease and ulcerative colitis patients compared with healthy controls in the Guangxi Han population *n* (%)

Mutant	Genotype	Allele	Control	CD		UC	
					P value		*P* value
P268S					[b]*P*[1]		NS[1]
	CC		78 (100.0)	46 (92.0)		48 (96.0)	
	CT		0 (0.0)	4 (8.0)		2 (4.0)	
	TT		0 (0.0)	0 (0.0)		0 (0.0)	
		T	0 (0.0)	4 (4.0)	[d]*P*[2]	2 (2.0)	NS[2]
JW1					NS[1]		NS[1]
	CC		78 (100.0)	50 (100.0)		50 (100.0)	
	CT		0 (0.0)	0 (0.0)		0 (0.0)	
	TT		0 (0.0)	0 (0.0)		0 (0.0)	
		T	0 (0.0)	0 (0.0)	NS[2]	0 (0.0)	NS[2]
N852S					NS[1]		NS[1]
	AA		78 (100.0)	50 (100.0)		50 (100.0)	
	AG		0 (0.0)	0 (0.0)		0 (0.0)	
	GG		0 (0.0)	0 (0.0)		0 (0.0)	
		G	0 (0.0)	0 (0.0)	NS[2]	0 (0.0)	NS[2]

[1]Comparisons of genotype frequencies; [2]Comparisons of allele frequencies. [b]$P < 0.05$ *vs* control using Fisher's exact test; [d]$P < 0.05$ *vs* control using Fisher's exact test. CD: Crohn's disease; UC: Ulcerative colitis; NS: No significance.

Paneth cells[21]. The NOD2/CARD15 protein has two caspase recruitment domains and includes a nucleotide-binding domain and a leucine-rich repeat (LRR). The LRR may stimulate the secretion of defensin through the identification of bacterial muramyl dipeptide. The level of defensin decreased markedly in patients with CD and gene mutations[22]. LRR may cause a defensive inflammatory reaction by combining bacterial lipopolysaccharide and activating NF-κB[23]. *NOD2/CARD15* is the first confirmed predisposing gene for CD, and the R702W, G908R, and L1007fs SNPs of the *NOD2/CARD15* gene were found to be significantly associated with CD in Caucasian populations[12-14]. The mutant allele frequencies of these three mutations accounted for approximately 81% of the total CD mutations[24]. Nevertheless, these SNPs were not associated with CD in Japanese, Malaysian, Indian, or Hong Kong, Zhejiang, and Guangxi populations in China, and none of the patients with CD had heterozygous or homozygous variants of R702W, G908R, and L1007fs SNPs[10,11,15,17,25,26]. In addition, the R702W, G908R, L1007fs, P268S, and JW1 SNPs were not correlated with IBD patients in Turkey, instead, the R702W mutation was significantly lower in the IBD group (1.5%) than in the control group (4.8%) ($P < 0.05$)[27]. Thus, these findings indicate that the *NOD2/CARD15* genotype distribution has significant ethnic differences.

This is the first study to report the P268S, JW1, and N852S mutations of the *NOD2/CARD15* gene in patients with CD from the Guangxi Zhuang population of China, where the ethnic background is heteroge-

Table 5 Clinical characteristics of Crohn's disease patients in Guangxi with and without P268S mutations

Phenotype	*n*	P268S+	P268S-	*P* value
Age of onset				[a]*P*
⩽ 40 years	45	8 (17.8)	37 (82.2)	
> 40 years	53	2 (3.8)	51 (96.2)	
Location				NS
Ileum	58	6 (10.3)	52 (89.7)	
Colon/ileocolon	40	4 (10.0)	36 (90.0)	
Gender				NS
Male	51	7 (13.7)	44 (86.3)	
Female	47	3 (6.4)	44 (93.6)	
Ethnic groups				NS
Han	50	4 (8.0)	46 (92.0)	
Zhuang	48	6 (12.5)	42 (87.5)	
Comorbidities				NS
Luminal stenosis	31	4 (12.9)	27 (87.1)	
No luminal stenosis	67	6 (9.0)	61 (91.0)	
Severity				NS
Severe	42	3 (7.1)	39 (92.9)	
Mild-moderate	56	7 (12.5)	49 (87.5)	

NS: No significance. P268S+ Mutant P268S; P268S- Wild-type P268S. [a]$P < 0.05$ using Fisher's exact test.

neous with Han, Zhuang, and other ethnic groups. In this study, the P268S mutation genotype of *NOD2/CARD15* was found in some Zhuang and Han patients with CD and was detected only sporadically in healthy individuals and patients with UC in Zhuang and Han. The JW1 and N852S mutations of the *NOD2/CARD15* gene were not detected in Guangxi Zhuang or Han patients with IBD.

In recent years, several studies have reported that the P268S mutation of the *NOD2/CARD15* gene was found in Ashkenazi Jewish and Irish patients with CD[16,28]. The population-attributable risk of the P268S-JW1 haplotype was 15.1% in Jewish patients with CD[16]. Gasche *et al*[29] reported that the evolution of P268S occurred in the Middle East and that the mutant was associated with CD in Chinese Tu and Pakistani populations. The P268S SNP of the *NOD2/CARD15* gene was also reported to be closely related to CD in Indian patients[17,30]. Similarly, the P268S mutant was confirmed to contribute to CD susceptibility and clinical features in a Han population in Guangdong, China[19]. However, that finding was not in accordance with those of Juyal *et al*[31], in which the P268S mutant of the *NOD2/CARD15* gene was correlated with UC in North India. In our study, we confirmed that the P268S SNP may be involved in the susceptibility of Zhuang or Han patients to CD in Guangxi, China. Our results are in agreement with those from studies on Han and Tu patients with CD from other areas in China[19,29]. However, the P268S homozygous variant was found in Han patients in Guangdong, China, and was not detected in our study population, which may be due to racial heterogeneity or our relatively small sample size. Compared to Europeans (31.2%)[16], we found a lower frequency (12.5%) of mutant P268S in our Zhuang CD patients.

The N852S mutation of the *NOD2/CARD15* gene was found to be significantly associated with CD in Ashkenazi Jewish populations[18]; however, since it did not appear as a haploid with R702W, G908R, and L1007fs of the *NOD2/CARD15* gene, it is thought to be an independent risk factor for CD[32]. Our results indicated that N852S mutations of the *NOD2/CARD15* gene were not detected in Guangxi Zhuang patients with IBD. The JW1 mutant of the *NOD2/CARD15* gene was confirmed in Chinese Han in Malaysia[17]. However, we did not find any heterozygous or homozygous mutations of JW1 in the Chinese Zhuang population from the Guangxi Zhuang Autonomous Region. These two novel loci have rarely been reported in China, and further studies are necessary to explore these loci in a larger cohort in China. In summary, the differences in these results may be attributed to the differences in race, geography, environment, and population.

Several studies have proved that the *NOD2/CARD15* gene is related to the clinical features of CD including onset location, age, complications, and disease severity[33-35]. It was reported that P268S was related to ileal lesions ($P = 0.003$), lumen stenosis ($P = 0.007$), and age ⩽ 20 years ($P = 0.028$) in a Chinese Han population with CD from Guangdong, China[19]. In addition, Chua *et al*[17] reported that the JW1 mutant tended to correlate with luminal stenosis ($P = 0.055$) and age < 41 years ($P = 0.095$) in patients with CD in Malaysia. The results of the present study confirmed that P268S was only related to age ⩽ 40 years ($P = 0.040$) in CD patients from the Chinese Zhuang population in the Guangxi Zhuang Autonomous Region. No important relationship was detected between mutant P268S and location, gender, ethnic group, lumen stenosis, and severity of CD. Our results were not in agreement with those studies on Chinese Han patients with CD from Guangdong or patients with CD from Malaysia. This difference may be due to racial heterogeneity, geographic environment, and a relatively small sample size.

In conclusion, this study is the first to demonstrate the relationship between the P268S SNP of the *NOD2/CARD15* gene and susceptibility to CD in a Zhuang population from the Guangxi Zhuang Autonomous Region, China. JW1 and N852S SNPs of the *NOD2/CARD15* gene were not found in the Zhuang population. Thus, we emphasize that genetic predisposition may be vital in the pathogenesis of IBD. However, the power of this conclusion may be limited by the relatively small sample size in this study. Further studies investigating risk factors and genetic susceptibility to IBD in a larger cohort of patients and in different ethnic groups are needed.

COMMENTS

Background

Inflammatory bowel disease (IBD) is a multifactorial disease with different susceptibility genes in various races. The P268S, JW1, and N852S polymorphisms of *NOD2/CARD15* have been confirmed in Crohn's disease (CD) susceptibility in Chinese Han and Ashkenazi Jewish populations, but there are no reports of a correlation between these three polymorphisms and the Chinese Zhuang CD population in the Guangxi Zhuang Autonomous Region.

Research frontiers

Nucleotide-binding oligomerization domain containing 2/caspase-activation and recruitment domain gene 15 (*NOD2/CARD15*) is the first confirmed predisposing gene for CD, and the P268S mutation of the *NOD2/CARD15* gene was found in Ashkenazi Jewish, Irish, Indian, Pakistani, and Chinese Han and Tu patients with CD, but not in CD in North India. The JW1 SNP of the *NOD2/CARD15* gene was shown to be associated with CD in Chinese Han, Malay, and Indians in Malaysia. The N852S mutation of the *NOD2/CARD15* gene was only found to be significantly associated with CD in Ashkenazi Jewish populations. The present study assessed whether these known SNPs were associated with IBD in Zhuang patients from Guangxi, China.

Innovations and breakthroughs

The Guangxi Zhuang Autonomous Region of China has the largest Zhuang population, thus genetic diseases and gene polymorphisms are unique. This study is the first to demonstrate the relationship between the P268S, JW1, and N852S polymorphisms of the *NOD2/CARD15* gene and susceptibility to CD in the Zhuang population from the Guangxi Zhuang Autonomous Region, China.

Applications

The P268S polymorphism may contribute to CD susceptibility in the Zhuang population in the Guangxi Zhuang Autonomous Region, China. However, JW1 and N852S SNPs may be absent or rare in this population.

Terminology

NOD2/CARD15 is located on chromosome 16q12, and encodes a protein with homology to plant disease resistance-related gene products. Mutant *NOD2/CARD15* responds to bacterial muramyl dipeptide and decreases NF-kappaB activation, and these results implicate *NOD2/CARD15* in susceptibility to Crohn's disease. Polymerase chain reaction-restriction fragment length polymorphism is a popular technique used in genetic analysis. It has been used for the detection of intraspecies as well as interspecies variation.

Peer review

This brief paper demonstrates the relationship between the P268S polymorphism in *NOD2/CARD15* and Crohn's disease susceptibility in an ethnic Zhuang population. While the scientific findings in this paper are limited, documentation of genetic variation in CD susceptibility among various ethnic and regional groups is useful.

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