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• BRIEF REPORTS •

Association of *Fas-670* gene polymorphism with inflammatory bowel disease in Chinese patients

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Abstract

AIM: Recent studies suggest that Fas-mediated apoptosis is involved in the pathogenesis of inflammatory bowel disease (IBD). It has been hypothesized that either increased apoptosis of intestinal epithelium or decreased apoptosis of lamina propria lymphocytes may induce inflammation of gut. The aim of this study was to determine whether the *Fas* gene promoter polymorphism at position-670 was associated with IBD in Chinese patients.

METHODS: Fifty unrelated Chinese patients with IBD (38 patients with ulcerative colitis and 12 with Crohn's disease) and 124 healthy controls were genotyped for the *Fas-670* polymorphism by PCR-restriction fragment length polymorphism method. The PCR product was digested by *Mva* I restriction enzyme.

RESULTS: Distribution of the *Fas-670* gene polymorphism was 33% for the AA genotype, 52% for the AG genotype and 15% for the GG genotype in 124 healthy subjects. In patients with IBD, 30% was for the AA genotype, 42% for the AG genotype and 28% for the GG genotype respectively. However, there was no significant difference in the genotype ($P = 0.1498$), allele frequencies ($P = 0.3198$) and carriage frequencies ($P = 0.4133$) between healthy controls and IBD patients. Furthermore, we did not find any difference between the left-sided colitis and total colitis ($P = 0.8242$).

CONCLUSION: *Fas-670* polymorphism is not associated with IBD in Chinese patients.

Key words: Inflammatory bowel disease; Fas-670 gene; Apoptosis; Polymorphism

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INTRODUCTION

Fas (Apo-1/CD95) antigen is a 45-kDa type I membrane protein, which is expressed in various tissues and cells. Fas is a member of the tumor necrosis factor superfamily and mediates apoptosis when cross-linked with agonistic anti-Fas antibody or Fas ligand (FasL)[1]. Although the best-characterized physiological system involving Fas/FasL-mediated apoptosis is observed in the immune system, a role of Fas/FasL in non-lymphiod tissues is becoming increasingly evident. Fas-mediated apoptosis is thought to be involved in autoimmune disease and inflammatory disorders.

Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD) is characterized by chronic, relapsing intestinal inflammation with unknown etiology. Recent studies have suggested that immune dysregulation and genetic factors play important roles in the pathogenesis of IBD. Defective apoptosis of lamina propria T cells (LPT) may be a factor in mucosal immune dysregulation and tissue inflammation. Bu *et al*[2] found 15% of LPT cells underwent apoptosis in normal individuals. There was a marked reduction in apoptosis of LPT cells in patients with UC and CD and those with specific colitis. In normal gastrointestinal tract, LPT cells are shown to be more susceptible than periphery T cells to Fas-mediated apoptosis[3]. Fas-mediated apoptosis is implicated in the intestinal inflammation, especially in UC[4,5]. The role of Fas/FasL in UC is centered on the hypothesis that Fas-positive intestinal epithelial cells (IEC) are targeted by FasL-positive lymphocytes resulting in IEC apoptosis. Apoptosis of IEC in the crypts has been reported in UC[6] and FasL has been shown to be upregulated on intestinal lymphocytes in UC[5]. Although Fas/FasL-mediated apoptosis may contribute to intestinal tissue damage, resistance of LPT to apoptosis is probably more important to perpetuation of chronic inflammation[7]. Suzuki *et al*[8] demonstrated that CD45RO+CD4+T cells were less sensitive to apoptotic signals mediated by Fas in UC patients.

The *Fas/Apo-1* gene has been mapped to the chromosome 10q24.1 region[9]. The gene consists of nine exons and eight introns. Two polymorphisms located in the promoter region of the *Fas* gene have recently been reported[10]. One of these polymorphisms is a single nucleotide substitution at the -670 position that alters the *Mva* I restriction enzyme cutting site, creating a restriction fragment length polymorphism (RFLP). This polymorphism is situated at the consensus sequence site, the gamma interferon activation site (GAS). This site can bind to transcription factors such as signal transducers and activator of transcription (STAT), and thus may exert an effect on the level of transcription of the Fas protein. The aim of the present study was to investigate the distribution of *Fas* gene-670 polymorphism and its association with IBD in Chinese patients.

MATERIALS AND METHODS

Patients

Fifty patients with IBD (32 male and 18 female), mean age 39.4±14.4 years, 38 patients with UC and 12 patients with CD, were registered in Wuhan University Zhongnan Hospital. A total of 124 healthy controls (82 male and 42 female), mean age

44.8±17.4 years, were healthy physical examiners in the hospital. All patients and healthy controls were of unrelated Chinese Han nationality. The diagnosis of CD and UC was based on clinical symptoms and endoscopic, radiographic and histopathological findings according to conventional criteria by Lennard-Johns[11]. UC was classified to left-sided colitis and total colitis according to location of the disease. All patients gave informed consent to participate in the study that was approved by the Ethics Committee of Wuhan University Medical School.

Fas-670 polymorphism genotyping

Genomic DNA was obtained from peripheral blood by proteinase K digestion and phenol-chloroform extraction and ethanol precipitation. The *Fas-670* polymorphism was genotyped by polymerase chain reaction (PCR) amplification[10] and the *Mva* I digestion. The oligonucleotides 5'-CTACCTAAGAGCTAT CTACCGTTC-3' and 5'-GGCTGTCCATGTTGTGGCTGC-3' flanking this region were used as primers. PCR was performed using a thermal cycle Perkin-Elmer 2400 as follows: initial denaturation at 94 ℃ for 5 min, followed by 30 amplification cycles, each consisting of denaturation at 94 ℃ for 30 s, annealing at 60 ℃ for 30 s and extension at 72 ℃ for 1min, and final extension at 72 ℃ for 5 min and cooled to 4 ℃. The PCR products were analyzed by electrophoresis on 1% agarose gels containing 0.1% ethidium bromide. Ten microlitre of the PCR products was digested with 5 U of *Mva* I restriction enzyme for 5 h at 37 ℃ and electrophoresed on 6% non-denaturing polyacrylamide gels, and visualized by sliver staining. Two polymorphic alleles, *G* (189+99+44 bp) and *A* (233+99 bp) could be distinguished.

Statistical analysis

Hardy-Weinberg equilibrium was tested by χ^2 test. The distribution of *Fas-670* genotypes and alleles and carriers in IBD was compared which that in healthy controls by χ^2 test and Fisher's exact test. Associations were expressed as odd ratios (*OR*) with 95% confidence interval (95%*CI*). A *P* value of <0.05 was considered statistically significant. Statistical analysis was performed with SPSS version 9.0 for windows.

RESULTS

As shown in Table 1, genotypes in IBD and healthy control groups were in Hardy-Weinberg equilibrium. There were no differences in the *Fas-670* genotypes, allele frequencies and carriage rates between healthy controls and IBD groups. There were also no significant differences in genotype and allele frequencies between left-sided colitis and total colitis (Table 2). Meanwhile, there were no significant differences in the *Fas-670* genotype distribution and allelic frequencies between the Chinese healthy controls and the other three ethnic healthy control groups (Table 3).

Table 1 *Fas-670* genotypes, allele frequencies and carriage rates in healthy controls and inflammatory bowel disease (IBD) in Chinese Han patients

	Healthy controls (*n* = 124)	IBD (*n* = 50)	UC (*n* = 38)	CD (*n* = 12)
Genotypes *n* (%)				
A A	41 (33)	15 (30)	12 (32)	3 (25)
AG	64 (52)	21 (42)	15 (40)	6 (50)
GG	19 (15)	14 (28)	11 (28)	3 (25)
Allele frequencies %				
A	59	51	51	50
G	41	49	49	50
Carriage %				
A	85	72	71	75
G	67	70	68	75

Table 2 *Fas-670* genotypes and allele frequencies and location of ulcerative colitis

	Genotypes *n* (%)			Allele frequencies (%)	
	AA	AG	GG	A	G
Left-sided colitis (*n* = 20)	7 (35)	7 (35)	6 (30)	52	48
Total colitis (*n* = 18)	5 (28)	8 (44)	5 (28)	50	50

Table 3 *Fas-670* genotypes and allele frequencies in several ethnic healthy controls

	Genotype frequency *n* (%)			Allele frequency (%)	
	AA	AG	GG	A	G
Chinese (*n* = 124)	41 (33)	64 (52)	19 (15)	59	41
Dutch (*n* = 206)[24]	46 (23)	118 (57)	42 (20)	51	49
Australian (*n* = 183)[25]	46 (25)	97 (53)	40 (22)	52	48
Korean (*n* = 84)[28]	25 (30)	46 (55)	13 (15)	57	43

DISCUSSION

In the present study, we genotyped *Fas-670* polymorphism in Chinese patients with IBD and healthy controls, and found that the polymorphism was not associated with UC and CD. The study suggested that *Fas-670* polymorphism might not play a role in susceptibility of IBD in Chinese patients.

Fas-670 polymorphism within the promoter region is situated at a transcriptional binding site and may potentially have a functional effect on gene regulation. The substitution of *G* to *A* in the position-670 (TTCCAGG/AAA) will change the interferon gamma activated site (GAS). GAS is involved in interferon gamma (IFN-γ) signaling pathway[12-14]. The interaction with interferon receptor at the cell surface leads to the activation of kinase of the Jak family and then phosphorylation of the substrate STATs. The phosphorylated STATs move to the nuclei, bind to GAS and transcript GAS-containing genes[15-17]. Mutagenesis of the GAS element may decrease or even completely abolish responsiveness to IFN-γ-mediated gene activation[18]. Previous studies showed that IFN-γ significantly regulated Fas expression and increased Fas-induced human intestinal epithelial apoptosis in a dose-dependent manner[19]. Although several studies[20,21] have shown that resistance to Fas-mediated apoptosis can be overcome by administration of IFN-γ, this activation-induced sensitization appeared not to depend on only the enhancement of CD95 surface expression.

In recent years, increased serum concentration of soluble form of Fas (sFas) has been reported in several autoimmune diseases, which may involve the similar mechanism of pathogenesis of IBD. The *Fas-670* polymorphism has been shown to be associated with several autoimmune diseases, such as celiac disease, SLE, rheumatoid arthritis[22,23] and multiple sclerosis[24,25]. Vetuschi *et al*[26] have found that in SD rats, which have many structural and ultrastructural features similar to those seen in human ulcerative colitis, the epithelial apoptotic index increased 20-fold after the first cycle and 120-fold after the second and third cycles compared with the controls, as well as expression index of proapoptotic proteins (Fas, FasL) dramatically increased. This result indicates that the Fas might have a key role in IBD. Several studies also reported that Fas was conservatively expressed in the epithelia of both normal colon and that with UC lesions[5], and sFas level was significantly lower in active UC than in controls[27]. These results indicate that Fas-mediated apoptosis may involve in the pathogenesis of IBD, especially UC.

Although expression and functional effects of the Fas antigen have been found to be associated with IBD, the

relationship between *Fas-670* polymorphism and IBD has not been reported yet. In our study, we could not find any significant association between *Fas-670* polymorphism and IBD, which indicates genetic heterogeneity of the diseases. Since *Fas-670* polymorphism does not contribute to IBD, there may be other genes that are involved in the pathogenesis of IBD, and other mechanisms of gene regulation may influence Fas-mediated epithelial apoptosis in IBD.

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