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

Bing Xia, Yu-Hong Yu, Qiu-Sha Guo, Xiang-Yin Li, Li Jiang, Jin Li

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-lymphoid 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 [7]. 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 [8]. Suzuki et al. [9] 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 [10]. The gene consists of nine exons and eight introns. Two polymorphisms located in the promoter region of the Fas gene have recently been reported [11]. 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'-CTACCTAAAGGGTATCTACGGTTCACTGTTGTGCCTG-3' flanking this region were used as primers. PCR was performed using a thermal cycle Perkin-Elmer 2400 as follows: initial denaturation at 94°C for 5 min, followed by 30 amplification cycles, each consisting of denaturation at 94°C for 30 s, annealing at 60°C for 30 s and extension at 72°C for 1 min, and final extension at 72°C for 5 min and cooled to 4°C. The PCR products were analyzed by electrophoresis on 1% agarose gels containing 0.1% ethidium bromide. Ten microlitre of the PCR products was visualized by silver 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 χ² test. The distribution of Fas-670 genotypes and alleles and carriers in IBD was compared which that in healthy controls by χ² 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 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 2 | Fas-670 genotypes and allele frequencies and location of ulcerative colitis |
|---------|--------------------------------------------------------------------------|
| Genotypes n (%) | Allele frequencies (%) |
| AA | AG | GG |
| A | G | A | G |
| Left-sided colitis (n = 20) | 7 (35) | 5 (25) | 6 (30) | 52 | 48 |
| Total colitis (n = 18) | 5 (28) | 4 (22) | 5 (28) | 50 | 50 |

| Table 3 | Fas-670 genotypes and allele frequencies in several ethnic healthy controls |
|---------|--------------------------------------------------------------------------|
| Genotype n (%) | Allele frequency (%) |
| AA | AG | GG |
| A | G | 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)[20] | 46 (25) | 97 (53) | 40 (22) | 52 | 48 |
| Korean (n = 84)[20] | 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 A to T in the position-670 (TTCCAGGA/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 nucleus, 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[20] 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[21], 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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