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Association of Fas/Apo1 gene promoter (-670 A/G) polymorphism in Tunisian patients with IBD

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Abstract

AIM: To detect a possible association between the polymorphism of the (-670 A/G) Fas/Apo1 gene promoter and susceptibility to Crohn's disease (CD) and ulcerative colitis (UC) in the Tunisian population.

METHODS: The (-670 A/G) Fas polymorphism was analyzed in 105 patients with CD, 59 patients with UC, and 100 controls using the polymerase chain reaction restriction fragment length polymorphism method.

RESULTS: Significantly lower frequencies of the Fas -670 A allele and A/A homozygous individuals were observed in CD and UC patients when compared with controls. Analysis of (-670 A/G) Fas polymorphism with respect to sex in CD and UC showed a significant difference in A/A genotypes between female patients and controls (*P* corrected = 0.004 in CD patients and *P* corrected = 0.02 in UC patients, respectively). Analysis also showed a statistically significant association between genotype AA of the (-670 A/G) polymorphism and the ileum localization of the lesions (*P* corrected = 0.048) and between genotype GG and the colon localization (*P* corrected = 0.009). The analysis of inflammatory bowel disease patients according to clinical behavior revealed no difference.

CONCLUSION: Fas-670 polymorphism was associated with the development of CD and UC in the Tunisian population.

Key words: Fas/Apo1; Gene polymorphisms; Inflammatory bowel disease; Crohn's disease; Ulcerative colitis

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INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic disorder of the gastrointestinal tract characterized by immune dysregulation and leukocyte recruitment[1]. IBD may manifest as either Crohn's disease (CD) or ulcerative colitis (UC), which are two distinct forms of IBD with some common clinical, epidemiological, and immunological features, but they can be distinguished by anatomical and histological features as well as by serologic markers[2,3].

The etiology of IBD is unknown, but the condition seems to be the result of a combination of environmental, genetic, and immunologic factors in which an uncontrolled immune response within the intestinal lumen leads to inflammation in genetically predisposed individuals[4].

Genetic factors are known to play an important role in determining an individual's susceptibility to IBD. Significant linkages in chromosomes 1, 3, 6, 7, 12, 14, 16 and 19 have been reported[5-8]. However, IBD-1, a susceptibility locus in chromosome 16, was the first gene to be clearly associated with CD[9,10].

Although the underlying genetic and environmental causes remain to be elucidated, CD4+ T helper 1 (Th1) lymphocytes play a pivotal role in the pathogenesis of these diseases. A Th1-like phenotype, with its signature cytokines interferon (IFN)-γ and tumor necrosis factor (TNF)-α, is shared among many colitis models and is found in patients with active CD[1,11,12]. Moreover, IFN-γ not only cooperates with TNF-α to cause colonic cell death, it also modulates epithelial Fas expression and sensitizes colonic epithelial cells to Fas-induced apoptosis[1].

Programmed cell death, or apoptosis, is regulated by tightly controlled intracellular signaling events in response to pathological cytotoxic stimuli including TNF-α, TNF-related apoptosis-inducing ligand (TRAIL), and Fas[13-15]. Fas, a member of the tumor necrosis factor superfamily, is constitutively expressed by the basolateral membrane of normal colon and small intestinal epithelium[11,12,16-19]. Fas ligation induces apoptosis in colonic epithelial cells and is implicated in the epithelial damage seen in ulcerative colitis[11,12,16-19]. The ligand for Fas (FasL) is expressed by intraepithelial and lamina propria lymphocytes, and its expression is increased in the lamina propria of UC patients, suggesting that Fas-FasL-induced apoptosis participates in the mucosal damage of UC[11,12]. Inhibition of cytokine-regulated apoptosis may therefore be useful in preventing or treating intestinal lesions in patients with IBD.

The Fas/Apo-1 gene has been mapped to the chromosome 10q24.1 region[20]. The gene consists of nine exons and eight introns. Two polymorphisms located in the promoter region of the Fas gene have been reported[21]. One of these polymorphisms is a single nucleotide substitution (A/G) at the -670 position. Several studies have shown an association between this A/G single nucleotide polymorphism (SNP) and autoimmune diseases such as type 1 diabetes[22], multiple sclerosis[6], Sjögren's syndrome[23], rheumatoid arthritis[24], and systemic lupus erythematosus[6,24].

In this study, we have analyzed the Fas/Apo1 gene promoter (-670 A/G) polymorphism in unrelated Tunisian patients with CD and UC to evaluate the contribution of the Fas/Apo1 (CD95) gene to genetic susceptibility to IBD.

MATERIALS AND METHODS

Patients

Blood samples were obtained from 164 subjects with IBD, composed of 13 cases of familial forms and 151 cases of sporadic forms. These patients were classified into 105 patients with CD (50 men, 55 women) with a mean age of 36.07 years (range, 23-60 years), and 59 patients with UC (17 men, 42 women) with a mean age of 37.89 years (range, 25-74 years). A total of 100 healthy individuals (52 men and 48 women) matched for age, sex and ethnicity were included as controls. None of the healthy controls had any evidence of autoimmune diseases such as inflammatory bowel disease, diabetes, or other autoimmune diseases. All subjects were unrelated Tunisians treated at the Department of Gastroenterology of Charles Nicolle and La Rabta Hospitals in Tunis. The diagnoses of CD and UC were determined according to conventional endoscopic, radiological, and histological criteria[25]. Data obtained from each patient included age at diagnosis, disease location, disease characteristics, and extraintestinal location, which were used to group the patients according to the Vienna classification[26]. All patients and controls gave informed consent to participate in this study which was approved by the Ethics Committee of Charles Nicolle Hospital in Tunis.

Methods

Transition A/G, in position -670 of the promoter of the Apo1/Fas gene, was analyzed by the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method. The Apo1/Fas marker frames an area of 232 Pb in the area of the promoter and was able to contain a site of cut for the enzyme Bst I (Promega). The digestion of the amplified fragment gave rise to two types of allele: the allele G marked by the presence of two fragments 188 Pb and 232 Pb and the allele A characterized by the absence of cut (A), therefore a fragment of 232 Pb. The PCR was carried out in a final volume of 20 μL containing 5 μL of genomic DNA at 40 ng/μL, 1 U of *Taq* polymerase, 1.5 mmol/L of $MgCl_2$, 0.2 mmol/L of dNTP and 10 pmol of each primer.

The primers used were: Fas (1); 5'-CTACCTAAGA GCTATCTACCGTTC-3' and Fas (2); 5'-GGCTGTCC ATGTTGTGGCTGC-3'. The amplification of product was carried out on 2% agarose gel and visualized under UV. Ten microliter of the amplified fragments were then digested by 5 U of Bst I in a final volume of 20 μL and the mixture was left 2.5 h at 60℃. The separation of the products of digestion was performed on 3% agarose gel and visualized under UV.

Statistical analysis

Frequencies of the genotypes, alleles, and phenotypes were analyzed by using the χ^2 test. The odds ratio (OR) and 95% confidence interval were calculated to measure the strength of the association observed. Hardy Weinberg equilibrium was tested by calculating the χ^2 for reliability of fit. Calculations were made by using Internet programs from www.myatt.demon.co.uk/epicalc.htm. Statistical significance was defined as $P < 0.05$. P values were corrected by Bonferroni correction for multiple comparisons, taking into account the number of alleles studied.

RESULTS

As shown in Table 1, Crohn's disease (64%) was more frequent than ulcerative colitis (36%) in the study. The peak of the disease is between 20 and 40 years. The familial forms represent 8.57% of the CD cases and 6.77% of the UC cases. The principal clinical manifestations observed during evolutionary flares of

Table 1 Clinical characteristics of study subjects *n* (%)

	CD (*n* = 105)	UC (*n* = 59)
Sex (Male/female)	50/55	17/42
Age average	36.07	37.89
Age (Min/max)	23/60	25/74
Familial forms	9 (8.57)	4 (6.77)
Surgical history	11 (10.47)	4 (6.77)
Activity of the disease (*n*)	In remission (75)	In remission (46)
	Moderated (18)	Moderated (13)
	Severe (12)	Severe (0)
Location of lesion, (*n*)	Ileum (27)	Distal (21)
	Colon (24)	Left-sided colitis (26)
	Ileocolon (54)	Pancolitis (12)
Forms, *n* (%)	Stenosing, 43 (40.95)	
	Fistula, 37 (35.23)	
Extra-intestinal manifestation	31 (29.53)	5 (8.47)
Evolution, *n* (%)	Favorable, 89 (84.16)	Favorable, 58 (98.30)
	Cortico dependency, 12 (11.42)	Cortico dependency, 1 (1.69)
	Cortico-resistance, 2 (1.92)	Cortico-resistance, 0
	Unfavourable, 2 (1.92)	Unfavourable, 0
Production of pANCA	43	28
Production of ASCA	22	9
Treatments	5-ASA, 32 (30.47)	5-ASA, 58 (98.30)

Table 2 Allelic and genotypic frequency of (-670 A/G) Apo1/Fas gene polymorphisms in patients with inflammatory bowel disease and controls *n* (%)

Apol/Fas (-670)	Controls (*n* = 100)	IBD (*n* = 164)	UC (*n* = 59)	CD (*n* = 105)
Genotypic frequencies				
A/A	49 (49)	46 (28.04)[c]	15 (25.42)[b]	31 (29.52)[a]
A/G	32 (32)	70 (42.68)	30 (50.85)	40 (38.10)
G/G	19 (19)	48 (29.26)	14 (23.73)	34 (32.38)
Allelic frequencies				
A	130 (70)	162 (49.39)[d]	60 (50.84)[f]	102 (48.57)[e]
G	70 (35)	166 (50.61)	58 (49.16)	108 (51.43)

[a]Comparing AA genotype frequencies in CD patients with controls: *P* corrected = 0.004, OR: 0.44, 95% CI, 0.24 < OR < 0.81; [b]Comparing AA genotype frequencies in UC patients with controls: *P* corrected = 0.0034, OR: 0.35, 95% CI, 0.16 < OR < 0.76; [c]Comparing AA genotype frequencies in IBD patients with controls: *P* corrected = 0.0005, OR: 0.41, 95% CI, 0.23 < OR < 0.70; [d]Comparing A/Allele frequencies in IBD patients with controls: *P* corrected = 0.0004, OR: 0.53, 95% CI, 0.36 < OR < 0.77; [e]Comparing A/Allele frequencies in CD patients with controls: *P* corrected = 0.0007, OR: 0.57, 95% CI, 0.34 < OR < 0.77; [f]Comparing A/Allele frequencies in UC patients with controls: *P* corrected = 0.01, OR: 0.56, 95% CI, 0.34 < OR < 0.91. IBD: Inflammatory bowel disease; UC: Ulcerative colitis; CD: Crohn's disease; OR: Odds ratio; CI: Confidence interval.

the disease depended on the site: Ileum (25.71%), Colon (22.85%) and Ileocolon (51.42%) for CD patients and Pancolitis (20.33%), Distal (35.59%) and Left-sided colitis (44.06%) for UC patients. Results for the genotyping of the Fas/Apo1 gene promoter (-670 A/G) SNP in 105 patients with CD, 59 subjects with UC, and 100 healthy controls are summarized in Table 2. The allele frequencies were in Hardy-Weinberg equilibrium both in the patients and in controls. We have compared the A/A genotype frequencies found in various subgroups of IBD patients with those in the entire control population. When comparing CD patients with the control group, the frequency of the -670 A allele was found to be significantly lower in CD than in controls. Also, the distribution of the -670 A/A genotype was significantly lesser in CD patients than in controls. When comparing UC patients with the control group, the frequencies of the -670 A allele and the homozygous -670 A/A genotype were lower in UC patients than in controls and these differences were statistically significant. When we analyzed the Fas/Apo1 gene promoter (-670 A/G) polymorphism with respect to gender (Table 3), we found that Fas/Apo1 genotype frequencies were similar in controls of both sexes (48 females and 52 males). However, the frequency of CD female patients carrying the -670 A/A genotype was significantly less when compared with the control group. The same result was obtained in women with UC when compared with those in the control group. Thus, the homozygosity for the A allele could be a protective factor for development of IBD in female patients. An analogous analysis has been done on male patients with CD and UC. The results did not show a statistically significant association for either CD or UC patients compared with the control group. Subsequently, we sought to investigate whether this polymorphism could be linked to a particular clinical phenotype. When stratifying CD patients according to the Vienna classification, as shown in Table 4, we found a statistically significant association ($\chi^2 = 3.89$, two degrees of freedom, *P* corrected = 0.048) odds ratio of 2.48 (0.90 < OR < 6.87, CI = 95%) between genotype AA of the Fas/Apo1 gene promoter (-670 A/G) polymorphism and the ileum localization of the lesions. In addition, we found a statistically significant association ($\chi^2 = 6.74$, two degrees of freedom, *P* corrected = 0.009) odds ratio of 3.38 (1.19 < OR < 9.67, CI = 95%) between genotype GG of the polymorphism and the colon localization of the lesions. Analysis of IBD patients according to clinical behavior revealed no difference between those carrying

Table 3 Incidence of (-670 A/G) Apo1/Fas gene polymorphism analyzed with respect to sex in IBD patients *n* (%)

Apo1/Fas	Controls (*n* = 100)	CD females (*n* = 55)	CD males (*n* = 50)	UC females (*n* = 42)	UC males (*n* = 17)
Genotypic frequencies					
A/A	49 (49)	14 (25.45)[a]	17 (34)	12 (28.57)[b]	5 (29.41)
A/G	32 (32)	24 (43.64)	16 (32)	23 (54.76)[c]	7 (41.18)
G/G	19 (19)	17 (30.91)	17 (34)	7 (16.67)	5 (29.41)
Allelic frequencies					
A	130 (70)	52 (47.27)	50 (50)	47 (55.95)	17 (50)
G	70 (35)	58 (52.73)	50 (50)	37 (44.05)	17 (50)

Fas genotypes were similar in controls of both sexes: 52 males and 48 females; $\chi^2 = 0.06$, $P = 0.8$, two degrees of freedom; [a]χ^2 test of heterogeneity between CD females and controls ($\chi^2 = 8.15$, two degrees of freedom, P corrected = 0.004) odds ratio for AA genotype is 0.36 (0.16 < OR < 0.77, CI = 95%); [b]χ^2 test of heterogeneity between UC females and controls ($\chi^2 = 5.04$, two degrees of freedom, P corrected = 0.02) odds ratio for AA genotype is 0.42 (0.18 < OR < 0.96, CI = 95%); [c]χ^2 test of heterogeneity between UC females and controls ($\chi^2 = 6.46$, two degrees of freedom, P corrected = 0.01) odds ratio for AG genotype is 2.57 (1.15 < OR < 5.76, CI = 95%).

Table 4 Association between (-670 A/G) Apo1/Fas gene polymorphism and location of lesion

Apo1/Fas	UC (*n* = 59)				CD (*n* = 105)		
	G/G	G/A	A/A		G/G	G/A	A/A
Distal	4	9	8	Ileum	5	10	12[a]
Left-sided colitis	5	15	6	Colon	13[b]	8	3
Pancolitis	3	8	1	Ileocolon	16	22	16

[a]χ^2 test between AA genotype and ileum location of lesion in CD patients ($\chi^2 = 3.89$, two degrees of freedom, P corrected = 0.048) odds ratio is 2.48 (0.90 < OR < 6.87, CI = 95%); [b]χ^2 test between GG genotype and colon location of lesion in CD patients ($\chi^2 = 6.74$, two degrees of freedom, P corrected = 0.009) odds ratio is 3.38 (1.19 < OR < 9.67, CI = 95%).

or not carrying the (-670 A/G) allele (data not shown). We did not find an association between (-670 A/G) polymorphism and the severity of disease in any of the IBD patients, as defined by the need for surgery (data not shown).

DISCUSSION

There are several candidate genes potentially involved in the pathogenesis of IBD because of chromosomal location or function within inflammatory processes or both[27]. Much investigation has surrounded the FasL system, a major pathway responsible for inducing apoptosis of T cells and enterocytes in the colonic mucosa[28]. Fas is a type I transmembrane protein and a member of the TNF receptor superfamily that may be expressed constitutively by gut lamina propria T (LPT) cells[29]. FasL is a type II transmembrane protein that is expressed on cytotoxic T cells and that induces apoptosis of cells expressing Fas[30]. Defective apoptosis of LPT cells may be a factor in mucosal immune dysregulation and tissue inflammation. Rioux *et al*[31] found that 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 the present study, we genotyped Fas-670 polymorphisms in Tunisian patients with IBD and healthy controls, and found that the frequencies of the A allele and the AA genotype were significantly lower in patients with IBD compared with those in the control group, and in UC patients compared to CD patients, but here the difference was not significant. Thus, we have demonstrated an association between a promoter polymorphism of the (-670 A/G) Fas/Apo1 gene and IBD in this study. The significance of this association may differ according to the population studied and the type of inflammatory bowel disease. In fact, no association of (-670 A/G) Fas/Apo1 SNP with IBD in Chinese, Dutch, Australian and Korean patients exists[32-35]. Confusingly, in some studies the A variant was found as a susceptibility factor for rheumatoid and multiple sclerosis disease. Stratification analyses revealed that the association was stronger in males than in females. Sex effects in IBD have already been reported by Huang *et al*[36] who have identified several putative regions of sex-specific linkage. Regions on chromosomes 6, 11, 14 and 18 showed strong evidence of linkage in male-affected families but not in female-affected families. Moreover, oral contraceptives have been shown to be associated with increased risk for CD[37]. It has been proposed that epigenetic factors play an important role in the pathogenesis of IBD[38], and that sex effects are mediated by sexual hormones, which have an effect on gene expression and consequently could lead to differential expression of disease susceptibility genes in males and females. According to our findings, it appears that Fas/Apo1 gene polymorphisms decrease susceptibility to CD and UC in females in the Tunisian population. However, because there was no evidence of linkage of the Fas/Apo1 gene with the X chromosome, it is possible that this difference could be a random variation. On the other hand, it appears that CTLA-4 +49 gene polymorphisms increase susceptibility to CD in females in the Tunisian population and in the same patients as have Fas/Apo1 polymorphisms. No explanations have been presented for the discrepancy between positive findings, like ours, and the negative findings of others. The most plausible is the known genetic diversity of the different populations at the haplotype level. Our present results and the lack of previous studies on the sex distribution of Fas/Apo1 polymorphisms in patients with IBD warrant further investigation. The reason for this divergence is not clear but might reflect an ethnic difference in the contribution of genetic factor(s).

Fas-670 polymorphism within the promoter region is situated at a transcriptional binding site (the γ interferon activation site) and may potentially have a functional effect on gene regulation of factors such as STATs as well as on expression of proapoptotic proteins (Fas, FasL). In fact, in patients with UC, concentration of a systemic soluble form of Fas was found to be significantly lower in active UC than in controls[35]. Garcia Rodriguez *et al*[39] have studied Fas/FasL expression on activated colonic T

cells of UC patients, as well as the susceptibility of such T cells to activation-induced cell death, and have reported that CD4+ and CD8+ T cells in UC mucosa expressing FasL were significantly enhanced, suggesting that T cells in UC are less sensitive to apoptotic signals mediated by Fas. A few studies reported the association between the Fas/Apo1 gene promoter (-670 A/G) polymorphism and the localization of the lesions in IBD. Xia *et al*[40] have not found a difference between left-sided and total colitis (P = 0.8242). Moreover, in our study we found an association between ileum and colon localization of the lesions in CD patients and (-670 A/G) polymorphism.

Regarding the Fas-670 polymorphism, it has been hypothesized that either increased apoptosis of intestinal epithelium or decreased apoptosis of lamina propria lymphocytes may induce inflammation of the gut and, 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. However, in this study, a Fas-670 polymorphism was associated with the development of CD and UC, providing strong support for an IBD susceptibility gene in the region surrounding ApoI/Fas. It remains to be determined precisely how the Fas alleles influence the pathogenesis of IBD. Finally, it is possible that haplotypes exist in our population which we may have missed. We conclude that our results must await confirmation by other investigators.

COMMENTS

Background

In recent years, a few studies have been published addressing the question of where and under what conditions ligand for Fas (FasL) is produced in the gut in the normal and neoplastic situation. Some of these studies have considerably influenced our view of the role of the CD95/CD95L system. That is why it is necessary to analyze the Fas/Apo1 polymorphism in unrelated Tunisian patients with Crohn's disease (CD) and ulcerative colitis (UC) to evaluate the contribution of the CD95 gene to genetic susceptibility to inflammatory bowel disease (IBD).

Research frontiers

Inhibition of cytokine-regulated apoptosis may be useful in preventing or treating intestinal lesions in patients with IBD.

Innovations and breakthroughs

The relationship between Fas-670 polymorphism and IBD has not been reported yet. This is probably the first report on the association of Fas-670 polymorphisms in Tunisian IBD patients. However, this polymorphism was associated with the development of CD and UC, providing strong support for an IBD susceptibility gene in the region surrounding ApoI/Fas.

Applications

By understanding how the Fas-670 polymorphism is associated with the development of CD and UC, this study may indicate a future strategy for therapeutic intervention in patients with IBD.

Peer review

The manuscript by Ben Aleya *et al* reports on the association of a Fas/Apo1 promoter polymorphism with IBD in Tunisian patients. The experiments are performed well and the results are interesting.

REFERENCES

1 **Fiocchi C**. Inflammatory bowel disease: etiology and pathogenesis. *Gastroenterology* 1998; **115**: 182-205

2 **Hendrickson BA**, Gokhale R, Cho JH. Clinical aspects and pathophysiology of inflammatory bowel disease. *Clin Microbiol Rev* 2002; **15**: 79-94

3 **Peeters M**, Joossens S, Vermeire S, Vlietinck R, Bossuyt X, Rutgeerts P. Diagnostic value of anti-Saccharomyces cerevisiae and antineutrophil cytoplasmic autoantibodies in inflammatory bowel disease. *Am J Gastroenterol* 2001; **96**: 730-734

4 **Karlinger K**, Gyorke T, Mako E, Mester A, Tarjan Z. The epidemiology and the pathogenesis of inflammatory bowel disease. *Eur J Radiol* 2000; **35**: 154-167

5 **Ahmad T**, Satsangi J, McGovern D, Bunce M, Jewell DP. Review article: the genetics of inflammatory bowel disease. *Aliment Pharmacol Ther* 2001; **15**: 731-748

6 **De Maria R**, Boirivant M, Cifone MG, Roncaioli P, Hahne M, Tschopp J, Pallone F, Santoni A, Testi R. Functional expression of Fas and Fas ligand on human gut lamina propria T lymphocytes. A potential role for the acidic sphingomyelinase pathway in normal immunoregulation. *J Clin Invest* 1996; **97**: 316-322

7 **Alnemri ES**, Livingston DJ, Nicholson DW, Salvesen G, Thornberry NA, Wong WW, Yuan J. Human ICE/CED-3 protease nomenclature. *Cell* 1996; **87**: 171

8 **Ashkenazi A**, Dixit VM. Death receptors: signaling and modulation. *Science* 1998; **281**: 1305-1308

9 **Fiorucci S**, Santucci L, Cirino G, Mencarelli A, Familiari L, Soldato PD, Morelli A. IL-1 beta converting enzyme is a target for nitric oxide-releasing aspirin: new insights in the antiinflammatory mechanism of nitric oxide-releasing nonsteroidal antiinflammatory drugs. *J Immunol* 2000; **165**: 5245-5254

10 **Iwamoto M**, Koji T, Makiyama K, Kobayashi N, Nakane PK. Apoptosis of crypt epithelial cells in ulcerative colitis. *J Pathol* 1996; **180**: 152-159

11 **Strater J**, Wellisch I, Riedl S, Walczak H, Koretz K, Tandara A, Krammer PH, Moller P. CD95 (APO-1/Fas)-mediated apoptosis in colon epithelial cells: a possible role in ulcerative colitis. *Gastroenterology* 1997; **113**: 160-167

12 **Ueyama H**, Kiyohara T, Sawada N, Isozaki K, Kitamura S, Kondo S, Miyagawa J, Kanayama S, Shinomura Y, Ishikawa H, Ohtani T, Nezu R, Nagata S, Matsuzawa Y. High Fas ligand expression on lymphocytes in lesions of ulcerative colitis. *Gut* 1998; **43**: 48-55

13 **Inazawa J**, Itoh N, Abe T, Nagata S. Assignment of the human Fas antigen gene (Fas) to 10q24.1. *Genomics* 1992; **14**: 821-822

14 **Huang QR**, Morris D, Manolios N. Identification and characterization of polymorphisms in the promoter region of the human Apo-1/Fas (CD95) gene. *Mol Immunol* 1997; **34**: 577-582

15 **Thornberry NA**, Lazebnik Y. Caspases: enemies within. *Science* 1998; **281**: 1312-1316

16 **Nolsoe RL**, Kristiansen OP, Sangthongpitag K, Larsen ZM, Johannesen J, Karlsen AE, Pociot F, Nerup J, Verge CF, Mandrup-Poulsen T. Complete molecular scanning of the human Fas gene: mutational analysis and linkage studies in families with type I diabetes mellitus. The Danish Study Group of Diabetes in Childhood and The Danish IDDM Epidemiology and Genetics Group. *Diabetologia* 2000; **43**: 800-808

17 **Cascino I**, Ballerini C, Audino S, Rombola G, Massacesi L, Colombo G, Scorza Smeral di R, d'Alfonso S, Momigliano Richiardi P, Tosi R, Ruberti G. Fas gene polymorphisms are not associated with systemic lupus erythematosus, multiple sclerosis and HIV infection. *Dis Markers* 1998; **13**: 221-225

18 **Bolstad AI**, Wargelius A, Nakken B, Haga HJ, Jonsson R. Fas and Fas ligand gene polymorphisms in primary Sjogren's syndrome. *J Rheumatol* 2000; **27**: 2397-2405

19 **Huang QR**, Danis V, Lassere M, Edmonds J, Manolios N. Evaluation of a new Apo-1/Fas promoter polymorphism in rheumatoid arthritis and systemic lupus erythematosus patients. *Rheumatology* (Oxford) 1999; **38**: 645-651

20 **Lennard-Jones JE**. Classification of inflammatory bowel disease. *Scand J Gastroenterol Suppl* 1989; **170**: 2-6; discussion 16-19

21 **Gasche C**, Scholmerich J, Brynskov J, D'Haens G, Hanauer SB, Irvine EJ, Jewell DP, Rachmilewitz D, Sachar DB, Sandborn WJ, Sutherland LR. A simple classification of Crohn's disease: report of the Working Party for the World Congresses of Gastroenterology, Vienna 1998. *Inflamm Bowel Dis* 2000; **6**: 8-15

22 **Nagata S**. Fas ligand-induced apoptosis. *Annu Rev Genet* 1999; **33**: 29-55

23 **French LE**, Tschopp J. Constitutive Fas ligand expression in several non-lymphoid mouse tissues: implications for immune-protection and cell turnover. *Behring Inst Mitt* 1996; 156-160

24 **Hampe J**, Shaw SH, Saiz R, Leysens N, Lantermann A, Mascheretti S, Lynch NJ, MacPherson AJ, Bridger S, van Deventer S, Stokkers P, Morin P, Mirza MM, Forbes A, Lennard-Jones JE, Mathew CG, Curran ME, Schreiber S. Linkage of inflammatory bowel disease to human chromosome 6p. *Am J Hum Genet* 1999; **65**: 1647-1655

25 **Satsangi J**, Parkes M, Louis E, Hashimoto L, Kato N, Welsh K, Terwilliger JD, Lathrop GM, Bell JI, Jewell DP. Two stage genome-wide search in inflammatory bowel disease provides evidence for susceptibility loci on chromosomes 3, 7 and 12. *Nat Genet* 1996; **14**: 199-202

26 **Lawrance IC**, Fiocchi C, Chakravarti S. Ulcerative colitis and Crohn's disease: distinctive gene expression profiles and novel susceptibility candidate genes. *Hum Mol Genet* 2001; **10**: 445-456

27 **Hugot JP**, Laurent-Puig P, Gower-Rousseau C, Olson JM, Lee JC, Beaugerie L, Naom I, Dupas JL, Van Gossum A, Orholm M, Bonaiti-Pellie C, Weissenbach J, Mathew CG, Lennard-Jones JE, Cortot A, Colombel JF, Thomas G. Mapping of a susceptibility locus for Crohn's disease on chromosome 16. *Nature* 1996; **379**: 821-823

28 **Hugot JP**, Chamaillard M, Zouali H, Lesage S, Cezard JP, Belaiche J, Almer S, Tysk C, O'Morain CA, Gassull M, Binder V, Finkel Y, Cortot A, Modigliani R, Laurent-Puig P, Gower-Rousseau C, Macry J, Colombel JF, Sahbatou M, Thomas G. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 2001; **411**: 599-603

29 **Boughton-Smith NK**, Evans SM, Hawkey CJ, Cole AT, Balsitis M, Whittle BJ, Moncada S. Nitric oxide synthase activity in ulcerative colitis and Crohn's disease. *Lancet* 1993; **342**: 338-340

30 **Fisher SA**, Hampe J, Macpherson AJ, Forbes A, Lennard-Jones JE, Schreiber S, Curran ME, Mathew CG, Lewis CM. Sex stratification of an inflammatory bowel disease genome search shows male-specific linkage to the HLA region of chromosome 6. *Eur J Hum Genet* 2002; **10**: 259-265

31 **Rioux JD**, Silverberg MS, Daly MJ, Steinhart AH, McLeod RS, Griffiths AM, Green T, Brettin TS, Stone V, Bull SB, Bitton A, Williams CN, Greenberg GR, Cohen Z, Lander ES, Hudson TJ, Siminovitch KA. Genomewide search in Canadian families with inflammatory bowel disease reveals two novel susceptibility loci. *Am J Hum Genet* 2000; **66**: 1863-1870

32 **Low JH**, Williams FA, Yang X, Cullen S, Colley J, Ling KL, Armuzzi A, Ahmad T, Neville MJ, Dechairo BM, Walton R, Lench NJ, Jewell DP. Inflammatory bowel disease is linked to 19p13 and associated with ICAM-1. *Inflamm Bowel Dis* 2004; **10**: 173-181

33 **Satsangi J**, Morecroft J, Shah NB, Nimmo E. Genetics of inflammatory bowel disease: scientific and clinical implications. *Best Pract Res Clin Gastroenterol* 2003; **17**: 3-18

34 **Gaya DR**, Russell RK, Nimmo ER, Satsangi J. New genes in inflammatory bowel disease: lessons for complex diseases? *Lancet* 2006; **367**: 1271-1284

35 **Bu P**, Keshavarzian A, Stone DD, Liu J, Le PT, Fisher S, Qiao L. Apoptosis: one of the mechanisms that maintains unresponsiveness of the intestinal mucosal immune system. *J Immunol* 2001; **166**: 6399-6403

36 **Huang QR**, Teutsch SM, Buhler MM, Bennetts BH, Heard RN, Manolios N, Stewart GJ. Evaluation of the apo-1/Fas promoter mva I polymorphism in multiple sclerosis. *Mult Scler* 2000; **6**: 14-18

37 **Lee YH**, Ji JD, Sohn J, Song GG. Polymorphsims of CTLA-4 exon 1 +49, CTLA-4 promoter -318 and Fas promoter -670 in spondyloarthropathies. *Clin Rheumatol* 2001; **20**: 420-422

38 **van Veen T**, Kalkers NF, Crusius JB, van Winsen L, Barkhof F, Jongen PJ, Pena AS, Polman CH, Uitdehaag BM. The FAS-670 polymorphism influences susceptibility to multiple sclerosis. *J Neuroimmunol* 2002; **128**: 95-100

39 **Garcia Rodriguez LA**, Gonzalez-Perez A, Johansson S, Wallander MA. Risk factors for inflammatory bowel disease in the general population. *Aliment Pharmacol Ther* 2005; **22**: 309-315

40 **Xia B**, Yu YH, Guo QS, Li XY, Jiang L, Li J. Association of Fas-670 gene polymorphism with inflammatory bowel disease in Chinese patients. *World J Gastroenterol* 2005; **11**: 415-417

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