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Analysis of Single Nucleotide Polymorphisms in CD14 and Tumor Necrosis Factor α Gene Promoters in Inflammatory Bowel Disease

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ABSTRACT

To clarify the genetic predisposition to two inflammatory bowel diseases, ulcerative colitis (UC) and Crohn's disease (CD), we analyzed the allele and genotype frequencies of the single nucleotide polymorphisms (SNPs) at -159 (T/C) of CD14 gene and at -1031 (C/T), -863(C/A) and -857 (C/T) of the tumor necrosis factor α (TNF-α) gene in 98 patients with UC and 79 patients with CD. The frequency of homozygous genotype -159 (C/C) of CD14 gene was decreased in both UC and CD. The T allele frequency at position -857 of TNF-α gene was higher in UC and lower in CD than in control subjects. The frequencies of genotypes -1031(T/T) and -863(C/C) of the TNF-α gene were higher in patients with UC with proctitis, and the frequency of the C allele at position -863 of the TNF-α gene was decreased in patients with UC involving the colon. The allele and genotype distributions at position -159 of CD14 gene were similar among the subgroups of patients with UC and CD. The SNP at position -159 of the CD14 gene promoter may indicate a genetic predisposition factor for inflammatory bowel disease, where as the SNP at position -857 of the TNF-α gene promoter may represent the linkage disequilibrium with HLA-DR. (Jikeikai Med J 2003; 50: 149-58)

Key words: inflammatory bowel disease, single nucleotide polymorphism, CD14, tumor necrosis factor α

INTRODUCTION

Ulcerative colitis (UC) and Crohn's disease (CD) belong to a group of inflammatory diseases of the large and small intestines whose interrelations remain obscure. Both these chronic diseases of unknown origin are considered types of inflammatory bowel disease (IBD). UC is characterized by diffuse and continuous inflammation involving the mucosa of the large intestine, whereas CD can appear with mucosal to transmural inflammation in every part of the gastrointestinal tract but is most common in the ileocolonic area. UC and CD are defined by typical clinical, pathologic, radiologic, endoscopic, and laboratory features. Although the development and progression of symptoms differ between UC and CD, the pathogenesis of IBD remains unknown.

Recent genome-wide linkage analysis has shown that several candidate loci, designated IBD1 to IBD7, increase susceptibility to IBD[1]. IBD1 is located on chromosome 16q12-13, IBD2 on chromosome 12p13.2-q24.1, IBD3 on chromosome 6p, also known as the human leukocyte antigens (HLA) region, IBD4 on chromosome 14q11-12, IBD5 on chromosome 5q31,

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Table 1. Characteristics of patients with ulcerative colitis and Crohn's disease

Ulcerative colitis (n=98)		Crohn's disease (n=79)	
Gender			
Male	42	61	
Female	56	18	
Age (years)			
mean	40.8±7.5	36.8±9.2	
range	16−72	19−62	
Disease location			
Proctitis	(n=22)	Small bowel	(n=18)
Left-side colitis	(n=32)	Ileocolon	(n=47)
Total colitis	(n=44)	Colon	(n=14)

IBD6 on 19p13, and IBD7 on chromosome 1p36. The loci IBD1, 4, and 5 are believed to be involved in the pathogenesis of CD, whereas IBD2, 3, 6, and 7 are believed to be involved in the pathogenesis of both UC and CD. Many additional genes have been proposed to participate in the susceptibility to IBD[2−11].

CD14 is a lipopolysaccharide (LPS) receptor expressed on the surfaces of monocytes, neutrophils, and macrophages[12]. Upon stimulation with LPS, the main endotoxin-derived from Gram-negative bacteria, large amounts of proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1, and IL-6, are produced and released from CD14-expressing cells[13]. The response to LPS occurs through activation of toll-like receptor 4 (TLR4) and nuclear factor kappa B (NF-kB)[14]. There are two forms of CD14, a soluble form (sCD14) found in the serum and a membrane-binding form (mCD14)[15].

The CD14 gene is located on chromosome 5q31.1 at locus IBD5[16] and shows an association with CD. A single nucleotide polymorphism (SNP; T/C at position −159) is present in the promoter region of the CD14 gene[17], and T/T homozygotes have significantly higher serum levels of sCD14[18].

TNF-α is an important proinflammatory cytokine that is thought to play a pivotal role in the development of IBD[18]. A large amount of TNF-α is secreted from cells expressing mCD14 following stimulation with LPS[19]. The TNF-α gene is located on chromosome 6p in the human leukocyte antigen (HLA) class III region and is located between the HLA DR (class II) and HLA B (class I) loci[20]. Furthermore, the IBD3 locus is also on chromosome 6p and is believed to play a role in the susceptibility to both CD and UC[5].

In the present study, we evaluated the effect of SNPs on the susceptibility to IBD by direct sequencing of single nucleotide substitutions at −159 (T/C) in the promoter of the CD14 gene and at −1031 (C/T), −863 (C/A), and −857 (C/T) in the promoter of the TNF-α gene.

Materials and Methods

Patients

Ninety-eight patients with UC and 79 patients with CD treated at The Jikei University Hospital from April 2001 through December 2002 were enrolled. In addition, 102 unrelated healthy volunteers were enrolled as control subjects. The diagnosis of UC or CD was made on the basis of clinical symptoms and endoscopic, radiographic, and histologic criteria developed by the Japanese Research Committee of Inflammatory Bowel Disease supported by the Ministry of Health, Labor and Welfare. Other intestinal disorders, such as intestinal Behcet's disease, were carefully ruled out. Patients with suspected but unconfirmed UC or CD were excluded. All patients and control subjects were unrelated Japanese. Only one patient with UC had a family history of IBD.

Written informed consent was obtained from all subjects. The study was approved by the Ethics Committee of The Jikei University School of Medicine.

Classification of UC and CD

The 98 patients with UC were divided into the following subgroups according to the location and extent of inflammatory lesions: proctitis ($n=22$), left-sided colitis ($n=32$), or total colitis ($n=44$). Patients with CD were divided into the following subgroups according to the location of lesions: small bowel ($n=18$), ileocolon ($n=47$), or colon ($n=14$). The characteristics of the patients are shown in Table 1.

DNA Extraction

Heparinized peripheral blood was obtained, and mononuclear cells were collected by standard sediment centrifugation. Genomic DNA was extracted from peripheral mononuclear cells using a DNA extraction kit (Talent srl, Trieste, Italy) according to the manufacturer's instructions.

Sequencing CD14 and TNF-α genes promoter regions

Polymerase chain reactions (PCRs; total volume, 50 μl) were performed according to the manufacturer's instructions with AmpliTaq Gold (Applied Biosystems, Foster, CA, USA), 50 to 100 ng of each genomic DNA template, and the following PCR primer pairs for the indicated SNPs: polymorphism at -159 (T/C) of CD14 gene was amplified with the forward primer (5′-GCAGAGTATGGTACTGGCCTAAGGC-3′) and reverse primer (5′-AGCTTCTTTCCTACACAGCGGC-3′). Polymorphisms at -1031 (C/T), -863 (C/A), and -857 (C/T) of the TNF-α gene were amplified with the forward primer (5′-CAAAAGGATAAGGGCTCAGAGAGC-3′) and reverse primer (5′-CGTCCCCTGTATTCCATACCTGG-3′). Amplification was done as follows: initial denaturation at 94(C for 30 seconds, annealing at 60° C for 30 seconds, and extension at 72°C for 30 seconds, with a final extension at 72°C for 5 minutes PCR products were purified through a Sephadex G-50 column (AM Giken Co., Tokyo) and sequenced according to the manufacturer's instructions on an ABI 3100 automated sequencer using the ABI PRISM Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems) with sequence primers for CD14 (5′-GCAGAGTATGGTACTGGCCTAAGGC-3′) or TNF-α (5′-CAAAAGGATAAGGGCTCAGAGAG C-3′).

Estimating haplotype frequencies at -1031 (C/T), -863 (C/A), and -857 (C/T) of the TNF-α gene

The haplotype frequencies of the TNF-α gene based on three SNPs at positions -1031, -863 and -857 could not be directly determined in this study. Instead, we applied the genotypic data to a computer program to estimate the haplotype frequencies in the population. This program was designed for determining haplotypes with a maximum-likelihood estimation method based on the expectation maximization (EM) algorithm of Dempster et al.[21] using population genetics analysis software (Arlequin, available at http://acasun1.unige.ch/arlequin/). This method estimates the frequencies of haplotypes in the population from which the sample was obtained.

Statistical analysis

Allele and genotype frequencies were calculated using direct counts. The allele counts were compared between patients with IBD and healthy control subjects using the chi-square test. Yates's correction was used for groups of less than 5 subjects. The strength of association was assessed by odds ratios (OR) with 95% confidence intervals (95% CI). Statistical significance was indicated by a probability less 0.05. Hardy-Weinberg equilibrium in control subjects was tested using the chi-square test.

Results

The association of IBD with polymorphisms in the promoter regions of the CD14 and TNF-α gene was analyzed with direct sequencing. Genotype frequencies at -159 of the CD14 gene promoter and -1031, -863, and -857 of the TNF-α gene promoter in control subjects were in Hardy-Weinberg equilibrium.

Table 2. Allele and genotype frequencies at −159 of CD14 gene in patients with ulcerative colitis (UC) or Crohn's disease (CD)

	Allele frequency		Genotype frequency		
	T (%)	C (%)	T/T (%)	T/C (%)	C/C (%)
UC (n=98)	120 (61.2)*	76 (38.8)	36 (36.7)	48 (49.0)	14 (14.3)*
CD (n=79)	95 (60.1)	63 (39.9)	27 (34.2)	41 (51.9)	11 (13.9)*
Controls (n=102)	104 (51.0)	100 (49.0)	33 (32.3)	38 (37.3)	31 (30.4)

*p<0.05 vs Control

Table 3. Allele and genotype frequencies at −1031, −863 and −857 of TNF-α gene in UC and CD patients

	−1031					−863					−857				
	Allele frequency		Genotype frequency			Allele frequency		Genotype frequency			Allele frequency		Genotype frequency		
	T (%)	C (%)	T/T (%)	T/C (%)	C/C (%)	C (%)	A (%)	C/C (%)	C/A (%)	A/A (%)	C (%)	T (%)	C/C (%)	C/T (%)	T/T (%)
UC	167	29	75	17	6	170	26	78	14	6	178	18*	81	16	1[a]*
(n=98)	(85.2)	(14.8)	(76.5)	(17.4)	(6.1)	(86.7)	(13.3)	(79.6)	(14.3)	(6.1)	(90.8)	(9.2)	(82.7)	(16.3)	(1.0)
CD	131	27	53	25	1	133	25	55	23	1	112	46**	35**	42	2
(n=79)	(82.9)	(17.1)	(67.1)	(31.6)	(1.3)	(84.2)	(15.8)	(69.6)	(29.1)	(1.3)	(70.9)	(29.1)	(44.3)	(53.2)	(2.5)
Control	163	41	73	21	8	163	41	76	20	6	169	35	75	19	8
(n=102)	(79.9)	(20.1)	(71.6)	(20.5)	(7.8)	(79.9)	(20.1)	(74.5)	(19.6)	(5.9)	(82.8)	(17.2)	(73.5)	(18.6)	(7.9)

*p<0.05 vs Control
**p<0.01 vs Control
[a]*p<0.05 vs Control with Yates's correction

Polymorphism in −159 (T/C) of the CD14 gene in patients with UC or CD

The allele frequency of −159T was significantly higher in patients with UC than in control subjects (OR=1.52, 95% CI=1.02 to 2.26, p<0.05) but did not differ significantly between patients with CD and control subjects (Table 2).

The distributions of genotypes T/T, T/C, and C/C were similar in patients with UC and patients with CD, and the frequency of homozygote −159(C/C) in patients with UC and patients with CD was significantly lower than in control subjects (OR=0.38, 95% CI=0.19 to 0.77, p<0.05, and OR=0.37, 95% CI=0.17 to 0.80, p<0.05, respectively). The frequencies of −159(T/T) and −159(T/C) did not differ statistically between patients with IBD and control subjects.

Polymorphisms in −1031 (T/C), −863 (C/A), and −857 (C/T) of the TNF-α gene in patients with UC or CD

There were no significant differences in the allele and genotype frequencies at positions −1031 and −863 (Table 3).

The frequency of −857T was significantly lower in patients with UC than in control subjects (OR=0.49, 95% CI=0.27 to 0.90, p<0.05) but was significantly higher in patients with CD than in control subjects (OR=1.98, 95% CI=1.20 to 3.27, p<0.01). Furthermore, the frequency of the −857T allele was significantly higher in patients with CD than in patients with UC (OR=4.06, 95% CI=2.24 to 7.36, p<0.01).

The frequency of homozygote −857(C/C) was significantly lower in patients with CD than in control subjects or patients with UC (OR=0.29, 95% CI=0.15

Table 4. Allele and genotype frequencies at −159 of CD14 gene by subgroups of UC and CD patients

	Allele frequency		Genotype frequency		
	T (%)	C (%)	T/T (%)	T/C (%)	C/C (%)
UC					
Proctitis (n=22)	24 (54.5)	20 (45.5)	5 (22.7)	14 (63.7)	3 (13.6)
Left-sided colitis (n=32)	41 (64.1)	23 (35.9)	13 (40.6)	15 (46.9)	4 (12.5)
Total colitis (n=44)	52 (59.1)	36 (40.9)	15 (34.1)	22 (50.0)	7 (15.9)
CD					
Small bowel (n=18)	23 (63.9)	13 (36.1)	6 (33.3)	11 (61.1)	1 (5.6)
Ileocolon (n=47)	53 (56.4)	41 (43.6)	17 (36.2)	21 (44.7)	9 (19.1)
Colon (n=14)	17 (60.7)	11 (39.3)	4 (28.6)	9 (64.3)	1 (7.1)

Table 5. Allele and genotype frequencies at −1031, −863 and −857 of TNF-α gene by subgroups of UC patients

	−1031					−863					−857				
	Allele frequency		Genotype frequency			Allele frequency		Genotype frequency			Allele frequency		Genotype frequency		
	T (%)	C (%)	T/T (%)	T/C (%)	C/C (%)	C (%)	A (%)	C/C (%)	C/A (%)	A/A (%)	C (%)	T (%)	C/C (%)	C/T (%)	T/T (%)
Proctitis (n=22)	43* (97.7)	1 (2.3)	21* (95.5)	1 (4.5)	0 (0.0)	43* (97.7)	1 (2.3)	21* (95.5)	1 (4.5)	0 (0.0)	41 (93.2)	3 (6.8)	19 (86.4)	3 (13.6)	0 (0.0)
Other than proctitis (n=76)	124 (81.5)	28 (18.5)	54 (71.1)	16 (21.1)	6 (7.8)	127 (83.5)	25 (16.5)	57 (75.0)	13 (17.1)	6 (7.9)	138 (90.8)	14 (9.2)	62 (81.6)	14 (18.4)	0 (0.0)
Left-sided colitis (n=32)	46	18	18	10	4	48	16	20	8	4	59	5	27	5	0
Total colitis (n=44)	78	10	36	6	2	79	9	37	5	2	79	9	35	9	0

*p<0.05 vs Other than proctitis

to 0.53 p<0.01 and OR=0.17, 95% CI=0.08 to 0.33, p<0.01). Moreover, the frequency of homozygous genotype −857 (T/T) in UC patients was significantly lower than in control subjects (OR=0.12, 95% CI=0.01 to 0.99, p<0.05).

Polymorphisms in UC and CD subgroups

The allele and genotype frequencies of CD14 gene promoter −159 were similar among subgroups of both UC and CD (Table 4).

The frequencies of alleles −1031T and −863C of the TNF-α gene promoter and the frequencies of genotypes −1031 (T/T) and −863 (C/C) were significantly higher in patients with proctitis than in other patient subgroups. In contrast, the allele and genotype frequencies at −857 were similar in all subgroups of UC (Table 5).

In subgroups of CD, the allele frequency of −863C was significantly lower in the colon subgroup than in the small bowel or ileocolon subgroup (OR=0.24, 95% CI=0.07 to 0.85, p<0.05 and OR=0.33, 95% CI=0.13 to 0.84, p<0.05). However, there was no significant difference in the distribution of genotypes at −863, owing in part to the small number of patients in the colon subgroup. There were no differences in allele or genotype frequencies at −1031 or −857 (Table 6).

Table 6. Allele and genotype frequencies at −1031, −863 and −857 of TNF-α gene by subgroups of CD patients

Subgroup (Type)	−1031					−863					−857				
	Allele frequency		Genotype frequency			Allele frequency		Genotype frequency			Allele frequency		Genotype frequency		
	T (%)	C (%)	T/T (%)	T/C (%)	C/C (%)	C (%)	A (%)	C/C (%)	C/A (%)	A/A (%)	C (%)	T (%)	C/C (%)	C/T (%)	T/T (%)
Small bowel	31	5	13	5	0	32	4*	14	4	0	27	9	9	9	0
(n=18)	(86.1)	(13.9)	(72.2)	(27.8)	(0.0)	(88.9)	(11.1)	(77.8)	(22.2)	(0.0)	(75.0)	(25.0)	(50.0)	(50.0)	(0.0)
Ileocolon (n=47)	78 (83.0)	16 (17.0)	31 (66.0)	16 (34.0)	0 (0.0)	80 (85.1)	14* (14.9)	33 (70.2)	14 (29.8)	0 (0.0)	71 (78.5)	23 (24.5)	26 (55.3)	19 (40.4)	2 (4.3)
Colon (n=14)	19 (67.9)	9 (32.1)	6 (42.9)	7 (50.0)	1 (7.1)	21 (75.0)	7 (25.0)	8 (57.1)	5 (35.7)	1 (7.2)	24 (85.7)	4 (14.3)	10 (71.4)	4 (28.6)	0 (0.0)

*$p<0.05$ vs Colon type

Table 7. The maximum-likelihood of haplotype frequencies comprised of −1031 (C/T), −863 (C/A) and −857 (C/T) in the promoter of TNF-α gene in UC and CD patients

	Haplotype frequency				
	TCC	CAC	TCT	CCC	Others
UC	0.763	0.127	0.088	0.012	0.010
CD	0.610	0.139	0.225	0.010	0.016
Control	0.654	0.138	0.155	0.032	0.021

Estimated haplotype frequencies in the TNF-α promoter region

Maximum-likelihood haplotype frequencies based on SNPs at −1031, −863 and −857 in the TNF-α gene promoter are shown in Table 7. There were three major haplotypes — TCC, CAC and TCT — and assumed minor haplotypes, including CCC. In patients with UC, the frequency of the TCC haplotype tended to be high and the frequency of the TCT haplotype was extremely low. The frequency of the CAC haplotype did not differ among patients with UC, patients with CD, and control subjects.

DISCUSSION

In the present study, we found the allele frequency of T at −159 of the CD14 gene was significantly higher in patients with UC than in control subjects and that the allele frequency of T at −857 of TNF-α gene was significantly higher in patients with CD than in control subjects. Furthermore, the genotype frequency of −159 (C/C) of the CD14 gene in patients with UC or CD was significantly lower than in control subjects. The frequency of homozygote −857(C/C) of the TNF-α gene was significantly lower in patients with CD than in control subjects and the frequency of −857(T/T) in patients with UC was significantly lower than in control subjects.

These results suggest that the polymorphisms at position −159 of the CD14 gene and at position −857 of the TNF-α gene are genetic predisposition factors for UC and CD in the Japanese population.

CD14, a receptor of LPS, is attached to the plasma membrane by a glycosylphosphatidyl inositol anchor and lacks an intracellular domain[13]. Signal transduction of CD14 is mediated by interaction with TLR4 through activation of NF-kB[14]. In IBD, the inflammatory mucosa shows elevated expression of mCD14 on mononuclear cells[22] and the mucosal barrier is thought to be disrupted. These conditions may allow LPS derived from intestinal bacteria to invade the inflamed mucosa and stimulate mononuclear cells, causing large quantities of proinflammatory cytokines to be released. In this situation, the amount of expressed mCD14 may contribute to the intestinal inflammation[12,23,24].

The SNP at position −159 of the CD14 gene promoter affects the expression of CD14. The T allele is associated with high levels in serum of sCD14 and elevated expression of mCD14 on circulating

monocytes[25]. Although the mechanism has not been determined, the T substitution for C at position -159 might affect the promoter activity of the CD14 gene because position -159 is adjacent to a putative AP-2 binding site of the promoter region. An *in vitro* promoter assay with luciferase has shown significant enhancement of promoter activity from -227 to -128[26].

Klein et al. report that the frequencies of the T allele and the T/T genotype at position -159 of CD14 gene are increased in white patients with CD[16]. Obana et al. report that frequencies of the T allele and the T/T genotype at -159 of the CD14 gene are increased in Japanese patients with UC but not in patients with CD[27]. These findings suggest that polymorphisms at -159 in the promoter of the CD14 gene play an important role in the development of UC or CD by regulating the expression of CD14. The difference in allele frequencies may be a racial difference.

In the present study, the frequency of the T allele at -159 was significantly higher in patients with UC and that of the C/C genotype was lower in patients with UC or CD. These findings were similar to those of a previous study[27]. However, to confirm the significance of the SNP at -159 in the development of CD in the Japanese population, even larger numbers of patients with CD should be examined.

Many candidate genes have been proposed near the CD14 gene locus, including those for IL-3, 4, 5, 9, and 13 and platelet-derived growth factor receptor. Therefore, we cannot rule out the possibility that the association is the result of linkage disequilibrium with a responsible gene[27]. However, our findings strongly suggest that the polymorphism at position -159 in the CD14 gene is responsible for the association with both UC and CD.

The TNF-α gene is located in the class III region of the major histocompatibility complex (MHC), the 250-kb centromeric of the class I HLA-B locus and the 850-kb telomeric of the class II HLA-DR locus[28]. TNF-α is produced mainly by activated lymphocytes and macrophages. In IBD, TNF-α participates in disease progression and functions as a potent proinflammatory cytokine to stimulate acute-phase reactants, to activate epithelial cells with HLA II antigen expression, and to produce other cytokines[29].

Many SNPs have been reported in the promoter region of the TNF-α gene[30-33], with the most common ones at positions -1031 (C/T), -863 (C/A), -857 (C/T), -308 (G/A), and -238 (G/A). These SNPs are common in the Japanese population[33]. Other rare polymorphisms are defined in the proximal promoter of the TNF-α gene at positions -163, -376, and -574[31-33]. Among these SNPs, those at -1031, -863, and -857 are reportedly associated with transcriptional activity[34]. In addition, the SNP at -308 of the TNF-α gene is believed to be responsible for transcriptional regulation[35,36].

In Japanese patients Negoro et al. have reported that the TNF-α gene polymorphisms -1031C, -863A, and -857T are associated with CD, but not with UC[37], and Kawasaki et al. have reported that the U03 TNF-α promoter haplotype (-1031C, -863A, -857C) is more frequent and the U04 haplotype (-1031C, -863C, -857C) is less frequent in Japanese patients with CD[38]. The association of -857T with CD agreed in part with the findings of Negoro et al[37]. However, our finding that the frequency of haplotype CAC is not higher in patients with CD disagrees with the findings of Kawasaki et al.[38].

The LPS-stimulated production of TNF-α in whole blood ex vivo is higher in healthy whites homozygous for -857C. In addition, transcription factor OCT1 binds selectively at -857T and interacts with the NF-kB transcription factor p65 subunit at an adjacent binding site[39]. A luciferase assay has shown that the -1031C, -863A and -857T alleles are associated with high production of TNF-α and high transcriptional promoter activity in response to concanavalin A[34]. Therefore, the identity of the allele or genotype responsible for the ability to produce TNF-α remains unclear.

Recent studies have suggested that the transition from G to A at position -308 is associated with the higher production of TNF-α[35,36]. The SNP at -308 was not related to susceptibility to CD[40] but was related to the intense inflammatory activity and risk of arthritis in fistulizing CD[41]. Thus, whether the correlation between SNPs in the TNF-α gene pro-

moter and IBD actually imply genetic susceptibility to IBD is controversial. The correlation may simply reflect a disequilibrium linkage with a nearby, truly associated gene, for instance HLA DR or HLA B.

Kawasaki et al. have reported that HLA-DR B1* 0405 and 0410 and TNF-α promoter SNPs independently contributed to the susceptibility to CD[38]. However, Higuchi et al. have reported strong disequilibrium with HLA and SNPs of the TNF-α promoter. In the general Japanese population the -1031C and -863A alleles of the TNF-α gene are in significant linkage disequilibrium with HLA-B61 and -DRB1*0901 and the -857T allele is in linkage disequilibrium with HLA-B54, -B35, -B59, or -DRB1* 0405[34]. Furthermore, Nakajima et al. have demonstrated that HLA-DRB1*0405/*0401 and *0802 are associated with CD in the Japanese population[42]. These findings suggest that HLA DR may be responsible for the susceptibility to CD. In addition, Futami et al. have shown that the HLA-DRB1*1502 allele is strongly associated with susceptibility to UC[43]; however, the linkage disequilibrium with TNF-α promoter has not been clarified.

In view of recent microsatellite polymorphism findings that the TNF-α gene is not involved in the pathogenesis of CD[44], SNPs in TNF-α promoter might not participate in the development of IBD but simply reflect the linkage disequilibrium with HLA DR. Instead, the SNPs at -1031, -863, and -867 of the TNF-α gene may be related to the extent of inflammatory lesions in UC or to the disease type of CD through regulation of the ability to produce TNF -α.

In conclusion, the -159T allele of the CD14 gene promoter may be a genetic predisposition factor for IBD, whereas the association of the SNP at -857 of the TNF-α gene promoter and IBD may represent the linkage disequilibrium with HLA-DR. To analyze the significance of SNPs in the TNF-α gene promoter for the genetic predisposition of IBD, disequilibrium with HLA DR must considered.

REFERENCES

1. OMIM Home page http://www3.ncbi.nlm.nih.gov/omim/
2. Hugot JP, Laurent-Puig P, Gower-Rousseau C, Olson JM, Lee JC, Beaugerie L, et al. Mapping of susceptibility locus for Crohn's disease on chromosome 16. Nature 1996; 379: 821-3.
3. Duerr RH, Barmada MM, Zhang L, Davis S, Preston RA, Chensny LJ, et al. Linkage and association between inflammatory bowel disease and a locus on chromosome 12. Am J Hum Genet 1998; 63: 95-100.
4. Hampe J, Schreiber S, Shaw SH, Lau KF, Bridger S, Macpherson AJS, et al. A genomewide analysis provides evidence for novel linkages in inflammatory bowel disease in a large European cohort. Am J Hum Genet 1999; 64: 808-16.
5. Hampe J, Shaw SH, Saiz R, Lddeysens N, Lantermann A, Mascheretti S, et al. Linkage of inflammatory bowel disease to human chromosome 6p. Am J Hum Genet 1999; 65: 1647-55.
6. Duerr RH, Barmada MM, Zhang L, Pfutzer R, Weeks DE. High-density genome scan in Crohn disease shows confirmed linkage to chromosome 14q11-12. Am J Hum Genet 2000; 66: 1857-62.
7. Ahmad T, Satsangi J, Mcgovern D, Bunce M, Jewell DP. Review article: the genetics of inflammatory bowel disease. Aliment Pharmacol Ther 2001; 15: 731-48.
8. Rioux JD, Silverberg MS, Daly MJ, Steinhart AH, McLeod RS, Griffiths AM, et al. Genomewide search in Canadian families with inflammatory bowel disease reveals two novel susceptibility loci. Am J Hum Genet 2000; 66: 1863-70.
9. Rioux JD, Daly MJ, Silverberg MS, Lindblad K, Steinhart H, Cohen Z, et al. Genetic variation in the 5q31 cytokine gene cluster confers susceptibility to Crohn disease. Nat Genet 2001; 29: 223-8.
10. Negoro K, McGovern DP, Kinouchi Y, Takahashi S, Lench NJ, Shimosegawa T, et al. Analysis of the IBD5 locus and potential gene-gene interactions in Crohn's disease. Gut 2003; 52: 541-6.
11. Cho JH, Nicolae DL, Gold LH, Fields CT, LaBuda MC, Rohal PM, et al. Linkage and linkage disequilibrium in chromosome band 1p36 in American Chaldeans with inflammatory bowel disease. Hum Mol Genet 2000; 9: 1425-32.
12. Rugtveit J, Haraldsen G, Hogasen AK, Bakka A, Brandtzaeg P, Scott H. Respiratory burst of intestinal macrophages in inflammatory bowel disease is mainly caused by $CD14^+L1^+$ monocyte derived cells. Gut 1995; 37: 367-73.
13. Dentener MA, Bazil V, Von Asmuth EJ, Ceska M, Buunrman WA. Involvement of CD14 in lipopolysaccharide-induced tumor necrosis factor-alpha, IL-6 and IL-8 release by human monocytes and alveolar macrophages. J Immunol 1993; 150: 2885-91.
14. Cario E, Rosenberg IM, Brandwein SL, Beck PL, Reinecker HC, Podolsky DK. Lipopolysaccharide activates distinct signaling pathways in intestinal epithelial

cell lines expressing toll-like receptors. J Immunol 2000 ; 164 : 966-72.
15. Pugin J, Schurer-Maly CC, Leturcq D, Moriarty A, Ulevitch RJ, Tobias PS. Lipopolysaccharide activation of human endothelial and epithelial cells is mediated by lipopolysaccharide-binding protein and soluble CD14. Proc Natl Acad Sci USA 1993 ; 90 : 2744-8.
16. Klein W, Tromm A, Griga T, Fricke H, Folwaczny C, Hocke M, et al. A polymorphism in the CD14 gene is associated with Crohn disease. Scand J Gastroenterol 2002 ; 37 : 189-91.
17. Zee RYL, Lindpaintner K, Struk B, Hennekens CH, Ridker PM. A prospective evaluation of the CD14 C(-260) T gene polymorphism and the risk of the myocardial infarction. Atherosclerosis 2001 ; 154 : 699-702.
18. Paradakis KA, Targan SR. Tumor necrosis factor : Biology and therapeutic inhibitors. Gastroenterology 2000 ; 119 : 1148-57.
19. Rugtveit J, Nilsen EM, Bakka A, Carlsen H, Brandtzaeg P, Scott H. Cytokine profiles differ in newly recruited and resident subsets of mucosal macrophages from inflammatory bowel disease. Gastroenterology 1997 ; 112 : 1493-505.
20. Wilson BAG, Vries ND, Pociot F, Giovine FS, Putte LBA, Duff GW. An Allelic polymorphism within the human tumor necrosis factor α promoter region is strongly associated with HLA A1, B8, and DR3 alleles. J Exp Med 1993 ; 177 : 557-60.
21. Dempster AP, Laird NM, Rubin DB. Maximum likelihood from incomplete data via the EM algorithm. J Roy Statist Soc 1977 ; 39B : 1-38.
22. Grimm MC, Pavli P, Pol EVD, Doe WF. Evidence for a $CD14^{s}$ population of monocytes in inflammatory bowel disease mucosa : implications for pathogenesis. Clin Exp Immunol 1995 ; 100 : 291-7.
23. Reinecker HC, Steffen M, Witthoeft T, Pflueger I, Schreiber S, Macdermott RP. Enhanced secretion of tumour necrosis factor-alpha, IL-6, and IL-1β by isolated lamina propria mononuclear cells from patients with ulcerative colitis and Crohn's disease. Clin Exp Immunol 1993 ; 94 : 174-81.
24. Beatty WL, Sansonetti PJ. Role of lipopolysaccharide in signaling to subepithelial polymorphonuclear leukocytes. Infect Immun 1997 ; 65 : 4395-404.
25. Hubacek JA, Pit'ha J, Skodova Z, Stanek V, Poledne R. C(-260)→T Polymorphism in the promoter of the CD14 monocyte receptor gene as a risk factor for myocardial infarction. Circulation 1999 ; 99 : 3218-20.
26. Zhang D, Hetherington CJ, Tan S, Dziennis SE, Gonzalez DA, Chen H. Sp1 is a critical factor for the monocytic specific expression of human CD14. J Biol Chem 1994 ; 269 : 11425-34.
27. Obana N, Takahashi S, Kinouchi K, Negoro K, Takagi S, Hiwatashi N, et al. Ulcerative colitis is associated with a promoter polymorphism of lipopolysaccharide receptor gene, CD14. Scand J Gastroenterol 2002 ; 37 : 699-704.
28. Yang H, Plevy SE, Taylor K, Tyan D, Fischel-Ghodsian N, McElree C, et al. Linkage of Crohn's disease to the major histocompatibility complex region is detected by multiple non-parametric analysis. Gut 1999 ; 44 : 519-26.
29. Breese EJ, Michie CA, Nicholls SW, Murch SH, Williams CB, Domizio P. Tumor necrosis factor-α producing cells in the intestinal mucosa of children with inflammatory bowel disease. Gastroenterology 1994 ; 106 : 1455-66.
30. Wilson AG, Giovine FS, Blakemore AIF, Duff GW. Single base polymorphism in the human Tumor Necrosis Factor alpha (TNF-α) gene detestable by Ncol resection of PCR product. Hum Mol Gene 1992 ; 1 : 353.
31. Hamann A, Mantzoros C, Vidal-Puig A, Flier JS. Genetic variability in the TNF-α promoter is not associated with type II diabetes mellitus (NIDDM). Biochem Biophys Res Commun 1995 ; 211 : 833-9.
32. Zimmermann PA, Guiderian RH, Nutman TB. A new TNFA promoter allele identified in South American Blacks. Immunogenetics 1996 ; 44 : 485-6.
33. Uglialoro AM, Turbay D, Pesavento PA, Delgado JC, McKenzie FE, Gribben JG. Identification of three new single nucleotide polymorphisms in the human tumor necrosis factor-α gene promoter. Tissue Antigens 1998 ; 52 : 359-67.
34. Higuchi T, Seki N, Kamizono S, Yamada A, Kimura A, Kato H, et al. Polymorphism of the 5′-flanking region of the human tumor necrosis factor (TNF)-α gene in Japanese. Tissue Antigens 1998 ; 51 : 605-12.
35. Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW. Effect of a polymorphism in the human tumor necrosis factor α promoter on transcription activation. Proc Natl Acad Sci USA 1997 ; 94 : 3195-9.
36. Kroeger KM, Carville KS, Abraham LJ. The -308 tumor necrosis factor-α promoter polymorphism effect transcription. Mol Immunol 1997 ; 34 : 391-9.
37. Negoro K, Kinouchi Y, Hiwatashi N, Takahashi S, Takagi S, Satoh J, et al. Crohn's disease is associated with novel polymorphisms in the 5′-flanking region of the tumor necrosis factor gene. Gastroenterology 1999 ; 117 : 1062-8.
38. Kawasaki A, Tsuchiya N, Hagiwara K, Takazoe M, Tokunaga K. Independent contribution of HLA-DRB1 and TNF alpha promoter polymorphisms to the susceptibility to Crohn's disease. Gene Immun 2000 ; 1 : 351-7.
39. Von Heel DA, Udalova IA, Silva AP, McGovern DP, Kinouchi Y, Hull J, et al. Inflammatory bowel disease is associated with a TNF polymorphism that affects an interaction between the OCT1 and NF-κB transcription factors. Hum Mol Genet 2002 ; 11 : 1281-9.
40. Sashio H, Tamura K, Ito R, Yamamoto Y, Bamba H, Kosaka T, et al. Polymorphisms of the TNF gene and the TNF receptor superfamily member 1B gene are

associated with susceptibility to ulcerative colitis and Crohn's disease, respectively. Immunogenetics 2002; 53: 1020–7.
41. Bouma G, Poen AC, Garcia-Gonzalez MA, Schreuder GMT, Felt-Bersma RJF, Meuwissen SGM. HLA-DRB1*03, but not the TNFA-308 promoter gene polymorphism, confer protection against fistulising Crohn's disease. Immunogenetics 1998; 47: 451–5.
42. Nakajima A, Matsuhasi N, Kodama T, Yazaki Y, Takazoe M, Kimura A. HLA-linked susceptibility and resistance genes in Crohn's disease. Gastroenterology 1995; 109: 1462–7.
43. Futami S, Aoyama N, Honsako Y, Tamura T, Morimoto S, Nakashima T, et al. HLA-DRB1*1502 allele, subtype of DR15, is associated with susceptibility to ulcerative colitis and its progression. Dig Dis Sci 1995; 40: 814–8.
44. Heresbach D, Ababou A, Bourienne A, Alizadeh M, Quelvennec E, Pagenault M. Polymorphism of the microsatellites and tumor necrosis factor genes in chronic inflammatory bowel diseases. Gastroenterol Clin Biol 1997; 21: 555–61.