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Molecules and Cells

Identification of Single Nucleotide Polymorphisms in the *TNFRSF17* Gene and Their Association with Gastrointestinal Disorders

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TNFRSF17 is preferentially expressed in mature B lymphocytes, and may be important for the development of B cells. *TNFRSF17* is selected as a candidate susceptibility gene to IBD pathogenesis by our cDNA microarray analysis, and we showed the specific expression of *TNFRSF17* in resting and activated CD19$^+$ cells obtained from human blood. We identified four SNPs (g-1729G>A, g.2295T>C, g.2445G>A and g.2493G>A) and one variation site (g.-894delT) in the *TNFRSF17* gene using direct sequencing analysis. In addition, the association of the genotype and allelic frequencies of these SNPs was studied in healthy controls and in patients with ulcerative colitis (UC) or irritable bowel syndrome (IBS). Although, the genotype and allelic frequencies of these SNPs, in the UC and IBS patients, were not significantly different from those in the healthy controls, the distribution of the AAG, GGA, AGG and AAA haplotypes, of the SNPs (g.-1729G>A, g.2445G>A and g.2493G>A) associated with the *TNFRSF17* gene, in the UC patients, were notably different from those of the healthy controls (*P* = 0.002, 0.002, 4.7E-4 and 3.3E-6, respectively). Moreover, the frequencies of the AAG, AGG, GAG and GAA haplotypes were significantly different in the IBS patients compared to the healthy controls (*P* = 4.2E-5, 4.4E-17, 1.8E-22 and 1.6E-10, respectively). These results suggest that the haplotypes of the *TNFRSF17* polymorphisms might be associated with UC and IBS susceptibility.

INTRODUCTION

Inflammatory bowel disease (IBD) is a complex multifactorial disorder involving genetic, environmental and microbial factors (Fiocchi, 1998; Podolsky, 2002). Crohn's disease (CD) and ulcerative colitis (UC) are the two main clinical forms of IBD in humans (Blumberg et al., 1999). Within these subgroups there are many differences regarding disease extension, localization, behavior and the occurrence of extra-intestinal manifestations (Forbes, 2003; Travis and Jewell, 2003). While the inflammation in UC starts from the anal margin and is limited to the mucosa of the colon, the inflammation in CD is trans-mucosa, can occur throughout the gastrointestinal tract, and is characterized by a T helper 1 cell (Th1) response (Fuss et al., 1996). IBD and irritable bowel syndrome (IBS) are a chronic disorders frequently encountered in the gastrointestinal tract with similar symptoms. IBS is characterized by chronic abdominal symptoms including pain or discomfort and altered bowel habits without structural or biochemical abnormalities (Longstreth et al., 2006; Talley et al., 2002). Although microscopic inflammation (Collins et al., 2001; Spiller, 2004) and episodes of gastrointestinal infection (Spiller, 2003) have been proposed to be associated with the development of IBS symptoms, the exact cause and underlying genetic factors of IBS remain unknown.

Multiple susceptibility loci have been implicated in IBD by genome-wide linkage analysis in siblings with IBD or in relative-paired families (Ahmad et al., 2004). Many studies have suggested the existence of at least nine IBD loci (referred to as *IBD 1-9*) in the human genome. Whereas some loci appear to be specific to CD (IBD1) (Cavanaugh, 2001; Hugot et al., 1996) or UC (IBD2) (Parkes et al., 2000), others seem to confer susceptibility to both CD and UC (IBD3) (Hampe et al., 1999; Van Heel et al., 2004). DNA microarray analysis can be used to identify susceptibility candidate genes in complex genetic disorders and identify novel disease-related genes. This technology has been used to study gene expression profiling in mucosal biopsies from the UC or CD patients and healthy controls, and identified the genes with altered expression, including *CD74*, *NGAL*, *GRO*, and calcium-binding *S100* genes (Lawrance et al., 2001; Warner et al., 2002).

Member of the tumor necrosis factor (TNF) superfamily (TNFSF) and TNF receptor (TNFR) superfamily (TNFRSF) are involved in host immune responses; they regulate cell proliferation, survival, differentiation, and apoptosis (Locksley et al., 2001). Three TNFSF ligands, TNFSF13B (also known as BAFF and BLyS) (Moore et al., 1999; Schneider et al., 1999), TNFSF13

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(also known as APRIL and CD256) (Hahne et al., 1998) and TNFSF8 (also known as CD30L) (Phillips et al., 2003) have been identified. Two of the three ligands, TNFSF13 and TNFSF13B are critical for immune system function (Mackay et al., 2002). There are three, currently identified, receptors associated with TNFSF13 and TNFSF13B: TNFRSF17 (also known as BCMA and CD269) (Thompson et al., 2000), TNFRSF13B (also known as TACI) (Wu et al., 2000), and TNFRSF13C (also known as BAFF-R) (Thompson et al., 2001). TNFSF13 binds to TNFRSF17 with high affinity, whereas TNFSF13B binds with a lower affinity. The low affinity of monomeric TNFRSF17 for TNFSF13B is overcome by TNFRSF17 dimerization (Day et al., 2005). TNFRSF17 is located on chromosome 16p13.1 and was initially cloned as a translocation product in human T cell lymphoma; it is preferentially expressed in mature B lymphocytes (Laabi et al., 1992). TNFRSF17 is involved in the late stages of B cell maturation; it is important for the survival of plasmablasts and long-lived plasma cells in the bone marrow (Bossen and Schneider 2006; O'Connor et al., 2004). In contrast to TNFRSF13C and TNFRSF13B, the functional consequences of TNFRSF17 on B cells remain unclear.

In this study, we examined the gene expression profiles of peripheral blood in the IBD patients (4 UC and 4 CD), and tested whether the single nucleotide polymorphisms (SNPs) of identified gene with the altered expression might be associated with susceptibility to the gastrointestinal disorders including UC. Twelve immune and inflammatory response related genes with over 3-fold up- or down-expression both the UC and CD patients were identified. After validation of the expression of these genes, a receptor gene, *TNFRSF17*, was selected as a candidate susceptibility gene to IBD pathogenesis. In an attempt to understand the genetic influences of *TNFRSF17* in UC, we identified possible variation sites and SNPs of the *TNFRSF17* in the genomic DNAs isolated from 48 unrelated individual (24 UC patients and 24 healthy controls) by direct sequencing. To determine whether these *TNFRSF17* SNPs are associated with the susceptibility to gastrointestinal disorders, we have analyzed and compared their frequencies in 124 UC patients, 106 IBS patients and 536 healthy controls. We further investigated the haplotype frequencies by these SNPs between the UC or IBS patient group and the healthy control group.

MATERIAL AND METHOD

Patients and DNA Samples

Blood samples were obtained from 124 UC patients (67 males and 57 females), 106 IBS patients (47 males and 59 females) and 536 healthy controls (334 males and 202 females). The mean ages of the UC, IBS patients and the healthy controls were about 40.5, 44.9 and 40.7 years, respectively. Genomic DNA was extracted from the leukocytes of the peripheral blood by a standard phenol-chloroform method or by using a Genomic DNA Extraction kit (iNtRON Biotechnology, Korea). The UC and IBS patients were recruited from the outpatient clinic at Wonkwang University Hospital. The patients were classified according to the clinical features, the endoscopic findings and the histopathologic examinations. The healthy controls were recruited from the general population and they had undergone comprehensive medical screening at the Wonkwang University Hospital. All the subjects employed in this study were Koreans.

RNA extraction

Total RNA from peripheral blood mononuclear cells (PBMC) was isolated from the Buffy coats by density gradient centrifugation using Ficoll-Paque Plus (Amersham Biosciences, Sweden), washed with the phosphate-buffered saline (GIBCO BRL, Invitrogen, USA), and lysed in TRIzol reagent (Invitrogen) according to the manufacturer's directions (Jin et al., 2009).

DNA Microarrays analysis

Fluorescent-labeled cRNA for oligo micorarray analysis was prepared by amplification of the total RNA isolated from four healthy controls and four UC or four CD patients in the presence of aminoallyl-UTP (Amersham Pharmacia) followed by the coupling of Cy3 or Cy5 dyes. Agilent human 22 K DNA microarray chip (Agilent, USA) was hybridized with the fluorescently labeled cRNA at 60°C for 16 h and then washed. DNA chips were scanned using GenePix 4000B (Axon Instruments, USA). The scanned images were analyzed with GenePix Pro 3.0 software (Axon Instruments) to obtain gene expression ratios. The logged gene expression ratios were normalized by LOWESS regression (Yang et al., 2002).

Validation by reverse transcription- polymerase chain reaction (RT-PCR)

To validate the gene expression data obtained by the cDNA microarray analysis, approximately 1 μg of total RNA was used in the first-strand cDNA synthesis with a sequence specific primer using the M-MLV reverse transcriptase (Bioneer, Korea) for reverse transcription-polymerase chain reaction (RT-PCR). The PCR samples were prepared in a 20 μl reaction volume containing 50 ng cDNA, 10× buffer 2 μl, 10 mM dNTP 0.5 μl, EF-taq 0.2 U (Solgent, Korea) and 0.5 μM of each primer (Table 1) under the following conditions: 10 min at 95°C for initial denaturation, 30 cycles of PCR consisting of denaturation for 10 s at 98°C, annealing for 15 s at 55°C, extension for 30 s at 72°C, and a final extension for 10 min at 72°C in a PCR Thermal Cycler DICE Gradient (TaKaRa, Japan). The expression assay for *TNFRSF17* mRNA was performed using the cDNA obtained from various human tissues or blood cells (Clontech, USA).

Polymerase chain reaction (PCR) and sequence analysis

The entire coding regions of *TNFRSF17*, including 1.8 kb promoter regions, were partially amplified using two primer pairs (Table 1). The PCR reaction mixtures were prepared by previously described condition (Chae et al., 2009). Amplification was carried out in a PCR Thermal Cycler DICE Gradient (TaKaRa) at 95°C for 5 min to pre-denature the template DNA, followed by 30 cycles of denaturation at 98°C for 10 s, annealing at 68°C for 15 s and extension at 72°C for 2.0 or 2.5 min. The final extension was completed at 72°C for 10 min. After purification using PCR purification kit (Millipore, USA), the PCR products were employed as template DNA for the sequencing analysis using the ABI Prism BigDye Terminator cycle sequencing system (Applied Biosystems, USA) on the ABI 3100 automatic sequencer (Applied Biosystems). Both sense and antisense strands of PCR products were directly sequenced using the same primers used for the PCR amplification, and eight primers were additionally used for DNA sequence analysis (Table 1). The SNPs and variation sites of *TNFRSF17* gene were detected by direct sequence analysis. The reference sequence for *TNFRSF17* gene was based on the sequence of human chromosome 16, clone RP11-166B2.

Genotype analysis by high resolution melting (HRM)

Genotype analysis for the g.-1729G>A of *TNFRSF17* gene was performed by high resolution melting (HRM) analysis. The 10 μl reaction mixtures were made up using the 1× QuantiTect Probe PCR Kit (Qiagen, USA) and consisted of 50 ng of genomic

Table 1. Primer sequences used for PCR amplification, sequencing analysis, RT-PCR and genotyping in the *TNFRSF17* gene

Applications	Primers	Primer sequence (5′ → 3′)	Regions
PCR analysis	SF17-PF1	TCCTTGGCACACTCCTTGGGCCT	Promoter
	SF17-PR1	TGGCCTCAAACTGCCGACCTCA	Intron
	SF17-PF2	ACGAATGCGATTCTCTGGACCTGT	Exon
	SF17-PR2	AGGAAGTTTCTACCATTAAGCTCCCA	Exon
Sequencing analysis	SF17-SF1	ACCAGGCGTGCTGGCGCAT	Promoter
	SF17-SF2	AGCACGTCTCCCTGCCTCA	Promoter
	SF17-SR1	TCCTGACCTCAAGTGATCCACCT	Promoter
	SF17-SF3	TGATGCTGTGGGCTTGTCTGCA	Promoter
	SF17-SF4	TGCAGATGGCTGGGCAGTGCT	Exon
	SF17-SF5	ACCTGTGAAGACTGCATCAAGAGCA	Exon
	SF17-SR2	CTTCCTCCATAGCTGGGAGTGGA	Exon
	SF17-SR3	TGGTGACAAGAATGGTTGCGCCT	Exon
High resolution melting	SF17-HF1	TCCTTGGCACACTCCTTGGGCCT	g.-1729G>A
	SF17-HR1	TCTCTGAGGTAAGTGGTGGGGAA	
Taq-Man analysis	SF17-GAF1	GGAAGGCGCAACCATTCTTG	g.2445G>A
	SF17-GAR1	GCAGCTGGCAGGCTCTT	
	SF17-GAF2	AGGCGCAACCATTCTTGTCA	g.2493G>A
	SF17-GAR2	ACTGCTCGAGTCGAAATGGTTAATT	
Probe	SF17-GAV1	CACCACGAAAACGA	g.2445G>A
	SF17-GAM1	TCACCACAAAAACGA	
	SF17-GAV2	TTTGAGTGCTACGGAGATA	g.2493G>A
	SF17-GAM2	TGAGTGCTACAGAGATA	
RT-RCT	SF17-MPF2	ACGAATGCGATTCTCTGGACCTGT	mRNA
	SF17-MPR3	ACCTGTGAAGACTGCATCAAGAGCA	

DNA, 100 nM of each primer (Table 1), and 1× Evagreen solution (Biotium, USA). The PCR cycling and HRM analysis was carried out using a Rotor-Gene thermal cycler RG6000 (Corbett Research, Australia). The PCR cycling conditions were as follows: one cycle of 95°C for 15 min; 45 cycles of 95°C for 10 s, and the annealing conditions were 58°C for 10 s, 72°C for 30 s; HRM analysis was performed at 77 to 95°C increasing at 0.1°C per second and the fluorescence was recorded.

Genotype analysis by Taq-Man probe

The Taq-Man assay probes for g.2445G>A and g.2493G>A SNPs were designed by Applied Biosystems (Applied Biosystems). The reagents consisted of a 40X mix of un-labeled PCR primer and TaqMan MGB probes were labeled with the FAM dye and the other with the fluorescent VIC dye (Shin et al., 2008). The reaction was performed in 10 μl that contained 0.125 μl 40X reagents, 5 μl 2X TaqMan Genotyping Master mix (Applied Biosystems), and 2 μl of 50ng genomic DNA. The PCR conditions were as follows: one cycle at 95°C for 15 min; 50 cycles at 95°C for 10 s and 60°C for 45 s. The PCR reaction was performed in the Rotor-Gene thermal cycler RG6000 (Corbett Research). The samples were read and analyzed using the software Rotor-Gene 1.7.40 (Corbett Research).

Statistic analysis

χ^2 tests were applied to estimate the Hardy-Weinberg equilibrium (HWE). Pair-wise comparison of the biallelic loci was employed for the analyses of the Linkage Disequilibrium (LD). The haplotype frequencies of *TNFRSF17* for multiple loci were estimated using the expectation maximization (EM) algorithm with SNPAlyze software (DYNACOM, Japan). Logistic regression analyses (SPSS 11.5) were used to calculate the odds ratios (with the 95% confidence intervals). A *P*-value less than 0.05 were considered to indicate statistical significance.

RESULTS

Identification of up- and down-expressed genes in IBD

The peripheral blood from eight patients with IBD (4 UC and 4 CD) and four healthy controls were collected, and their total RNAs were isolated for use on a 22 K DNA microarray chip for the microarray analysis. We identified 771 (410 up-expressed) and 449 (266 up-expressed) genes that were expressed differently by a measure of 2-fold in the UC and CD patients. At our cutoff level (3-fold differently expressed) for the UC patients, 170 genes were up-expressed and 103 genes were down-expressed compared to the healthy controls. In order to delineate the molecular fingerprint of each of the two disease subtypes, the differentially regulated genes were assigned to functional groups based on classification by the DAVID criteria (http://david.abcc.ncifcrf.gov/). Among them, 49 genes in the UC and 51 genes in the CD were listed as immune and inflammatory response related genes. Finally, there were 12 genes that were up- or down-expressed in both the UC and CD patients (Table 2). Among these genes, the *TNFRSF17* was selected as an IBD candidate gene that was down-expressed

Table 2. Inflammation-related genes which are up- or down-regulated in the peripheral blood mononuclear cells of the UC and CD patients

Gene name	Gene symbol	Chromosome location	Functions	Expression level (UC, CD)
FCGR1A	Fc fragment of IgG, high affinity Ia, receptor (CD64)	1q21.2-q21.3	IgG binding	Up (4.1, 2.1)
SOD2	Superoxide dismutase 2, mitochondrial	6q25.3	Manganese ion binding	Up (3.6, 2.8)
FTH1	Ferritin, heavy polypeptide 1	11q13	Ferric iron binding	Up (3.4, 3.1)
S100A9	S100 calcium binding protein A9	1q21	Calcium ion binding	Up (4.0, 2.0)
HP	Haptoglobin	16q22.1	Trypsin activity	Up (5.0, 3.9)
IFI27	Interferon, alpha-inducible protein 27	14q32	-	Up (4.4, 2.0)
AQP9	Aquaporin 9	15q22.1-22.2	Amine transporter activity	Up (4.5, 2.5)
BCL2	B-cell CLL/lymphoma 2	18q21.33	Protein binding	Down (3.2, 2.0)
TNFRSF17	Tumor necrosis factor receptor superfamily, member 17	16p13.1	Receptor activity	Down (5.0, 2.0)
GPR183	G protein-coupled receptor 183	13q32.3	Purinergic nucleotide receptor activity, G-protein coupled	Down (4.3, 2.3)
TPD52	Tumor protein D52	8q21	-	Down (3.6, 2.2)
KLRF1	Killer cell lectin-like receptor subfamily F, member 1	12p13.2-12.3	Transmembrane receptor activity	Down(3.4, 2.2)

in both the UC and CD patients compared to the healthy controls.

Validation and expression of *TNFRSF17* mRNA

To validate the accuracy of the microarray expression profiling data, the expression levels of *TNFRSF17* were compared in three CD and UC patients and three healthy controls by RT-PCR. As shown in Fig. 1A, the expression level of *TNFRSF17* mRNA was down-expressed by about 2.0 fold in the peripheral blood of the CD patients compared to the healthy controls, while the expression level of *TNFRSF17* mRNA in UC patients was somewhat down-expressed by about 1.4 fold. The expression pattern of *TNFRSF17* mRNA, in 12 normal tissues obtained from the digestive tract, was examined. The results showed that the expression level of *TNFRSF17* mRNA, in the tissues from the human digestive system, was highest in the stomach, while the expression level of *TNFRSF17* mRNA in the esophagus was barely detectable (Fig. 1B). Furthermore, we examined the expression levels of *TNFRSF17* mRNA in the cells derived from human blood. While *TNFRSF17* mRNA was highly expressed in the resting and activated $CD19^+$ cells, the expression of *TNFRSF17* mRNA in the resting and activated $CD4^+$, $CD8^+$ and $CD14^+$ cells was undetectable (Fig. 1C).

Identification of SNPs

The human *TNFRSF17* gene is a member of the TNF-receptor superfamily, which is located on chromosome 16p13.1 and consists of three exons. To determine the possible variation sites, in the entire coding regions, and the boundary intron sequences of *TNFRSF17* that include about 1.9 kb of the promoter region, we first scanned the genomic DNAs isolated from 24 unrelated UC patients and 24 healthy controls. We identified four SNPs and one variation site by direct sequencing methods, g.-1729G>A (rs12926535) and g.-894delT (novel) in the promoter region, and g.2295T>C (novel), g.2445G>A (rs2017662) and g.2493G>A (rs2071336) in exon 3 (Fig. 2). The LD coefficients (|D′|) between all SNP pairs was calculated, and there was no absolute LD (|D′| = 1 and r^2 = 1) among the SNPs of the *TNFRSF17* gene (data not shown). Among the identified polymorphisms, three SNPs (g.-1729G>A, g.2445G>A and g.2493G>A) were selected for large sample genotyping based on their locations, LD and frequencies. The SNP, g.982A>G (rs373496, but not detected in our study), from the NCBI SNP database, was also genotype analyzed; however, when 96 samples were analyzed, there was only a GG genotype. These results indicate that the g.982A>G (rs373496) of *TNFRSF17* might be a very rare polymorphism or monomorphism in the Korean population.

Genotype analysis and association studies

To determine whether the *TNFRSF17* SNPs identified are associated with UC or IBS susceptibility, the genotypes of the *TNFRSF17* polymorphisms were analyzed by the HRM or TaqMan probe method, and the genotype and allelic frequencies between the groups were compared. The genotype frequencies of all loci were in HWE ($P > 0.05$, data not shown). The genotype and allelic frequencies of the SNPs in the UC patient group were not significantly different from those of the healthy control group (Table 3). The genotype and allele frequencies of the SNPs were also analyzed and compared in the IBS patients and the healthy controls (Table 4). There were also no significant differences between these two groups. These results suggest that the SNPs identified in *TNFRSF17* appear not to be associated with UC and IBS susceptibility.

To determine the possible correlation between the haplotypes associated with g.-1729G>A, g.2445G>A and g.2493G>A of the *TNFRSF17* gene and UC or IBS susceptibility, we further analyzed haplotype frequencies of the SNPs in patients with UC, IBS and the healthy controls (Table 5). While three major (more than 5%) haplotypes explaining more than 95.8% of the distributions were identified in the healthy controls, four and five major haplotypes (96.2% and 93.9%, respectively), out of eight possible haplotypes, were identified in the patients with UC and IBS, respectively (Table 5). The distribution of the four haplotypes (AAG, GGA, AGG and AAA) were notably different in the UC patients compared to the healthy controls ($P = 0.002$, 0.002, 4.7E-4 and 3.3E-6, respectively). Interestingly, while the major haplotype (GGG) frequency in the healthy controls was 70.8%, that of the patients with IBS was 55.1% (P = 2.8E-5). Furthermore, the distribution of the four haplotypes (AAG, AGG,

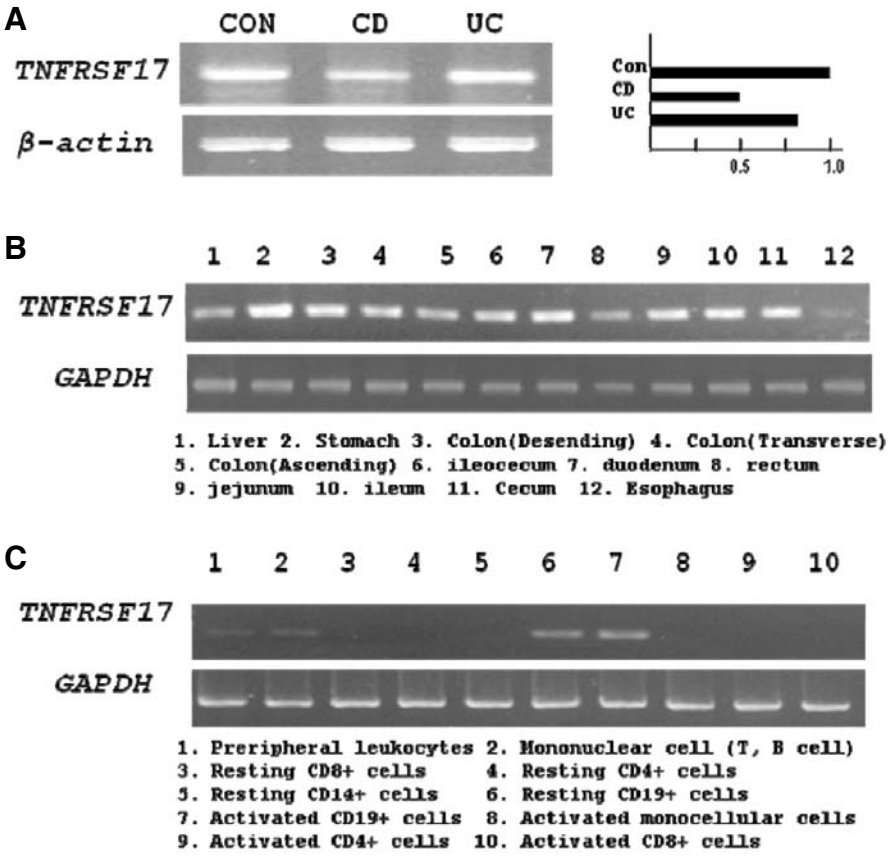

Fig. 1. Expression patterns of the *TNFRSF17* mRNA by RT-PCR. (A) The validation of *TNFRSF17* mRNA expression in the UC, CD patients and the healthy controls, (B) in the tissues of human digestive system and (C) in the various human blood cells.

GAG and GAA) was also significantly different in the patients with IBS compared to the healthy controls (P = 4.2E-5, 4.4E-17, 1.8E-22 and 1.6E-10, respectively). These results suggest that *TNFRSF17* polymorphisms might be an important genetic factor associated with UC and IBS susceptibility.

DISCUSSION

The precise etiology of IBD and IBS are unclear. However, both IBS and IBD are thought to result from both environmental and genetic factors that increase susceptibility to these disorders. Multiple IBD susceptibility loci (referred to as *IBD 1-9*) have been implicated in genomic studies in human. The most extensively studied genetic region, associated with IBD, among these loci is the *IBD1* locus (16p13.1-16q12.2). The *CARD15*/*NOD2* gene, which has been widely shown to influence both the susceptibility and phenotype of patients with CD, is located at the *IBD1* locus. Three missense mutations, Arg702Trp, Gly908Arg and Leu1007 finsC, have been identified as being significantly associated with an increased risk for CD and this association has been replicated in multiple studies (Hampe et al., 2001; Hugot et al., 2001; Ogura et al., 2001). Most studies have shown an association between *CARD15* mutations and the susceptibility to CD, but not to UC. We previously reported that an exon 4 variation of the *Tim-1* gene and the SNPs of the *eotaxin-2* and *eotaxin-3* genes were associated with UC in a Korean population (Park et al., 2005; Song et al., 2004).

In this study, we identified the *TNFRSF17* gene as a candidate gene associated with the pathogenesis of IBD, by cDNA microarray analysis. To validate the cDNA microarray results, the expression levels of *TNFRSF17* were compared, by RT-PCR, in total RNAs isolated from three CD or UC patients and three healthy controls. The expression levels of *TNFRSF17* in the CD patients were shown to be similar to the results of the cDNA microarray analysis, while the expression levels (about 1.4-fold down regulated) in the UC patients were found to be somewhat different from that (about 5.0-fold down regulated) of the cDNA microarray results (Fig. 1A). TNFRSF17 is known to play an important role in the regulation of B cell immunity. It is involved in the late stages of B cell maturation in the bone marrow (Bossen and Schneider, 2006; O'Connor et al., 2004). The *TNFRSF17* mRNA was highly expressed in the resting and activated $CD19^+$ cells in this study, while their expression was not detected in the resting and activated $CD4^+$, $CD8^+$ and $CD14^+$ cells (Fig. 1C). Our results support the findings of a previous report that showed that the expression of *TNFRSF17* was observed only in some CD19+ cells (Smirnova et al., 2008).

The polymorphisms of the *TNF* gene and the *TNFRSF1B* gene have been reported to be associated with UC and CD susceptibility, respectively (Sashio et al., 2002). However, other *TNFRSF* polymorphisms, including the *TNFRSF17* gene in the etipathogenesis of UC or IBS have not been elucidated. To determine whether the *TNFRSF17* variations were associated with the susceptibility to gastrointestinal disease, such as UC and IBS, we identified four SNPs and one variation site (Fig. 2) in the *TNFRSF17* gene using direct sequencing methods and analyzed the genotype frequencies of these SNPs in patient with UC and IBS as well as the healthy controls. Although, the genotype and allelic frequencies of these SNPs in both the UC and IBS patients were not significantly different from the healthy controls (Tables 3 and 4), the distribution of the four haplotypes (AAG, GGA, AGG and AAA) of the *TNFRSF17* SNPs, g.-1729G

Table 3. Genotype and allele analyses of the *TNFRSF17* gene polymorphisms in the UC patients and the healthy controls

Position[a]	Genotype/Allele	Control n (%)	UC n (%)	Odds ratio[b] (95% CI)	*P*[c]
g.-1729G>A (rs12926535)	GG	322 (63.51)	73 (61.86)	1.00	0.389
	GA	162 (31.95)	36 (30.51)	0.98 (0.63-1.52)	
	AA	23 (4.54)	9 (7.63)	1.73 (0.77-3.89)	
	G	806 (79.49)	182 (77.12)	1.00	0.425
	A	208 (20.51)	54 (22.88)	1.15 (0.82-1.62)	
g.2445G>A (rs2017662)	GG	340 (64.03)	83 (66.94)	1.00	0.791
	GA	169 (31.83)	37 (29.84)	0.87 (0.58-1.36)	
	AA	22 (4.14)	4 (3.23)	0.74 (0.25-2.19)	
	G	849 (79.94)	203 (81.85)	1.00	0.535
	A	213 (20.06)	45 (18.15)	0.88 (0.62-1.26)	
g.2493G>A (rs2071336)	GG	428 (80.45)	96 (78.69)	1.00	0.891
	GA	97 (18.23)	24 (19.67)	1.10 (0.67-1.82)	
	AA	7 (1.32)	2 (1.98)	1.27 (0.26-6.23)	
	G	953 (89.57)	216 (87.13)	1.00	0.645
	A	111 (10.43)	28 (12.87)	0.99 (0.64-1.55)	

[a]Calculated from the translation start site
[b]Logistic regression analyses were used for calculating OR (95% CI; confidence interval).
[c]Value was determined by Fisher's exact test or χ^2 test from 2 × 2 contingency table.

Table 4. Genotype and allele analyses of the *TNFRSF17* gene polymorphisms in the IBS patients and the healthy controls

Position[a]	Genotype/Allele	Control n (%)	IBS n (%)	Odds ratio[b] (95% CI)	*P*[c]
g.-1729G>A	GG	322 (63.51)	66 (64.71)	1.00	0.303
	GA	162 (31.95)	28 (27.45)	0.84 (0.52-1.36)	
	AA	23 (4.54)	8 (7.84)	1.70 (0.73-3.96)	
	G	806 (79.49)	160 (78.43)	1.00	0.776
	A	208 (20.51)	44 (21.57)	1.07 (0.74-1.54)	
g.2445G>A	GG	340 (64.03)	61 (63.54)	1.00	0.188
	GA	169 (31.83)	27 (28.13)	0.88 (0.54-1.44)	
	AA	22 (4.14)	8 (8.33)	2.00 (0.85-4.71)	
	G	849 (79.94)	149 (77.60)	1.00	0.496
	A	213 (20.06)	43 (22.40)	1.15 (0.80-1.67)	
g.2493G>A	GG	428 (80.45)	77 (76.24)	1.00	0.601
	GA	97 (18.23)	22 (21.78)	1.26 (0.75-2.13)	
	AA	7 (1.32)	2 (1.98)	1.59 (0.32-7.73)	
	G	953 (89.57)	176 (87.13)	1.00	0.267
	A	111 (10.43)	26 (12.87)	1.14 (0.72-1.79)	

[a]Calculated from the translation start site
[b]Logistic regression analyses were used for calculating OR (95% CI; confidence interval)
[c]Value was determined by Fisher's exact test or χ^2 test from 2 × 2 contingency table.

>A, g.2445G>A and g.2493G>A, in the UC and IBS patients were notably different from the healthy controls (Table 5). These results suggest that the haplotypes of the *TNFRSF17* polymorphisms might be associated with UC and IBS susceptibility.

In conclusion, the results of this study suggest that *TNFRSF17* might be a candidate gene associated with the pathogenesis of IBD. The haplotypes of the *TNFRSF17* polymorphisms might be one of the markers for UC and IBS susceptibility. Although it is not clear how the *TNFRSF17* polymorphisms are related to susceptibility for gastrointestinal disease such as UC and IBS, our results provide useful information for further functional studies of the *TNFRSF17* gene and gastrointestinal disease such as colorectal cancer and inflammatory responses.

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Table 5. Haplotype frequencies of the *TNFRSF17* SNPs in the patients with UC or IBS and the healthy controls

Haplotype			Frequency[a]			Chi-square		*P*[b]	
g.-1729G>A	g.2445G>A	g.2493G>A	Control	UC	IBS	vs. UC	IBS	vs. UC	IBS
G	G	G	0.708	0.738	0.551	0.83	17.55	0.306	2.8E-5
A	A	G	0.168	0.088	0.050	9.19	16.79	0.002	4.2E-5
G	G	A	0.082	0.024	0.076	9.57	0.08	0.002	0.777
A	G	G	0.015	0.053	0.138	21.65	70.59	4.7E-4	4.4E-17
A	A	A	0.022	0.083	0.016	12.22	0.25	3.3E-6	0.611
G	A	G	0.005	0.005	0.124	-	95.13	0.928	1.8E-22
G	A	A	2.4E-38	0.009	0.041	9.19	40.95	0.002	1.6E-10
A	G	A	8.9E-9	1.5E-20	0.004	-	-	-	

[a]Values were constructed by EM algorithm with genotyped SNPs.
[b]Values were analyzed by Chi-square.

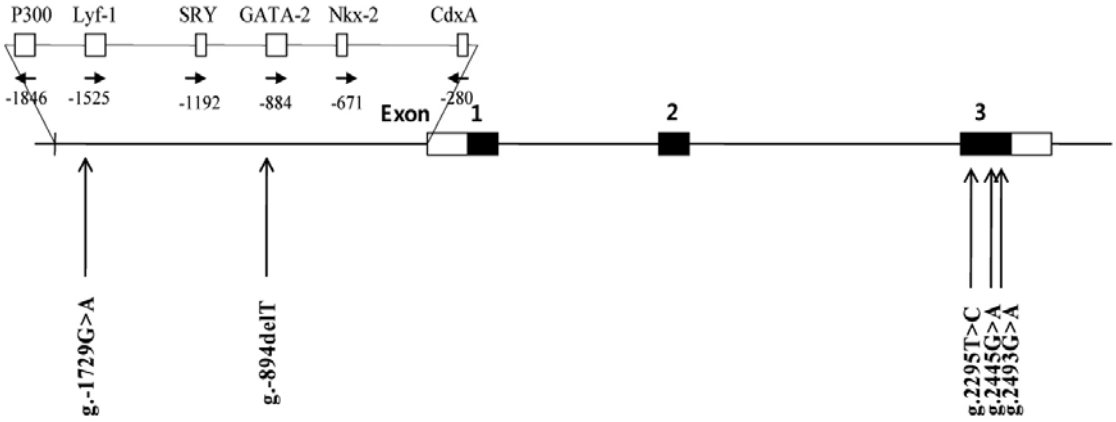

Fig. 2. The locations of each SNPs and variation sites in the *TNFRSF17*. Coding exons are marked by black blocks and 5′- and 3′-UTR by white blocks. The positions of SNPs were calculated from the translation start site. Putative transcription factor sites were searched at www.cbrc.jp/research/db/TFSEARCH.html. The reference sequence for *TNFRSF17* was based on the sequence of human chromosome 16, clone RP11-166b2.

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