Identification of the Polymorphisms in \textit{IFITM2} and \textit{IFITM5} Genes and their Association with Ulcerative Colitis

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Interferon inducible transmembrane protein (IFITM) family genes have been implicated in various cellular processes such as the homotypic cell adhesion functions of IFNs and cellular anti-proliferative activities. The present study aimed to investigate whether the polymorphisms of the \textit{IFITM2} and \textit{IFITM5} genes are associated with susceptibility to UC. We identified a total of thirteen polymorphisms (eleven SNPs and two variations) in the \textit{IFITM2} gene and twelve polymorphisms (eleven SNPs and one variation) in the \textit{IFITM5} gene, by the direct sequencing method. Genotype analysis in the \textit{IFITM2} and \textit{IFITM5} SNPs was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and Taq-Man probe analysis, and the haplotype frequencies of \textit{IFITM2} and \textit{IFITM5} SNPs for multiple loci were estimated using the expectation maximization (EM) algorithm. The genotype and allele frequencies of \textit{IFITM2} SNPs, as well as \textit{IFITM5} SNPs, in UC patients were not significantly different from those of the healthy controls. We also analyzed the combined frequencies of rs77537847 of \textit{IFITM1}, rs909097 of \textit{IFITM2}, and rs56069858 of \textit{IFITM5} in the UC patients and the healthy controls. Although the distribution of the major combined genotype frequency did not differ significantly between the healthy controls and the UC patients, the GGT combined frequency in the healthy controls was significantly different from that in the UC patients (P=0.002). This result suggests that the combined genotype of the IFITMs polymorphisms may be associated with a susceptibility to UC and could be a useful genetic marker for UC.

\textbf{Key words :} IBD, \textit{IFITM2} (Interferon inducible transmembrane protein 2), \textit{IFITM5}, UC, polymorphism

\textbf{Introduction}

Inflammatory bowel disease (IBD) is a chronic disease that is frequently encountered in the gastrointestinal tract and it can profoundly affect the quality of life. Ulcerative colitis (UC) and crohn’s disease (CD) in humans are the two major forms of IBD [10]. IBDs are complex and multifactorial involving genetic, environmental and microbial factors [1, 13, 14]. Cytokine production in lamina propria CD4\(^+\) T lymphocytes differs between CD and UC. Whereas CD is associated with increased production of T helper 1 cell (Th1) type cytokines, such as interferon-gamma (IFN-\(\gamma\)) and tumor necrosis factor alpha (TNF-\(\alpha\)), UC is associated with T cells that produce large amounts of the Th2 type cytokine IL-5, however, IFN-\(\gamma\) production is not affected [17, 21, 22].

The human IFITM genes are located on chromosome 11p15.5, and composed of five genes: \textit{IFITM1}, \textit{IFITM2}, \textit{IFITM3}, \textit{IFITM5} [15] and \textit{IFITM10}, which is newly identified as a member of IFITM family [2]. Expression levels of IFITM genes have been found to be up-regulated in gastric cancer cells and colorectal tumors [4, 29]. The IFITM family potently inhibits human immunodeficiency virus type 1 (HIV-1) [23], SARS coronavirus [16], West Nile virus and dengue virus infections [12]. \textit{IFITM1} was initially cloned from a human lymphoid cell cDNA library [15], and is located on chromosome 11p15.5 [24]. IFITM2 (also known as 1-8D) is associated with both cell cycle arrest and subsequent p53-independent apoptosis [14]. IFITM5 (also known as O15, BRIL and DSPA1) is highly expressed in osteoblasts and thought to have a function in bone formation and osteoblast maturation [7]. Actually, the \textit{IFITM5} gene has been found to be mutated in patients with osteogenesis imperfecta (OI) type V [11, 26]. Some of the OI patients have been identified with the heterozygous mutation in the 5’-UTR of \textit{IFITM5}. This mutation
creates the addition of five new amino acids to the N-terminus of the protein by frame alternative start-codon.

We have previously identified single nucleotide polymorphisms (SNPs) and multiple variation regions in the IFITM1 and IFITM3 gene, and have suggested that IFITM1 and IFITM3 polymorphisms are associated with susceptibility to UC [20, 25]. However, other IFITM family including the IFITM2 and IFITM5 genes in the epipathogenesis of UC has not been elucidated. In an attempt to understand the genetic influences of IFITM2 and IFITM5 on UC, we have identified possible variation sites and SNPs through the two exons of IFITM2 and IFITM5 and their boundary intron sequences, including the ~2.2 kb promoter regions. Genotype and allele frequencies of sequences, including the ~2.2 kb promoter regions. Genotype and allele frequencies of these SNPs in both groups.

Materials and Methods

Patients and DNA Samples

The DNA samples used in this study were provided by the Biobank of Wonkwang University Hospital, a member of the National Biobank of Korea, which is supported by the Ministry of Health and Welfare. On the basis of approval and informed consent from the institutional review board (WKUH-1157), we obtained the genomic DNAs from 126 UC patients (70 males and 56 females) and 532 healthy controls (334 males and 198 females). Mean ages of IBD patients and controls were 41.3 years and 40.9 years, respectively. Genomic DNA was extracted from peripheral blood leukocytes by using a standard phenol-chloroform method or by using a Genomic DNA Extraction kit (iNTRON Biotechnology, Korea) according to the manufacturer’s directions. IBD patients were recruited from the outpatient clinic at Wonkwang University Hospital. Patients were classified into the IBD group according to clinical features, endoscopic findings, and histopathologic examinations. Healthy controls were recruited from the general population, and had received comprehensive medical testing at the Wonkwang University Hospital. All subjects in this study were Korean.

Polymerase chain reaction (PCR) and sequence analysis

The entire coding regions of the IFITM2 and IFITM5 gene, including the ~2.2 kb promoter regions, were partially amplified by PCR using the two primer pairs (Table 1). PCR reactions were prepared by previously described procedures [27]. Amplification was carried out in a GeneAmp PCR system 9700 thermocycler (PE Applied Biosystem, USA) at 95°C for 5 min in order to pre-denature the template DNA, followed by 30 cycles of denaturation at 98°C for 10 s, annealing at 68°C for 30 s and extension at 72°C for 2.0 or 2.5 min. The final extension was completed at 72°C for 7 min. PCR products purified by use of a PCR purification kit (Millipore, USA) were used template DNA for sequencing analysis. Purified PCR products were sequenced using the ABI Prism BigDye Terminator cycle sequencing system (PE Applied Biosystems, USA) on the ABI 3100 automatic sequencer (PE Applied Biosystem). Both sense and antisense strands of PCR products were directly sequenced using the same primers used for the PCR amplification, and seven primers were additionally used to sequence the promoter and intron 1 region (Table 1). SNPs and variation sites of the IFITM2 and IFITM5 gene were detected by direct sequence analysis. The reference sequence for the IFITM2 and IFITM5 gene was based on the sequence of human chromosome 11, clone RP13-317D12 and RP11-326C3, respectively.

Genotype analysis by Taq-Man probe

The assay reagents for g.384G>A (rs909097) in the IFITM2, and for g.-874C>A (rs7111803) and g.-96T>C (rs56069858) in the IFITM5 gene was designed by Applied Biosystems (Applied Biosystems, USA). 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 [5]. The reaction in 10 μl was optimized to work with 0.125 μl 40x reagents, 5 μl 2x TaqMan Genotyping Master mix (Applied Biosystems, USA), and 2 μl (50 ng) of genomic DNA. The PCR conditions were as follows: one cycle at 95°C for 15 min; 40 cycles at 95°C for 15 s and 60°C for 45 s. The PCR was performed in the ABI plus (Applied Biosystems, USA). The samples were read and analyzed using the software ABI plus (Applied Biosystems, USA).

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis

IFITM2 containing g.-15T>C (rs1058873) and g.122T>C (rs14408), and IFITM5 containing g.-1115G>C (rs72636981) polymorphic sites were partially amplified by PCR. The spe-
cific primer pairs were used for PCR amplification (Table 1). An initial polymerase chain reaction (PCR) denaturation step was performed at 95°C for 5 min, followed by 30 cycles of denaturation at 98°C for 10 s, annealing at the melting temperature of each primer pair for 20 s and extension at 72°C for 30 s, with a final 10 min extension at 72°C. The PCR products for g.-15T>C was digested with 1 U of MboI (Takara, Japan), g.122T>C was digested with 1U of AatII (Takara, Japan), g.-1115G>C was digested with 1 U of BalI (Takara, Japan), for 16 h at 37°C and then separated on 2% agarose gel and visualized under UV with ethidium bromide. After restriction enzyme digestion, the PCR products for g.-15T>C (240 bp), g.122T>C (441 bp), and g.-1115G>C (525 bp) took the form of two fragments, that is, 150 bp and 90 bp, 271 bp and 170 bp, and 372 bp and 153 bp, respectively.

### Statistical analysis

UC patients and healthy control groups were compared using case-control association analysis. The χ² test was used to estimate Hardy-Weinberg equilibrium (HWE). Allele frequency was defined as the percentage of individuals carrying the allele among the total number of individuals. Logistic regression analyses were used to calculate odds ratios (95% confidence interval) for SNP sites. Linkage disequilibrium (LD) analyses by pair-wise comparison of biallelic loci and haplotype frequencies of the *IFITM2* and *IFITM5* gene for multiple loci were estimated using the expectation maximization (EM) algorithm with SNPAlyze software (DYNACOM, Japan). A P-value of less than 0.05 was considered an indication of statistical significance.
Results

To determine the possible variation sites, in the entire coding regions, and the boundary intron sequences of IFITM2 and IFITM5 that include about 2.2 kb of the promoter region, we scanned the genomic DNAs isolated from 24 unrelated UC patients and 24 healthy controls. We identified eleven SNPs and two variation sites in IFITM2 by direct sequencing methods, g.-1402T>C (rs72867735), g.-1315G>A (rs11246061), g.-1309T>C (rs10751647), g.-1157T>C (rs3809112), g.-688delA (novel), g.-467A>G (rs2031090) and g.-281T>C (rs7480474) in the promoter region, g.-254delA (rs34498415), g.-15T>C (rs1058873) and g.-13A>G (rs10398) in 5'UTR, g.384G>A (rs909097) in intron 1 and g.935A>G (rs1059091; V212I) in exon 2 (Fig. 1). We also identified eleven SNPs and one variation site in IFITM5, g.-1675G>A (rs146940957), g.-1550delA (novel), g.-1115G>C (rs72636981), g.-874G>A (rs7111803), g.-644T>C (rs7105970), g.-557G>A (rs7112167) and g.-96T>C (rs56089838) in the promoter region, g.80G>C (rs57285449; G27A) in exon 1, g.2015C>T (rs116899068), g.400C>G (rs4758636) and g.733C>T (rs2293745) in intron 1 and g.1175G>A (rs2293744) (Fig. 1). The LD coefficients (|D'|) between all SNP pairs were calculated, and there was no absolute LD (|D'| = 1 and r^2 = 1) among the SNPs of the IFITM2 or IFITM5 gene (data not shown). Among the identified polymorphisms, two SNPs (g.-15T>C and g.384G>A) of IFITM2, and three SNPs (g.-1115G>C, g.-874G>A and g.-96T>C) of IFITM5 were selected for large sample genotyping analysis. A SNP, g.122T>C (rs14408) of IFITM2, was also selected from public database for genotype analysis. Two SNPs, g.935A>G (V212I) of IFITM2, and g.80G>C (G27A) of IFITM5, were not analyzed because of it were difficult to make the Taq-Man probe.

To find out whether the IFITM2 and IFITM5 SNPs are
associated with UC susceptibility, the genotypes of the \textit{IFITM2} and \textit{IFITM5} polymorphisms were analyzed by the PCR-RFLP or TaqMan probe method, and the genotype and allelic frequencies between the groups were compared. The genotype and allelic frequencies of the \textit{IFITM2} and \textit{IFITM5} SNPs in the UC patient group were not significantly different from those of the healthy control group (Table 2). These results suggest that the \textit{IFITM2} and \textit{IFITM5} SNPs appear to be not associated with UC susceptibility.

To judge the possible correlation between the haplotypes associated with rs1058873, rs14408 and rs909097 of the \textit{IFITM2} gene and UC susceptibility, we further analyzed haplotype frequencies of the SNPs in the UC patients and the healthy controls (Table 3). However, there are no significant differences between the two groups. These results suggest that the haplotype frequency of \textit{IFITM2} polymorphisms might be not associated with UC susceptibility.

We also analyzed haplotype frequencies of the \textit{IFITM5} SNPs, rs72636981, rs7111803 and rs56069858, in the UC patients and the healthy controls (Table 4). Although, the distribution of the GGC haplotype frequency was significantly different between the healthy controls and the UC patients ($P = 0.05$), the major GGT, CAT and GAT haplotypes frequency were not significantly different between two groups (Table 4). These results suggest that the haplotype frequency of \textit{IFITM5} polymorphisms might be not associated with UC susceptibility.

To evaluate whether the combined frequencies of \textit{IFITM1}, \textit{IFITM2} and \textit{IFITM5} SNPs are associated with UC susceptibility, we analyzed the combined frequencies of rs77337847

\begin{table}[h]
\centering
\begin{tabular}{lccccc}
\hline
\textbf{Position}\textsuperscript{a} & \textbf{Genotype / allele} & \textbf{Control n (%)} & \textbf{UC n (%)} & \textbf{Odds ratio}\textsuperscript{b} & \textbf{P}\textsuperscript{c} \\
\hline
\textbf{IFITM2} & & & & & \\
\textbf{g.-15T>C} & CC & 263 (51.3) & 62 (49.2) & 1.00 & 0.81 \\
& CT & 217 (42.3) & 57 (45.2) & 1.11 (0.75-1.67) & \\
& TT & 33 (6.4) & 7 (5.6) & 0.90 (0.38-2.1) & \\
& C & 743 (72.4) & 181 (71.8) & 1.00 & \\
& T & 283 (27.6) & 71 (28.2) & 1.03 (0.76-1.40) & \\
\textbf{g.122T>C} & CC & 467 (91.6) & 120 (95.2) & 1.00 & 0.03 \\
& CT & 43 (8.4) & 5 (4.0) & 0.45 (0.18-1.17) & \\
& TT & 0 (0.0) & 1 (0.8) & - & \\
& C & 977 (95.8) & 245 (97.2) & 1.00 & 0.37 \\
& T & 43 (4.2) & 7 (2.8) & 0.65 (0.29-1.46) & \\
\textbf{g.384G>A} & GG & 479 (91.8) & 113 (96.6) & 1.00 & 0.07 \\
& GA & 43 (8.2) & 4 (3.4) & 0.39 (0.14-1.12) & \\
& AA & 0 (0.0) & 0 (0.0) & - & \\
& G & 1001 (95.9) & 230 (98.3) & 1.00 & 0.08 \\
& A & 43 (4.1) & 4 (1.7) & 0.41 (0.14-1.14) & \\
\textbf{IFITM5} & & & & & \\
\textbf{g.-1115G>C} & GG & 297 (58.3) & 71 (56.3) & 1.00 & 0.92 \\
& GC & 186 (36.5) & 48 (38.1) & 1.08 (0.72-1.63) & \\
& CC & 26 (5.1) & 7 (5.6) & 1.13 (0.47-2.70) & \\
& G & 780 (76.6) & 190 (75.4) & 1.00 & 0.68 \\
& A & 238 (23.4) & 62 (24.6) & 1.07 (0.78-1.48) & \\
\textbf{g.-874G>A} & GG & 190 (40.3) & 47 (39.2) & 1.00 & 0.97 \\
& GA & 221 (46.9) & 57 (47.5) & 1.04 (0.68-1.61) & \\
& AA & 60 (12.7) & 16 (13.3) & 1.08 (0.57-2.04) & \\
& G & 601 (63.8) & 151 (62.9) & 1.00 & 0.82 \\
& A & 341 (36.2) & 89 (37.1) & 1.04 (0.77-1.39) & \\
\textbf{g.-96T>C} & TT & 381 (71.6) & 93 (78.2) & 1.00 & 0.11 \\
& TC & 138 (25.9) & 21 (17.6) & 0.62 (0.37-1.04) & \\
& CC & 13 (2.4) & 5 (4.2) & 1.58 (0.55-4.53) & \\
& T & 900 (84.6) & 207 (87.0) & 1.00 & 0.42 \\
& C & 164 (15.4) & 31 (13.0) & 0.82 (0.54-1.24) & \\
\hline
\end{tabular}
\caption{Genotype and allele analyses of the \textit{IFITM2} and \textit{IFITM5} gene polymorphisms in the UC patients and the healthy controls}
\textsuperscript{a}Calculated from the translation start site.
\textsuperscript{b}Logistic regression analyses were used for calculating OR (95% CI; confidence interval).
\textsuperscript{c}Value was determined by Fisher's exact test or $\chi^2$ test from a 2×2 contingency table.
\end{table}
Table 3 Haplotype frequencies between UC patients and healthy controls in IFITM2 SNPs

| Haplotype | Frequency | Chi-square | \( p^b \) |
|-----------|-----------|------------|-----------|
| rs1058873 | rs14408   | rs909097   | Control   | UC       |          |
| C         | C         | G          | 0.65      | 0.67     | 0.35     | 0.55     |
| T         | C         | G          | 0.27      | 0.28     | 0.26     | 0.61     |
| Others    |           |            | 0.08      | 0.05     |          |          |

\( ^a \)Values were constructed by EM algorithm with genotyped SNPs.
\( ^b \)Values were analyzed by Chi-square.

Table 4. Haplotype frequencies between UC patients and healthy controls in IFITM5 SNPs

| Haplotype | Frequency | Chi-square | \( p^b \) |
|-----------|-----------|------------|-----------|
| rs72636981| rs7111803 | rs56069858 | Control   | UC       |          |
| G         | G         | T          | 0.55      | 0.55     | 1.41E-5  | 0.10     |
| C         | A         | T          | 0.19      | 0.21     | 0.24     | 0.62     |
| G         | A         | T          | 0.11      | 0.11     | 1.725E-4 | 0.99     |
| G         | G         | C          | 0.09      | 0.05     | 3.91     | 0.05     |
| Others    |           |            | 0.10      | 0.08     |          |          |

\( ^a \)Values were constructed by EM algorithm with genotyped SNPs.
\( ^b \)Values were analyzed by Chi-square.

Table 5. Combined genotype frequencies of IFITMs SNPs in UC patients and healthy controls

| Combined genotypes | Frequency | Chi-square | \( p^b \) |
|--------------------|-----------|------------|-----------|
| rs77537847 rs909097 rs56069858 | Control | UC |          |
| A                  | G         | T          | 0.54      | 0.50     | 1.17     | 0.28     |
| G                  | G         | T          | 0.26      | 0.36     | 9.57     | 0.002    |
| A                  | G         | C          | 0.09      | 0.08     | 0.69     | 0.41     |
| Others             |           |            | 0.11      | 0.06     |          |          |

\( ^a \)Values were constructed by EM algorithm with genotyped SNPs.
\( ^b \)Values were analyzed by Chi-square.

of IFITM1, rs909097 of IFITM2 and rs56069858 of IFITM5 in the UC patients and the healthy controls (Table 5). Although, the distribution of the major combined genotype AGT frequency was not significantly different between the healthy controls and the UC patients, the GGT combined frequency in the healthy controls was significantly different from that in the UC patients \( (p=0.002) \). This result suggests that the combined genotypes of the IFITMs polymorphisms might be associated with a susceptibility to UC and could be useful genetic marker for UC.

Discussion

IBD is a chronic inflammatory disease of the gastrointestinal tract [10]. CD and UC are the principal types of IBD, which fall into the class of autoimmune diseases. Although great advances have been made in the management of IBD with the introduction of immune-modulators and monoclonal antibodies, the precise etiology of IBD is unclear [1, 13]. However, IBD is thought to be the result of the interaction of environmental and genetic factors. 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 NOD2 gene, which has been widely shown to influence both the susceptibility and phenotype of patients with CD, is located at the IBD1 locus [3, 8, 18]. We previously reported that an exon 4 variation of the Tim-1 gene and the SNPs of the IL27, TNFRSF17 and EED genes were associated with UC in a Korean population [5, 6, 9, 19].

The human IFITM2 and IFITM5 gene, a member of the IFITM family, consist of two exons and one intron. IFITM proteins were first discovered in T98G neuroblastoma cells
that express the proteins in response to interferon stimulation [28]. We have previously identified 4 polymorphisms in the IFITM1 and 7 polymorphisms in IFITM3, and have suggested that the IFITM1 or IFITM3 polymorphisms are associated with a susceptibility to UC [20, 25]. These results led us to determine whether or not the IFITM2 and IFITM5 SNPs are associated with susceptibility to UC in this study. In this study, we identified a total of thirteen polymorphisms (eleven SNPs and two variations) in the IFITM2 gene and twelve polymorphisms (eleven SNPs and one variation) in IFITM5 gene (Fig. 1). However, the genotype and allele frequencies of IFITM2 SNPs as well as IFITM5 SNPs in UC patients were not significantly different from those of the healthy control group (Table 2). These results suggest that SNPs of IFITM2 and IFITM5 may be not associated with susceptibility to UC. Although it is not clear how the IFITM1 and IFITM3 gene polymorphisms are related to the susceptibility of UC, our previous [20, 25] and this present study suggest that the IFITM gene family might be differently associated with the pathogenesis of UC.

We also analyzed haplotype frequencies using the IFITM2 and IFITM5 SNPs in the UC patients and the healthy controls (Table 3). However, there are no significant differences between the two groups. These results suggest that the haplotype frequency of IFITM2 and IFITM5 polymorphisms might be not associated with UC susceptibility. These results led us to evaluate whether the combined frequencies of IFITM1, IFITM2 and IFITM5 SNPs are associated with UC susceptibility. Therefore, we analyzed the combined frequencies of rs77537847 of IFITM1, rs909097 of IFITM2 and rs56069858 of IFITM5 in the UC patients and the healthy controls (Table 5). Although, the distribution of the major combined genotype AGT frequency was not significantly different between the healthy controls and the UC patients, the GGT combined frequency in the healthy controls was significantly different from that in the UC patients (Table 5). This result suggests that the combined genotype of the IFITMs polymorphisms might be associated with a susceptibility to UC and could be useful genetic marker for UC.

There are several limitations to our study. We did not check the expression levels of IFITMs in UC patients and did not show the clinical impact of IFITMs SNPs on UC. Although there are several limitations, our results provide useful information for further functional studies of the IFITM gene family, and gastrointestinal disease such as colorectal cancer and inflammatory responses.

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초록: IFITM2 및 IFITM5 유전자 다형성의 발굴과 궤양성 대장염의 감수성과의 연관성

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Interferon inducible transmembrane protein (IFITM) family 유전자는 인터페론(IFNs)의 동형 세포부착 기능 및 세포의 항-증식 활성과 같은 몇 가지 세포증식 과정에 연관되어 있다. 본 연구에서는 IFITM2 및 IFITM5 SNPs가 궤양성 대장염의 감수성과 연관되어 있는지 알아보고자 했다. 본 연구에서 직접 염기서열 분석법을 사용하여 IFITM2 유전자에서 총 13개, IFITM5 유전자에서는 12개의 유전적 변이를 발굴하였다. 이들의 SNPs의 유전자형 분석은 PCR-RFLP 법과 Taq-Man probe 분석법을 사용하였고, 일배체형 빈도 분석은 EM algorithm을 사용하여 분석하였다. 궤양성 대장염 환자에서 IFITM2 및 IFITM5 SNPs의 유전자형과 대립유전자 빈도는 건강한 대조군과 비교했을 때 유의성이 없었다. 궤양성 대장염 환자와 정상인 대조군에서 IFITM1의 rs77537847, IFITM2의 rs909097, IFITM5의 rs56069858을 지표로 하는 유전자형 조합 빈도를 분석한 결과 주된 유전자형 조합비도에서는 유의성이 없는 것으로 나타났으나, 궤양성 대장염 환자와 건강한 대조군의 GGT 유전자형 조합 빈도 분석에서는 유의하게 다른 차이를 보였다(p=0.002). 이러한 결과에 의거하여 IFITMs의 SNPs 유전자형 조합이 궤양성 대장염의 감수성과 연관성이 있고, 궤양성 대장염의 유용한 유전자 마커로 사용할 수 있다고 생각된다.