Genetic polymorphisms of the interleukin 23 receptor and interleukin 17A and their associations with inflammatory bowel diseases

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Directed by Professor Won Ho Kim

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ABSTRACT

Genetic polymorphisms of the interleukin 23 receptor and interleukin 17A and their associations with inflammatory bowel diseases

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(Directed by Professor Won Ho Kim)

Inflammatory bowel disease (IBD), which includes mainly ulcerative colitis (UC) and Crohn's disease (CD), is characterized by chronic relapsing intestinal inflammation. Currently, IBD is considered to be caused by a complex interaction of genetic, environmental, and other processes involving immunoregulatory factors of which play a principal role in the pathogenesis of IBD. Recently, a particular subset of T helper cells (Th17 cells) characterized by interleukin 17A (IL-17A or further referred to as IL-17) production was implicated as a critical mediators of autoimmune disease including IBD and the

IL-23R gene is known to be a susceptibility gene related to IBD in Caucasian IBD patients, although it has not been detected in Asian populations. Moreover, while there are a few reports on the associations of *IL-17A* gene polymorphisms, it is still unknown whether *IL-17A* SNPs are associated with IBD susceptibility except rs2275913 and, if so, how these *IL-17A* SNPs exactly modulate IBD susceptibility.

Thus, It was investigated the associations of genetic and epigenetic variations in *IL-23R* and *IL-17A* with the development of IBD in this study. The promoters and exon regions encompassing the intron junctions of *IL-23R* and *IL-17A* were analyzed in 728 subjects including 201 CD patients, 268 UC patients, and 259 healthy controls using DNA sequencing and denaturing high performance liquid chromatography. Associations of *IL-23R* and *IL-17A* polymorphisms with IBD susceptibility were analyzed and their gene-gene interactions including *STAT4* SNPs were tested using logistic regression analysis. Jurkat cells and peripheral blood mononuclear cells (PBMC) were used for *in vitro* assay as follows. The transcription factor binding activity was determined using electrophoretic mobility shift assay, *IL-17A* mRNA expression levels by reverse-transcriptase polymerase chain reaction, and methylation status of *IL-17A* promoter by bisulfite sequencing and pyrosequencing.

In CD, a case-control analysis showed that disease development was associated with the *IL-23R* variant G149R (odds ratios [OR] 0.32, 95% confidence intervals [CI] 0.15–0.68) and *IL-17A* variant IVS1+18G>C (OR

10.65, 95% CI 1.32–85.89). The analysis for UC showed an association with *IL-23R* variants G149R (OR 0.41, 95% CI 0.21–0.76), IVS4+17C>T (OR 2.89, 95% CI 1.20–6.96), and Q3H (OR 0.61, 95% CI 0.38-0.99), and *IL-17A* variants -737C>T (OR 1.50, 95% CI 1.06–2.13), -197G>A (OR 0.63, 95% CI 0.40–0.97), and IVS1+18 G>C (OR 8.93, 95% CI 1.12–70.99). As further evidence of the synergistic effect of the genes in this pathway in the development of IBD, a significant statistical gene-gene interaction among *IL-23R*, *IL-17A* and *STAT4* were observed. The -877G, -737T, and -444A risk alleles of *IL-17A* displayed higher binding affinity of transcription factor complex and higher expression levels of *IL-17A* transcripts. DNA hypomethylation of the *IL-17A* promoter was observed in PBMCs from IBD patients with a methylation extent of IVS1+17 site and significant inverse correlation between *IL-17A* mRNA level. Finally, *IL-17A* mRNA expression was restored after exposure to demethylating agent in Jurkat cells. The results of this study provide insights into the genetic and epigenetic interactions in the *IL-23R/IL-17* axis including *STAT4* that are associated with elevated expression of IL-17 and IBD pathogenesis.

Key words : interleukin 23 receptor, interleukin 17A, genetic polymorphism, inflammatory bowel disease, epigenetics

Genetic polymorphisms of the interleukin 23 receptor and interleukin 17A and their associations with inflammatory bowel diseases

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I. INTRODUCTION

Inflammatory bowel disease (IBD), which includes mainly ulcerative colitis (UC) and Crohn's disease (CD) is characterized by chronic relapsing intestinal inflammation of unknown etiology.[1] Currently, IBD is considered to be caused by complex interactions of genetic, environmental, and other processes involving immunoregulatory factors.[2,3] Of these, studies in twins and family members suggest that genetic factors play a principal role in the pathogenesis of IBD. Recently, several IBD susceptibility genes

have been identified,[4] and linkage studies have implicated multiple regions within the human genome.[5,6] Since the caspase-recruitment domain 15 gene (NOD2) was identified as the first CD susceptibility gene in 2001, genome-wide association studies or linkage analyses uncovered several susceptibility genes related to IBD in Caucasian populations including *DLG5*, *SLC22A4*, *SLC22A5*, *TNFSF15*, *IL-23R*, and *ATG16L1*.[4,7-10] However, the genetic polymorphisms located within these genes do not fully explain the pathogenesis of IBD or the variations in disease phenotypes. Furthermore, the precise genetic pathogenesis of IBD still remains poorly understood.

Recently, a subset of T helper cells (Th17 cells) characterized by interleukin 17A (IL-17A or further referred to as IL-17) production was implicated as a critical mediator of autoimmune diseases such as bronchial asthma, experimental autoimmune encephalomyelitis (EAE), rheumatoid arthritis (RA), psoriasis, systemic lupus erythematosus (SLE), systemic sclerosis (SSc), and IBD.[11-13] Accumulating evidence has demonstrated that IL-17 plays an important pathologic role in the development of IBD.[13-15]

Interleukin 23 (IL-23) is essential for maintaining the Th17 response[12] and is characteristically associated with Th17 cell lineage differentiation.[16,17] Moreover, IL-23 receptors (IL-23Rs) are expressed on a variety of cells and may directly activate a subset of macrophages, natural killer cells, monocytes, and dendritic cells that secrete IL-17A.[18,19] Interestingly, although the *IL-23R* gene is known to be a susceptibility gene related to IBD, functional variants in the *IL-23R* gene, which was identified as a susceptibility locus in Caucasian IBD patients, has not been detected in Asian

populations.[10,20-23] These results reveal that there are distinct ethnic differences in the genetic background of IBD between Asian and Caucasian populations. Therefore, it is necessary to identify new functional variation in coding region of *IL-23R* in Asian patients with IBD. Moreover, while there are a few reports on the associations of *IL-17A* gene polymorphisms with several autoimmune diseases such as RA and bronchial asthma,[24,25] it is still unknown whether *IL-17A* SNPs (single nucleotide polymorphism) with the exception of rs2275913 are associated with IBD susceptibility and, if so, how these *IL-17A* SNPs exactly modulate IBD susceptibility. In another side, STAT4 (signal transducer and activator of transcription 4) is another critical transcription factor transducing IL-12-, IL-23-, and type-1 interferon (IFN)-mediated signals into Th1 and Th17 differentiation, monocyte activation, and IFN-production.[26-29] *STAT4* polymorphisms have been found to be associated with autoimmune diseases, including IBD, RA, SLE, asthma, and Sjögren's syndrome (SS).[30-33] IL-23 activates not only STAT3 but also to a lesser degree STAT4. STAT4 is also required for cytokine-stimulated production of IL-17. Moreover, evidence for weak gene-gene interaction of *STAT4* with the *IL-23R* SNPs in Caucasian was recently reported.[34]

In this study, the roles of *IL-23R* and *IL-17A* SNPs in the genetic susceptibility to IBD in the Korean population were assessed to elucidate their association with IBD. Potential epistasis (gene-gene interaction) among the IBD susceptibility gene *IL-23R*, *IL-17A* and *STAT4* was next investigated. It was also investigated the functional consequences of genetic and epigenetic factors that interact to increase the susceptibility to IBD .

II. MATERIALS AND METHODS

1. Study Subjects

A total of 258 healthy controls, and 469 unrelated Korean IBD participants (201 patients with CD, 268 with UC) were surveyed, and IBD patients were diagnosed and managed at the gastroenterology clinics of Yonsei University College of Medicine, Severance Hospital, Seoul, Korea between June 2006 and February 2008. The diagnosis of CD or UC was based on established clinical, radiologic, endoscopic, and histopathologic criteria.[35,36] Cases of indeterminate colitis, or patients with concomitant immune-mediated diseases such as RA, SLE, primary SS, type 1 diabetes, SSc, or asthma were excluded from the study. The demographic and clinical characteristics of the subjects were obtained through the review of medical records and detailed questionnaires. CD phenotypes were classified by age at diagnosis, location, and disease behavior according to the Montreal classification.[37] In patients with UC, anatomic locations were subgrouped using the Montreal classification as ulcerative proctitis (E1), left-sided UC (E2) or extensive UC (E3). Differences in the frequencies of disease characteristics such as age at diagnosis, gender, extra-intestinal manifestations (EIMs), total follow-up period, the use of immunosuppressive drugs, and the need for surgical intervention were also assessed. This study was approved by the Institutional Review Board of Severance Hospital. Written informed consent was obtained from all participants or their guardians.

2. DNA Extraction and Polymerase Chain Reaction (PCR)

Genomic DNA was isolated from whole blood samples from each subject using commercially available kits (Qiagen, Hilden, Germany). Specific primers were designed to amplify the promoter and exons spanning splice junctions (Table 1).

Table 1. Oligonucleotides for genotyping of the *IL-17A* and *IL-23R* genes

Target gene	Regions	Forward sequence	Reverse sequence
IL-17A	Promoter 4	TTCAGGATCCGGCAAACTAAT	GATAGAGACTGGACAAAGGTG
	Promoter 3	CCATCATGTTCCTCTCCTT	CCTCACAGATTCCTTGGCC
	Promoter 2	TTGAGTAGTTTCCGGAATTGTC	TGTCGCAGTGGGTTCAGGG
	Promoter 1 ~ exon 1	ACATGAATTTCTGCCCTTCCC	CCACGGTCCAGAAATACTATC
	Exon 2	AACCACATAGTAATCTAATCTCC	GATGCATAAGAGGGATGAAGAAT
	Exon 3	CTCTCTTCATGTATTCCTGTTT	GGATGAGGGTTCCTGAGGG
IL-23R	Exon 1	CCAAATAGTGACACGAGAGCCAG	AACAACTTGGCCCATGTATTTCC
	Exon 2	CCCTAATCAAAGGTTCCCATC	CATTTGGAAGGAGATTGGGC
	Exon 3	TCTTCTGAATCTCTTGATTTAATGTTT	TGACCAGTTAATTGTTAAACTAGC
	Exon 4	GACTGGAGCTGTTCCTATTCAGC	GATTTCTTGGATCTCTGCTGGAGG
	Exon 5	GAGCCACCATGCCTGGCCAATTA	AGACCTCATTTGACAAGGTCAGCA C
	Exon 6	TGCCAGTTTCTCCCTAGGCAAGT	AAATAGACATAATAAGTCCACTGTAGCC
	Exon 7	GGTGTTCCATACATTTCTGCTAA	ATTATAGGTGTGAGCCACTGTGC
	Exon 8	CAAGGGAAGAAACTCCGTTGGGA	CCTATGGAAGACATAAGGCATATC
	Exon 9	CACCCTTTCTCCTTTGAGACCT	TCTAGTAAACAACTGAAATGACTAAAT
	Exon 10	AATCCTCCCACCTCAGCC	TGATTTGAAACAGGTACAGTTTTG
	Exon 11-1	TCATTCAATTATCCAGTTGGTTC	TATTGCTGAGATGGCTTCCC
	Exon 11-2	GAGACTACCCGCAAAACTCG	ATTTTCCAAAAGCATGGTGG
	Exon 11-3	TGAATTCACTAAGCAACACAATATTTC	GATTGCAAGGCAGCTTTCTC

Genotyping was performed using PCR. PCR was carried out in a total volume of 25 μl containing 200 μM dNTPs, 50 ng of template DNA, 10 pmol of each primer, and 0.5 unit of i-StarmaxTM II DNA Taq Polymerase (Intron Biotechnology, Seoul, Korea) in 1 × reaction buffer. The PCR was performed with an initial denaturation step at 94℃ for 5 min; 35 cycles of 94℃ for 30 sec, 60℃ for 30 sec, and 72℃ for 30-90 sec; and a final extension step at 72℃ for 7 min.

3. Genotyping

DNA sequencing was used for preliminary identification of candidate IBD risk SNPs of *IL-23R* in 50 patients and 50 controls; all the remaining subjects enrolled in the study were genotyped using full DNA sequencing of promoter regions and denaturing high performance liquid chromatography (DHPLC) of exons and all splice junctions (Figure 1).

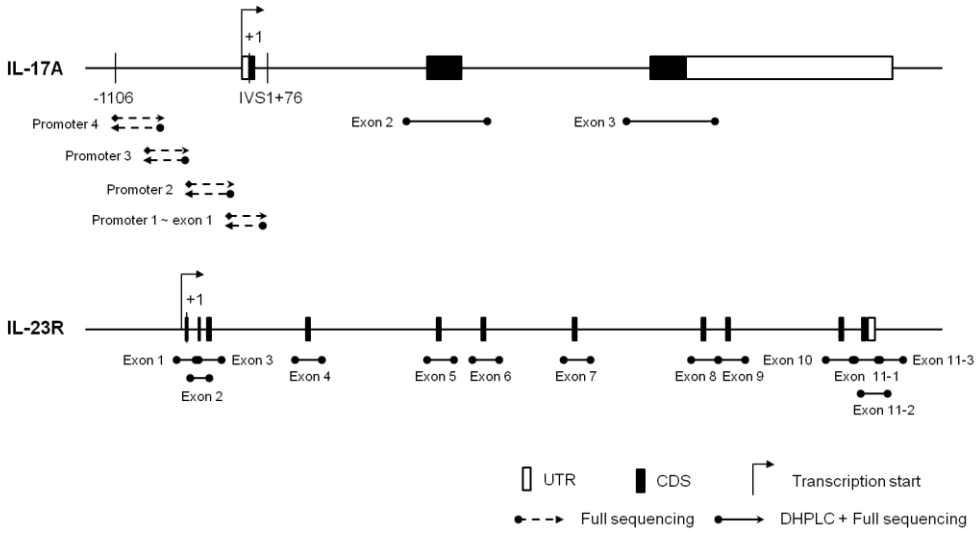

Figure 1. Schematic representation of the genotyping strategies for the *IL-17A* and *IL-23R* genes. The genomic region of the human *IL-17A* gene encompassing the promoter and three exons and that of the *IL-23R* gene encompassing 11 exons are schematically shown. +1 indicates the first nucleotide of the start codon. Coding exons are marked by filled blocks and untranslated regions (UTR) by open blocks. Filled circles show the primer sites.

After purification using a PCR purification kit (Qiagen), the PCR products were used as template DNA for sequencing analysis (ABI Prism Big Dye Terminator cycle sequencing system; ABI 3100 automatic sequencer, Applied Biosystems, Foster City, CA, USA). Unpurified PCR products were mixed 3:1 with a sequence-confirmed wild-type reference before being subjected to a 5 min, 95℃ denaturing step, followed by gradual cooling to 65℃ with a temperature change of 1℃/min before DHPLC analysis. The addition of wild-type DNA to the sample before the denaturation step enabled reliable detection of homozygous alteration to identify homozygous sequence variations. Five microliters of each mixture were loaded onto a DNASep-HT column (Transgenomic, Omaha, NE, USA) and the amplicons were eluted in 0.1 M triethylammonium acetate (pH 7) with a linear acetonitrile gradient at a flow rate of 1.5 ml/min. Heteroduplex mismatches were recognized by the appearance of aberrant patterns in the elution profiles under appropriate temperature conditions, which were calculated by the Navigator Software of the Wave Nucleic Acid Fragment Analysis system device (Transgenomic). The most important criteria for assigning the presence of a sequence alteration in each DHPLC fragment were numbered and the shapes of the elution peaks compared to a sequence-confirmed wild-type control subject elution profiles that were used as references (Figure 2). All detected variations from DHPLC analysis were validated using subsequent full DNA sequencing.

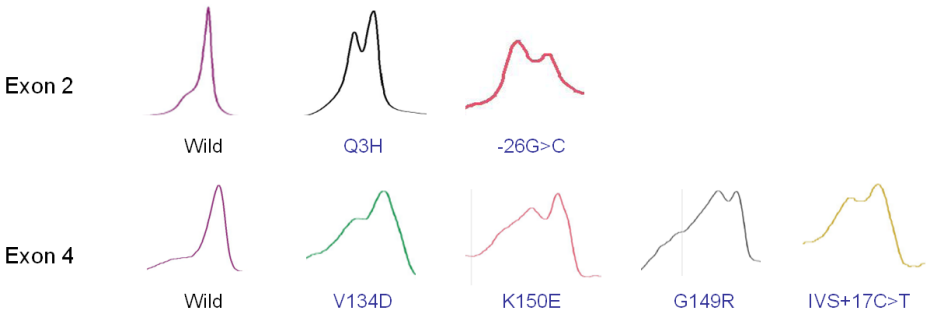

Figure 2. Representative DHPLC elution profiles for SNPs in the *IL-23R* gene. Seven SNPs were found in intron 1, exon 2, exon 4, and intron 4. From top to bottom and left to right, each peak shows the absorbance and elution time, respectively.

The genotype data for eight IBD-associated *STAT4* SNPs (rs11889341, rs7574865, rs8179673, rs6752770, rs925847, rs10168266, rs10181656, rs11685878) were available from a previous study.[30]

4. Colonic Mucosal Tissue Preparation, Peripheral Blood Mononuclear Cell (PBMC) Isolation and Cell Culture

Colonic mucosal tissues were collected during colonoscopy and PBMCs were isolated using Ficoll-paque plus density gradient centrifugation (GE Health Care,

Piscataway, NJ, USA) from healthy donors and IBD patients. Jurkat cells (American Type Culture Collection, Manassas, VA, USA) were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 1 mM glutamine and 1% penicillin/streptomycin (Invitrogen, Carlsbad, CA, USA). Jurkat cells at a density of 0.5-1.0 $\times 10^6$ cells/ml were then activated with anti-CD3 mAb (2 μg/ml; eBioscience, San Diego, CA, USA) and anti-CD28 mAb (1 μg/ml; eBioscience) for four days, and were stimulated with tumor growth factor β (TGF-β; 2.5 ng/ml; R&D Systems, Minneapolis, MN, USA), IL-6 (30 ng/ml; R&D Systems), IL-23 (10 ng/ml; R&D Systems), or with phorbol 12-myristate 13-acetate (PMA, 20 nM; Sigma-Aldrich St. Louis, MO, USA) and ionomycin (2 μM; Sigma-Aldrich) for six h. To assess re-activation of *IL-17A* expression, Jurkat cells were plated in 6-well tissue plates 16 hr before treatment, 5-azadeoxycytidine (5-Aza-dC; Sigma-Aldrich) at final concentrations of 0.4 μM or phosphate buffered saline in duplicate was added to the fresh medium and the cells were harvested after three or four days of treatment.

5. Electrophoretic Mobility Shift Assay (EMSA)

Nuclear and cytoplasmic extracts were prepared as follows. Briefly, harvested cells were lysed for 5 min in lysis buffer [5 mM KCl, 25 mM HEPES, 0.5 mM $MgCl_2$, 1 mM EDTA, 0.5 mM spermidine, 0.15 mM spermine, 0.2% Triton X-100, complete protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany)], and then nuclei were pelleted via centrifugation at 6,000 g for 15 min. Nuclei were lysed in hypotonic buffer (350 mM NaCl, 10 mM HEPES, 25% glycerol, 0.1 mM EDTA, 0.5

mM spermidine, 0.15 mM spermine, and protease inhibitors as above) for 30 min, and debris were removed via centrifugation at 13,000 rpm for 5 min. Bradford reagent (Bio-Rad Laboratories, Hercules, CA, USA) was used to measure protein content. EMSA was performed using a Lightshift™ chemiluminiscent EMSA kit (Pierce, Rockford, IL, USA) following the manufacturer's protocol. Complementary oligonucleotides (Table 2) were biotin-labeled separately using the Biotin End-labeling kit (Pierce) and annealed before use.

Table 2. Oligonucleotides for EMSA

Region	Sense sequence	Antisense sequence
-877-wild type	CTATTCTCAAGGACCTGAGTCCAAG	CTTGGACTCAGGTCCTTGAGAATAG
-877-mutant type	CTATTCTCGAGGACCTGAGTCCAAG	CTTGGACTCAGGTCCTCGAGAATAG
-737-wild type	CCTTTTCTCCATCTCCATCACCTTTGTC	GACAAAGGTGATGGAGATGGAGAAAAGG
-737-mutant type	CCTTTTCTCCATCTTCATCACCTTTGTC	GACAAAGGTGATGAAGATGGAGAAAAGG
-444-wild type	AGGAATCTGTGAGGAAAAGAAAGATCAA	TTGATCTTTCTTTTCCTCACAGATTCCT
-444-mutant type	AGGAATCTGTGAGGGAAAGAAAGATCAA	TTGATCTTTCTTTCCCTCACAGATTCCT
-197-wild type	CCTTCAGAAGGAGAGATTCTTCTATGA	TCATAGAAGAATCTCTCCTTCTGAAGG
-197-mutant type	CCTTCAGAAGAAGAGATTCTTCTATGA	TCATAGAAGAATCTCTTCTTCTGAAGG

The oligonucleotides of *IL-17A* promoter regions with the normal or variant sequence for SNPs were used as probe. Complementary oligonucleotides were biotin-labeled separately and annealed before use. The variation sequences are underlined.

Each binding reaction contained 1 × binding buffer (100 mM Tris, pH 7.5, 500 mM KCl, 10 mM dithiothreitol), 2.5% glycerol, 5 mM $MgCl_2$, 50 ng/μl poly (dIdC), 0.05% NP-40, 5 μg of nuclear extract, and 40 fM of biotin end-labeled target oligonucleotide probe. The reaction mixture was incubated at room temperature for 20 min, and the complexes were separated on 4% non-denaturing polyacrylamide gel and then were transferred to a nylon membrane. When the transfer was complete, DNA was crosslinked to the membrane at 120 mJ/cm^2 using a UV crosslinker at 254 nm. The biotin end-labeled DNA was detected using streptavidin-horseradish peroxidase conjugate and a chemiluminescent substrate. The membrane was exposed to X-ray film and developed using a Kodak film processor (Eastman Kodak, Rochester, NY, USA). RAR-related orphan receptor C (RORC) antibody (Abcam, Cambridge, MA, USA) was used for the supershift assay.

6. Quantitative Real-time Reverse-transcription Polymerase Chain Reaction (RT-PCR)

Total RNA was extracted using TRIzol Reagent (Invitrogen) and 1 μg of RNA was reverse transcribed using the SuperScript First-Strand Synthesis kit (Invitrogen) according to the manufacturer-recommended protocol. The cDNAs were mixed with SYBR Green master mix (Applied Biosystems) and the primer pairs (4 pmol of each primer) in triplicate. PCR was performed using primers (Table 3) for *IL-17A*, RORC, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Table 3. Oligonucleotides for real-time RT-PCR

Target	Sense sequence	Antisense sequence
IL-17A	CAATCCCACGAAATCCAGGATG	GGTGGAGATTCCAAGGTGAGG
RORC	TTTTCCGAGGATGAGATTGC	CTTTCCACATGCTGGCTACA
STAT4	CACCTGCCACATTGAGTCAACTA	TAAGACCACGACCAACGTACGA
GAPDH	TGATGACATCAAGAAGGTGG	TTTCTTACTCCTTGGAGGCC

Samples were amplified in a 7500 real-time PCR System (Applied Biosystems) for 40-45 cycles using the following PCR variables: 95℃ for 30 sec, 60-62℃ for 1 min, and 72℃ for 1 min. Finally, quantitative analysis was performed using the relative standard curve method and the results were reported as the relative expression or fold change as compared to that of the calibrator after normalization of the transcript level with regard to the control, namely *GAPDH,* or *RORC.*

7. Determination of DNA Methylation using Bisulfate Sequencing and Pyrosequencing

To determine the methylation status of CpG sequences in the *IL-17A* gene promoter, bisulfite modification was accomplished using an Epitect bisulfite kit (Qiagen) according to the manufacturer's instructions. The promoter region of the *IL-17A* gene from PBMCs was PCR-amplified with primers (Table 4 and 5) that were designed using a PSQ assay design program.

Table 4. Oligonucleotides for pyrosequencing and bisulfate sequencing of the *IL-17A* gene

Application	Regions	Forward primer	Reverse primer
1st PCR	-969~-684	Biotin-ATTATTTATTTTAGTGGGGGTAGG	ACCCTACATACTACCAAACAACTT
	-234~IVS1+77	Biotin-TGAAAAGAGGATATGGTTTTTAGG	CCTAAATCTCCATAATCAAAACCC
2nd PCR sequencing	-969~-876	Biotin-ATTATTTATTTTAGTGGGGGTAGG	ATAAACTTAAACTCAAATCC
	-234~IVS1+21	Biotin-TGAAAAGAGGATATGGTTTTTAGG	CCAAATCAACAAAAACATC
Pyrosequencing	-877		ATAAACTTAAACTCAAATCC
	-2, IVS1+17, IVS1+21		CCAAATCAACAAAAACATC

Table 5. Oligonucleotides for pyrosequencing and bisulfate sequencing of the *STAT4* gene

Application	Regions	Forward primer	Reverse primer
PCR	-266~-58	Biotin- GGTTGAGTGGAGTTTTATATTA	TCTATTCTAAAAATACTAAC
	-176~ +49	Biotin- GGAAATTATTGAAGAAAATGTAT	CCTAAATCTCCATAATCAAAACCC
Pyrosequencing	-266~-147		CAACTACAAATAACTCAAC
	-146~-58		TCTATTCTAAAAATACTAAC
	-57~-42		CCACTTAAAACTTTCCTATA
	-42~-+49		ACTCAAATCCAAAATCAA

Bisulfite sequencing analyses were performed as described previously,[38] and the pyrosequencing reactions were performed on a PyroMark Q24 system (Qiagen) following the manufacturer's instruction. The subsequent data were analyzed using the methylation-analysis software.

8. Statistical Analysis

All SNPs investigated in this study were tested for Hardy-Weinberg equilibrium (HWE) in controls and associations of SNPs with disease susceptibility were determined by comparing allele and genotype frequencies between cases and controls using the Chi-square test. Logistic regression analyses were performed to calculate the odds ratios (OR), 95% confidence intervals (CI), and corresponding *P*-values of each SNP under four alternative models (additive, codominant, dominant, and recessive). In addition, relationships between disease genotypes and phenotypes were assessed according to clinical characteristics, such as mean age at diagnosis, gender, disease location and behavior, EIMs, and a history of immunosuppressive drug use and surgical intervention.

Haploview software version 4.1 (Broad Institute of MIT and Harvard, Cambridge, MA, USA) was used to analyze the linkage disequilibrium (LD) structure and to test for associations between haplotypes and IBD. Haplotypes with a frequency > 3% were numbered in order of frequency (*H1*, *H2*, and so on). Gene–gene interactions were tested using an interaction test in logistic regression models.[39] For all calculations, SPSS version 12.0 (SPSS, Chicago, IL, USA) was used. A two-tailed test was used for all analyses and two-sided *P*-values of < 0.05 were considered significant.

III. RESULTS

1. Baseline Clinical Characteristics of the Study Subjects

A total of 727 subjects including 201 patients with CD, 268 patients with UC, and 258 healthy controls were analyzed in this study. The demographic and clinical characteristics of patients with CD and UC are summarized in Table 6. The mean patient age at diagnosis of CD was 26.0±11.2 years at a gender ratio ratio of 1.6:1 (male: female), and that of UC was 36.4±12.4 years at a gender ratio of 1.0:1.0 Based on the Montreal Classification, 87.1% of CD patients had only ileal or ileocolonic disease, with or without upper gastrointestinal involvement. As for disease behavior, penetrating phenotype was observed in 78 (39.2%) patients and a stricturing phenotype in 37 (18.6%) patients. With respect to disease location at diagnosis in UC, extensive colitis was noted in 113 (42.1%) patients and left-sided colitis in 91 (34.0%) patients.

Table 6. Clinical and demographic characteristics of CD and UC patients

	CD (n = 201)	UC (n = 268)
Gender (male/female)	123/78 (1.6:1)	135/133 (1.0:1)
Age (years)[a]	26.0±11.2	36.4±12.4
Follow-up duration (months)[a]	89.3±51.7	103.6±61.3
Disease location (%)[b]		
Ileum (L1)±L4	61 (30.7)	
Colon (L2)±L4	24 (12.1)	
Ileocolon (L3)±L4	114 (57.3)	
Rectum (E1)		64 (23.9)
Left-side (E2)		91 (34.0)
Extensive (E3)		113 (42.1)
Disease behavior (%)[b]		
Inflammatory (B1)±p	81 (41.3)	
Stricturing (B2)±p	37 (18.6)	
Penetrating (B3)±p	78 (39.2)	
EIMs (%)		
Joint	19 (9.5)	30 (14.9)
Skin	10 (5.0)	20 (10.0)
Eye	11 (5.5)	28 (13.9)
Immunosuppressive drugs		
Steroid	137 (68.2)	97 (51.7)
Azathioprine	107 (53.2)	42 (20.9)
Infliximab	36 (17.9)	1 (0.5)

CD, Crohn's disease; UC, ulcerative colitis; EIMs, extra-intestinal manifestations. [a]Mean±standard deviation. [b]Disease localization and behavior according to the Montreal classification (L4, upper gastrointestinal involvement; p, perianal disease).

2. Association of *IL-23R* Genetic Polymorphisms with IBD Susceptibility

According to DHPLC and DNA sequencing of the exon regions including exon/intron junctions of *IL-23R* to uncover functional variations, seven SNPs in HWE($P > 0.05$) was found, of which five (intron 1: IVS1-26G>C; exon 4: V134D, K150E, K160L; intron 4: IVS4+17C>T) had never been reported (Table 7). CD showed a significant association with one marker in exon 4, G149R ($P < 0.002$, 95% CI 0.15–0.68 in codominant models) and UC with three markers, rs1884444 (named "Q3H" hereafter) in exon 2 ($P < 0.045$, 95% CI 0.38–0.99 in recessive), G149R ($P < 0.004$, 95% CI 0.21–0.77 in additive and dominant), and IVS4+17C>T ($P < 0.012$, 95% CI 1.20–6.96 in codominant). G149R and Q3H showed a protective association and IVS4+17C>T was responsible for disease risk. The variant rs11209026 which was described to be responsible for CD in Caucasians, appears to be absent in the Korean population.

Table 7. Distributions of genotype and allele frequencies of *IL-23R* SNPs

		CD					UC					Control
		No.(%)	Additive	Codominant	Dominant	Recessive	No. (%)	Additive	Codominant	Dominant	Recessive	No. (%)
Position	Genotype		*P* value	*P* value	*P* value	*P* value		*P* value	*P* value	*P* value	*P* value	
(dbSNP ID)				OR (95% CI)					OR (95% CI)			
			OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)		OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	
IVS1-26	GG	199 (99.0%)	NA	0.069	NA	NA	268 (100.0%)	NA	NA	NA	NA	258 (100.0%)
(Novel)	GC	2 (1.0%)		NA (0.00-NA)			0 (0.0%)					0 (0.0%)
	CC	0 (0.0%)					0 (0.0%)					0 (0.0%)
Q3H	GG	70 (34.8%)	0.820	0.240	0.300	0.370	107 (39.9%)	0.270	0.110	0.930	**0.045**	102 (39.5%)
(rs1884444)	GT	100 (49.8%)		1.35 (0.90-2.03)			128 (47.8%)		1.13 (0.78-1.64)			108 (41.9%)
	TT	31 (15.4%)	1.03 (0.80-1.34)	0.94 (0.55-1.62)	1.22 (0.83-1.79)	0.80 (0.49-1.31)	33 (12.3%)	0.87 (0.68-1.11)	0.66 (0.39-1.10)	0.98 (0.69-1.40)	**0.61 (0.38-0.99)**	48 (18.6%)
V134D	TT	200 (99.5%)	NA	0.260	NA	NA	262 (97.8%)	NA	0.056	NA	NA	254 (98.5%)
(Novel)	TA	1 (0.0%)					6 (2.2%)		1.45 (0.41-5.21)			4 (1.6%)
	AA	0 (0.0%)		0.32 (0.04-2.86)			0 (0.0%)					0 (0.0%)
G149R	GG	192 (95.5%)		**0002**			253 (94.4%)		**0.004**			225 (87.2%)
	GA	9 (4.5%)		**0.32 (0.15-0.68)**			15 (5.6%)					33 (12.8%)
	AA	0 (0.0%)					0 (0.0%)		**0.41 (0.21-0.76)**			0 (0.0%)
K150E	AA	200 (99.5%)	NA	0.200	NA	NA	266 (99.2%)	NA	0.100	NA	NA	258 (100.0%)
(Novel)	AG	1 (0.5%)		NA (0.00-NA)			2 (0.8%)		NA (0.00-NA)			0 (0.0%)
	GG	0 (0.0%)					0 (0.0%)					0 (0.0%)
K160L	GG	200 (99.5%)	NA	0.200	NA	NA	268 (100.0%)	NA	NA	NA	NA	258 (100.0%)
(Novel)	GC	1 (.5%)		NA (0.00-NA)			0 (0.0%)					0 (0.0%)
	CC	0 (0.0%)					0 (0.0%)					0 (0.0%)
IVS4+17	CC	193 (96.0%)		0.450			248 (92.5%)		**0.012**			251 (97.3.%)
(Novel)	CT	8 (4.0%)		1.49 (0.53-4.17)			20 (7.5%)					7 (2.7%)
	TT	0 (0.0%)					0 (0.0%)		**2.89 (1.20-6.96)**			0 (0.0%)

"Novel" indicates SNPs that had not been reported previously. Associations significant at $P < 0.05$ are shown in bold. NA, Not applicable; IVS, intervening sequence.

3. Associations of *IL-17A* Genetic Polymorphisms with IBD Susceptibility

Based on DNA sequencing and DHPLC of the promoter and exon regions of *IL-17A*, 14 SNPs in HWE from promoter regions was found, of which seven SNPs (-861G>A, -851C>G, -759C>G, -604delT, -265G>A, -168C>G, and -112G>A) had never been reported (Table 8).

Table 8. Distributions of genotype and allele frequencies of *IL-17A* SNPs

		CD					UC					Control
		No. (%)	Additive	Codominant	Dominant	Recessive	No. (%)	Additive	Codominant	Dominant	Recessive	No. (%)
Position†	Genotype		*P* value	*P* value	*P* value	*P* value		*P* value	*P* value	*P* value	*P* value	
(dbSNP ID)				OR (95% CI)					OR (95% CI)			
			OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)		OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	
-877	AA	104 (51.7%)	0.170	0.300	0.340	0.140	131 (48.9%)	0.087	0.220	0.093	0.370	145 (56.2%)
(rs4711998)	AG	77 (38.3%)		1.10 (0.75- 1.64)			115 (42.9%)		1.30 (0.92-1.88)			97 (37.6%)
	GG	20 (9.9%)	1.22 (0.92-1.63)	1.74 (0.86- 3.52)	1.20 (0.83-1.73)	1.67 (0.84-3.32)	22 (8.2%)	1.27 (0.96-1.67)	1.52 (0.77-3.02)	1.34 (0.95-1.89)	1.35 (0.69-2.64)	16 (6.2%)
-861	GG	200 (99.5%)	NA	0.860	NA	NA	267 (99.6%)	NA	.980	NA	NA	257 (99.6%)
(Novel)	GA	1 (0.5%)		1.28 (0.08-20.67)			1 (0.4%)		0.96 (0.06-15.47)			1 (0.4%)
	AA	0 (0.0%)					0 (0.0%)					0 (0.0%)
-851	CC	200 (99.5%)	NA	0.2	NA	NA	268 (100.0%)	NA	NA	NA	NA	258 (100.0%)
(Novel)	CG	1 (0.5%)		NA (0.00-NA)			0 (0.0%)					0 (0.0%)
	GG	0 (0.0%)					0 (0.0%)					0 (0.0%)
-759	CC	201 (100.0%)	NA	NA	NA	NA	266 (99.2%)	NA	.100	NA	NA	258 (100.0%)
(Novel)	CG	0 (0.0%)					2 (0.8%)		NA (0.00-NA)			0 (0.0%)
	GG	0 (0.0%)					0 (0.0%)					0 (0.0%)

Continued

-737	CC	106 (52.7%)	0.290	0.420	0.510	0.200	106 (39.6%)	**0.033**	0.067	**0.020**	0.450	128 (49.6%)
(rs8193036)	CT	84 (41.8%)		0.94 (0.64- 1.38)			134 (50.0%)		1.50 (1.04-2.15)			108 (41.9%)
	TT	11 (5.5%)	0.85 (0.63-1.15)	0.60 (0.28- 1.30)	0.88 (0.61-1.28)	0.62 (0.29-1.31)	28 (10.4%)	**1.34 (1.02-1.74)**	1.54 (0.83-2.84)	**1.50 (1.06-2.13)**	1.25 (0.70-2.25)	22 (8.5%)
-604	TT	201 (100.0%)	NA	NA	NA	NA	267 (99.6%)	NA	0.250	NA	NA	258 (100.0%)
(Novel)	T-	0 (0.0%)					1 (0.4%)		NA (0.00-NA)			0 (0.0%)
	--	0 (0.0%)					0 (0.0%)					0 (0.0%)
-525	AA	171 (85.1%)	0.190	0.260	0.240	0.200	227 (84.7%)	0.140	0.230	0.170	0.250	229 (88.8.%)
(rs9791323)	AC	29 (14.4%)		1.34 (0.77- 2.32)			40 (14.9%)		1.39 (0.83-2.32)			29 (11.2%)
	CC	1 (0.5%)	1.42 (0.83-2.42)	NA (0.00-NA)	1.39 (0.80-2.40)	NA (0.00-NA)	1 (.4%)	1.45 (0.88-2.39)	NA (0.00-NA)	1.43 (0.86-2.37)	NA (0.00-NA)	0 (0.0%)
-444	AA	47 (23.4%)	0.320	0.490	0.230	0.690	80 (29.9%)	0.230	0.250	0.690	0.095	73 (28.3%)
(rs3819024)	AG	108 (53.7%)		1.29 (0.83-2.02)			146 (54.5%)		1.02 (0.69-1.52)			130 (50.4%)
	GG	46 (22.9%)	1.14 (0.88-1.49)	1.30 (0.76-2.22)	1.29 (0.85-1.98)	1.10 (0.70-1.71)	42 (15.7%)	0.86 (0.67-1.10)	0.70 (0.42-1.16)	0.93 (0.64-1.35)	0.69 (0.44-1.07)	55 (21.3%)
-265	GG	200 (99.5%)	NA	0.200	NA	NA	268 (100.0%)	NA	NA	NA	NA	258 (100.0%)
(Novel)	GA	1 (0.5%)		NA (0.00-NA)			0 (0.0%)					0 (0.0%)
	AA	0 (0.0%)					0 (0.0%)					0 (0.0%)
-197	GG	55 (27.4%)	0.690	0.590	0.390	0.800	90 (33.6%)	0.110	0.110	0.530	**0.036**	80 (31.0%)
(rs2275913)	GA	102 (50.8%)		1.25 (0.81-1.92)			136 (50.8%)		1.02 (0.69-1.50)			119 (46.1%)
	AA	44 (21.9%)	1.05 (0.81-1.36)	1.08 (0.64-1.82)	1.19 (0.79-1.79)	0.95 (0.61-1.47)	420 (15.7%)	0.82 (0.64-1.05)	0.63 (0.38-1.04)	0.89 (0.62-1.28)	**0.63 (0.40-0.97)**	59 (22.9%)
-168	CC	201 (100.0%)	NA	.280	NA	NA	268 (100.0%)	NA	.230	NA	NA	257 (99.6%)
(Novel)	GC	0 (0.0%)					0 (0.0%)		0.00 (0.00-NA)			1 (0.4%)
	GG	0 (0.0%)		0.00 (0.00-NA)			0 (0.0%)					0 (0.0%)

Continued

-121	GG	156 (77.6%)	0.630	0.880	0.610	0.960	198 (73.9%)	0.630	0.890	0.650	0.780	195 (75.6%)
(rs8193037)	GA	42 (20.9%)		0.89 (0.57-1.39)			65 (24.2%)		1.09 (0.72-1.63)			59 (22.9%)
	AA	3 (1.5%)	0.91 (0.61-1.35)	0.94 (0.21-4.25)	0.89 (0.58-1.39)	0.96 (0.21-4.35)	5 (1.9%)	1.09 (0.76-1.56)	1.23 (0.33-4.65)	1.09 (0.74-1.62)	1.21 (0.32-4.55)	4 (1.6%)
-112	GG	198 (98.5%)	NA	0.720	NA	NA	263 (98.1%)	NA	0.950	NA	NA	253 (98.1%)
(Novel)	GA	3 (1.5%)					5 (1.9%)		0.96 (0.28-3.36)			5 (1.9%)
	AA	0 (0.0%)					0 (0.0%)					0 (0.0%)
IVS1+18	GG	139 (69.2%)	0.760	**0.009**	0.630	**0.004**	172 (64.2%)	.200	**0.027**	0.490	**0.007**	173 (67.0%)
(rs3819025)	GA	54 (26.9%)		.80 (0.53-1.20)			87 (32.5%)		1.04 (0.72-1.50)			84 (32.6%)
	AA	8 (4.0%)	1.06 (0.74-1.51)	**9.96 (1.23-80.56)**	.91 (0.61-1.35)	**10.65 (1.32-85.89)**	9 (3.3%)	1.24 (0.89-1.73)	**9.05 (1.13-72.21)**	1.14 (0.79-1.63)	**8.93 (1.12-70.99)**	2 (0.4%)

"Novel" indicates SNPs that had not been reported previously. †The positions of SNPs were calculated from the translation start site. Associations significant at $P < 0.05$ are shown in bold. NA, not applicable; IVS, intervening sequence.

A Chi-square test of homogeneity with regard to allelic distributions between cases and controls showed significant associations with CD for one marker, rs3819025 ("IVS1+18" hereafter; P = 0.009, 95% CI 1.23–80.56 in codominant model; P = 0.004, 95% CI 1.32–85.89 in recessive) and with UC for three markers, rs8193036 ("-737" hereafter; P= 0.033, 95% CI 1.02–1.74 in additive; P = 0.020, 95% CI 1.06–2.13 in dominant), rs2275913 ("-197" hereafter; P = 0.036, 95% CI 0.40–0.97 in recessive), and IVS1+18G>A (P = 0.027, 95% CI 1.13–72.21 in codominant; P = 0.007, 95% CI 1.12–70.99 in recessive).

4. Haplotypes, Gene-gene Interaction and Risk of IBD

The LDs of the analyzed SNPs were calculated and plotted (Figure 3A). Single LD block between the G149R and IVS4+17C>T ($D' = 1.0$, $r^2 = 0.31$) was identified at *IL23-R*. Additionally, the haplotype frequencies were calculated and both protective (H1) and risk (H2) haplotypes. The H2 haplotype showed a significant associattion with CD ($P = 0.003$) and UC ($P = 0.005$) (Table 9). One LD block was identified at *IL-17A*; -197 of *IL-17A* showed an association only with UC was located on the same strong LD block with rs8193037 ("-121" hereafter) and IVS1+18 ($D' = 0.864$, $r^2 = 0.09$ between -197 and -121; $D' = 0.895$, $r^2 = 0.142$ between -197 and IVS1+18; $D' = 1.0$, $r^2 = 0.033$ between -121 and IVS1+18). Furthermore, the haplotype frequencies of *IL-17A* were calculated but no correlation was observed between haplotype patterns and disease susceptibility to CD or UC (data not shown).

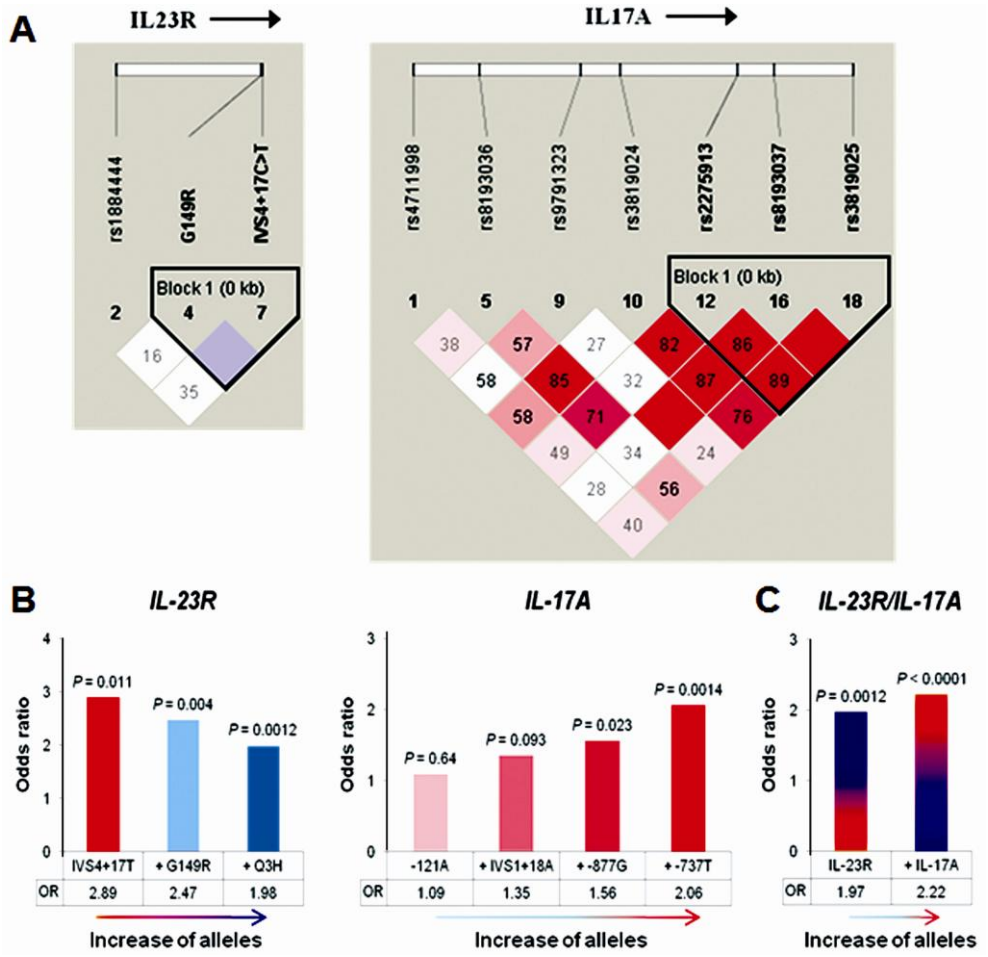

Figure 3. Genetic analysis of *IL-23R* and *IL-17A* in IBD. (*A*) LD and haplotype block structures of *IL-23R* and *IL-17A* for the combined case-control dataset obtained via Haploview 4.1 analysis. The upper bars represent SNPs and the physical distances between them. The numbers within the squares indicate LD coefficient D′ values between respective SNPs (only LD values < 100% are reported). Dark colors indicate high LD and light colors indicate low LD. The SNPs are ordered according to their position in the gene, the direction of transcription is shown above, and internal

references are used for polymorphisms not present in dbSNP. (*B*) Odds ratios for UC with increasing protective (G149R or Q3H) or risk (IVS1+18A, -877G, or -737T) alleles in *IL-23R* or *IL-17A*, respectively. The x-axis shows the accumulations of corresponding alleles. (*C*) Odds ratios for UC with protective (IVS4+17, G149R or Q3H) alleles from *IL-23R* without and with risk (-121, IVS1+18, -877, or -737) alleles from *IL-17A*. A dominant model was applied to (*B*) and (*C*), and the *IL-23R* gene is considered as dominant.

Table 9. Haplotype Frequencies of *IL-23R* among cases and controls and their association with IBD

Gene	Haplotype	G149R	IVS4+17C>T	CD			UC		
				Frequency	Case, Control ratios	*P* value	Frequency	Case, Control ratios	*P* value
IL-23R	H1	G	C	0.938	0.958, 0.923	0.219	0.929	0.935, 0.922	0.441
	H2	A	C	**0.042**	**0.033, 0.064**	**0.005**	**0.046**	**0.028, 0.064**	**0.005**

Haploview software version 4.1 (Broad Institute of MIT and Harvard, Cambridge, MA, USA) was used to analyze the linkage disequilibrium (LD) structure and to test for associations between haplotypes and IBD. Haplotypes with a frequency > 3% are numbered in order of frequency. Associations significant at $P < 0.05$ are shown in bold.

Significant dose-dependent increases in the frequencies of protective (*IL-23R*) or risk (*IL-17A*) alleles were found in UC patients compared to wild type patients (Figure 3B) but the increases were not significant for CD (data not shown). Consequently, cumulative risk alleles of *IL-17A* diminished the protective OR of *IL-23R* in IBD patients, which indicates that as the number of risk allele of *IL-17A* increases, so does the additive risk for UC (Figure 3C).

The large number of IBD candidate genes allowed us to evaluate gene-gene interactions among susceptibility genes to gain insight into disease pathology and to help predict disease risk. As further evidence of the synergistic effect of the genes in this pathway in the development of IBD, a significant statistical gene-gene interaction between Q3H in *IL-23R* and IVS1+18G>A in *IL-17A* was observed in UC (*P*

interaction = 0.003 in codominant model; *P* interaction = 0.003 in dominant model; *P* interaction = 0.045 in recessive model; Table 10) but not in CD (Table 11). These data suggest that there is a gene dosage effect on the *IL23R* risk associations of UC patients who also carry other genetic variants within *IL-17A*.

Table 10. Synergistic interactions between *IL-23R* and *IL-17A* genotypes in UC

UC	IVS1+18								
	Codominant			Dominant			Recessive		
Q3H	GG	GA	AA	GG	GA	AA	GG	GA	AA
GG	1.00	**2.21 (1.21-4.01)**		1.00	**2.21 (1.21-4.01)**		1.00	1.10 (0.74-1.65)	
GT	1.73 (1.09-2.75)	**1.04 (0.59-1.83)**					0.74 (0.41-1.33)	0.56 (0.25-1.27)	0.00
TT	0.98 (0.52-1.87)	0.75 (0.32-1.77)	0.00	1.50 (0.97-2.31)	0.96 (0.57-1.60)	6.42 (0.73-56.39)			
Interactive *P* value	**0.003**			**0.003**			0.045		

Associations significant at $P < 0.05$ are shown in bold.

Table 11. Synergistic interactions between *IL-23R* and *IL-17A* genotypes in CD

CD	IVS1+18								
	Codominant			Dominant			Recessive		
Q3H	GG	CA	AA	GG	CA	AA	GG	CA	AA
GG	1.00	1.38 (0.69-2.73)	3.28 (0.29-37.14)	1.00	1.38 (0.69-2.73)	3.28 (0.29-37.14)	1.00	0.78 (0.50-1.23)	7.15 (0.85-60.22)
GT	1.81 (1.11-2.96)	0.90 (0.48-1.68)							
TT	1.04 (0.53-2.05)	0.96 (0.41-2.28)	3.28 (0.29-37.14)	1.57 (0.99-2.49)	0.92 (0.52-1.60)	9.83 (1.15-84.21)	0.75 (0.40-1.41)	0.70 (0.31-1.59)	2.38 (0.21-26.61)
Interactive *P* value	0.150			0.130			0.830		

Our groups previously genotyped 8 SNPs (rs11889341, rs7574865, rs8179673, rs6752770, rs925847, rs10168266, rs10181656, and rs11685878) in the *STAT4* gene in IBD patients and controls and reported the association of *STAT4* gene (rs11889341, $P = 0.029$; rs925847, $P = 0.023$; rs10168266, $P = 0.044$) with IBD.[30] In addition, weak epistasis between the CD-protective *IL23-R* variant rs11209026 with several *STAT4* SNPs in Caucasian was demonstrated.[34] Given that IL-23 activates not only STAT3 but also to a lesser degree STAT4, It next was investigated potential gene-gene interaction (epistasis) with the IBD susceptibility gene *IL-23R* and *IL-17A*. As further evidence of the synergistic effect of the genes in this pathway in the development of IBD, a significant statistical gene-gene interaction between *IL-23R* and *STAT4* was observed in UC (between Q3H and rs6752770: *P* interaction = 0.005 in codominant model; *P* interaction = 0.010 in dominant model; *P* interaction = 0.010 in recessive model; between Q3H and rs11685878: *P* interaction = 0.003 in codominant model; *P* interaction = 0.020 in dominant model; *P* interaction = 0.048 in recessive model; Table 12) in UC (Table 12) but not in CD (data not shown).

Table 12. Gene-gene interactions between *IL-23R* and *STAT4* genotypes in UC

UC	rs6752770								
	Codominant			Dominant			Recessive		
Q3H	AA	AG	GG	AA	AG	GG	AA	AG	GG
GG	1.00	**0.34 (0.18-0.64)**	0.61 (0.14-2.55)	1.00	**0.34 (0.18-0.64)**	0.61 (0.14-2.55)	1.00	**0.58 (0.38-0.89)**	1.01 (0.39-2.58)
GT	0.71 (0.43-1.17)	0.67 (0.36-1.24)	1.06 (0.29-3.84)						
TT	**0.35 (0.17-0.73)**	0.80 (0.35-1.79)	0.00	**0.61 (0.38-0.97)**	0.71 (0.41-1.23)	0.71 (0.22-2.24)	**0.42 (0.21-0.83)**	0.96 (0.45-2.05)	0.00
Interactive *P* value	**0.005**			**0.010**			**0.010**		

UC	rs11685878								
	Codominant			Dominant			Recessive		
Q3H	CC	CT	TT	CC	CT	TT	CC	CT	TT
GG	1.00	0.52 (0.27-1.00)	0.70 (0.31-1.57)	1.00	0.52 (0.27-1.00)	0.70 (0.31-1.57)	1.00	0.77 (0.50-1.19)	1.08 (0.60-1.95)
GT	0.60 (0.31-1.18)	0.73 (0.40-1.33)	1.19 (0.49-2.86)						
TT	**0.23 (0.09-0.63)**	0.77 (0.35-1.72)	0.64 (0.17-2.42)	**0.47 (0.26-0.88)**	0.74 (0.42-1.31)	1.02 (0.46-2.23)	**0.29 (0.11-0.72)**	0.92 (0.44-1.89)	0.78 (0.22-2.80)
Interactive *P* value	**0.034**			**0.020**			**0.048**		

A significant statistical gene-gene interaction was observed between -444 in *IL-17A* and rs6752770 in *STAT4* (*P* interaction = 0.040 in codominant model; *P* interaction = 0.020 in dominant model), between -197 in *IL-17A* and rs11889341 in *STAT4* (*P* interaction = 0.035 in codominant model; *P* interaction = 0.022 in codominant model; *P* interaction = 0.142 in recessive model), between -197 in *IL-17A* and rs7574865 in *STAT4* (*P* interaction = 0.022 in codominant model), between -197 in *IL-17A* and rs8179673 in *STAT4* (*P* interaction = 0.017 in codominant model; *P* interaction = 0.041 in recessive model), between -197 in *IL-17A* and rs10168266 in *STAT4* (*P* interaction = 0.038 in codominant model; *P* interaction = 0.042 in dominant model; *P* interaction = 0.041 in recessive model), between -197 in *IL-17A* and rs10181656 in *STAT4* (*P* interaction = 0.021 in codominant model; *P* interaction = 0.045 in recessive model), between -197 in *IL-17A* and rs11685878 in *STAT4* (*P* interaction = 0.018 in recessive model), between IVS1+18 in *IL-17A* and rs11889341 in *STAT4* (*P* interaction = 0.003 in codominant model; *P* interaction = 0.004 in dominant model), and between IVS1+18 in *IL-17A* and rs10168266 in *STAT4* (*P* interaction = 0.015 in codominant model; *P* interaction = 0.021 in dominant model) in CD (Table 13).

Table 13. Gene-gene interactions between *IL-17A* and *STAT4* genotypes in CD

CD	rs6752770								
	Codominant			Dominant			Recessive		
-444	AA	AG	GG	AA	AG	GG	AA	AG	GG
AA	1.00	2.08 (0.92-4.69)	0.47 (0.05-4.43)	1.00	2.08 (0.92-4.69)	0.47 (0.05-4.43)	1.00	0.97 (0.61-1.56)	1.29 (0.51-3.23)
AG	1.75 (0.94-3.25)	1.15 (0.58-2.29)	2.80 (0.89-8.86)						
GG	1.93 (0.94-3.97)	1.08 (0.44-2.66)		**1.81 (1.01-3.24)**	1.13 (0.60-2.15)	2.80 (0.89-8.86)	1.34 (0.74-2.40)	0.75 (0.34-1.66)	
Interactive *P* value	0.040			**0.020**			0.280		

CD	rs10168266								
	Codominant			Dominant			Recessive		
-197	GG	AG	AA	GG	AG	AA	GG	AG	AA
GG	1.00	1.10 (0.51-2.35)	3.97 (0.72-21.80)	1.00	1.10 (0.51-2.35)	3.97 (0.72-21.80)	1.00	0.73 (0.46-1.17)	0.80 (0.32-2.00)
GA	**1.97 (1.07-3.63)**	1.08 (0.57-2.06)	0.63 (0.18-2.21)						
AA	1.10 (0.50-2.43)	1.39 (0.66-2.93)	0.26 (0.03-2.31)	1.68 (0.95-2.97)	1.18 (0.66-2.13)	0.50 (0.16-1.50)	0.74 (0.37-1.50)	0.94 (0.49-1.81)	0.18 (0.02-1.51)
Interactive *P* value	**0.038**			0.042			0.220		
IVS1+18	GG	AG	AA	GG	AG	AA	GG	AG	AA
GG	1.00	1.12 (0.69-1.84)	0.44 (0.15-1.28)	1.00	1.12 (0.69-1.84)	0.44 (0.15-1.28)	1.00	0.82 (0.54-1.23)	0.65 (0.29-1.47)
GA	1.15 (0.65-2.05)	**0.41 (0.20-0.85)**	1.53 (0.39-5.93)						
AA	4.89 (0.53-44.86)			1.26 (0.72-2.20)	0.51 (0.26-1.01)	1.53 (0.39-5.93)	4.66 (0.51-42.40)		
Interactive *P* value	**0.015**			**0.021**			0.280		

CD	rs11889341								
	Codominant			Dominant			Recessive		
-197	CC	CT	TT	CC	CT	TT	CC	CT	TT
GG	1.00	1.18 (0.56-2.51)	6.48 (0.69-60.88)	1.00	1.18 (0.56-2.51)	6.48 (0.69-60.88)	1.00	0.81 (0.51-1.29)	0.92 (0.36-2.32)
GA	**1.90 (1.04-3.49)**	1.16 (0.61-2.21)	0.81 (0.25-2.61)						
AA	1.22 (0.56-2.62)	1.47 (0.68-3.18)	0.20 (0.02-1.70)	1.67 (0.94-2.94)	1.26 (0.70-2.26)	0.54 (0.19-1.52)	0.84 (0.43-1.66)	1.01 (0.51-2.02)	0.14 (0.02-1.14)
Interactive *P* value	**0.035**			0.022			0.140		
IVS1+18	CC	CT	TT	CC	CT	TT	CC	CT	TT
GG	1.00	1.21 (0.74-1.99)	0.36 (0.13-1.04)	1.00	1.21 (0.74-1.99)	0.36 (0.13-1.04)	1.00	0.87 (0.58-1.32)	0.63 (0.28-1.42)
GA	1.10 (0.62-1.95)	**0.43 (0.21-0.88)**	3.09 (0.58-16.44)						
AA	4.94 (0.54-45.28)			1.20 (0.69-2.10)	0.53 (0.27-1.05)	3.09 (0.58-16.44)	4.78 (0.53-43.51)		
Interactive *P* value	**0.003**			**0.004**			0.290		

CD	rs7574865								
	Codominant			Dominant			Recessive		
-197	GG	GT	TT	GG	GT	TT	GG	GT	TT
GG	1.00	1.30 (0.62-2.74)	4.30 (0.78-23.83)	1.00	1.30 (0.62-2.74)	4.30 (0.78-23.83)	1.00	0.76 (0.48-1.21)	1.29 (0.56-2.98)
GA	**2.17 (1.13-4.17)**	1.11 (0.58-2.14)	1.55 (0.55-4.32)						
AA	1.17 (0.53-2.57)	1.82 (0.80-4.16)	0.47 (0.12-1.84)	1.77 (0.97-3.24)	1.28 (0.70-2.36)	0.98 (0.41-2.33)	0.75 (0.38-1.51)	1.17 (0.56-2.45)	0.30 (0.08-1.13)
Interactive *P* value	0.022			0.058			0.056		

CD	rs8179673								
	Codominant			Dominant			Recessive		
-197	TT	CT	CC	TT	CT	CC	TT	CT	CC
GG	1.00	1.30 (0.62-2.74)	4.30 (0.78-23.83)	1.00	1.30 (0.62-2.74)	4.30 (0.78-23.83)	1.00	0.77 (0.48-1.22)	1.30 (0.56-2.99)
GA	**2.18 (1.14-4.20)**	1.13 (0.59-2.15)	1.55 (0.55-4.32)						
AA	1.10 (0.50-2.44)	1.92 (0.85-4.36)	0.47 (0.12-1.84)	1.75 (0.95-3.20)	1.31 (0.72-2.41)	0.98 (0.41-2.33)	0.71 (0.35-1.44)	1.24 (0.60-2.57)	0.30 (0.08-1.13)
Interactive *P* value	**0.017**			0.065			0.041		

CD	rs10181656								
	Codominant			Dominant			Recessive		
-197	CC	CG	GG	CC	CG	GG	CC	CG	GG
GG	1.00	1.30 (0.62-2.74)	4.30 (0.78-23.83)	1.00	1.30 (0.62-2.74)	4.30 (0.78-23.83)	1.00	0.79 (0.50-1.26)	1.31 (0.57-3.03)
GA	**2.14 (1.11-4.12)**	1.16 (0.61-2.21)	1.55 (0.55-4.32)						
AA	1.10 (0.50-2.44)	1.92 (0.85-4.36)	0.47 (0.12-1.84)	1.72 (0.94-3.15)	1.34 (0.73-2.45)	0.98 (0.41-2.33)	0.72 (0.36-1.46)	1.26 (0.61-2.61)	0.31 (0.08-1.15)
Interactive *P* value	**0.021**			0.071			0.045		

A significant statistical gene-gene interaction was observed between -737 in *IL-17A* and rs6752770 in *STAT4* (*P* interaction = 0.005 in dominant model; *P* interaction = 0.002 in recessive model), between -444 in *IL-17A* and rs7574865 in *STAT4* (*P*

interaction = 0.043 in dominant model), between -444 in *IL-17A* and rs8179673 in *STAT4* (*P* interaction = 0.045 in recessive model), between -444 in *IL-17A* and rs10181656 in *STAT4* (*P* interaction = 0.014 in codominant model; *P* interaction = 0.043 in recessive model), between -197 in *IL-17A* and rs11889341 in *STAT4* (*P* interaction = 0.031 in dominant model), between -197 in *IL-17A* and rs7574865 in *STAT4* (*P* interaction = 0.022 in codominant model; *P* interaction = 0.035 in dominant model), between -197 in *IL-17A* and rs8179673 in *STAT4* (*P* interaction = 0.019 in codominant model; *P* interaction = 0.026 in dominant model), between -197 in *IL-17A* and rs925847 in *STAT4* (*P* interaction = 0.035 in dominant model), between -197 in *IL-17A* and rs10181656 in *STAT4* (*P* interaction = 0.025 in codominant model; *P* interaction = 0.035 in recessive model), between IVS1+18 in *IL-17A* and rs11889341 in *STAT4* (*P* interaction = 0.023 in codominant model; *P* interaction = 0.042 in dominant model) in UC (Table 14).

Table 14. Gene-gene interactions between *IL-17A* and *STAT4* genotypes in UC

UC	rs6752770								
	Codominant			Dominant			Recessive		
-737	AA	AG	GG	AA	AG	GG	AA	AG	GG
CC	1.00	0.65 (0.36-1.17)	2.54 (0.48-13.53)	1.00	0.65 (0.36-1.17)	2.54 (0.48-13.53)	1.00	**0.57 (0.38-0.86)**	1.01 (0.36-2.79)
CT	**1.69 (1.04-2.74)**	0.83 (0.47-1.48)	0.81 (0.21-3.15)						
TT	0.74 (0.32-1.74)	**4.74 (1.30-17.22)**	0.68 (0.11-4.18)	1.48 (0.93-2.33)	1.11 (0.65-1.90)	0.76 (0.25-2.31)	0.58 (0.25-1.30)	**3.66 (1.03-13.03)**	0.52 (0.09-3.18)
Interactive *P* value	**0.005**			0.230			**0.002**		

UC	rs7574865								
	Codominant			Dominant			Recessive		
-444	GG	GT	TT	GG	GT	TT	GG	GT	TT
AA	1.00	1.00 (0.50-1.98)	1.16 (0.24-5.66)	1.00	1.00 (0.50-1.98)	1.16 (0.24-5.66)	1.00	1.26 (0.83-1.90)	1.22 (0.55-2.69)
AG	0.82 (0.44-1.56)	1.18 (0.63-2.23)	1.05 (0.39-2.80)						
GG	0.53 (0.23-1.23)	1.39 (0.61-3.18)	**0.17 (0.04-0.87)**	0.74 (0.40-1.36)	1.23 (0.67-2.26)	0.61 (0.26-1.43)	0.60 (0.29-1.25)	1.58 (0.78-3.22)	**0.20 (0.04-0.93)**
Interactive *P* value	0.140			0.360			**0.043**		
-197	GG	GT	TT	GG	GT	TT	GG	GT	TT
GG	1.00	**2.47 (1.29-4.75)**	3.58 (0.65-19.71)	1.00	**2.47 (1.29-4.75)**	3.58 (0.65-19.71)	1.00	1.31 (0.86-1.98)	1.22 (0.54-2.77)
GA	**2.00 (1.07-3.74)**	1.62 (0.89-2.93)	1.43 (0.53-3.87)						
AA	0.86 (0.39-1.90)	1.77 (0.80-3.91)	0.39 (0.10-1.52)	1.55 (0.87-2.76)	1.66 (0.94-2.91)	0.89 (0.39-2.04)	0.59 (0.29-1.19)	1.21 (0.59-2.45)	**0.27 (0.07-0.99)**
Interactive *P* value	**0.026**			**0.035**			0.190		

UC	rs8179673								
	Codominant			Dominant			Recessive		
-444	TT	CT	CC	TT	CT	CC	TT	CT	CC
AA	1.00	1.05 (0.53-2.10)	1.20 (0.25-5.85)	1.00	1.05 (0.53-2.10)	1.20 (0.25-5.85)	1.00	1.28 (0.84-1.94)	1.23 (0.56-2.72)
AG	0.85 (0.45-1.62)	1.22 (0.65-2.29)	1.08 (0.40-2.90)						
GG	0.55 (0.24-1.27)	1.44 (0.63-3.30)	**0.18 (0.04-0.90)**	0.76 (0.41-1.41)	1.27 (0.69-2.33)	0.63 (0.27-1.49)	0.61 (0.29-1.27)	1.60 (0.78-3.27)	**0.20 (0.04-0.94)**
Interactive *P* value	0.150			0.430			**0.045**		
-197	TT	CT	CC	TT	CT	CC	TT	CT	CC
GG	1.00	**2.61 (1.35-5.02)**	3.71 (0.67-20.42)	1.00	**2.61 (1.35-5.02)**	3.71 (0.67-20.42)	1.00	1.33 (0.87-2.02)	1.23 (0.54-2.80)
GA	**2.08 (1.11-3.92)**	1.67 (0.92-3.03)	1.48 (0.55-4.01)						
AA	0.89 (0.40-1.97)	1.83 (0.83-4.05)	0.40 (0.10-1.58)	1.60 (0.89-2.87)	1.71 (0.97-3.01)	0.92 (0.40-2.12)	0.59 (0.29-1.21)	1.22 (0.60-2.48)	0.27 (0.07-1.00)
Interactive *P* value	**0.019**			**0.026**			0.190		

UC	rs10181656								
	Codominant			Dominant			Recessive		
-444	CC	CG	GG	CC	CG	GG	CC	CG	GG
AA	1.00	**1.00 (0.50-1.98)**	1.16 (0.24-5.66)	1.00	1.00 (0.50-1.98)	1.16 (0.24-5.66)	1.00	1.25 (0.83-1.90)	1.22 (0.55-2.69)
AG	**0.82 (0.43-1.56)**	1.18 (0.63-2.21)	1.05 (0.39-2.80)						
GG	0.53 (0.23-1.23)	1.39 (0.61-3.18)	**0.17 (0.04-0.87)**	0.74 (0.40-1.36)	1.23 (0.67-2.25)	0.61 (0.26-1.43)	0.60 (0.29-1.25)	1.58 (0.77-3.23)	**0.20 (0.04-0.93)**
Interactive *P* value	**0.014**			0.360			**0.043**		
-197	CC	CG	GG	CC	CG	GG	CC	CG	GG
GG	1.00	**2.47 (1.29-4.75)**	3.58 (0.65-19.71)	1.00	**2.47 (1.29-4.75)**	**3.58 (0.65-19.71)**	1.00	1.30 (0.86-1.98)	1.22 (0.54-2.77)
GA	**2.01 (1.07-3.78)**	1.62 (0.89-2.92)	1.43 (0.53-3.87)						
AA	0.86 (0.39-1.90)	1.77 (0.80-3.91)	0.39 (0.10-1.52)	1.55 (0.87-2.77)	**1.65 (0.94-2.90)**	0.89 (0.39-2.04)	0.59 (0.29-1.19)	1.21 (0.59-2.45)	**0.27 (0.07-0.99)**
Interactive *P* value	**0.025**			**0.035**			0.190		

UC	rs11889341								
	Codominant			Dominant			Recessive		
-197	CC	CT	TT	CC	CT	TT	CC	CT	TT
GG	1.00	**2.38 (1.24-4.55)**	5.37 (0.57-50.18)	1.00	**2.38 (1.24-4.55)**	5.37 (0.57-50.18)	1.00	**1.53 (1.01-2.32)**	0.93 (0.38-2.29)
GA	1.63 (0.91-2.94)	**1.81 (1.01-3.23)**	0.81 (0.27-2.43)						
AA	0.90 (0.41-1.93)	1.34 (0.64-2.83)	0.34 (0.07-1.68)	1.38 (0.80-2.39)	1.66 (0.97-2.86)	0.60 (0.23-1.53)	0.68 (0.34-1.37)	1.02 (0.52-2.00)	0.26 (0.05-1.24)
Interactive *P* value	0.120			**0.031**			0.610		
IVS1+18	CC	CT	TT	CC	CT	TT	CC	CT	TT
GG	1.00	**1.65 1.03-2.63)**	0.39 (0.15-1.05)	1.00	**1.65 (1.03-2.63)**	**0.39 (0.15-1.05)**	1.00	1.45 (0.99-2.11)	0.70 (0.32-1.51)
GA	1.02 (0.58-1.80)	1.17 (0.68-2.04)	3.34 (0.65-17.07)						
AA	2.23 (0.20-25.07)			1.05 (0.60-1.84)	**1.38 (0.81-2.37)**	3.34 (0.65-17.07)	2.21 (0.20-24.72)		
Interactive *P* value	**0.023**			**0.042**			0.140		

UC	rs925847								
	Codominant			Dominant			Recessive		
-197	TT	CT	CC	TT	CT	CC	TT	CT	CC
GG	1.00	0.41 (0.18-0.96)	0.31 (0.12-0.81)	1.00	**0.41 (0.18-0.96)**	**0.31 (0.12-0.81)**	1.00	**0.59 (0.36-0.97)**	0.75 (0.42-1.33)
GA	0.54 (0.22-1.31)	0.38 (0.17-0.86)	0.72 (0.28-1.80)						
AA	0.43 (0.15-1.27)	0.19 (0.08-0.50)	0.50 (0.15-1.63)	0.51 (0.22-1.18)	**0.31 (0.14-0.69)**	0.65 (0.27-1.57)	0.65 (0.27-1.58)	**0.29 (0.14-0.60)**	0.75 (0.27-2.08)
Interactive *P* value	0.130			**0.035**			0.510		

5. Allelic-specific Binding Activities of Nuclear Proteins in the IL-17A Promoter Region and their Effects on Gene Expression

Levels of *IL-17A* and *STAT4* mRNA in colonic mucosa were measured by real-time RT-PCR. *IL-17A* expression was elevated in the colonic mucosa of IBD patients, as reported earlier[40-42] but levels of *STAT4* were not significantly different between cases and controls. (Figure 4).

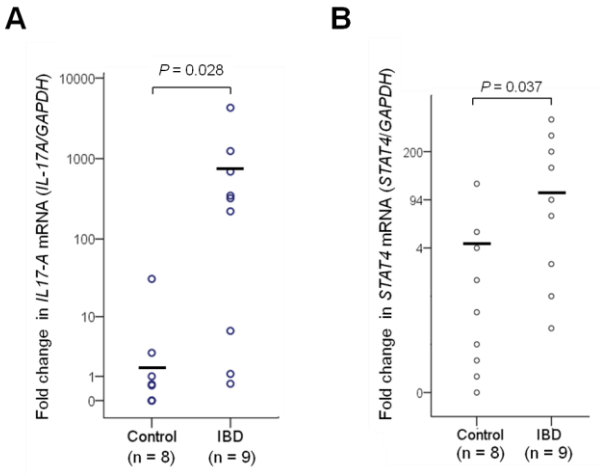

Figure 4. Expressions levels of *IL-17A* mRNA in the colonic mucosa of IBD patients. Elevated expression of *IL-17A* was observed in the colonic mucosa of IBD patients. RNA was extracted from mucosal biopsies from healthy donors (n = 9) and IBD patients (n = 9) and *IL-17A* expression was quantified by real-time RT-PCR and normalized to GAPDH expression. The horizontal lines represent the median values for each group.

Gene expression is regulated by a complex of transcriptional activator or inhibitor with transcriptional machinery and can be influenced by sequence variations in promoter regions.[43] Potential transcription factor binding sites in *IL17A* promoter region were identified by the TFSEARCH program (http://www.cbrc.jp/research/db/TFSEARCH) based on the TRANSFAC databases[44] with a threshold of over 80. putative T3R-B1 and RORE site in -877, Oct-1 site in -737, C/EBP-d in -444, and were identified. No known SNPs were found within the TFs binding sites of *IL-17A* promoter (Figure 5).

Figure 5. Structure of the putative human *IL-17* promoter. SNPs were indicated. No known SNPs were found within the binding sites of transcription factors. The predicted transcription factor binding sites (*box*) of the *IL-17* promoter are indicated. The variation locus (*underlined bold italics*), CpG sites (*yellow shaded*) and translation starting point (+1) are also shown. *Novel SNP.

EMSA was performed to investigate whether the allelic differences between the risk (-877G, -737T, -444A, and -197G) and the non-risk alleles of *IL-17A* are attributable to their binding activities for nuclear proteins from Jurkat cells, which were activated with anti-CD3/anti-CD28 mAb, and were stimulated with tumor growth factor β, IL-6, IL-23 to induce *IL-17A*. As shown in Figure 6A, a higher level of protein binding to the risk alleles than to the non-risk alleles was detected using EMSA, supporting the roles of the risk alleles in *IL-17A* gene expression. However, a protein binding to the -197 sites was not detected.

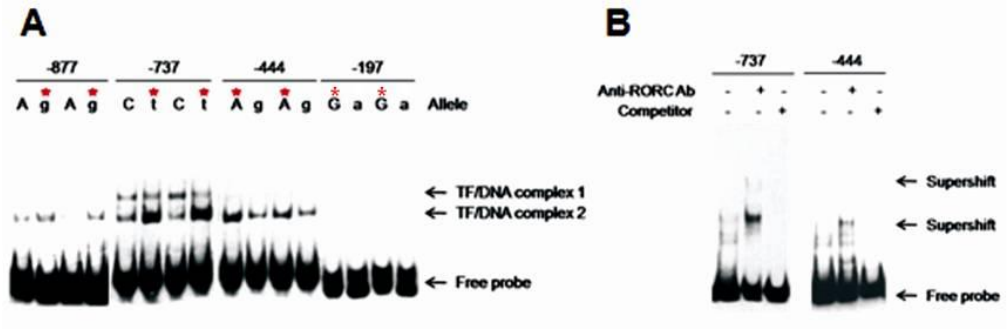

Figure 6. Preferential binding of the transcription factor complex to the risk alleles of *IL-17A*. (A) Electrophoretic mobility shift assays were used to determine the pattern of nuclear protein binding to major and minor allele probes. Nuclear extract was obtained from activated Jurkat cells incubated for 2 hr in media with IL-23 (10 ng/μL), TGF-β (10 ng/μL), and IL-6 (10 ng/μL) after activation with CD3 (1 mg/ml) and CD28 (1 μg/ml). Nuclear extracts from Jurkat cells were incubated with biotin-labeled oligonucleotide probes containing risk or no risk alleles of high frequency. Asterisks indicate the risk alleles. (B) A supershift assay was performed using antibodies against RORC. Competition experiments were performed using a 200-fold molar excess of the cold probe. The result of one representative experiment (out of five that produced identical results) are shown.

To determine the sequence specificity of the DNA-protein complex, competition experiments were performed using cold probes (Figure 6B). The bands were competed against a 200-fold molar excess of unlabeled probe, which clearly demonstrate specific binding of the probes to the nuclear proteins. Moreover, we investigated the binding of RORC(human ortholog of RORγt), a master regulator of IL-17, to the TF complexes.[45] Antibodies against RORC (human ortholog of RORγt) supershifted the DNA-protein complex, indicating that the observed DNA-protein complexes formed due to specific DNA-protein interactions (Figure 6B).

Although several *IL-17A* polymorphisms have been characterized at least partially, no biological explanation has been provided for the observed associations between *IL-17A* genotypes and IBD. *IL-17A* mRNA levels have previously been reported to be elevated in the PBMCs of IBD patients.[46] Therefore, the *in vivo* mRNA expression levels according to *IL-17A* variants was further evaluated by comparing the expression patterns of *IL-17A* in PBMCs of normal and IBD patients using quantitative real-time RT-PCR. In the first attempt to understand how risk alleles might influence IBD predisposition, it was considered to evaluate the effects of variants at -877, -737, -444, and -197 on different *IL-17A* mRNA expression levels. Although a large variability was found among individuals, the expressions of *IL-17A* transcripts generally higher in patients with risk alleles, variant -737T ($P = 0.004$) or -444A ($P = 0.016$), compared to those of patients with no risk alleles (Figure 7).

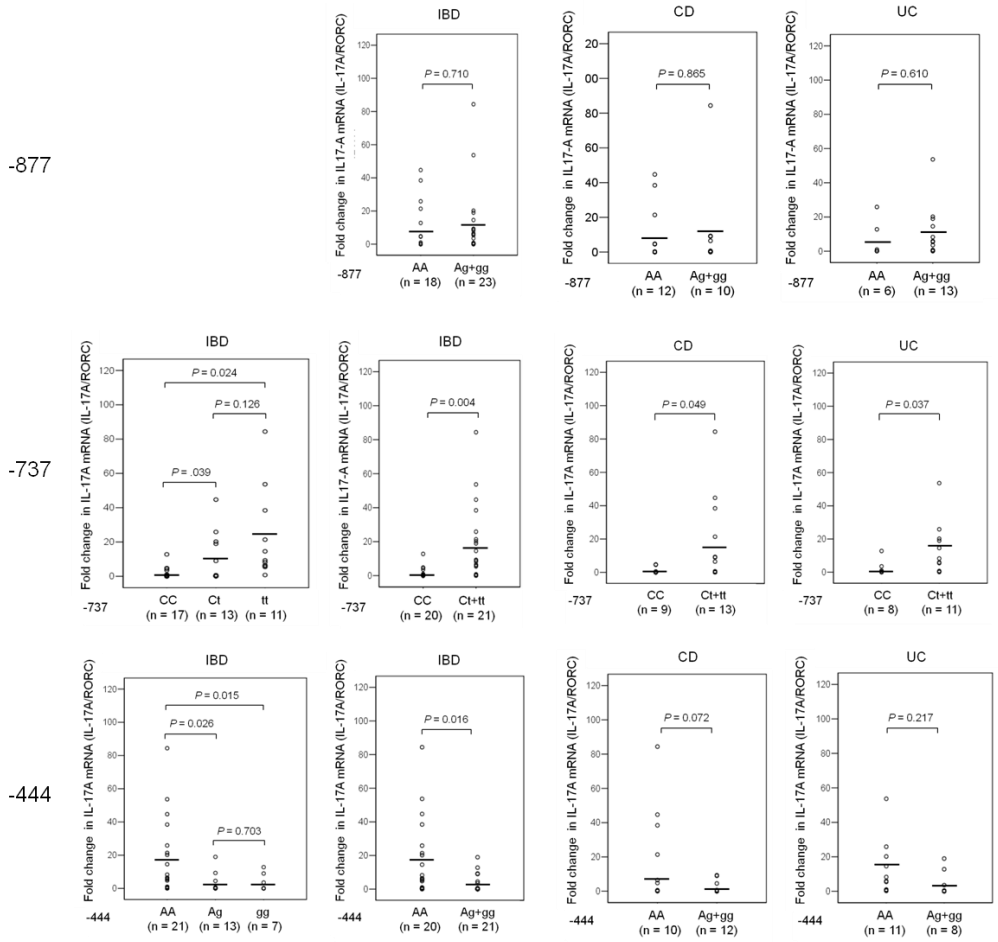

Figure 7. Analysis of *IL-17A* mRNA expression in PBMCs from IBD patients. *IL-17A* mRNA expression was measured using real-time PCR in PBMCs from CD and UC patients, and is expressed as normalized units according to RORC mRNA level. Controls had undetectable levels of *IL-17A*. Dots represent the means of triplicate assays, and the bars indicate the mean expression level of each group. *P* values were obtained via t- test analysis.

6. Associations of Aberrant Hypomethylation Status of IVS1+18 in *IL-17A* and Its Implication in IBD

Methylation of a single CpG site in a promoter region is sufficient to reduce gene expression.[38] Sequencing results in our study revealed that there is a cluster of putative CpG sites located near translation start codon that include many critical regulatory sites (Figure 5). However, the DNA methylation status of the exact CpG sites of *IL-17A* remains unknown. Thus, to identify differences in the methylation status of the putative CpG sites between control and IBD patients and to confirm the likelihood of the IVS1+18G/A mediated regulation of *IL-17A*, bisulfite sequencing of 12 putative CpG sites in the *IL-17A* locus (-969 to -684 bp and -234 to IVS1+21) including the variations was performed. Interestingly, it was found that two CpG sites (IVS1+17 and IVS1+21) in -234 to IVS1+21 region in healthy controls were significantly hypermethylated but were contrarily hypomethylated in IBD patients, based on the results of both bisulfite DNA sequencing (Figure 8A and 9A) and pyrosequencing (Figure 8B and 9B). In particular, IVS1+17 was significantly less methylated in CD (2.9%; n = 20) and UC (4.3%; n = 21) patients than that in the controls (44%; n = 21) (Figure 8B) and, were tightly correlated with IVS1+18G>A conversion in IBD patients but not significantly correlated in controls (Figure 8C).

It was investigated whether IVS1+18G>A conversion is correlated with *IL-17A* expression in PBMCs of IBD. A statistically significant inverse correlation was observed between the *IL-17A* mRNA levels and IVS1+18G>A conversion in IBD patients (Figure 8D), Based on these results, it can be proposed that in healthy

individuals, *IL-17A* expression is down-regulated by hypermethylation of CpG sites in the promoter region of *IL-17A*.

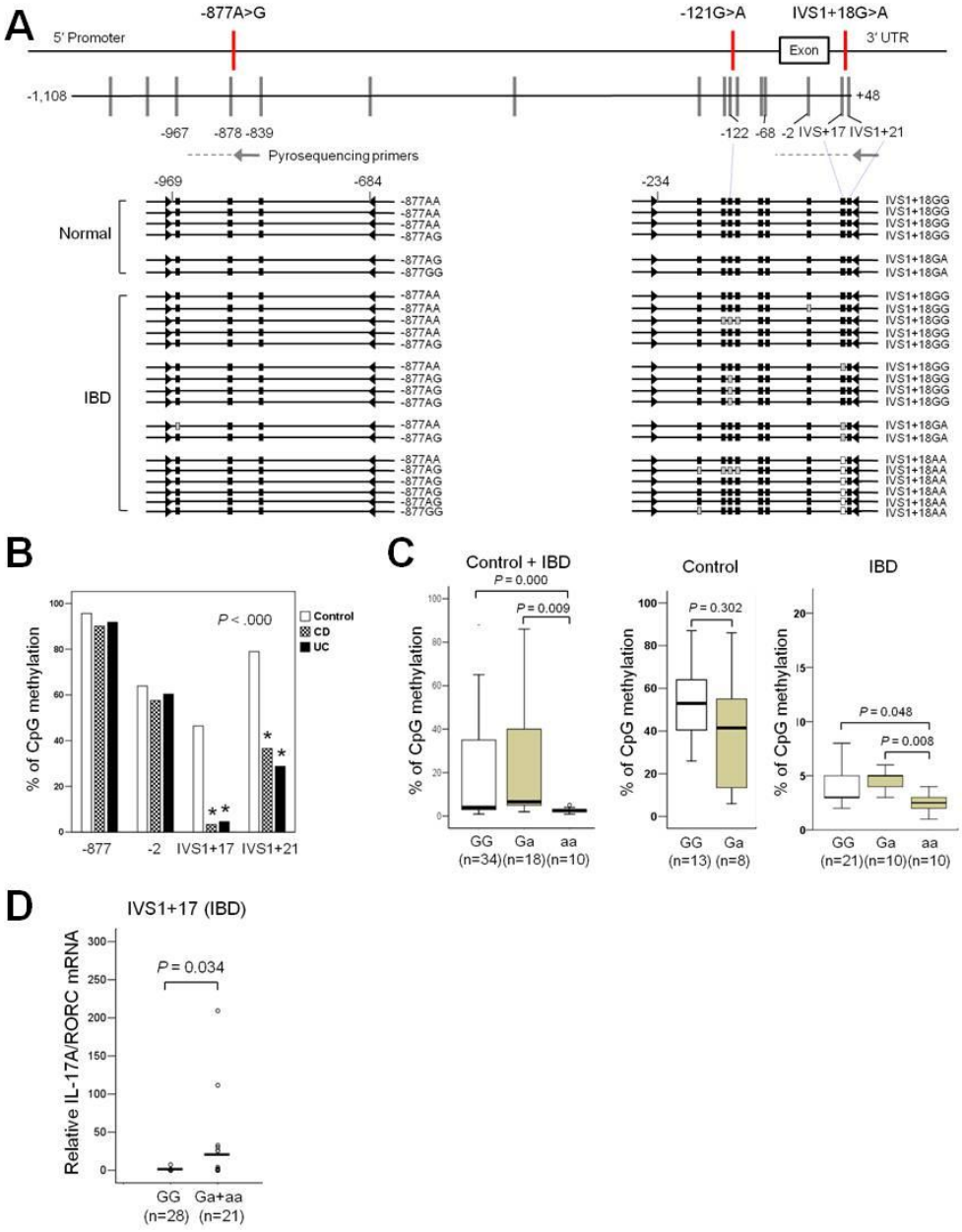

Figure 8. Promoter methylation status of the *IL-17A* promoter in IBD patients. Two CpG sites (IVS1+17 and IVS1+21) in the -234 to IVS1+21 region were significantly

hypermethylated in healthy controls but hypomethylated in IBD patients as shown by bisulfite DNA sequencing (A) and pyrosequencing (B). (A) Comparison of the methylation status of promoter CpG sites in PBMCs from healthy controls and IBD patients. Methylation status was analyzed using bisulfite sequencing. Black, gray, and white squares represent complete methylation, partial methylation, and no methylation, respectively. Position +1 is determinded by start codon. (B) Methylation status was analyzed using pyrosequencing. (C) Correlation between methylation status at IVS1+17C and allele types at IVS1+18 in IBD patients and health control. (D) Allele types at IVS1+18 and *IL-17A* mRNA expression levels in IBD patients. A statistically significant inverse correlation was observed between *IL-17A* mRNA levels and IVS1+18G>A conversion in IBD patients. Error bars indicate means ±SE. $^{*}P < 0.001$.

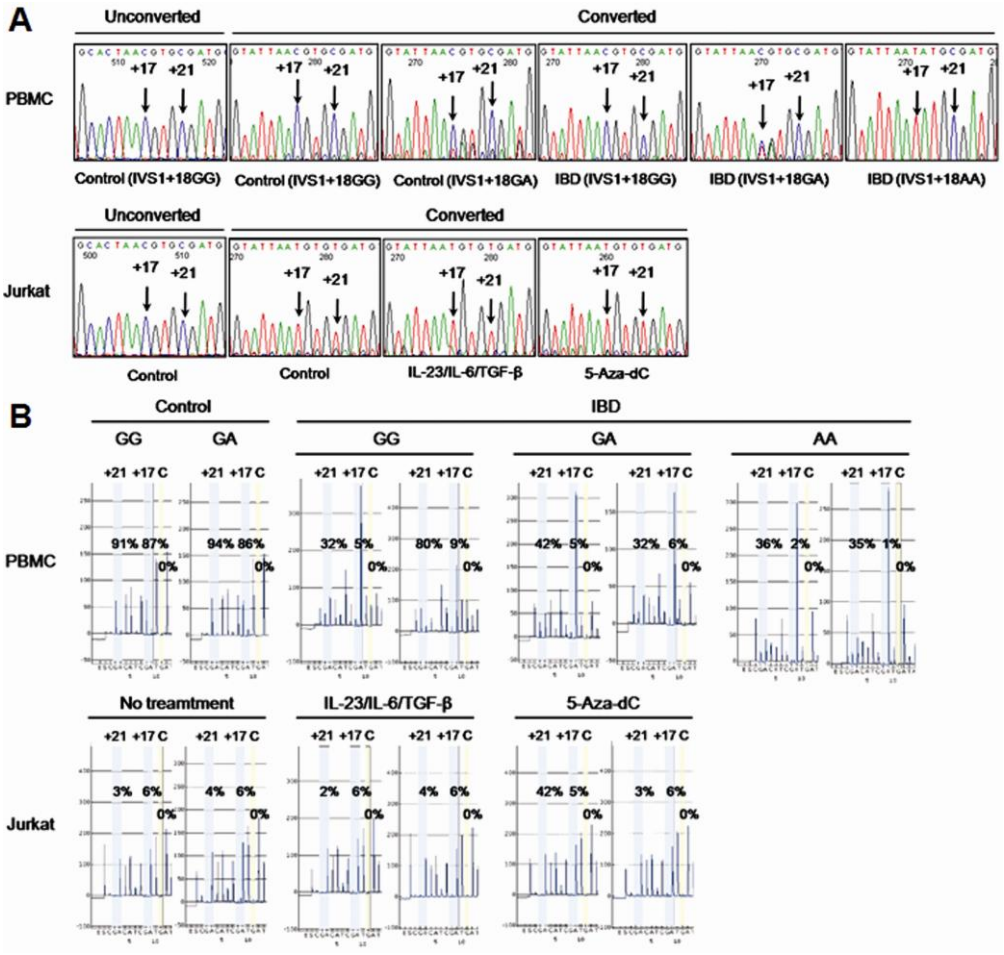

Figure 9. Representative bisulfite sequencing and pyrosequencing data for quantitation of methylated CpGs in the *IL-17A* promoter in patient PBMC. (A) After bisulfite treatment, all cytosine residues were converted to thymine. The cytosine residues of the CpG site located at IVS1+17 and 21 are designated as "+17" and "+21", respectively (B) Pyrosequencing of IVS1+17 and 21. The methylation percentage was calculated by comparing the magnitude of the "C" peak with that of the next "T" peak (blue box) subsequently read at the same nucleotide.

Our group reported that SNPs of *STAT4* was associated with IBD susceptibility.[30] Thus, *STAT4* mRNA expression and methylation status were also investigated (Figure 10). Compared with *STAT4*, *IL-17A* showed dramatic differences in the promoter methylation between healthy control and IBD patients.

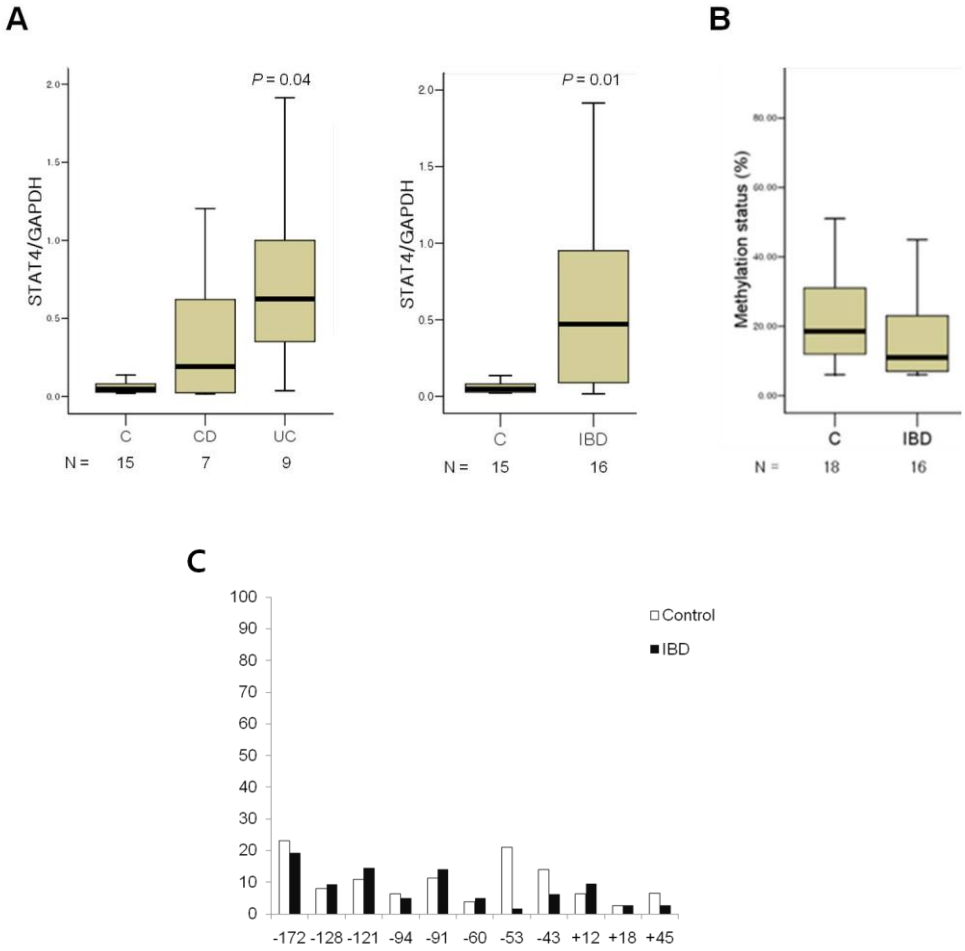

Figure 10. *STAT4* mRNA expression and promoter methylation status of the *STAT4* promoter in patients with IBD. (A) Analysis of *STAT4* transcript levels in PBMCs from normal (C) and IBD patients. (B) Comparison of *STAT4* promoter CpG site (+102) methylation in PBMC from healthy control and PBMC from IBD patients. Methylation status was analyzed by pyrosequencing.

7. Association of *IL-17A* Silencing with DNA Methylation in Jurkat cells

To decipher whether *IL-17A* induction is associated with aberrant DNA methylation, the effects of the demethylating agent 5-Aza-dC, a DNA methyltransferase (DNMT) inhibitor, on *IL-17A* expression in Jurkat cells was tested. *IL-17A* transcription was restored nearly to stimulated level following treatment of Jurkat cells which initially displayed no *IL-17A* expression (Figure 11).

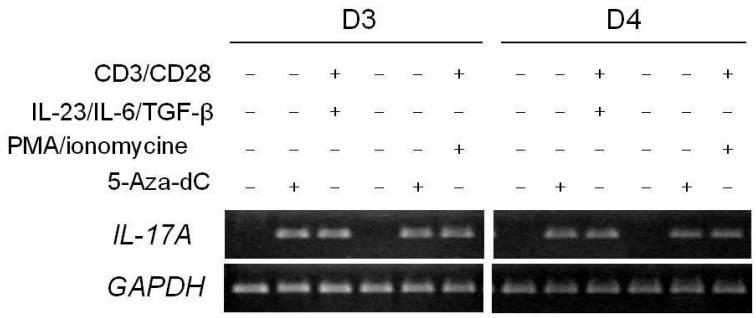

Figure 11. *IL-17A* is downregulated via DNA methylation in Jurkat cell lines. Jurkat cells were incubated for 12 h in media with IL-23/TGF-β/IL-6, or PMA (20 nM) and ionomycin (2 μM), or 5-Aza-dC (400 nM) for 72-96 h after activation with CD3 (1 mg/mL) and CD28 (1 μg/mL) for 48 hr. *IL-17A* mRNA levels were measured using quantitative real-time RT-PCR, in which RORC was used as the normalization control.

To evaluate the methylation statuses of CpG sites in the *IL-17A* promoter, bisulfate DNA sequencing and pyrosequencing analysis of 12 CpG sites at the *IL-17A* promoter region (nucleotides -969 to -684 bp and -234 to IVS1+21) including the variations was performed. Similar to the methylation patterns in PBMCs, CpG sites at -969 to -684 bp were found to be methylated extensively in Jurkat cells. Unexpectedly, however, CpG sites at -234 to IVS1+21 near the transcriptional start site were unmethylated in both the 5-Aza-dC-treated and untreated samples, consistent with the constant levels of *IL-17A* expression seen in 5-Aza-dC-treated Jurkat cells but in contrast to the results for PBMCs (Figure 12A and B). Although it was failed to explain the direct effect of IVS1+18G>A conversion on *IL-17A*, these results imply that more complicated chromatin conformation changes or other CpG sites may be involved in gene regulation via DNA methylation and indicate that DNA methylation is a critical mechanism through which *IL-17A* expression is regulated.

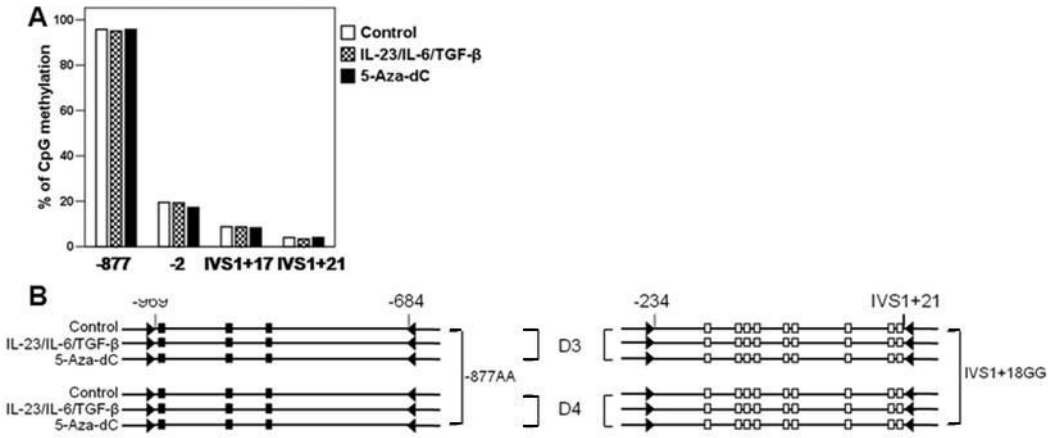

Figure 12. *IL-17A* methylation status in Jurkat cells. *IL-17A* methylation status analysis according to 5-Aza-dC treatment for 72-96 hr using pyrosequencing analysis (*A*) and bisulfite sequencing analysis (*B*). Black, gray, and white squares represent complete methylation, partial methylation, and unmethylation, respectively.

IV. DISCUSSION

The main objective of this study was to assess the contributions of *IL-23R* and *IL-17A* genetic polymorphisms to IBD susceptibility and to elucidate the underlying mechanisms. A growing body of evidence suggests that activation of the IL-23/IL-17 axis, as a critical regulatory system that bridges the innate and adaptive arms of the immune system, is fundamentally linked to the pathogenesis of autoimmune diseases, including IBD.[47-49] In this regard, recent studies in animal models and humans have demonstrated that Th17 cells promote intestinal inflammation[50] and that IL-17 exerts multiple inflammatory effects,[51] including the release of proinflammatory cytokines (IL-22, TNF-α, IL-6, and IL-8) and recruitment of monocytes and neutrophils.[52,53] IL-17 is able to induce the expression of two mucin genes in bronchial epithelial cells[54], and increased expression of IL-17 has been shown to be associated with enhanced mucin gene expression *in vivo*.[55] Furthermore, recent reports strongly suggested that the IL-23/IL-17 axis that is associated with Th17 is required for the development of TNBS-colitis as well as bacterially-induced intestinal inflammation.[56-58] IL-17-deficient mice are resistant to collagen-induced arthritis[59] and EAE.[60] In addition, IL-17- and IL-23-deficient mice are resistant to experimental glomerulonephritis.[61,62] In sharp contrast, the inflammatory function of *IL-17A* in different types of experimental colitis models are contradictory; some studies have reported that in the intestinal environment, *IL-17A* is protective,

while others have reported that it is proinflammatory.[63,64] IL-17 and IL-22 can be produced by non-T cells, and T cell responses may therefore not play indispensable roles, because IBD is inducible in T cell-deficient mice,[65] while monocyte and macrophage linage cells are important mediators of UC and DSS-induced colitis[66] and these cells also can produce IL-17A in inflamed colonic tissue.[15] CD, in contrast, shows a particularly strong connection to Th17 inflammation; genome-wide studies have shown an association between polymorphisms in *IL-23R* and increased CD susceptibility as well as between single nucleotide polymorphisms in other components of the Th17 pathway (i.e., *stat3, p40, jak2,* and *ccr6*) and CD susceptibility.[9] Therefore, IL-17 may be a key mechanistic factor that can account for the common features of IBD types that develop via different T cell-dependent and independent etiologies. IL-23 is expressed on activated myeloid cells, and a subset of T cells is the most potent inducer of IL-17. IL-23 functions as a terminal differentiator of Th17 cells, and IL-23R serves as an initial sensor of IL-23 and is therefore important in Th17-cell mediated autoimmune responses.[12,67] The main objectives of this study were to assess the contributions of genetic variants of *IL-23R* and *IL-17A* to genetic susceptibility to IBD and to elucidate their potential underlying mechanisms.

Although the exact mechanisms via which IL-23R modulates IBD susceptibility are not yet clear, the key role of IL-23 in IBD has been demonstrated by recent genetic studies in which genetic variants in the *IL-23R* gene were found to be associated with IBD.[10,20-23] Although candidate SNPs of *IL-23R* in Caucasian populations, including rs11209026, are not present as polymorphisms in Asians, including Japanese and

Koreans, intronic SNPs at intron 5 and in an intergenic region of *IL-23R* were recently reported to be associated with CD in the Korean population.[20,68] These results suggest that there are ethnic differences in this disease, and that entire exons of *IL-23R* need to be completely investigated in Asian population. Therefore, it was considered to identify new susceptible genetic SNPs of *IL-23R* in the Korean population through whole exon and junction instead of using common tag SNPs. Although rs11209026 was not polymorphic in either our Korean IBD patients or our healthy controls, that is different from Caucacian, it was found that the two novel SNPs, G149R (protective in CD and UC) and IVS4+17C>T (risk in UC) located in exon 4 showed significant associations with IBD development. It is interesting that the protective arginine 149 allele was present in ~13% of controls, 4% of CD patients, and 15% of UC patients in our study, whereas the protective glutamine 381 allele is known to present in ~7% of controls, ~2-4% of CD cases, and ~4% of UC in Caucasian populations.[10,69] However, the odds ratio of G149R in CD or UC is similar to that in Caucacian populations. Q3H in exon 2 of *IL-23R* showed a protective effect against UC. Furthermore, pairwise analysis suggests that there is a genetic interaction between Q3H in *IL-23R* and IVS1+18G>A in *IL-17A*. The functional variants, glycine 149 to arginine and glutamine 3 to histidine, may affect the structure and the biologic function or stability of IL-23R, and nucleotide alteration at intron 4 (IVS4+17C>T) may alter the stability of mRNA. These findings support the idea that *IL-23R* is responsible for the association with IBD in both Caucacian and Asian populations even though there are ethnic differences in specific SNPs affecting IBD risk. Further investigations are

warranted to validate these results.

IL-17A is located on 6p12.1, a genomic region reported to contain the putative susceptibility loci (IBD3) for IBD.[70] *IL-17A* mRNA levels in PBMCs and circulating IL-17 levels in plasma have been reported to be increased in IBD patients.[42,46] High levels of *IL-17A* mRNA have been detected in the mucosa of both CD and UC mucosa patients compared to healthy controls, and *IL-17A* has been shown to be produced by T cells or $CD68^+$ macrophages in the colonic mucosa of IBD patients, leading to significant elevation of serum *IL-17A* levels, in contrast to normal individuals.[15,42,71] Although CD and UC are collectively referred to as IBD, it has been hypothesized that different pathogenic mechanisms may lead to the clinical phenotypes of these two diseases. CD is characterized by a more penetrating, transmural inflammation that can affect any tract of mucosa in the gut than UC; UC shows progressive inflammation that does not extend beyond the large intestine.[1,36] These data suggest that there are subtle genetic and expression correlation differences of *IL-17A* in UC and CD and also provides evidence for different contributions of *IL-23R* and *IL-17A* polymorphisms to CD and UC although CD and UC shared some susceptibility genes in the epidemiologic study, concordant to immunologically different disease entities between UC and CD .

In addition, this study demonstrated epistasis among *IL23R*, *IL-17A* and *STAT4* variants, certain *STAT4* genotypes increased the risk for IBD in patients with the *IL-23R or IL-17A* risk alleles. The requirement for STAT4-dependent cytokine regulation has been established for the pathogenesis of autoimmune encephalomyelitis,[72,73]

RA,[31,74] and also IBD,[75-77] highlighting a critical role for STAT4 in autoimmune diseases.[29] Previous studies demonstrated constitutive STAT4 activation in the intestinal T cells of CD patients[75] and increased expression and activation of IL-12-induced STAT4 signaling in the mucosa of patients with UC.[76] The interaction between *STAT4* and *IL-23R* is intriguing given the fact that both of these genes are involved in IL-12 signaling and regulation of the Th1/Th17 cytokine balance. STAT4 pathway appears to be the major player in IL-12-induced Th1[78,79] and IL-23-mediated Th17 immune responses.[67] However, what has not been clear is the activational status of IL-12- or IL-23-induced STAT4 signaling in IBD, which is critical in orchestrating a sustained or chronic Th1 or Th17 inflammatory environment. Thus, the putative interaction in the context of the overall contribution of the *IL-23R*/*IL-17A*/*STAT4* genotype combination was analyzed, because this approach allows for determination of the extent to which the interaction contributes to IBD risk. If *IL-17A* was mutated, genetic alterations of *STAT4*, an upstream gene in the same pathway, could lead to changes in the Th17 cytokine profile. A recent study demonstrated a significant association of a *STAT4* risk allele with overexpression of STAT4 in primary cells of mesenchymal origin such as osteoblasts but not in B cells.[80] This indicates that tissue-specific intragenic enhancers could affect STAT4 expression levels and that different *STAT4* gene variants could have different cell type-specific effects. This line of reasoning is supported by a previous study that demonstrated that STAT4 isoforms differentially regulate Th1 cytokine production, with STAT4β promoting greater colonic inflammation and tissue destruction, correlating with STAT4 isoform-

dependent expression of TNF-α and GM-CSF *in vitro* and *in vivo*, but not in Th1 expression of IFN-γ or Th17 expression of IL-17.[77] Moreover, a previous study[81] reported that lupus patients carrying a risk variant of *STAT4* showed increased sensitivity to IFN-γ, which could contribute to increased mucosal inflammation in IBD patients and to patient responses to immunosuppressive and immunomodulatory therapies. A recent *in vivo* study demonstrated impaired development of human Th1 cells in patients with deficient expression of STAT4.[82] Furthermore, several recent studies that investigated the genetic background of STAT4 regulation suggested a significant association between genetic variants of *STAT4* with SLE and RA[29,31,83,84] as well as SD,[33] SSc,[29,85] psoriasis[86] and type-1 diabetes,[87] indicating common genetic and molecular pathways in multiple autoimmune diseases.

No nonsynonymous SNP of *IL-17A* is present in the NCBI SNP database, suggesting that regulatory polymorphisms rather than coding polymorphisms of *IL-17A* play a role in the pathophysiological processes of diseases with which *IL-17A* is associated. Moreover, a few reports have recently been published regarding the association between the -197 in the *IL-17A* promoter and the UC phenotype[88] and between -737 and pediatric asthma in Taiwanese children.[37] However, it has not yet been clarified whether *IL-17A* polymorphisms truly affect the risk of IBD development or how this polymorphisms influence the activity and expression of *IL-17A*. This study is the first finding to explain the mechanism underlying the disease development that is associated with the variants of -737 and IVS1+18 in addition to variant -197. Allele -737T is significantly associated with a higher level of *IL-17A* mRNA expression in IBD

patients and displays a higher binding activity to transcription factor complexes as a kind of regulatory mechanism. Although -444 and -877 showed no significant association with IBD, these sites may also contribute IBD susceptibility in terms of binding affinity to the TF complex. RORC was recently identified as a master regulator of Th17 cytokine production.[38] However, additional transcription factors (TFs) such as STAT3, RORα, and interferon regulatory factor 4 were reported to be involved in the promotion of Th17 cytokine production[39-42] while Foxp3, Ets1, Gfi1, T-bet, and Smad3 negatively regulate Th17.[43, 44] Nevertheless, the mechanisms underlying the roles of these factors in Th17 differentiation are not entirely clear, and more studies are required to understand the complex regulation of *IL-17A* expression. Of note, the variant at -197 was previously the only identified SNP explaining a possible association with autoimmune diseases in IBD; however, it did not show any affinity for TF complexes or methylation status and was located in LD with IVS1+18, an important positions that affected methylation status in this study. These results suggest that -197 may simply be a 'linked SNP' rather than a SNP with its own functional consequences.

DNA hypermethylation is strongly associated with heterochromatin and transcriptional silencing, and hypomethylation at CpG sites in the promoter region is a well-defined epigenetic phenomenon generally associated with active gene expression.[89] Differential methylation of DNA has been reported for T cells at different stages of cell differentiation. Moreover, Th17 cells show distinct chromatin remodeling of the *IL-17-A* gene locus consistent with the production of IL-17.[90] which is not stable

and is reversible through linkage with RORC.[91,92] These epigenetic modifications undoubtedly serve as an important regulatory mechanism during maintenance of lineage commitment in T cells. Hypermethylation at a small number of clustered CpGs, which later spreads to neighboring CpGs, has also been shown to contribute to transcriptional regulation.[93,94] The data presented in this study clearly indicate that CpG sites in the proximal promoter region of *IL-17A* in the healthy controls were dramatically hypermethylated with a negligible transcript level of *IL-17A* compared to that of the IBD patients. In particular, a significant polymorphism in the promoter region of *IL-17A*, IVS1+18G>A, was identified. As expected, changing a G nucleotide to an A at this site abrogated a DNA methylation site and induced aberrant hypomethylation of cytosine residues. In addition, the -121G>A in the same block with -197 showed a change in methylation status at CpG and recently was found to be polymorphic in other diseases,[24,88] which may also influence transcription through a similar mechanism. Interestingly, IVS1+17C including -122C is colocalized with 5′ untranslated regions of the *IL-17A* gene that are intensively marked by permissive modifications, histone H3 lysine residue 4 trimethylation (H3K4me3) in Th17 cells, but extensively marked by repressive H3K27me3 modification in other T cell lineages.[90,91] These data suggest that the irreversible hypomethylation of cytosine residues by IVS1+18G>A or -121G>A may create a far more highly “poised” state than normal for sustained expression of *IL-17A* through the H3K4me3 mark, which loosens the chromatin and recruits TFs. IVS1+18G>A including -121G>A could stably and irreversibly abrogate a binding site for methyl CpG binding protein that maintains

the epigenetic silencing of transcriptional activity. This change may also create potential TF–binding sites associated with genes that are independently activated by permissive modifications of histones in contrast to the phenotypic or developmental plasticity of Th17 cells.[91,92] The alteration of methylation status by genetic change may sustain the transcriptional competence of *IL-17A* in Th17 cell or induce lineage transitions to other IL-17 secreting cells. Moreover, the *IL-17A* expression could be restored by 5-Aza-dC, a DNMT inhibitor that reduces DNA methylation, in Jurkat cells that initially showed no expression of *IL-17A*. This is the first demonstration that a change in DNA methylation of the *IL-17A* promoter may play a critical role in *IL-17A* expression and pathogenesis of IBD. However, contrary to expectations, the nucleotide IVS1+18 in Jurkat cell was cytosine, which suggests involvement of an epigenetic modification of RORC or an unknown upstream repression site/upstream repression site including a conserved noncoding sequence or changes in higher-order chromatin structure.[91,95,96] Taken together, these results indicate that CpG dinucleotides and methylation in this region play an important role in the regulation of *IL-17A* transcription, though further research is needed to fully elucidate the exact mechanism. These aberrant methylations may result in increased sensitivity in IBD patients if intensive signals due to more sensitive *IL-23R* are introduced and cooperate with intensive transcriptional activation via modification of TF binding sites as unknown cis-elements (Figure 13). Genetic changes in multiple sites of the promoter can lead to physiological changes including epigenetic changes and may result in stringent or relaxed regulation of transcription. These findings shows for the first time that IL-17

expression is regulated by DNA methylation, which suggest novel mechanisms through which epigenetic change is coordinated with cis-regulation in IBD.

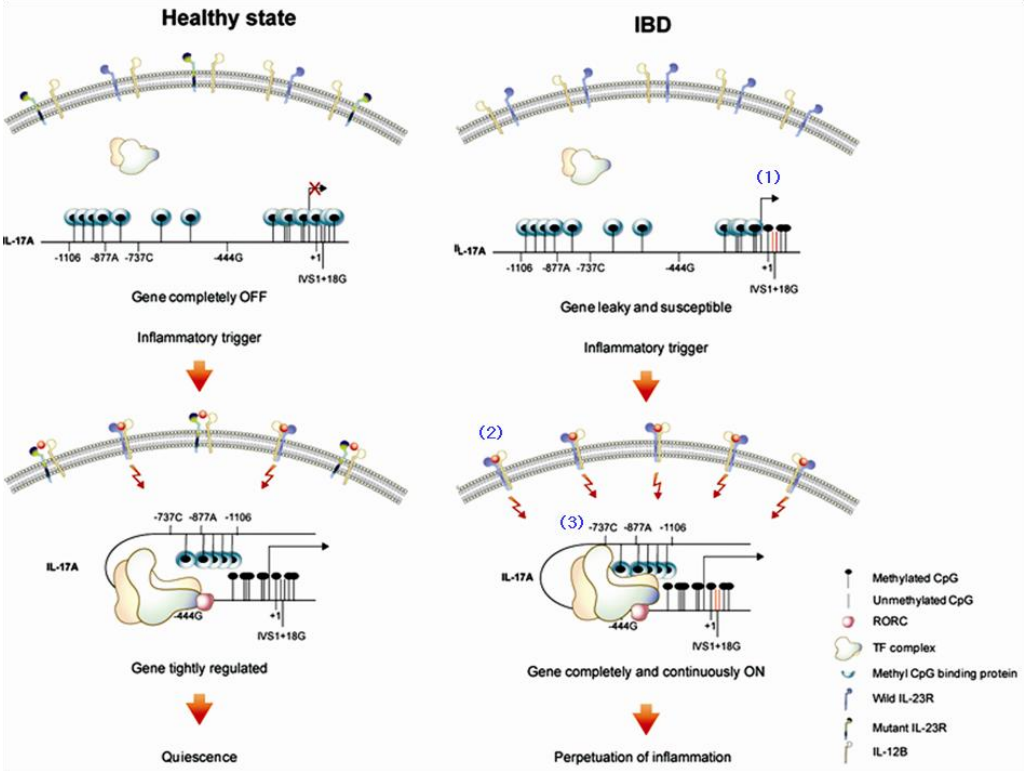

Figure 13. Proposed model depicting the interplay of the polymorphic sites in *IL-23R* and *IL-17A* genes in response to IL-23-mediated signals. Hypomethylation of the *IL-17A* promoter in IBD compared to that in controls leads to sustained signaling (1). In healthy controls, the protective allele of *IL-23R* is less sensitive to IL-23, but the *IL-23R* risk allele in IBD patients is more sensitive (2). The strong affinity of *IL-17A* risk alleles to the TF complexes results in higher transcriptional activity of the promoter, which strengthens and retains the signaling in IBD patients compared to that in the healthy control subjects (3).

In summary, new associated SNPs of *IL-23R* and *IL-17A* as a susceptibility gene in IBD through a case-control association study were identified. The current study clearly demonstrates also that *STAT4*, *IL-23R* and *IL-17A* contributed interactively to susceptibility to the development of IBD. Moreover, the functional consequences of the *IL-17A* variants which have an allele-specific effect on gene expression in PBMCs by examining the affinities of these variants for TF complexes and effects on DNA methylation profiles was characterized. This study also highlights the hypomethylation status of *IL-17A* in PBMC of IBD patients in contrast to that of healthy controls in an attempt to elucidate the regulation of *IL-17A* expression by DNA methylation. These findings suggest that the polymorphisms of both genes in *IL-23R/IL-17* including *STAT4* affect *IL-17A* gene expression and are fundamentally associated with the etiology of IBD. In addition, these findings provide new mechanistic insights into the IL-23/IL-17 axis by demonstrating that genetic and epigenetic interactions in *IL-17A* gene regulation are the basis for the elevated IL-17 expression in IBD patients, which could highlight a potential target for the treatment of IBD, although additional, larger multi-center replicated studies are required to confirm these findings.

V. **CONCLUSION**

New associated SNPs of *IL-23R* and *IL-17A* associated with susceptibility to IBD were identified using a case-control association study. Moreover, the functions of the SNPs of *IL-17A*, which had an allele-specific effect on *IL-17A* expression in PBMCs, were characterized by examining the affinity of the variants for TF complexes and effects of these variants on DNA methylation profiles. This study also highlights the hypomethylation status of *IL-17A* in PBMCs of IBD patient in contrast to healthy controls, suggesting that *IL-17A* expression may be regulated by DNA methylation. These findings suggest that polymorphisms in both *IL-23R* and *IL-17* genes including *STAT4* affect *IL-17A* gene expression and are fundamentally connected to the etiology of IBD. These findings provide new mechanistic insights into the IL-23/IL-17 axis and indicate that genetic and epigenetic interactions among these genes contribute to elevated expression of *IL-17A* in IBD patients, which could be a potential target for modifying T cell development, although further, larger multi-centre replicated studies are required to confirm these findings.

Genetic polymorphisms in IL-23R/IL-17 axis are essentially associated with IBD pathogenesis and provide new insights that genetic and epigenetic interactions are the basis for elevated expressions of IL-17 in IBD. This study would examine the intestinal inflammation physiology in IBD using genomic and epigenomic approachs, which will contribute to the advance in the diagnosis and treatment of various colonic disorders.

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<ABSTRACT (IN KOREAN)>

인터루킨 23 수용체 와 인터루킨 17A 유전자 다형성과 염증성 장질환과의 관련성 규명

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김 승 원

염증성 장질환은 만성적으로 대장에 염증을 나타내는 질환으로서 크게 궤양성 대장염과 크론병으로 나뉜다. 최근 염증성 장질환은 유전적, 환경적, 혹은 면역조절에 관련된 인자들의 복합적인 상호작용에 의하여 발병하는 것으로 추정하고 있다. 염증성 장질환과 관련하여 많은 연구가 이루어졌는데 이와 관련하여 서양에서

인터루킨 23 수용체(*IL-23R*)의 변이가 깊은 관련이 있음이 보고되었으나 동양에서는 관련성을 확인하지 못하였다. 또한 염증에 중요한 싸이토카인인 인터루킨 17A(*IL-17A*)의 변이의 관련성에 관한 연구가 최근 보고 되었나 세부적인 기전을 포함하는 연구가 필요하다.

본 연구에서는 *IL-23R*과 *IL-17A*의 유전학적, 후생유전학적 변화가 염증성 장질환의 발병과 관련 여부를 조사하였다. *IL-23R*과 *IL-17A*의 promoter와 exon을 크론병 201명, 궤양성 대장염 268명, 건강한 대조군 258명을 대상으로 DNA sequencing과 DHPLC를 이용하여 분석하였다. Jurkat 세포와 말초혈액 단핵구를 *in vitro* assay에 이용하였다. 전사인자의 결합력을 electro mobility shift assay를 통하여 분석하였으며 *IL-17A* mRNA 발현량은 RT-PCR로 확인하였고 *IL-17A* promoter의 methylation은 bisulfate sequencing과 pyrosequencing을 이용하여 분석하였다. 그 결과 크론병에서는, *IL-23R*의 변이 G149R (OR; 0.32, 95% CI 0.15-0.69), 와 *IL-17A* 의 변이 IVS1+18G>C (OR; 5.33, 95% CI 1.12-25.37)가 관련 있었다. 궤양성 대장염에서는 *IL-23R* 의 변이 Q3H (OR; 0.62, 95% CI 0.38-1.00), G149R (OR; 0.41, 95% CI 0.21-0.77), IVS4+17C>T (OR; 2.9, 95% CI 1.21-6.99), *IL-17A*의 변이 -737C>T (OR; 1.49, 95% CI 1.06-2.11), -197G>A (OR; 0.63, 95% CI 0.41-0.98), IVS1+18

G>C (OR; 4.47, 95% CI 0.96-20.86)가 관련되어 있었다. -877G, -737T, -444A risk allele 들은 전사인자 복합체와 강한 결합력을 나타냈으며 이들을 지닌 환자에서 말초혈액에서 높은 *IL-17A*의 발현과 상관성을 나타냈다. 염증성 장질환이 있는 환자의 말초혈액의 *IL-17A*는 현저한 DNA methylation을 나타내었고 이는 *IL-17A*의 발현과 IVS1+17의 methylation 정도와 상관성을 보였다. Jurkat 세포에서의 *IL-17A* 의 발현은 demethylating agent 처리시 회복됨을 확인하였다.

결론적으로 본 연구를 통하여 염증성 장질환은 *IL-23R/IL-17*축과 관계된 유전자 다형성과 깊이 연관되어 있으며 *STAT4*를 포함한 이들의 유전학적, 후생유전학적 상호작용이 *IL-17A*의 증가의 중요한 기전임을 밝혔다.

핵심되는 말 : 인터루킨 23 수용체, 인터루킨 17A, 유전자 다형성, 염증성 대장질환, 후생유전학