Research Article

Gene Expression-Genotype Analysis Implicates *GSDMA*, *GSDMB*, and *LRRC3C* as Contributors to Inflammatory Bowel Disease Susceptibility

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To investigate the biological foundation of the inflammatory bowel disease (IBD), ulcerative colitis and Crohn's disease, susceptibility locus rs2872507, we have investigated the expression of 13 genes using ileal and colonic biopsies from patients with IBD (inflamed and noninflamed mucosa) or from individuals without IBD (noninflamed mucosa). The susceptibility allele was consistently associated with reduced expression of *GSDMB* ($P = 4.1 \times 10^{-3}$–7.2×10^{-10}). The susceptibility allele was also associated with the increased expression of *GSDMA* ($P = 1.6 \times 10^{-4}$) and *LRRC3C* ($P = 7.8 \times 10^{-6}$) in colon tissue from individuals without IBD and with the reduced expression of *PGAP3* (IBD; $P = 2.0 \times 10^{-3}$) and *ZPBP2* (Crohn's disease; $P = 7.7 \times 10^{-4}$) in noninflamed ileum. Inflammation resulted in the reduced colonic expression of *ERBB2*, *GRB7*, *MIEN1*, and *PGAP3* ($P = 1.0 \times 10^{-4}$–1.0×10^{-9}) and the increased colonic expression of *IKZF3* and *CSF3* ($P = 2.4 \times 10^{-7}$–3.5×10^{-8}). Based on our results and published findings on *GSDMA*, *GSDMB*, *LRRC3C*, and related proteins, we propose that this locus in part affects IBD susceptibility via effects on apoptosis and cell proliferation and believe this hypothesis warrants further experimental investigation.

1. Introduction

Inflammatory bowel disease (IBD) is a group of intestinal disorders, subdivided into Crohn's disease (CD), ulcerative colitis (UC), and, in the absence of a confident diagnosis, unclassified colitis (IBD-U) [1, 2]. Susceptibility to IBD is to a large extent determined by genetic predisposition [3]. Consistent with that, genetic association studies have uncovered a large number of susceptibility loci in relation to IBD. Recently, a total of 163 IBD loci were identified in a genome-wide analysis, and 110 of those susceptibility loci were shared between CD and UC [4]. Within each susceptibility locus, candidate risk genes have been prioritized, based largely on bioinformatic evaluations of the relationships among genes, the presence of coding single nucleotide polymorphisms (SNPs), or the investigation of gene expression-genotype correlations [4–6].

A linkage disequilibrium block encompassing *IKZF3*, *ZPBP2*, *GSDMB*, and *ORMDL3*, which was previously associated with childhood-onset asthma [7], has been identified as an IBD susceptibility locus [6, 8]. Barrett et al. [6] and McGovern et al. [8] highlighted *ORMDL3* in relation to CD and IBD, respectively, based either on a correlation between genotype and gene expression in lymphoblastoid cell lines [7] or on the biological role and previous implication of *ORMDL3* in diseases involving dysregulated immune responses. More recent studies of UC and CD have emphasized several genes (*GSDMA*, *GSDMB*, *IKZF3*, *ORMDL3*, *PNMT*, and *ZPBP2*) within the same region, using information based on bioinformatics, the presence of coding SNPs, or correlations between genotype and gene expression [4, 5, 9].

The aim of this study was to investigate disease relevant intestinal tissue samples in order to further pinpoint the genes involved in IBD susceptibility. The ileal and colorectal

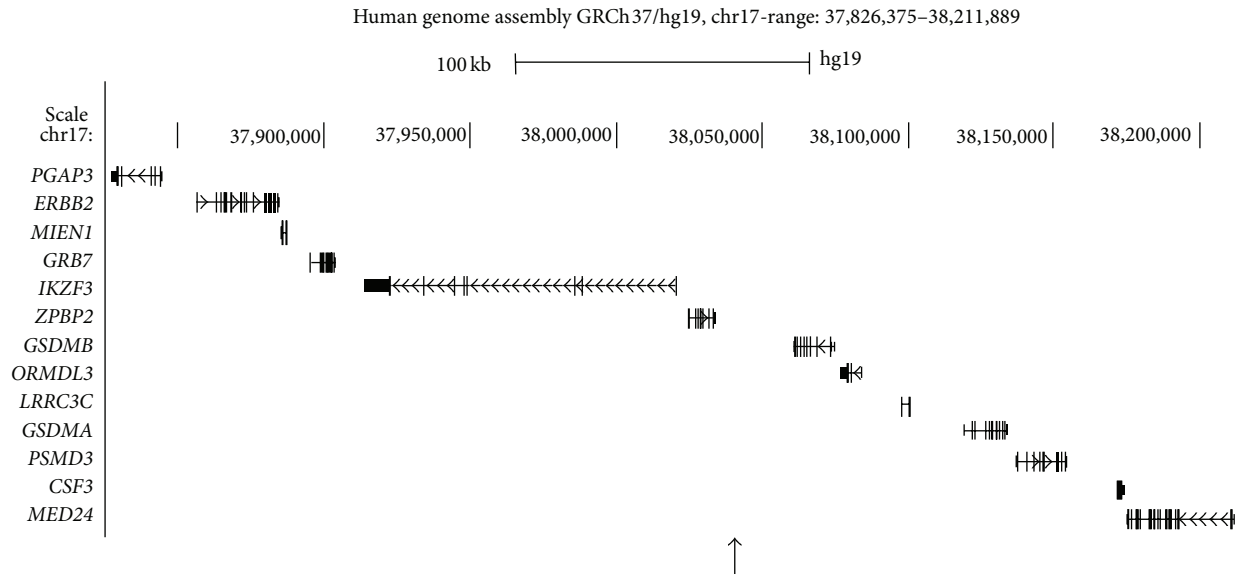Human genome assembly GRCh37/hg19, chr17-range: 37,826,375–38,211,889

FIGURE 1: Overview of the genes present within the investigated region. Location of genes in the vicinity of the IBD susceptibility marker rs2872507 (position marked with an upward-pointing arrow) according to the human genome assembly GRCh37/hg19 viewed using the UCSC Genome Browser (http://genome.ucsc.edu/).

expression of 13 genes within the genetic region of the shared (CD and UC) susceptibility locus rs2872507 (*PGAP3–MED24*; Figure 1) was analyzed in relation to genotype, inflammation, and sampling location.

2. Material and Methods

2.1. Study Samples. Colorectal and ileal mucosal biopsy specimens (n = 183) were collected during *routine* endoscopies of 85 adult patients who were being investigated for a known IBD diagnosis or were in the work-up for suspected gastrointestinal disorders (Table 1). Thirty-three patients not afflicted with IBD and without intestinal inflammation were included as noninflamed, non-IBD controls. Study biopsies were collected in parallel to and from the same areas as the biopsies collected for histopathologic assessment. Each biopsy was categorized as "inflamed" or "noninflamed" based on a compound evaluation of endoscopic findings as assessed by one experienced endoscopist (Sven Almer) and on a routine histopathologic assessment for inflammation.

The biopsy specimens used for RNA purification were immersed in RNA*later* RNA stabilization reagent (Qiagen, Hilden, Germany) and stored at 4°C overnight and at −20°C thereafter, awaiting RNA purification.

2.2. DNA and RNA Purification. The biopsies were homogenized using TissueRuptor and disposable probes (Qiagen). DNA and RNA were purified using the AllPrep DNA/RNA mini kit (Qiagen) according to the manufacturer's instructions, either manually or using the automated QIAcube system (Qiagen). Concentration and purity were spectrophotometrically measured using a Nanodrop ND-1000

TABLE 1: Clinical characteristics of participants.

	IBD (n = 52)	Non-IBD (n = 33)
Disease (CD/UC/IBDU)	21/29/2	
Disease (non-IBD)		a
Gender (male/female)	23/29	13/20
Age (years)[b]	40 (18–77)	49 (20–82)
Smoker (yes/previous/no/no data)	6/5/35/6	3/2/23/5
Immune-modulating drugs		
Aminosalicylates	24	0
Thiopurines	17	0
Corticosteroids	17	0
Anti-TNF-α-antibodies	6	1
Methotrexate	2	1
Tacrolimus	2	1
None	10	33

[a]Patients were referred for investigation of gastrointestinal symptoms (e.g., diarrhea, fecal blood, or abdominal pain) or for screening for colorectal adenomas with the following findings: diverticulosis (n = 4), polyps (n = 5), low-grade dysplasia adenomas (n = 2), colorectal cancer (n = 2), hemorrhoids (n = 3), radiation proctitis (n = 1), or without pathological findings (n = 19). There were two instances of simultaneous diverticulosis and polyps and one instance of simultaneous diverticulosis and hemorrhoids.
[b]Median (range) values are given.

(Thermo Fisher Scientific Inc., Waltham, MA, USA), and RNA integrity was assessed using the RNA integrity number with a 2100 Bioanalyzer (Agilent technologies, Santa Clara, CA). RNasin plus RNase inhibitor (Promega Corporation, Madison, WI, USA) was added to the RNA.

2.3. Reverse Transcription. Two preparations of $2\,\mu$g RNA from each biopsy were reverse-transcribed in a total volume of $20\,\mu$L each using the high capacity cDNA reverse transcription kit with RNase inhibitor (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. For each biopsy, the resulting cDNA libraries were pooled and stored at -80°C.

2.4. Gene Expression Analysis and Data Preprocessing. Assays for gene expression analysis (see S1 Table in Supplementary Material available online at http://dx.doi.org/10.1155/2015/834805) were preloaded onto TaqMan gene expression array cards (Life Technologies). All steps were in accordance with the manufacturer's instructions, using 300 ng sample cDNA per array port (48 different assays).

Threshold cycle (C_T) values were established using the ExpressionSuite software version 1.0.1 (Life Technologies). Missing C_T values, caused by low copy numbers, were replaced by the highest C_T value available, increased by one cycle, for the gene in question (GenEx software version 5.4.2, MultiD Analyses, Gothenburg, Sweden). The resulting C_T values were normalized to the average of the selected reference genes (S1 Table) using the GenEx software version 5.4.2. The resulting delta-C_T values were further processed to obtain the relative expression in relation to the sample with the lowest expression of each gene.

2.5. Genotyping. The genotype of the SNP susceptibility marker rs2872507 [5, 9] on chromosome 17q12 (http://www.ncbi.nlm.nih.gov/snp) was assessed using 5 ng genomic DNA per sample, a TaqMan SNP genotyping assay (assay ID C_11630970_20), and the TaqMan genotyping master mix (Life Technologies). All genotyping was conducted on the 7500 Fast real-time PCR system using the standard run mode, and the genotypes were generated using the 7500 Fast system SDS software version 2.0.6 (Life Technologies).

2.6. Data Analysis. Reference genes were evaluated for low sample-to-sample variation using the GeNorm [10] and NormFinder [11] algorithms in the GenEx software version 5.4.2.

In order to reduce potential confounding effects on the analysis of genotype and gene expression, the samples were stratified based on inflammatory status (inflamed versus noninflamed) and sampling location (ileum versus colon). Multiple colorectal biopsies within inflamed or noninflamed areas from single individuals were treated as biological replicates (see Effects of Sampling Location on Gene Expression in Results). Additionally, the samples were grouped based on disease status (CD, UC, overall IBD, and non-IBD).

The gene expression was investigated in relation to the genotypes using Spearman's rank correlation test. For group comparisons, the Mann-Whitney U test or the Kruskal-Wallis ANOVA were used, as appropriate. A Bonferroni-corrected P value < 0.00385 was considered significant. Statistical analyses were performed using Statistica version 10.0 (StatSoft Inc., Tulsa, OK, USA).

3. Results

Only biopsies with concordant results between the macroscopic appearance at endoscopy and the histopathological findings (i.e., "inflamed" or "noninflamed") were used for further analyses.

3.1. Correlation between Genotype and Gene Expression. The susceptibility allele (A) of the rs2872507 locus (Figure 1) was associated with the reduced expression of *Gasdermin B* (*GSDMB*) in intestinal biopsies from patients with IBD; the associations for noninflamed ileum and noninflamed colon were significant and that for inflamed colon was borderline significant (Table 2, Figure 2). The negative correlation between the number of susceptibility alleles and *GSDMB* expression was also noted separately for CD and UC, although the correlation only reached nominal significance ($P < 0.05$) in noninflamed ileal biopsies and inflamed colonic biopsies from patients with UC (Table 2). Among the patients with IBD, the expression of *GSDMB* was at least 2.17-fold lower in individuals homozygous for the susceptibility allele compared with that in individuals homozygous for the opposite allele (Figure 2). The susceptibility allele was further associated with the reduced expression of *PGAP3* in noninflamed ileum biopsies from the overall IBD group and with the reduced expression of *ZPBP2* in noninflamed ileum biopsies from the CD group (Table 2, Figure 3), with the expression of *PGAP3* reduced 1.81-fold (2 versus 0 susceptibility alleles) and that of *ZPBP2* reduced 2.27-fold (1 versus 0 susceptibility alleles). A correlation between the number of susceptibility alleles and *PGAP3* expression was also identified with nominal significance ($P < 0.05$) separately for CD and UC.

A negative correlation between *GSDMB* expression and the number of susceptibility alleles was also present in the noninflamed, non-IBD ileal and colonic biopsy samples (Table 2, Figure 4), and the expression was lower at least 1.84-fold in individuals homozygous for the IBD susceptibility allele compared with that in individuals homozygous for the opposite allele. Furthermore, the IBD susceptibility allele was associated with the increased expression of *GSDMA* and *LLRC3C* in noninflamed, non-IBD colonic samples (Table 2, Figure 5) and with expression levels increased 5.53-fold and 6.78-fold, respectively, in individuals homozygous for the susceptibility allele compared with those in individuals homozygous for the opposite allele.

3.2. Inflammatory Effects on Gene Expression. Five of the genes displayed either modestly decreased (*MIEN1*, *PGAP3*, *GRB7*, and *ERBB2*; 1.24–2.01-fold) or increased expression (*IKZF3*; 2.41-fold) in biopsies from inflamed, compared with noninflamed, colonic IBD mucosa (Table 3). Similar effects were seen separately for CD and UC, although only nominal significance ($P < 0.05$) was reached for *IKZF3* in CD and for *MIEN1* in both UC and CD. *CSF3* expression was increased at least 116-fold in inflamed, compared with noninflamed, biopsies from the overall IBD group and from the separate disease subentities (Table 3).

TABLE 2: Spearman's rank correlation between the genotype of rs2872507 (number of susceptibility alleles) and gene expression (dCt-values) in intestinal biopsy samples from different study groups[a].

Genes[b]	NI_N_il $n = 24$ P value	NI_N_co $n = 33$ P value	IBD_N_il $n = 24$ P value	IBD_N_co $n = 29$ P value	IBD_I_co $n = 28$ P value	CD_N_il $n = 11$ P value	CD_N_co $n = 12$ P value	UC_N_il $n = 12$ P value	UC_N_co $n = 16$ P value	UC_I_co $n = 19$ P value
PGAP3	0.37	6.8×10^{-03}	2.0×10^{-03}	0.019	0.017	0.030	0.43	0.29	0.060	0.59
ERBB2	0.64	0.57	0.69	0.27	0.23	0.91	0.31	0.50	0.68	0.89
MIEN1	0.66	0.89	0.034	0.15	0.50	0.79	0.35	0.085	0.023	0.27
GRB7	0.93	0.71	0.21	0.61	0.99	0.51	0.93	0.31	0.93	0.50
IKZF3	0.88	0.85	0.69	0.43	0.47	0.42	0.39	0.80	0.92	0.27
ZPBP2	0.24	0.59	0.84	0.33	0.17	7.7×10^{-04}	0.95	0.76	0.078	0.064
GSDMB	2.4×10^{-03}	3.7×10^{-04}	1.5×10^{-03}	7.2×10^{-10}	4.1×10^{-03}	3.8×10^{-03}	6.2×10^{-06}	0.035	1.9×10^{-04}	0.045
ORMDL3	0.92	0.77	0.037	0.35	0.47	0.016	0.63	0.24	0.045	0.22
LRRC3C	0.83	7.8×10^{-06}	0.92	0.058	0.42	0.79	0.58	0.21	0.24	0.27
GSDMA	0.016	1.6×10^{-04}	0.92	0.28	0.15	0.28	0.50	0.74	0.50	0.86
PSMD3	0.87	0.38	0.51	0.014	0.43	0.45	0.057	0.66	0.14	0.32
CSF3	0.42	0.45	0.61	0.66	0.81	0.77	0.31	0.25	0.17	0.10
MED24	0.39	0.43	0.16	6.9×10^{-03}	0.61	0.50	0.085	0.76	0.093	0.21

[a]Disease status_Inflammatory status_sampling location; the disease statuses are Crohn's disease (CD), inflammatory bowel disease (IBD), non-IBD (NI), and ulcerative colitis (UC); the inflammatory statuses are inflamed (I) and noninflamed (N); the sampling locations are colon (co) and ileum (il).
[b]Genes have been arranged (top to bottom) in the order in which they are positioned along the chromosome, and the risk locus (rs2872507) is located between ZPBP2 and GSDMB (closer to ZPBP2).

3.3. Drug-Mediated Effects on Gene Expression. Drug-mediated effects on gene expression were investigated to elucidate a potential explanation for the observed differences in the relationships between genotype and gene expression among patients with and without IBD, respectively. Colonic gene expression levels were compared among patients with IBD receiving a particular treatment versus those not receiving that treatment. No drug-mediated effects were observed in relation to treatment with thiopurines, aminosalicylates, or corticosteroids (S2 Table). There were too few individuals on treatment with anti-TNF-α-antibodies for a meaningful statistical analysis.

3.4. Effects of Sampling Location on Gene Expression. No differences in gene expression were observed among four different segments of colon (ascending, transverse, descending, and sigmoid) from the noninflamed intestinal mucosa of individuals without IBD (S3 Table; caecum and rectum were excluded because of a low number of samples). Four genes were differentially expressed between the ileum and the colon, however, with either lower (*PSMD3* and *LRRC3C*, 1.34- and 3.85-fold, resp.) or higher (*ORMDL3* and *IKZF3*, 1.34-fold and 2.23-fold, resp.) expression in the ileum compared with that in the colon (S4 Table).

4. Discussion

A large number of genetic susceptibility loci have been uncovered in relation to IBD, with pervasive sharing of genetic susceptibility loci between the two major disease subtypes, UC and CD [4]. In this study, we searched for an association between the risk allele of the shared (UC and CD) susceptibility locus rs2872507 and the expression of genes

in the intestinal mucosal. This locus has previously been associated with autoimmune diseases such as type-1 diabetes [12], rheumatoid arthritis [13], primary biliary cirrhosis [14], and childhood-onset asthma [7, 15] and has been shown to affect the number of white blood cells [16, 17].

We found that an increase in the number of susceptibility alleles was associated with the reduced expression of *GSDMB* in all of the investigated biopsy locations in noninflamed control individuals and in patients with IBD, irrespective of the presence of active inflammation. Additionally, the susceptibility allele was associated with the increased expression of *GSDMA* and *LRRC3C* in noninflamed colon biopsies from individuals without IBD and with the reduced expression of *PGAP3* and *ZPBP2* in noninflamed ileum biopsies from individuals in the overall IBD cohort and the CD cohort, respectively. Concurrent increase and decrease in the expression of neighboring genes in relation to a susceptibility haplotype for childhood asthma were previously shown for the same genomic region, possibly due to differential associations between the CCCTC-binding factor (CTCF) and different haplotypes [18]. CTCF function as an architectural protein that modifies the topology of the genome, which can result in differential regulation of neighboring genes [19]. A recent analysis of IBD susceptibility loci highlighted *ORMDL3* as a candidate gene in the same region based on a correlation between genotype and *ORMDL3* expression in lymphoblastoid cell lines, liver tissue, and adipose tissue [4]. Susceptibility alleles might exert tissue-dependent effects on gene expression, which are possibly modulated by disease type and medical treatment [20, 21]. It is conceivable that the more extensive alteration of gene expression in the colon (*GSDMA*, *GSDMB*, and *LRRC3C*) compared with that in the CD ileum (*ZPBP2*) reflects the fact that colonic inflammation
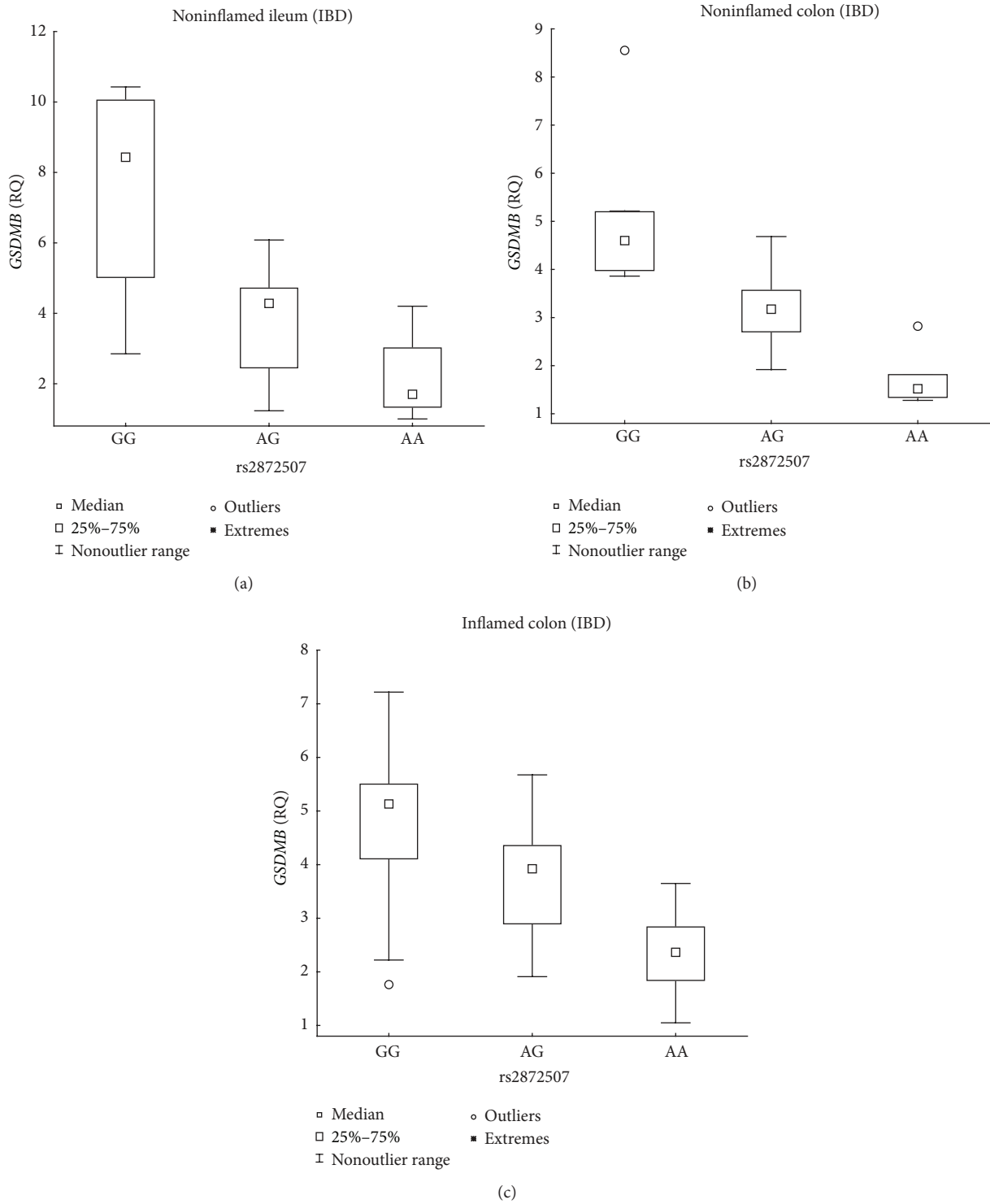
FIGURE 2: Association between rs2872507 genotype and intestinal *GSDMB* expression in IBD patients. Genotype (A is the IBD risk allele) versus the relative quantity (RQ) of *GSDMB* expression in intestinal biopsies from noninflamed ileal IBD mucosa ((a) $n_{GG} = 4$, $n_{AG} = 14$, $n_{AA} = 6$; $R_S = -0.61$, $P = 1.54 \times 10^{-3}$), noninflamed colonic IBD mucosa ((b) $n_{GG} = 8$, $n_{AG} = 14$, $n_{AA} = 7$; $R_S = -0.87$, $P = 7.18 \times 10^{-10}$), and inflamed colonic IBD mucosa ((c) $n_{GG} = 9$, $n_{AG} = 12$, $n_{AA} = 7$; $R_S = -0.53$, $P = 4.12 \times 10^{-3}$).
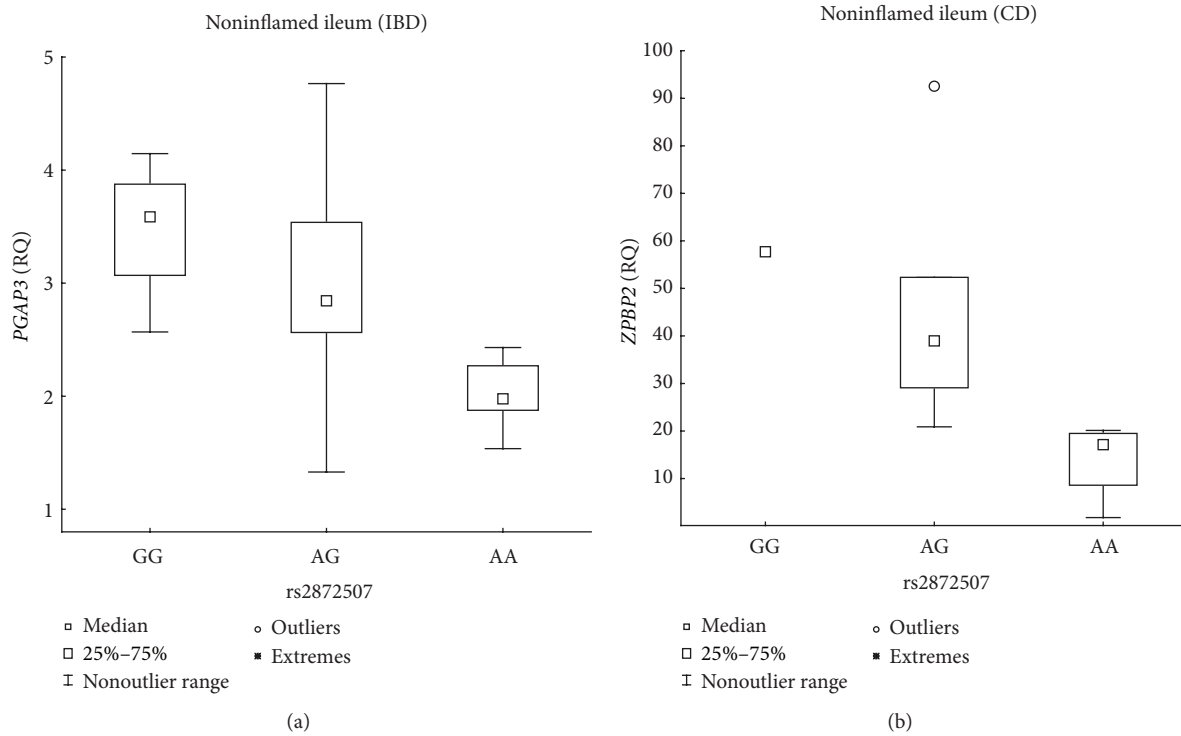
Figure 3: Association between rs2872507 genotype and ileal *PGAP3* expression in IBD patients. Genotype (A is the IBD risk allele) versus the relative quantity (RQ) of *PGAP3* expression in intestinal biopsies from noninflamed ileal IBD mucosa ((a) $n_{GG} = 4$, $n_{AG} = 14$, $n_{AA} = 6$; $R_S = -0.60$, $P = 2.00 \times 10^{-3}$) or the RQ of *ZPBP2* expression in noninflamed ileal CD mucosa ((b) $n_{GG} = 1$, $n_{AG} = 6$, $n_{AA} = 4$; $R_S = -0.86$, $P = 7.68 \times 10^{-4}$).
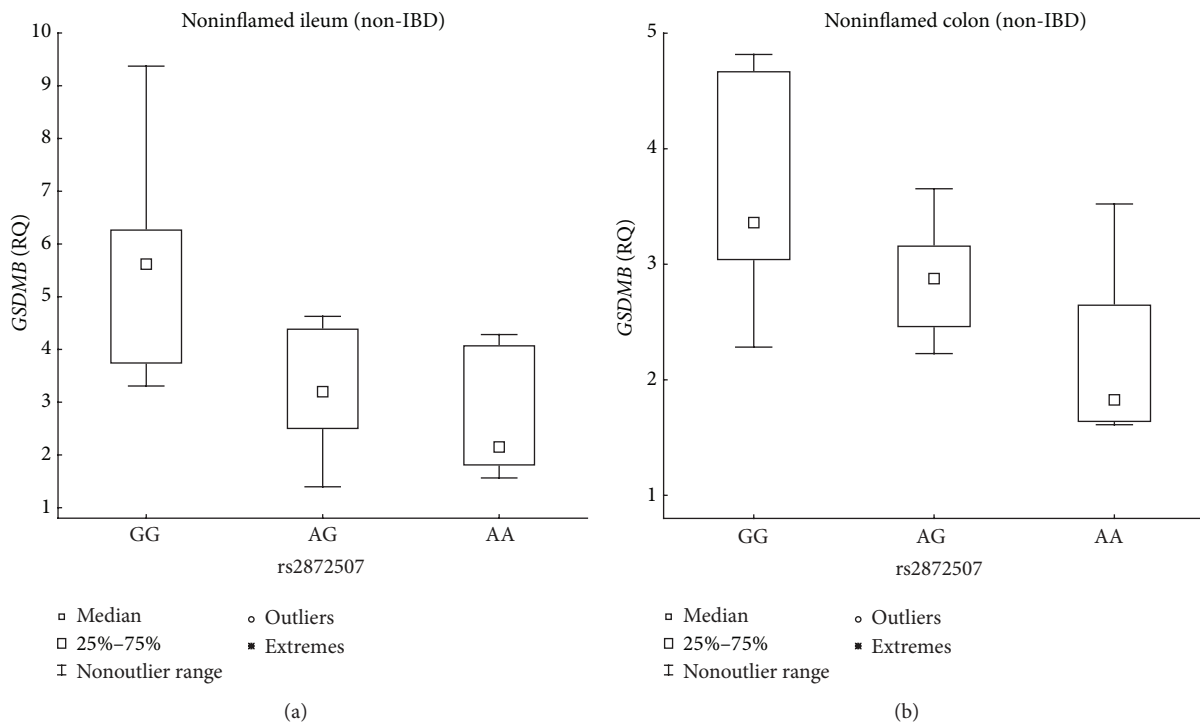

Figure 4: Association between rs2872507 genotype and intestinal *GSDMB* expression in non-IBD patients. Genotype (A is the IBD risk allele) versus the relative quantity (RQ) of *GSDMB* expression in intestinal biopsies from noninflamed ileal non-IBD mucosa ((a) $n_{GG} = 6$, $n_{AG} = 11$, $n_{AA} = 7$; $R_S = -0.59$, $P = 2.38 \times 10^{-3}$) or noninflamed colonic non-IBD mucosa ((b) $n_{GG} = 10$, $n_{AG} = 15$, $n_{AA} = 8$; $R_S = -0.58$, $P = 3.69 \times 10^{-4}$).

TABLE 3: Inflammatory effects on the colonic mucosal expression of genes in the IBD risk locus (rs2872507)[a].

Genes[b]	IBD_N_co ($n = 29$) versus IBD_I_co ($n = 28$)		UC_N_co ($n = 16$) versus UC_I_co ($n = 19$)		CD_N_co ($n = 12$) versus CD_I_co ($n = 7$)	
	P value	Fold change[c]	P value	Fold change[c]	P value	Fold change[c]
PGAP3	3.7×10^{-09}	−1.98	9.8×10^{-06}	−1.92	4.5×10^{-04}	−2.05
ERBB2	1.0×10^{-09}	−2.12	3.9×10^{-06}	−2.08	6.2×10^{-04}	−2.11
MIEN1	1.0×10^{-04}	−1.24	0.014	−1.23	0.010	−1.21
GRB7	1.7×10^{-07}	−2.01	2.9×10^{-04}	−1.93	8.4×10^{-04}	−2.01
IKZF3	2.4×10^{-07}	2.41	6.6×10^{-05}	2.21	6.0×10^{-03}	2.56
ZPBP2	0.21	2.05	0.27	1.96	0.58	2.07
GSDMB	0.31	1.11	0.33	1.12	0.97	−1.03
ORMDL3	0.65	−1.02	0.56	−1.07	0.64	1.01
LRRC3C	0.016	−1.82	0.18	−1.52	0.083	−2.32
GSDMA	0.48	1.27	0.96	1.10	0.29	1.71
PSMD3	0.024	−1.14	0.030	−1.18	0.33	−1.08
CSF3	3.5×10^{-08}	148.53	4.3×10^{-05}	115.72	2.7×10^{-03}	127.34
MED24	6.5×10^{-03}	−1.28	9.3×10^{-03}	−1.33	0.42	−1.19

[a]Disease status_Inflammatory status_sampling location; the disease statuses are Crohn's disease (CD), inflammatory bowel disease (IBD), and ulcerative colitis (UC); the inflammatory statuses are inflamed (I) and noninflamed (N); the sampling locations are colon (co) and ileum (il).
[b]Genes have been arranged (top to bottom) in the order in which they are positioned along the chromosome.
[c]A negative fold change indicates reduced expression in the inflamed tissue compared with that in the noninflamed tissue.
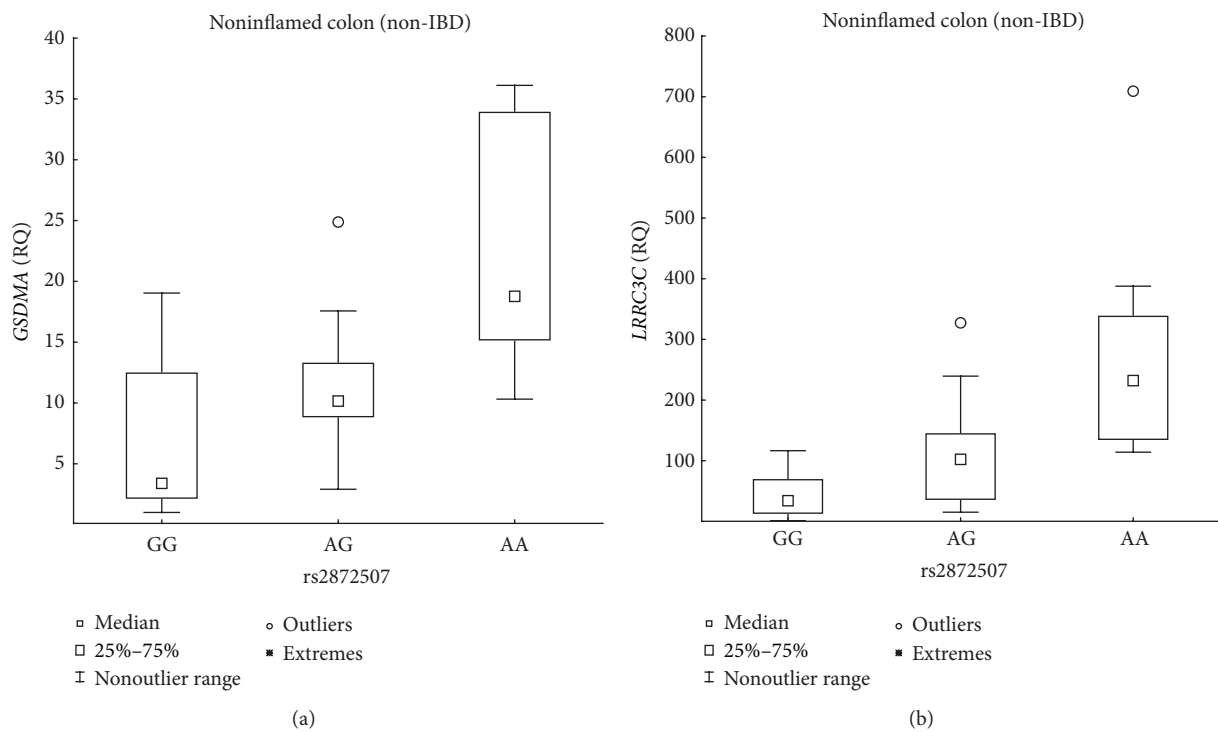

FIGURE 5: Association between rs2872507 genotype and colonic expression of GSDMA and LRRC3C in non-IBD patients. Genotype (A is the IBD risk allele) versus the relative quantity (RQ) of GSDMA ((a) $n_{GG} = 10$, $n_{AG} = 15$, $n_{AA} = 8$; $R_S = 0.61$, $P = 1.61 \times 10^{-4}$) expression or the RQ of LRRC3C ((b) $n_{GG} = 10$, $n_{AG} = 15$, $n_{AA} = 8$; $R_S = 0.69$, $P = 7.79 \times 10^{-6}$) expression in noninflamed colonic biopsies from non-IBD mucosa.

may occur in both CD and UC, whereas ileal engagement is restricted to CD. Recently, our group demonstrated an effect of thiopurine treatment on the expression of genes present in networks involving candidate IBD susceptibility genes [22]. Although it is likely that such therapeutic effects also extend to the actual IBD susceptibility genes, no such effects of ongoing medication were observed in the current study.

GSDMA and GSDMB belong to the Gasdermin family of genes. The expression of GSDMA and GSDMB has been associated with differentiated epithelial cells and with regions

containing proliferating cells or stem cells, respectively, of the esophagus and the gastric mucosa [23, 24]. *GSDMA*, but not *GSDMB*, expression was capable of inhibiting cell growth in a colony formation assay and could induce apoptosis [24]. Additionally, *GSDMA* expression was frequently suppressed in esophageal cancer cell lines and gastric cancer cell lines, whereas *GSDMB* was expressed in all investigated cancer cell lines and also showed evidence of gene amplification and overexpression in some cases of gastric cancer [24].

No functional studies have, to our knowledge, been published regarding *LRRC3C* and the single-pass membrane protein that it encodes: leucine-rich repeat- (LRR-) containing protein 3C (LRRC3C). Within the human genome, LRRC3C was most similar to the protein encoded by *LRRC3B*. Based on experiments using transformed carcinoma cell lines, a role in the suppression of cell proliferation has been suggested for *LRRC3B* [25, 26].

ZPBP2 encodes a protein paralogue of the zona pellucida binding protein [27] and harbors a missense SNP associated with primary biliary cirrhosis [14].

The colonic mucosa is a complex tissue, and the biopsies represent a heterogeneous collection of cell types. We did not determine the cellular origin of gene expression. However, all the genes that were differentially expressed with respect to inflammation were of potential interest in relation to either the inflammatory response or the turnover of epithelial cells. Inflamed, compared with noninflamed, colonic biopsies showed strongly increased transcript levels of *CSF3* and modestly increased transcript levels of *IKZF3*, whereas the transcript levels of *ERBB2*, *GRB7*, *MIEN1*, and *PGAP3* were modestly decreased. The granulocyte colony-stimulating factor (encoded by *CSF3*) stimulates the production, survival, and activity of neutrophils [28], and the granulocyte colony-stimulating factor produced by nonhematopoietic cells promotes neutrophil trafficking to the synovial tissue during collagen-induced arthritis in a murine model of rheumatoid arthritis [29]. Neutrophil infiltration of the intestinal mucosa is a feature of IBD [30]. The transcription factor zinc-finger protein Aiolos (encoded by *IKZF3*) is important for lymphocyte differentiation and shows higher expression in B cells [31]. The overexpression of *IKZF3* inhibited proliferation and suppressed apoptosis in a pre-B acute lymphoblastic leukemia cell line [32]. *Ikzf3* knockout mice developed a phenotype similar to human autoimmune systemic lupus erythematosus [33]. Also, *Pgap3* knockout mice developed autoimmune-like symptoms, possibly relating to impaired clearance of apoptotic cells by peritoneal macrophages [34]. *ERBB2* [35, 36], *GRB7* [37, 38], and *MIEN1* [39, 40] have all been associated with various carcinomas, affecting cell proliferation and/or cell migration.

Considering the risk genotype-gene expression relationships uncovered in our study, both a dysregulated intestinal epithelium [41–43] and aberrant T-cell proliferation and apoptosis [44] have been implicated in the pathogenesis of IBD. However, due to the relatively small sample size of our study the identified relationships need to be cautiously interpreted and confirmed in an independent, preferably larger, study cohort. The exact causes of IBD remain unclear, but the etiology likely includes complex interactions that depend on, at least, the intestinal microbiome and host factors such as genetic susceptibility, intestinal barrier function, and mucosal immune response [45, 46]. In this context it is notable that a joint effect of variants within the genetic region of our study and human rhinovirus wheezing illness has been observed on the subsequent risk of developing childhood-onset asthma. [47, 48]. Although the mechanism of action is not understood, it is possible that it is pertinent to IBD as well. Microbiome studies in IBD have, so far, mainly focused on alterations in the bacterial flora. However, recent studies have also described alterations in the viral component of the intestinal microbiome of IBD patients [49, 50].

Based on our results and published findings on *GSDMA*, *GSDMB*, *LRRC3C*, and related proteins, we propose that the rs2812507 locus in part affects IBD susceptibility via effects on apoptosis and cell proliferation and believe this hypothesis warrants further experimental investigation.

Ethical Approval

The study was approved by the Regional Ethical Review Board in Linköping, Sweden (Dnr 2011/201-31).

Consent

Written and informed consent was obtained from all participants.

Conflict of Interests

Acknowledgment

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