Haplotypes of *PADI4* susceptible to rheumatoid arthritis are also associated with ulcerative colitis in the Japanese population

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Abstract

Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), is a chronic inflammatory disorder characterized by intractable inflammation specific to the gastrointestinal tract. The precise etiology of IBD remains unknown. Recently, haplotypes of peptidylarginine deiminase type 4 (*PADI4*) have been identified as the rheumatoid arthritis (RA)-susceptible gene. *PADI4* is located at 1p36, which is one of chromosomal loci susceptible for IBD. Then, we examined whether haplotypes and diplotypes of *PADI4* are associated with IBD in the Japanese population. We studied haplotypes of *PADI4* in 114 patients with UC, 83 patients with CD, and 200 gender-matched healthy controls by PCR-restriction fragment length polymorphism. Frequencies and distributions of haplotypes and diplotypes were compared statistically between patients and controls by logistic regression analysis. The frequency of haplotype 1 was significantly decreased in patients with UC, compared to that in controls ($P = 0.037$; odds ratio (OR) = 0.702). In contrast, the frequency of haplotype 2 in patients with UC was significantly higher than that in controls ($P = 0.003$; OR = 1.722). Moreover, of a total of 114 patients with UC, 15 (13.2%) had a diplotype homozygous for haplotype 2, the frequency being significantly higher than in controls (9/200, 4.5%; $P = 0.008$, OR = 3.215). Our results indicate that

haplotype 1 of *PADI4* is associated with non-susceptibility to UC, whereas haplotype 2 is susceptible to UC. Thus, it is likely that *PADI4* is one of genetic determinants of UC in the Japanese population.

Key words

Ulcerative colitis, *PADI4*, haplotype, polymorphism, disease-susceptible gene, Japanese population

Introduction

Chronic inflammatory bowel disease (IBD) is a multifactorial disorder that is characterized by inflammation specific to the gastrointestinal tract, which results in intestinal malabsorption, immune defense abnormalities, and an exaggerated inflammatory response (1,2). Various immune and inflammatory cells, such as lymphocytes, macrophages, and dendritic cells, play important roles in the development and progression of IBD (3-5). In addition, bacterial antigens have been implied in the pathological inflammation and may mediate both innate and adaptive responses underlying chronic inflammation (6-8). IBD consists of two main subtypes: ulcerative colitis (UC) and Crohn's disease (CD) (1,2). Although the precise etiology of IBD remains unknown, both several environmental factors, such as dietary components and microorganisms, and genetic factors may contribute to the occurrence of IBD (6-8). In order to identify the genes underlying the etiology of IBD, genome-wide linkage analyses and candidate gene-based association studies have launched and shown possible IBD-susceptibility loci at 16q12 (IBD1), 12q13 (IBD2), 6p13 (IBD3), 14q11 (IBD4), 5q31-q33 (IBD5), 19p13 (IBD6), 1p36 (IBD7), 16q11 (IBD8), 3p21 (IBD9), and other loci (8,9).

Recently, peptidylarginine deiminase type 4 (*PADI4*) located at 1p36 is identified as

the rheumatoid arthritis (RA)-susceptible gene (10). RA is involved in autoimmune diseases, of which pathoetiology is probably similar to UC in a number of respects with not only mechanisms of immune defense abnormalities, such as the elevated production of autoantibody (1,2), but also arthritic manifestations, e.g. ankylosing spondylitis and sacroiliitis (11). Although RA complicated by UC is uncommon, recent report has described the development of UC in patients with RA (12). More importantly, *PADI4* also lies within the IBD-susceptibility locus (IBD7)(8-10).

Based on the above findings, we hypothesized that *PADI4* could play a role in the pathogenesis of IBD as well as RA. Thus, in this study, we have examined whether haplotypes and diplotypes of *PADI4* are associated with IBD in the Japanese population.

Subjects and Methods

Subjects

The study subjects comprised unrelated 114 patients with UC, unrelated 83 patients with CD, and 200 gender-matched, unrelated, healthy volunteers as control. The characteristics of subjects are shown in Table 1. Age at onset is indicated as the mean ± standard deviation (SD). All participants were Japanese who were randomly recruited from 8 general health

clinics in the Nagasaki district of Japan. The study protocol was approved by the Committee for Ethical Issues dealing with the Human Genome and Gene Analysis at Nagasaki University, and written informed consent was obtained from each participant.

The diagnosis of IBD was made on the basis of the endoscopic, radiological, histological, and clinical criteria provided by the WHO Council for International Organizations of Medical Sciences and the International Organization for the Study of Inflammatory Bowel Disease (13-15). Patients with indeterminate colitis and autoimmune diseases, such as RA, multiple sclerosis, and systemic lupus erythematosus, were excluded from the subjects in this study.

Patients with UC were classified into subgroups according to age at onset (<40 or ≥40 years), extension of lesions (proctitis, left-sided colitis, or pancolitis), disease severity (mild, moderate, or severe) and activity (active or inactive) (Table 1). Likewise, patient with CD were divided into subgroups according to age at onset (<40 or ≥40 years), localization of lesions (ileal, ileocolonic, colonic, or isolated upper disease), behavior of disease (structuring and penetrating), and disease severity (mild, moderate, or severe) and activity (active or inactive) (Table 1). The extension and location of UC and CD, disease severity of UC and CD, and behavior of CD were stratified in accordance with Montreal classification (16) with

slight modification. A high clinical activity index (CAI>5) for UC (17) and a high Crohn's disease activity index (CDAI>150) (18) were regarded as active phase patients.

The source of polymorphisms studied

With regard to haplotypes of *PADI4* (GenBank accession number: NT_030584), three single nucleotide polymorphisms (SNPs), padi4_92, padi4_96, and padi4_102, were selected and analyzed, as reported by Caponi *et al.* (19). Subsequently, haplotypes were determined to be based on combinations of their three SNPs (10), as shown in Tables 2 and 3, because SNPs in *PADI4* lie in a strong linkage disequilibrium block.

Preparation of genomic DNA

Genomic DNA was extracted from whole blood of each subject using the DNA Extractor WB-Rapid Kit (Wako Pure Chemical Industries, Osaka, Japan), according to the manufacturer's protocol.

Determination of three SNPs in PADI4

The three SNPs, padi4_92, padi4_96, and padi4_102, were detected by the polymerase chain

reaction-based restriction fragment length polymorphism method. Polymorphic regions were amplified by PCR with a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA) using 25 ng of genomic DNA in a 25-µl reaction mixture containing 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM dNTPs, using 15 pmol each of forward primer: 5'-TCCAGTGGGTGTTTGTTGAA-3' and reverse primer: 5'-CATCCTGCAGGGATTAGGAG-3' for padi4_92; forward primer: 5'-AAACGACCTGCCCATTC-3' and reverse primer: 5'-GGAAATACATAAGCCAAAAT-3' for padi4_96 (19); forward primer: 5'-CTGGCCCAGGCACCACCAG-3' and reverse primer: 5'-AGGGTTTCGGCAGCTGTGCC-3' for padi4_102 (19), and 1 U Taq DNA polymerase (Invitrogen Co., Carlsbad, CA). The amplification protocol comprised initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 60°C for padi4_92, at 52°C for padi4_96, and at 68°C for padi4_102 for 30 sec, extension at 72°C for 30 sec, and final extension at 72°C for 5 min. The 192-base pair (bp) PCR products for padi4_92, 316-bp PCR products for padi4_96, and 400-bp PCR products for padi4_102 were digested at 37°C overnight by restriction enzymes, *Msp* I (Takara Bio, Shiga, Japan), *Hae* III (Toyobo, Osaka, Japan), and *Rsa* I (Toyobo), respectively (19). The digests were subjected

to electrophoresis on a 6% polyacrylamide gel (Nacalai Tesque, Kyoto, Japan) for padi4_92, or on a 2% agarose gel (Nacalai Tesque) for padi4_96 and padi4_102, then stained with ethidium bromide (Nacalai Tesque) and visualized with UV transilluminator (Alpha Innotech, San Leandro, CA).

Statistical analysis

Gender and age values between patients and control subjects were evaluated by chi-square test and unpaired Student's *t*-test, respectively. Expected allele frequencies were calculated from respective single allele frequencies according to the Hardy-Weinberg equilibrium. The observed and expected allele frequencies were compared by chi-square test with Yates' correction using SNP Alyze 6.01 standard (Dynacom Inc., Yokohama, Japan). The frequencies and distributions of haplotype and diplotype were compared between patients and control subjects by logistic regression analysis. SPSS 15.0 program package (SPSS Japan Inc., Tokyo, Japan) was used for all statistical analyses including calculation of odds ratio (OR) with 95% confidence interval (95% CI). A *P* value of 0.05 or less than was considered statistically significant.

Results and Discussion

The distributions of genotypes of each SNP on *PADI4* in each group corresponded to the Hardy-Weinberg equilibrium (Data not shown). We identified 7 haplotypes composed of 3 SNPs among the subjects in this study (Tables 2 and 3). However, the rare frequent haplotypes, Hap 5, Hap 6, and Hap 7, were excluded in Tables 2 and 3. The frequencies and distributions of haplotypes in our study population corresponded to that in previous studies on association with RA in other Japanese populations (10,20). These results imply that the population studied had a homogeneous genetic background.

The frequency of haplotype 1 was significantly decreased in patients with UC, compared to that in control subjects ($P = 0.037$; OR = 0.702) (Table 2). While, the frequency of haplotype 2 was significantly increased in patients with UC, compared to that in control subjects ($P = 0.003$; OR = 1.722) (Table 2). However, there were no differences in the frequency of haplotypes between patients with CD and control subjects (Tables 3).

With regard to genotype, referred to as diplotype, of *PADI4*, 12 diplotypes composed of 7 haplotypes were identified (Tables 4 and 5). However, the rare frequent diplotypes, Hap 2/Hap 6, Hap 3/Hap 3, Hap 3/Hap 3, Hap 3/Hap 6, and Hap 4/Hap 4, were excluded in Tables 4 and 5. Of a total of 114 patients with UC, 15 (13.2%) had a diplotype homozygous for

haplotype 2 (Hap 2/2 in Table 4), the frequency being significantly higher than in control subjects (9/200, 4.5%; $P = 0.008$, OR = 3.215) (Table 4). In contrast, although statistical analysis did not show to be significant, it was a tendency that the frequency of UC patients possessing haplotype 1 homozygosity (Hap 1/1 in Table 4) was lower than that of control subjects (34/114, 29.8% vs. 81/200, 40.5%; $P = 0.060$, OR = 0.624)(Table 4). Thus, this is the first report on the association of *PADI4* haplotypes as well as diplotypes with IBD.

Moreover, the frequency of another diplotype heterozygous for haplotype 1 and haplotype 4 (Hap 1/4 in Table 5) in CD was significantly lower than that in Control (3/83, 3.6% vs. 23/200, 11.5%; $P = 0.048$, OR = 0.289) (Table 5). With respect to CD, it remains to be confirmed why the diplotype, Hap 1/Hap 4 is associated with non-susceptibility to CD. It is one of the possibilities that these CD patients might become complicated by UC in the future, since pathoetiology of certain CD patients would be closely similar to that of UC patients in spite of unknown molecular mechanisms (21).

Again, we analyzed the distributions of haplotypes and diplotypes of *PADI4* among UC and CD subgroups (Tables 6 and 7). There were no significant differences in frequencies of the haplotypes and diplotypes among the subgroups of each IBD subtype.

PDAI enzymes post-translationally catalyze the conversion of arginine residues into

citrulline (22). Citrullinated epitopes are involved in a peptide linkage and are the most specific targets of RA-related autoantibodies (23-25). In fact, anti-cyclic citrullinated peptides (anti-CCPs) antibody has been specifically observed in the sera of RA patients and their presence is a useful diagnostic marker for RA (26). Suzuki and colleagues have identified that a haplotype (haplotype 2) of *PADI4* on IBD locus at 1p36 was closely associated with susceptibility to RA (10). *PADI4* mRNA from the susceptible haplotype 2 was significantly more stable than that from non-susceptible alleles (10), linking to more enhanced production of the citrullinated peptides including CCPs. With respect to a possible mechanism for the extra cellular immune sampling for PADIs of a cytoplasmic enzymatic activity, Ireland *et al.* demonstrated that when mice were immunized with hen egg-white lysozyme (HEL), a unique cohort of T cells selectively responded to its citrullinated epitopes. That is, dendritic cells and macrophages sampled the extra cellular antigen (HEL), presented the citrullinated ones of HEL bound to the MHC molecule and stimulated modification-specific T cells (27). Thus, the citrullinated proteins are antigenic and presentation of the citrullinated peptides-MHC complex is a feature of immune response to the protein antigens. It has been reported that PDAIs are exclusively expressed in synovial tissues, neutrophils and mononuclear cells infiltrating into arthritic joints, where the

citrullinated peptides can be overproduced, especially in the subjects with haplotype 2 of *PADI4* (25, 29), and, then processed into the CCP-MHC complex in such antigen-presenting cells and may serve as autoanti-antigens. Although serologic examinations including anti-CCPs antibody and anti-neutrophil cytoplasmic antibody (ANCA) were not performed in this study and ANCA antibody is associated with UC (30), there are no reports on a correlation between circulating anti-CCPs antibody and IBD. Furthermore, it is well known that autoimmune phenomena are involved in the pathogenesis of IBD (1,2,31). For example, serum and mucosal autoantibodies against intestinal epithelial cells, human tropomyosin fraction V, and human neutrophil cytoplasm are present in UC (30, 31); this implies that *PADI4* haplotypes may be associated with the onset and/or development of UC through an autoimmune reaction against citrullinated self-peptides.

The present study has several limitations. Enzyme activities of PADI4 and other PADI4 isoforms, serologic examinations including anti-CCPs and ANCA antibodies, and polymorphisms of other PADI4 isoforms should be elucidated. The number of subjects was relatively small. Therefore, the association observed here needs to be confirmed in a larger sample of Japanese patients, as well as in other populations. With regard to other populations, Asian studies in Japan and Korea (10,20,32) have shown a close correlation between the

PADI4 haplotypes and RA, but not in Caucasian RA patients (33-35). Inversely, mutations of the caspase activating recruitment domain 15/nucleotide oligomerization domain 2 gene *(CARD15/NOD2)* at 16q12 (IBD1 locus) were associated with Crohn's disease in the Caucasian, but not in the Japanese (36-39). These findings may be due to different genetic background between the races. Even if there is a difference in the molecular mechanism of the pathogenesis of UC between Caucasian and Japanese patients, our results suggest that *PADI4* may be one of genetic determinants of UC in the Japanese population, or that another new UC-determinant gene except *PADI4* may exist near the *PADI4* locus at 1p36 (IBD7 locus).

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Tables

Table 1: The characteristics of subjects studied

Characteristics	Patients with		Control
	UC	CD	
Number	114	83	200
Age (yrs)	44.2 ± 16.7*	34.3 ± 12.5	32.5 ± 11.2
Age range (yrs)	14-83	17-75	20-60
Male/female (%)	59/55 (51.7/48.3)	50/33 (60.2/39.8)	125/75 (62.5/37.5)
Age at onset			
<40 y	39**	11	
≥40 y	75	72	
Extent of UC			
Proctitis	14		
Left sided UC	43		
Pancolitis	57		
Location of CD			
Ileal		16	
Colonic		11	
Ileocolonic		55	
Upper		1	
Disease severity			
Mild	51	17	
Moderate	38	45	
Severe	21	9	
Unknown	4	12	
Disease activity			
Active	63	54	
Inactive	48	17	
Unknown	3	12	
Behavior of CD***			
Stricturing		44	
Penetrating		40	
Perianal diseases		36	

P<0.01, as compared with controls* and CD**, ***, number of the affected patients.

Table 2: The distributions and association of haplotypes of *PDAI4* between UC patients and control subjects

Haplotype	SNP ID (padi4_X)			Number (%) of haplotypes in*		Haplotype comparison*	
(Ref 10)	92	96	102	UC	Control	OR (95%CI)	*P*
Hap 1	C	T	C	126 (55.3)	255 (63.8)	0.702 (0.504-0.978)	0.037
Hap 2	G	C	C	76 (33.3)	90 (22.5)	1.722 (1.199-2.473)	0.003
Hap 3	G	C	T	14 (6.2)	22 (5.5)	1.124 (0.563-2.243)	0.740
Hap 4	G	T	C	11 (4.8)	30 (7.5)	0.625 (0.307-1.273)	0.195
Others				1 (0.4)	3 (0.7)	—	—
Total number of haplotypes				228	400		

*Each haplotype was compared with other haplotypes combined by logistic regression analysis.

Table 3: The distributions and association of haplotypes of *PDAI4* between CD patients and control subjects

Haplotype	SNP ID (padi4_X)			Number (%) of haplotypes in		Haplotype comparison*	
(Ref 10)	92	96	102	CD	Control	OR (95% CI)	*P*
Hap 1	C	T	C	100 (60.3)	255 (63.8)	0.862 (0.594-1.249)	0.432
Hap 2	G	C	C	47 (28.3)	90 (22.5)	1.360 (0.594-1.249)	0.142
Hap 3	G	C	T	10 (6.0)	22 (5.5)	1.101 (0.510-2.380)	0.806
Hap 4	G	T	C	8 (4.8)	30 (7.5)	0.624 (0.280-1.392)	0.250
Others				1 (0.6)	3 (0.7)	—	—
Total number of haplotypes				166	400		

*Each haplotype was compared with other haplotypes combined by logistic regression analysis.

Table 4: The distributions and association of diplotypes of *PDAI4* between UC patients and control subjects

Diplotype	Number (%) of diplotypes in		diplotype comparison*	
	UC	Control	OR (95% CI)	*P*
Hap 1/1	34 (29.8)	81 (40.5)	0.624 (0.382-1.020)	0.060
Hap 1/2	41 (36.0)	57 (28.5)	1.409 (0.863-2.301)	0.171
Hap 1/3	8 (7.0)	13 (6.5)	1.086 (0.436-2.704)	0.860
Hap 1/4	9 (7.9)	23 (11.5)	0.660 (0.294-1.479)	0.313
Hap 2/2	15 (13.2)	9 (4.5)	3.215 (1.359-7.609)	0.008
Hap 2/3	3 (2.6)	7 (3.5)	0.745 (0.189-2.940)	0.674
Hap 2/4	2 (1.7)	5 (2.5)	0.696 (0.133-3.649)	0.669
Others	2 (1.7)	5 (2.5)	—	—
Total number	114	200		

*Each diplotype was compared with other diplotypes combined by logistic regression analysis.

Table 5: The distributions and association of diplotypes of *PDAI4* between CD patients and control subjects

Diplotype	Number (%) of diplotypes in		diplotype comparison*	
	CD	Control	OR (95% CI)	*P*
Hap 1/1	33 (39.8)	81 (40.5)	0.970 (0.575-1.635)	0.908
Hap 1/2	26 (31.3)	57 (28.5)	1.144 (0.656-1.996)	0.635
Hap 1/3	5 (6.0)	13 (6.5)	0.922 (0.318-2.674)	0.881
Hap 1/4	3 (3.6)	23 (11.5)	0.289 (0.084-0.989)	0.048
Hap 2/2	7 (8.4)	9 (4.5)	1.955 (0.703-5.436)	0.199
Hap 2/3	4 (4.8)	7 (3.5)	1.396 (0.398-4.902)	0.603
Hap 2/4	3 (3.6)	5 (2.5)	1.463 (0.341-6.265)	0.609
Others	2 (2.4)	5 (2.5)	—	—
Total number	83	200		

*Each diplotype was compared with other diplotypes combined by logistic regression analysis.

Table 6: The distributions of haplotypes of *PDAI4* with respect to subtypes of UC and CD patients.

		Number (%) of haplotypes in					
		Hap1	Hap2	Hap3	Hap4	Others	Total
UC	Extent						
	Proctitis	16 (57.1)	9 (32.1)	2 (7.1)	1 (3.6)	0 (0)	28
	Left sided UC	47 (54.7)	30 (34.9)	2 (2.3)	7 (8.1)	0 (0)	86
	Pancolitis	63 (55.3)	37 (32.5)	10 (8.8)	3 (2.6)	1 (0.9)	114
	Disease severity						
	Mild	56 (54.9)	38 (37.3)	4 (3.9)	4 (3.9)	0 (0)	102
	Moderate	39 (51.3)	24 (31.6)	9 (11.8)	4 (5.3)	0 (0)	76
	Severe	24 (57.1)	13 (31.0)	1 (2.4)	3 (7.1)	1 (2.4)	42
	Unknown	7 (87.5)	1 (12.5)	0 (0)	0 (0)	0 (0)	8
	Disease activity						
	Active	70 (55.6)	41 (32.5)	6 (4.8)	8 (6.3)	1 (0.8)	126
	Inactive	51 (53.1)	34 (35.4)	8 (8.3)	3 (3.1)	0 (0)	96
	Unknown	5 (83.3)	1 (16.7)	0 (0)	0 (0)	0 (0)	6
CD	Location						
	Ileal	17 (53.1)	11 (34.4)	0 (0)	4 (12.5)	0 (0)	32
	Colic	13 (59.1)	7 (31.8)	1 (4.5)	1 (4.5)	0 (0)	22
	Ileocolic	69 (62.7)	29 (26.4)	8 (7.3)	3 (2.7)	1 (0.9)	110
	Upper	1 (50.0)	0 (0)	1 (50.0)	0 (0)	0 (0)	2
	Disease severity						
	Mild	16 (47.1)	13 (38.2)	1 (2.9)	4 (11.8)	0 (0)	34
	Moderate	54 (60.0)	26 (28.9)	7 (7.8)	3 (3.3)	0 (0)	90
	Severe	13 (72.2)	2 (11.1)	2 (11.1)	0 (0)	1 (5.6)	18
	Unknown	17 (70.8)	6 (25.0)	0 (0)	1 (4.2)	0 (0)	24
	Disease activity						
	Active	67 (62.0)	28 (25.9)	9 (8.3)	3 (2.8)	1 (0.9)	108
	Inactive	16 (47.1)	13 (38.2)	1 (2.9)	4 (11.8)	0 (0)	34
	Unknown	17 (70.8)	6 (25.0)	0 (0)	1 (4.2)	0 (0)	24
	Stricturing						
	Present	55 (62.5)	23 (26.1)	5 (5.7)	4 (4.5)	1 (1.1)	88
	Absent	45 (57.7)	24 (30.8)	5 (6.4)	4 (5.1)	0 (0)	78
	Penetrating						
	Present	49 (61.3)	23 (28.8)	5 (6.3)	2 (2.5)	1 (1.3)	80
	Absent	51 (59.3)	24 (27.9)	5 (5.8)	6 (7.0)	0 (0)	86
	Perianal diseases						
	Present	43 (59.7)	19 (26.4)	6 (8.3)	4 (5.6)	0 (0)	72
	Absent	57 (62.0)	26 (28.3)	4 (4.3)	4 (4.3)	1 (1.1)	92
	Unknown	0 (0.0)	2 (100.0)	0 (0)	0 (0)	0 (0)	2

Table 7: The distributions of diplotypes of *PDAI4* with respect to subtypes of UC and CD patients.

		Number (%) of diplotypes in								
		Hap 1/1	Hap 1/2	Hap 1/3	Hap 1/4	Hap 2/2	Hap 2/3	Hap 2/4	Others	Total
UC	Extent									
	Proctitis	6 (42.9)	2 (14.3)	2 (14.3)	0 (0)	3 (21.4)	0 (0)	1 (7.1)	0 (0)	14
	Left sided UC	11 (25.6)	18 (41.9)	1 (2.3)	6 (14.0)	5 (11.6)	1 (2.3)	1 (2.3)	0 (0)	43
	Pancolitis	17 (29.8)	21 (36.8)	5 (8.8)	3 (5.3)	7 (12.3)	2 (3.5)	0 (0)	2 (3.5)	57
	Disease severity									
	Mild	17 (33.3)	16 (31.4)	3 (5.9)	3 (5.9)	10 (19.6)	1 (2.0)	1 (2.0)	0 (0)	51
	Moderate	8 (21.5)	15 (39.5)	5 (13.2)	3 (7.9)	3 (7.9)	2 (5.3)	1 (2.6)	1 (2.6)	38
	Severe	6 (28.6)	9 (42.9)	0 (0)	3 (14.3)	2 (9.5)	0 (0)	0 (0)	1 (4.8)	21
	Unknown	3 (75.0)	1 (25.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4
	Disease activity									
	Active	19 (30.2)	20 (31.7)	5 (7.9)	7 (11.1)	10 (15.9)	0 (0)	1 (1.6)	1 (1.6)	63
	Inactive	13 (27.1)	20 (41.7)	3 (6.3)	2 (4.2)	5 (10.4)	3 (6.3)	1 (2.1)	1 (2.1)	48
	Unknown	2 (66.7)	1 (33.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3
CD	Location									
	Ileal	5 (31.3)	7 (43.8)	0 (0)	0 (0)	1 (6.3)	0 (0)	2 (12.5)	1 (6.3)	16
	Colic	4 (36.4)	3 (27.3)	1 (9.1)	1 (9.1)	2 (18.2)	0 (0)	0 (0)	0 (0)	11
	Ileocolic	24 (43.6)	16 (29.1)	3 (5.5)	2 (3.6)	4 (7.3)	4 (7.3)	1 (1.8)	1 (1.8)	55
	Upper	0 (0)	0 (0)	1 (100.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1
	Disease severity									
	Mild	2 (11.8)	9 (52.9)	1 (5.9)	2 (11.8)	2 (11.8)	0 (0)	0 (0)	1 (5.9)	17
	Moderate	19 (42.2)	12 (26.7)	3 (6.7)	1 (2.2)	4 (8.9)	4 (8.9)	2 (4.4)	0 (0)	45
	Severe	5 (55.6)	2 (22.2)	1 (11.1)	0 (0)	0 (0)	0 (0)	0 (0)	1 (11.1)	9
	Unknown	7 (58.3)	3 (25.0)	0 (0)	0 (0)	1 (8.3)	0 (0)	1 (8.3)	0 (0)	12
	Disease activity									
	Active	24 (44.4)	14 (25.9)	4 (7.4)	1 (1.9)	4 (7.4)	4 (7.4)	2 (3.7)	1 (1.9)	54
	Inactive	2 (11.8)	9 (52.9)	1 (5.9)	2 (11.8)	2 (11.8)	0 (0)	0 (0)	1 (5.9)	17
	Unknown	7 (58.3)	3 (25.0)	0 (0)	0 (0)	1 (8.3)	0 (0)	1 (8.3)	0 (0)	12
	Stricturing									
	Present	19 (43.2)	13 (29.5)	2 (4.5)	2 (4.5)	3 (6.8)	2 (4.5)	2 (4.5)	1 (2.3)	44
	Absent	14 (35.9)	13 (33.3)	3 (7.7)	1 (2.6)	4 (10.3)	2 (5.1)	1 (2.6)	1 (2.6)	39
	Penetrating									
	Present	16 (40.0)	13 (32.5)	2 (5.0)	2 (5.0)	4 (10.0)	2 (5.0)	0 (0)	1 (2.5)	40
	Absent	17 (39.5)	13 (30.2)	3 (7.0)	1 (2.3)	3 (7.0)	2 (4.7)	3 (7.0)	1 (2.3)	43
	Perianal diseases									
	Present	15 (41.7)	9 (25.0)	3 (8.3)	1 (2.8)	3 (8.3)	3 (8.3)	1 (2.8)	1 (2.8)	36
	Absent	18 (39.1)	17 (37.0)	2 (4.3)	2 (4.3)	3 (6.5)	1 (2.2)	2 (4.3)	1 (2.2)	46
	Unknown	0 (0)	0 (0)	0 (0)	0 (0)	1 (100.0)	0 (0)	0 (0)	0 (0)	1