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ORIGINAL ARTICLE

SMALL BOWEL LESIONS DETECTED WITH WIRELESS CAPSULE ENDOSCOPY IN PATIENTS WITH ACTIVE ULCERATIVE COLITIS AND WITH POST-PROCTOCOLECTOMY

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Background: Although rare, duodenal lesions have been reported in association with ulcerative colitis (UC); however, there have been very few reports on small bowel lesions, and many aspects of their pathology and frequency remain unknown. This study determined whether small bowel lesions are present in UC by using wireless capsule endoscopy (WCE).
Patients and Methods: WCE was performed on 20 patients with active UC and 10 who had undergone proctocolectomy.
Results: Small bowel lesions (e.g. edema or ulcers) were observed in 11 of the 30 patients (36.6%): in eight (40%) of the 20 patients with active UC and in three (33.3%) of the 10 post-proctocolectomy patients. Ulcers that extended over a long segment or whole tertile of the small bowel were observed in five patients, and the disease type was extensive colitis in three of these and pouchitis in the other two. Age at onset was significantly lower in the 20 active UC patients that had small bowel lesions.
Conclusion: WCE revealed the presence of ulcers that extended over a long segment or a whole tertile in the small bowel in active extensive colitis and pouchitis. In future, it will be necessary to assess the clinical significance of small bowel lesions in UC in detail.

Key words: ulcerative colitis, pouchitis, proctocolectomy, wireless capsule endoscopy, small bowel lesion.

INTRODUCTION

In contrast to Crohn's disease (CD), which gives rise to lesions throughout the gastrointestinal tract, ulcerative colitis (UC) only causes diffuse inflammation in the mucosa of the large bowel. However, in recent years, there have been scattered reports of gastric and duodenal lesions that do not conform to the disease concept of UC, and reports by ourselves[1] and Hori *et al.*[2] have shown that 4.7–7.6% of UC cases are complicated by ulcerative gastroduodenal lesions associated with UC. The disease type of the UC with such lesions is active extensive or post-proctocolectomy. Complications of UC by small bowel lesions, such as backwash ileitis and post-proctocolectomy pouchitis, have been known of for a long time, whereas widespread diffuse small bowel lesions have been reported only rarely.[3–5] Inflammation proximal to the pouch in the neo-terminal ileum, so-called pre-pouch ileitis, has also been observed.[6,7]

In the past, great importance was attached to upper gastrointestinal lesions as an aid to the diagnosis of CD, and the presence of small bowel lesions was even thought to rule out UC. They were said to be particularly useful in making the differential diagnosis from inflammatory bowel disease of unclassified type (IBDU).[8,9] However, when bowel lesions outside the large bowel are also present in definitively diagnosed, classical UC, the disease concepts of CD and UC and differentiation between them become complicated.

We previously reported three cases of UC in which we performed wireless capsule endoscopy (WCE) and observed small bowel lesions.[10] In the present study, we used WCE to assess the presence and distribution of small bowel lesions in patients who were in the active phase of UC and in UC patients who had undergone proctocolectomy.

METHODS

Patients

The patients were being treated at our hospital for active UC or had undergone proctocolectomy for UC. The study was conducted from November 2008 to December 2009. The diagnosis of UC was based on clinical, endoscopic, radiological, and histological criteria. We investigated 20 active UC and 10 post-proctocolectomy patients. The patients were enrolled regardless of their clinical manifestations or the nature of their treatment, and fulfilled the following criteria after undergoing lower gastrointestinal tract endoscopy: (i) endoscopic activity and clinical activity indicated active-phase UC or those who had undergone proctocolectomy for UC; (ii) bacterial infection was ruled out by stool culture; (iii) there was no history of taking non-steroidal anti-inflammatory drugs within the previous 6 months; and (iv) there was no history of taking antibiotics within the previous 2 months.

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The clinical activity of the disease was classified based on the Truelove and Witts severity index[11] and the clinically active stage was defined as moderate and severe. The endoscopic activity was determined based on the Baron score[12] and the endoscopically active stage was defined as grade >1. The modified pouchitis disease activity index[13] was used to determine the activity of pouchitis.

Ethical considerations

This study was approved by the Institutional Review Board of Fukuoka University Chikushi Hospital. Written informed consent was obtained from all participants.

WCE procedure

WCE was performed with a PillCam SB (GIVEN Imaging Ltd, Yoqneam, Israel) in all studies. In UC patients with active disease, endoscopic examinations and pre-examination preparation, such as the use of purgatives, have the potential of exacerbating intestinal lesions. We therefore decided to perform CE in all cases without any pre-examination preparation, because no CE study in UC patients has ever been performed before and we could not exclude the possibility of pre-examination preparation, such as the use of purgatives, causing deterioration of the lesions in the intestinal tract.

We used RAPID 5 software (GIVEN Imaging Ltd) to analyze the findings, and we scored them according to the Lewis scoring system.[14] All WCE images were reviewed by a single experienced endoscopist who was unaware of the clinical information. This scoring index was based on three CE variables: villous appearance, ulceration, and stenosis. Values for each parameter were created by using the descent gradient methodology. Villous appearance was defined as edema in which villous width was equal to or greater than villous height. Ulcers were defined as mucosal breaks with a white or yellow base surrounded by a red or pink collar (Fig. 1). Lesions were classified according to number as single, few (2–7), or multiple (≥8). Small bowel transit time was divided into three equal parts or tertiles. If a capsule did not enter the colon or the patients had undergone proctocolectomy, the small bowel transit time was calculated to the last image obtained. Small bowel segment length involvement was defined by the percentage of a particular tertile that was involved with the mucosal change. A short segment was defined as ≤10% of a tertile. A long segment was defined as 11–50% of a tertile, and a whole segment was defined as >50% of a tertile.

Statistical analysis

Comparison of clinical manifestations with small bowel lesions was assessed with the Student's *t*-test, the Wilcoxon ranked test, the χ^2-test and the Cochran–Mantel–Haenszel test. The probability (*P*-value) in each test of significance was judged at the significance level after adjustment with Bonferroni correction.

RESULTS

We cared for 307 UC patients and 41 post-proctocolectomy patients during the study period. There were 116 patients with clinically active UC. Seventy-nine UC patients and 23 post-proctocolectomy patients underwent baseline lower gastrointestinal tract endoscopy in our hospital. There were 51 patients with endoscopically active UC. Thirty of these 102 patients were enrolled in this study (Fig. 2). There were 20 active UC cases (extensive colitis in 11, left-sided colitis in five, and proctosigmoiditis in four) and 10 post-proctocolectomy cases (pouchitis in two, and non-pouchitis

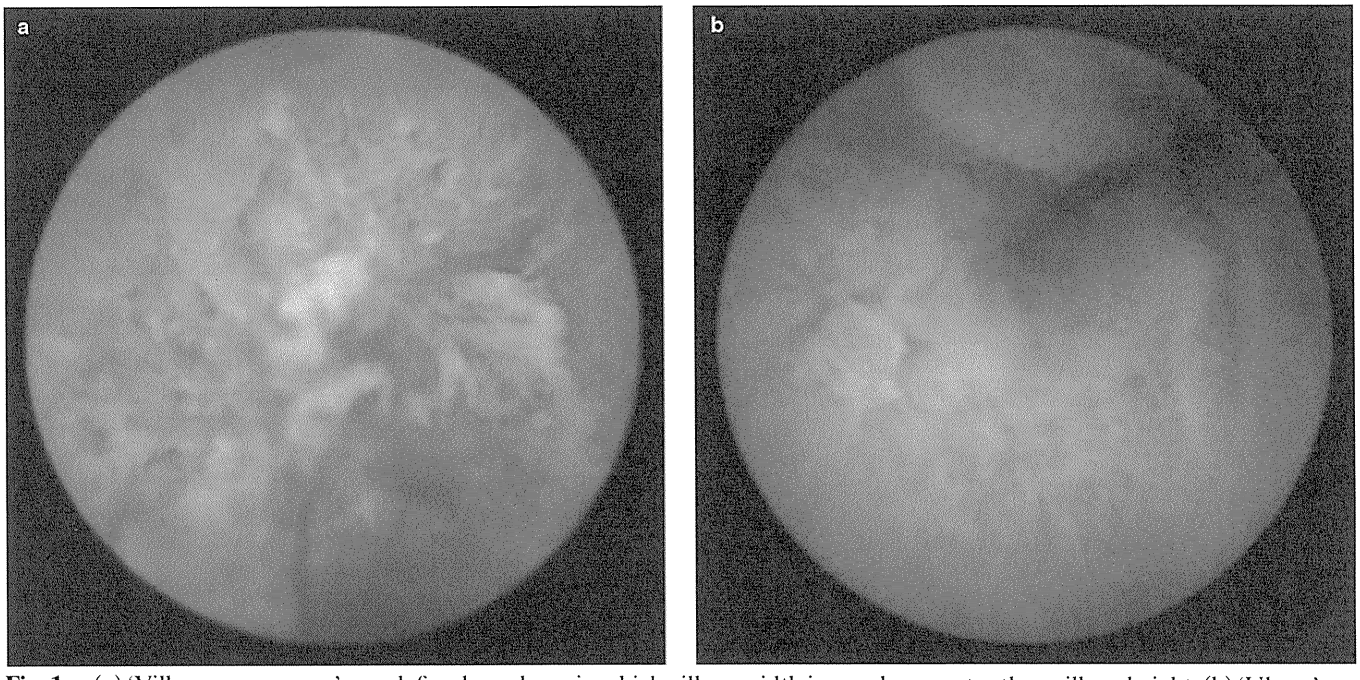

Fig. 1. (a) 'Villous appearance' was defined as edema in which villous width is equal or greater than villous height. (b) 'Ulcers' were defined as mucosal breaks having a white or yellow base surrounded by a red or pink collar.

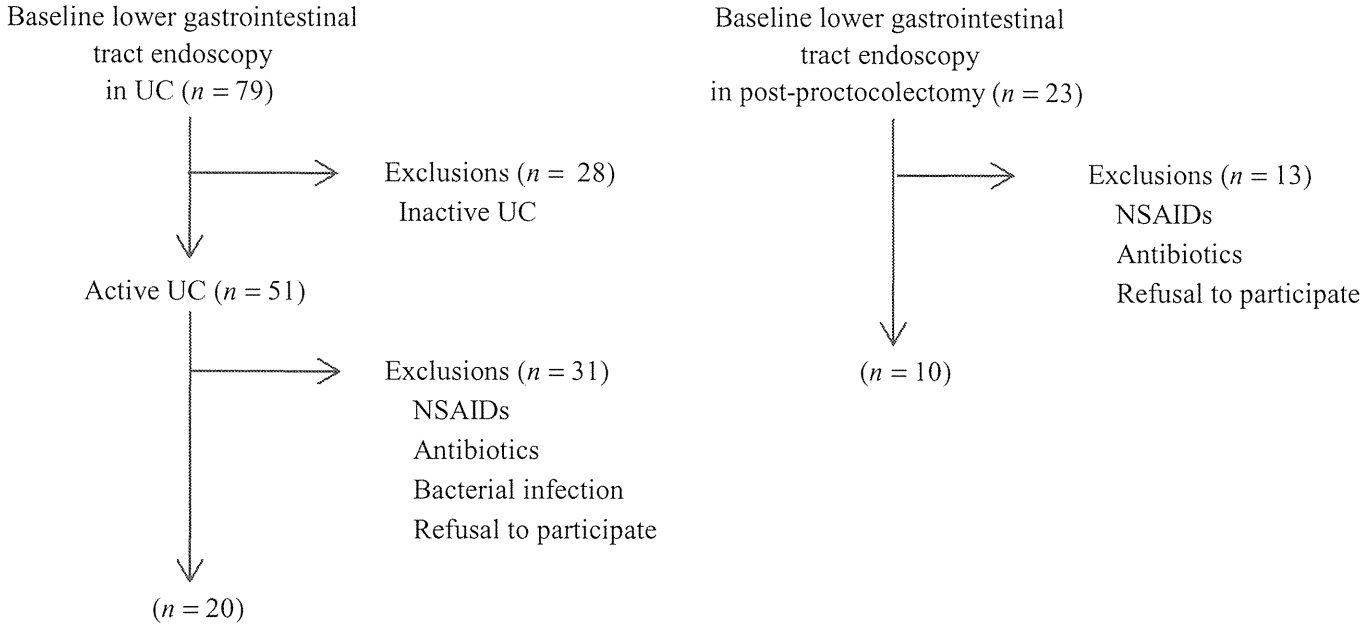

Fig. 2. Flow diagram of included and excluded patients. NSAIDs, non-steroidal anti-inflammatory drugs; UC, ulcerative colitis.

in eight). The male to female ratio was 16:14, and mean age was 37.3 ± 16.5 years. Mean age at onset of symptoms was 30.6 ± 12.3 years, and mean duration of illness was 6.7 ± 6.5 years. None of the patients had stenotic or tumorous lesions, and there were no complications, such as retention of the CE.

Small bowel lesions (e.g. edema or ulcers) were observed in 11 (36.6%) of the 30 patients, eight (40%) with active UC and three (33.3%) post-proctocolectomy patients. All of the 11 patients with small bowel lesions were negative for serum *Helicobacter pylori* immunoglobulin G antibody titer test. The endoscopic findings and scores of these 11 patients are shown in Table 1. The site of the lesions in the seven patients with extensive colitis was in the first tertile in six cases, the second tertile in three, and the third tertile in three, and the site of the lesions in the single case of proctosigmoiditis was in the second tertile. The site of the lesions in the three post-proctocolectomy cases was in the first tertile in one case, the second tertile in one, and the third tertile in three.

Ulcers were observed in eight patients (cases 1–3 and 7–11), but the circumferential extent of the ulcers was less than one-quarter in every case. Ulcers that extended over a long segment or a whole tertile were observed in five patients (1–3, 9 and 10), and the disease type was extensive colitis in three cases and pouchitis in two. Ulcers that started in the duodenum were observed in all three cases of extensive colitis (1–3) (Fig. 3), and in one of these, the lesions extended throughout the entire small bowel (Fig. 4).

We compared the clinical findings of 20 active UC and 10 post-proctocolectomy patients with and without small bowel lesions. The eight patients with small bowel lesions and the 12 without lesions were compared with active UC patients (Table 2). The significance level after adjustment with Bonferroni correction was 0.625%, and there were significant differences only in the age at onset between the UC patients with and without small bowel lesions. The three cases with small bowel lesions and the seven without lesions were compared with post-proctocolectomy patients (Table 3). The small bowel lesions were present in both of the pouchitis cases (Fig. 5) and in one of the eight (12.5%) non-pouchitis cases (Fig. 6). The significance level after adjustment with Bonferroni correction was 0.833%, and there were no obvious differences between the post-proctocolectomy patients with and without small bowel lesions.

DISCUSSION

Small bowel lesions were observed in 11 (36.6%) of the 30 patients in this study, and ulcers that extended over a long segment or a whole tertile in the small bowel were observed in five patients. As its name indicates, UC is a disease that has been considered to give rise to diffuse inflammation in the mucosa of the large bowel alone, and it is a disease that basically does not cause lesions to develop in the small bowel. It is said to be possible to diagnose IBD, particularly CD, based on the presence of multiple aphthae or ulcers in the small bowel detected by WCE, and WCE has been reported to be a useful method of examination.[15–17] It might be possible to classify the early endoscopic manifestations of small bowel CD on capsule images as notching of folds, a few small aphthous ulcers, larger ulcers, linear ulcers, circumferential involvement, abnormal vasculitis, and even a cobblestone appearance, before involvement can be demonstrated radiologically.[18] Recent studies have reported that the diagnostic yield of WCE can range between 43% and 71%.[19] Based on such reports, CE has been reported[8,9] to be useful in making the differential diagnosis in cases in which the diagnosis called 'indeterminate colitis' or 'inflammatory bowel disease of unclassified type (IBDU)' in Western countries is difficult to make. Whether small bowel lesions are present in UC or whether such cases should be diagnosed as IBDU or CD is an important issue. Mehdizadeh *et al.*[19] reported the diagnostic yield of WCE in CD patients, and 39% had active lesions in

Table 1. Endoscopic findings and Lewis score

Case no.	Type of disease	Score	First tertile	Second tertile	Third tertile
1	Extensive colitis	1540	1540 V: edematous, whole, patchy U: multiple, long, <1/4	280 V: edematous, whole, patchy –	135 – U: single, short, <1/4
2	Extensive colitis	1104	1104 V: edematous, long, diffuse U: multiple, long, <1/4	1068 V: edematous, long, patchy U: multiple, long, <1/4	0 – –
3	Extensive colitis	1015	565 V: edematous, whole, diffuse U: few, short, <1/4	415 V: edematous, whole, patchy U: single, short, <1/4	1015 V: edematous, whole, diffuse U: few, whole, <1/4
4	Extensive colitis	340	340 V: edematous, whole, diffuse –	0 – –	0 – –
5	Extensive colitis	204	204 V: edematous, long, diffuse –	0 – –	0 – –
6	Extensive colitis	168	168 V: edematous, long, patchy –	0 – –	0 – –
7	Extensive colitis	135	0 – –	0 – –	135 – U: single, short, <1/4
8	Proctosigmoiditis	135	0 – –	135 – U: single, short, <1/4	0 – –
9	Post-proctocolectomy (pouchitis)	1015	0 – –	0 – –	1015 V: edematous, whole, diffuse U: few, whole, <1/4
10	Post-proctocolectomy (pouchitis)	1012	0 – –	1012 V: edematous, short, patchy U: multiple, long, <1/4	562 V: edematous, short, patchy U: multiple, short, <1/4
11	Post-proctocolectomy (non-pouchitis)	143	135 – U: single, short, <1/4	0 – –	143 V: edematous, short, single U: single, short, <1/4

V,U: number, longitudinal extent, descriptors.
U, ulcer; V, villous appearance.

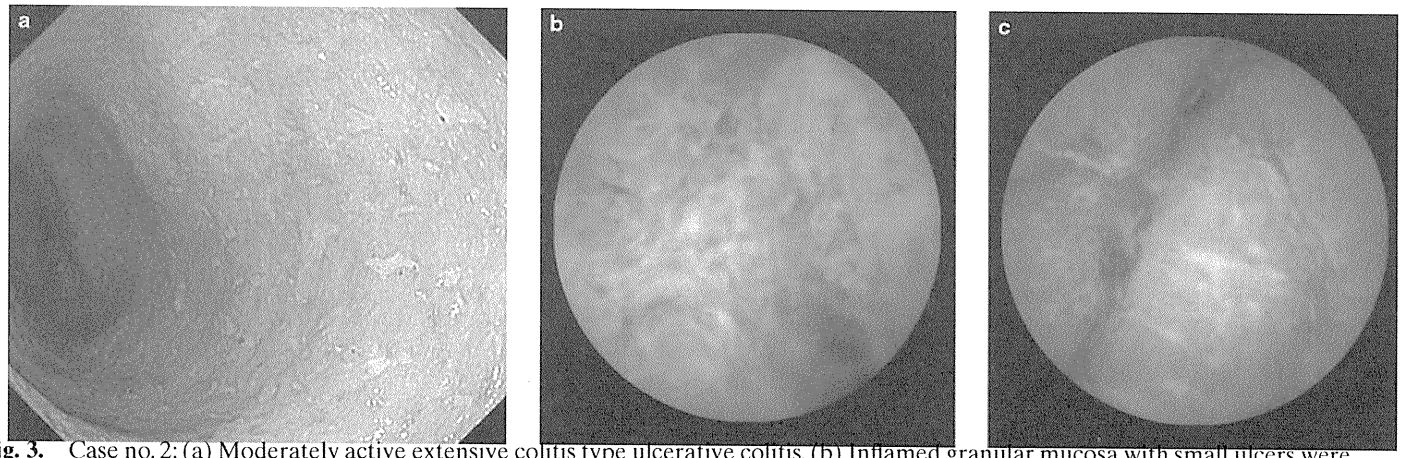

Fig. 3. Case no. 2: (a) Moderately active extensive colitis type ulcerative colitis. (b) Inflamed granular mucosa with small ulcers were seen in the duodenum of the first tertile. (c) Mucosal edema and shallow ulcers were observed in the jejunum of the first tertile.

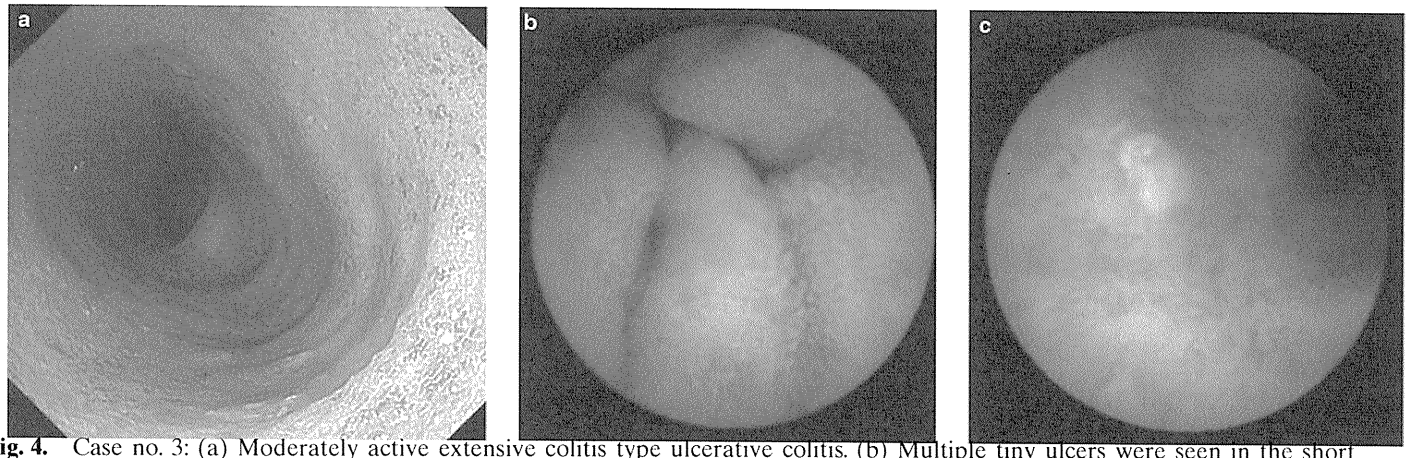

Fig. 4. Case no. 3: (a) Moderately active extensive colitis type ulcerative colitis. (b) Multiple tiny ulcers were seen in the short segment of the first tertile. (c) Mucosal edema with a few ulcers were observed throughout a whole segment of the third tertile.

the small intestine. The WCE images that they reported were similar to our WCE images in UC patients. There are some arguments on this similarity, but this remains to be resolved.

Calabrese *et al.* reported that when they performed CE in 15 patients with chronic refractory pouchitis after surgery for UC, they found small bowel lesions in the form of aphthae or erosion, edema, or ulcers in all cases.[20] However, they stated that the meaning of these small bowel lesions is still unclear, although the inflammatory involvement suggests CD. There have been reports of six previous cases of small bowel lesions associated with UC.[3–5,21] All of them were in post-proctocolectomy patients, and granular-like mucosa, erosion, and ulcers resembling the large bowel lesions were observed diffusely and continuously in all of them. In our previous study,[1] the ulcerative gastroduodenal lesions of UC were correlated with pouchitis. The present study also demonstrated that small bowel lesions occurred in two cases of pouchitis and most small bowel lesions were located in the third tertile. It is suggested that pouchitis is predicted by small bowel lesions revealed by WCE. Recently, inflammation proximal to the pouch in the neo-terminal ileum, so-called pre-pouch ileitis, has been reported.[7] Two cases of pouchitis with small bowel lesions might have been pre-pouch ileitis.

However, associations with gastroduodenal lesions that do not fit the disease concept of UC have been reported in recent years.[3–5,22–25] Such clinical features as the following can be cited: (i) findings resembling the lesions in the large bowel, including diffuse and continuous granular-like mucosa, erosions, hemorrhagic fragile mucosa, and ulcers; (ii) improvement in the lesions in response to the same treatment as for UC, for example, treatment with steroids or powdered mesalazine in patients resistant to treatment with antiulcer drugs, such as H2-blockers and proton pump inhibitors; (iii) findings, such as diffuse inflammatory cell infiltration, cryptitis, and crypt abscesses, that resemble the findings in the large bowel in biopsy specimens; and (iv) UC disease types of active extensive colitis or status post-proctocolectomy. Studies by ourselves[1] and Hori *et al.*[2] have shown that 4.7–7.6% of UC cases in Japan are complicated by ulcerative gastroduodenal lesions associated with UC. In the present study, ulcers that started in the duodenum were observed in three of the five patients in whom ulcers extended over a long segment or a whole tertile of the small bowel. When ulcerative gastroduodenal lesions associated with UC are observed, it is necessary to perform a test with the presence of small bowel lesions in mind.

Table 2. Comparison of clinical manifestation in active ulcerative colitis patients with and without small bowel lesion

	With small bowel lesion ($n = 8$)	Without small bowel lesion ($n = 12$)	P-value
Male : Female	2:6	9:3	$P = 0.06$†
Mean age (years)	23.0 ± 9.7	39.0 ± 13.6	$P = 0.01$‡
Age at onset (years)	20.6 ± 6.9	33.8 ± 10.1	$P = 0.005$‡
Duration of disease (years)	2.4 ± 3.0	5.3 ± 4.7	$P = 0.09$§
Type of disease			
Extensive colitis	7	4	$P = 0.03$†
Left-sided colitis + proctosigmoiditis	1	8	
Disease activity			
Baron			
Mild	2	3	$P = 0.88$¶
Moderate	5	8	
Severe	1	1	
Truelove and Witts			
Mild	2	4	$P = 0.7$¶
Moderate	6	8	
Severe	0	0	
Treatment at examination			
Aminosalicylic acid	2	5	$P = 0.19$†
Prednisolone	3	5	
Azathioprine	0	3	
No treatment	3	0	

†χ^2-test.
‡Student's t-test.
§Wilcoxon's ranked test.
¶Cochran–Mantel–Haenszel test.
The significance level in each test was adjusted with Bonferroni correction.

Table 3. Comparison of clinical manifestation in post-proctocolectomy patients with and without small bowel lesion

	With small bowel lesion ($n = 3$)	Without small bowel lesion ($n = 7$)	P-value
Male : Female	2:1	3:4	$P = 1.0$†
Mean age (years)	31.7 ± 12.7	53.0 ± 15.2	$P = 0.07$‡
Age at onset (years)	23.0 ± 12.2	39.7 ± 13.0	$P = 0.09$‡
Duration of disease (years)	8.7 ± 3.5	13.3 ± 8.2	$P = 0.36$§
Disease activity			
Pouchitis (mPDAI ≥ 5)	2	0	$P = 0.07$†
Treatment at examination			
Aminosalicylic acid	0	1	$P = 0.53$†
Prednisolone	1	0	
Azathioprine	0	0	
No treatment	2	6	

†χ^2-test.
‡Student's t-test.
§Wilcoxon's ranked test.
The significance level in each test was adjusted with Bonferroni correction.

CE in the present study also demonstrated the presence of minute lesions in the small bowel in UC, but it is not easy to make a diagnosis of UC-associated small bowel lesions by CE alone, without performing a pathological examination. There has also been a report that 10% of healthy persons have been found to have some sort of mucosal disorder in the small bowel,[26] and a few atypical aphthae and erosions have been found in the small bowel. However, we observed ulcers that extended over a long segment or a whole tertile of the small bowel, similar to the upper gastrointestinal lesions in active extensive colitis and pouchitis. These lesions are suspected of being associated with UC.

The present study had the following limitations: (i) the extent of the lesion was determined on the basis of capsule passage time, and was therefore not always accurate because it could be affected by capsule retention; and (ii) morphological judgment of the lesion was sometimes difficult with WCE. It is desirable to confirm the present findings in an additional study using double balloon enteroscopy and assessment of the histopathological findings from biopsied

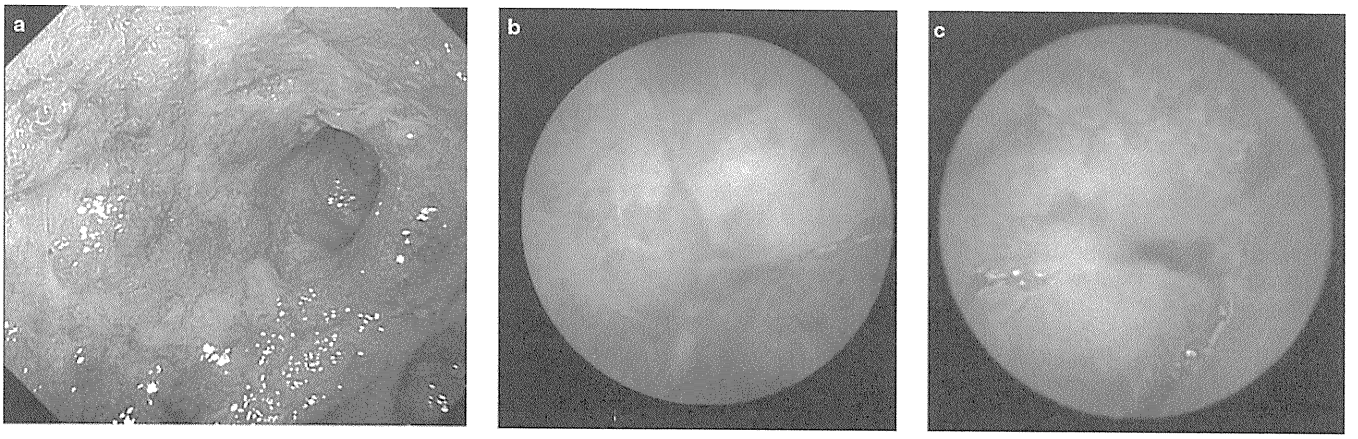

Fig. 5. Case no. 9: (a) Pouchitis. (b, c) Mucosal edema and ulcers were observed throughout a whole segment of the third tertile.

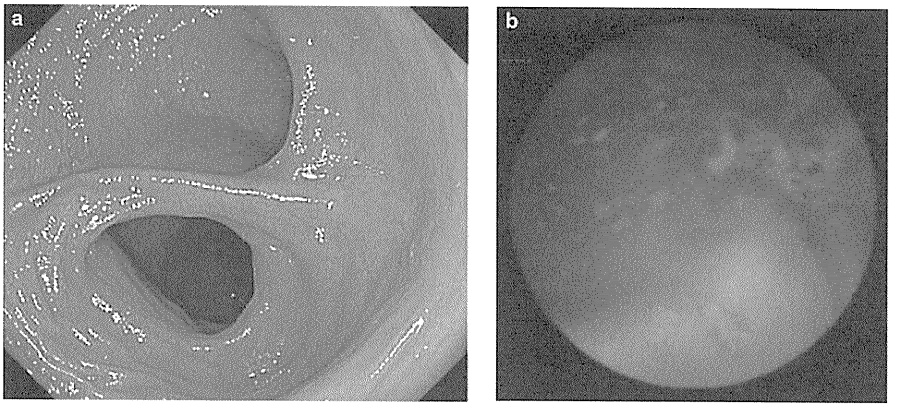

Fig. 6. Case no. 11: (a) Non-pouchitis. (b) A single ulcer was observed in the third tertile.

specimens. Furthermore, ulcers that extended over a long segment or a whole tertile were noted in only three (15%) of the 20 cases of active UC, and this percentage was not particularly high compared to that for healthy individuals (10%). In any event, the number of patients studied was not large enough, and evaluation of additional cases is desirable.

The lesions in UC, especially extensive colitis type, are not limited to the large bowel, and gastroduodenitis and, more rarely, enteritis, occur in UC. This suggests that the inflammation extends to the entire gastrointestinal tract, which is exposed to the pathogenic and inflammatory mechanisms of UC. However, these small bowel lesions seen in UC might be transient. It is possible that these small bowel lesions are not targets for treatment and have little impact in determining the outcome of a patient's large bowel lesions.

Based on the results of our present study using WCE, it is now clear that small bowel lesions are also present in UC, but the results might be non-specific. Further study, including in relation to the course of treatment, long-term course, and association with pouchitis, is necessary with regard to whether the presence of small bowel lesions is associated with UC or whether a diagnosis of IBDU should be made.

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CLINICAL AT

HLA-Cw*1202-B*5201-DRB1*1502 Haplotype Increases Risk for Ulcerative Colitis but Reduces Risk for Crohn's Disease

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BACKGROUND & AIMS: There are many genetic factors that are associated with both ulcerative colitis (UC) and Crohn's disease (CD). However, genetic factors that have distinct effects on UC and CD have not been examined. **METHODS:** We performed a comparative genome-wide association study (GWAS) and a replication study using data from 748 patients with UC and 979 with CD, selected from a Japanese population. We conducted high-resolution (4-digit) genotyping of human leukocyte antigen (HLA) alleles in patients with UC and CD and additional 905 healthy individuals (controls). We performed haplotype-based analysis using data from the GWAS and HLA alleles to associate them with UC or CD. **RESULTS:** The comparative GWAS and the replication study identified significant associations in the major histocompatibility complex region at 6p21 with UC and CD (rs9271366, $P = 1.6 \times 10^{-70}$; odds ratio [OR] = 4.44). Haplotype-based analysis in the major histocompatibility complex region showed that HLA-Cw*1202-B*5201-DRB1*1502 haplotype was significantly associated with increased risk of UC compared with CD ($P = 1.1 \times 10^{-33}$; OR = 6.58), accounting for most of the associations observed in the GWAS. Compared with the controls, this HLA haplotype significantly increases susceptibility to UC ($P = 4.0 \times 10^{-21}$; OR = 2.65), but reduces risk for CD ($P = 1.1 \times 10^{-7}$; OR = 0.40). Distinct effects of this HLA haplotype on UC and CD were independent of other HLA alleles and haplotypes ($P = 2.0 \times 10^{-19}$ and $P = 7.2 \times 10^{-5}$, respectively). **CONCLUSIONS: The HLA-Cw*1202-B*5201-DRB1*1502 haplotype increases susceptibility to UC but reduces risk for CD, based on a GWAS of a Japanese population.**

Keywords: Inflammatory Bowel Disease; Genetics; Risk Factor; Susceptibility.

Ulcerative colitis (UC) and Crohn's disease (CD), the 2 main subtypes of inflammatory bowel disease (IBD), are chronic relapsing inflammatory disorders of the digestive tract. Although aberrant responses of the intestinal immune system in genetically predisposed individuals play an important role in the pathogenesis of both diseases, typical features of UC and CD differ with respect to disease localization and histological findings.[1,2] CD most commonly involves the ileum and colon, but can affect any region of the gut, whereas UC always involves the rectum and extends as far as the cecum. Pathologically, inflammatory change is transmural and often discontinuous in CD, but it typically involves only superficial mucosal and submucosal layers of the intestinal wall with a continuous pattern in UC. Moreover, Th1- and Th17-associated cytokines are markedly increased in the inflamed mucosa of CD, whereas Th2-associated cytokines seem to be increased in that of UC.[3] These findings suggest that some genetic or environmental factors that differentiate UC and CD might exist.

A number of genome-wide association studies (GWAS) have identified numerous susceptibility loci for UC and CD.[4-17] Among them, *NKX2-3* and multiple genes involved in the interleukin-23 signaling pathway have been reported to be associated with both UC and CD.[16,18-20] In contrast, alterations in genes of innate immune system and autophagy are considered to be specific to CD.[14,18,19] However, current evidence is insufficient to explain the differences in the clinical manifestations between UC and CD. Previous studies have shown that the same alleles in particular genes sometimes have opposite directions of effects on different autoimmune disorders.[15,21] Therefore, finding additional variants with distinct effects on UC and CD will provide important clues for further understanding of the pathogenesis of both diseases.

To identify the genetic factors that have distinct role between UC and CD, we performed a comparative GWAS that directly compared UC and CD cases in the Japanese

Abbreviations used in this paper: CD, Crohn's disease; CI, confidence interval; DC, dendritic cell; GWAS, genome-wide association study; HLA, human leukocyte antigen; IBD, inflammatory bowel disease; LD, linkage disequilibrium; MHC, major histocompatibility complex; OR, odds ratio; SNP, single nucleotide polymorphisms; UC, ulcerative colitis.

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Table 1. Basic Characteristics of Study Subjects

	CD cases		UC cases		
Set	GWAS	Replication	GWAS	Replication	Control
No. of samples	372	607	372	376	905
Male, n (%)	266 (71.5)	416 (68.5)	172 (46.2)	188 (50.0)	671 (74.1)
Age at sampling (*y*), mean ± SD	33.9 ± 9.4	39.1 ± 12.7	42.6 ± 16.1	43.8 ± 15.6	52.5 ± 14.4
Characteristics of CD					
Age at disease onset, n (%)					
≤16 (A1)	61 (16.5)	68 (11.2)			
17–40 (A2)	296 (80.2)	465 (76.6)			
>40 (A3)	12 (3.3)	74 (12.2)			
Disease location, n (%)					
Ileal disease (L1)	153 (41.6)	235 (38.8)			
Colonic disease (L2)	54 (14.7)	98 (16.2)			
Ileocolonic disease (L3)	158 (42.9)	272 (44.9)			
Upper gastrointestinal disease (L4)	3 (0.8)	1 (0.2)			
Disease behavior, n (%)					
Nonstricturing, nonpenetrating (B1)	101 (27.5)	171 (28.2)			
Stricturing (B2)	185 (50.4)	229 (37.7)			
Penetrating (B3)	81 (22.1)	207 (34.1)			
Perianal disease modifier	150 (40.9)	316 (52.1)			
Characteristics of UC					
Disease extent, n (%)					
Ulcerative proctitis (E1)			51 (14.0)	68 (18.4)	
Left-sided UC (E2)			141 (38.7)	133 (36.0)	
Extensive UC (E3)			172 (47.3)	168 (45.5)	

SD, standard deviation.

population. This approach effectively enabled the identification of the genetic factor with distinct effects. Because previous studies reported that the several HLA alleles were associated with UC or CD,[22–25] we genotyped high-resolution HLA alleles of the subjects. Through an intensive analysis integrating the GWAS data and HLA allele genotypes, our study provided evidence that a particular HLA haplotype independently confers opposite directions of genetic effects on UC and CD.

Materials and Methods

Subjects

A total of 752 individuals with UC and 983 individuals with CD, all of Japanese descent, were enrolled in the study. Subjects with UC were collected from the Kyushu University and 25 affiliated hospitals as described previously[15] and randomly divided into the GWAS set (n = 376) and replication set (n = 376). Subjects with CD were collected at the Social Insurance Chuo General Hospital (n = 376 for GWAS set, overlapping with the cases of the previous study[4]) and the Kyushu University with 16 affiliated hospitals (n = 607 for replication set). The diagnosis of UC or CD in all subjects was made by expert gastroenterologists in accordance with clinical, radiological, endoscopic, and histological features according to the Lennard-Jones' criteria.[26] Patients with indeterminate colitis were excluded in advance. After applying quality control measures (see the next section), we analyzed a total of 748 UC cases and 979 CD cases (Table 1). For the control subjects, we used healthy volunteers recruited at the Midosuji and other related Rotary Clubs (n = 905). These subjects had been included in our previous studies.[4,15,27] The subjects who were determined to be of non-Japanese origin, by self-report or by principal component analysis, were excluded. All individuals enrolled in the study gave their written informed consent, and approval was obtained from the ethical committees at Kyushu University, Social Insurance Chuo General Hospital, and RIKEN Yokohama Institute.

Genotyping and Quality Control in the Comparative GWAS

In the comparative GWAS, 376 UC cases and 376 CD cases were genotyped with >550,000 single nucleotide polymorphisms (SNPs) using Illumina HumanHap550v3 Genotyping BeadChip (Illumina, San Diego, CA). After excluding subjects with call rates <0.98, SNPs with call rates <0.99 in UC cases or CD cases or nonautosomal SNPs were excluded. We excluded closely related subjects using identity-by-descent estimated by PLINK version 1.06.[28] For pairs in a first or second degree of kinship, we excluded the subjects who had lower call rate than the other. To evaluate potential population stratification in our study population, we performed principal component analysis for the GWAS data along with European, African, and East-Asian (Japanese and Han Chinese) individuals obtained from Phase II HapMap database (release 22)[29] using EIGENSTRAT version 2.0.[30] We excluded SNPs with minor allele frequency <0.01 in UC cases or CD cases.

Genotyping and Quality Control in the Replication Study

To validate the associations observed in our comparative GWAS, we performed a replication study using independent individuals of 376 UC and 607 CD. We selected the most significantly associated SNPs for each of the loci that showed $P < 1.0 \times 10^{-4}$ in the GWAS. Genotyping of the SNPs was performed for UC and CD cases using multiplex polymerase chain reaction–based Invader assay. To evaluate how the signif-

icant associations between UC and CD reflected the risk of UC and CD, we additionally genotyped the SNPs with those of 905 healthy controls. Genotyping for the healthy controls were performed using Illumina HumanHap550v3 Genotyping BeadChip, and the same quality control criteria in the GWAS were applied.

Genotyping of HLA Alleles

To comprehensively evaluate the associations with UC and CD in the major histocompatibility complex (MHC) region, we performed a high-resolution (4-digit) genotyping of HLA-C, HLA-B, HLA-DRB1, and HLA-DPB1 alleles for UC and CD cases enrolled in the GWAS and the healthy controls. Genotyping of the HLA alleles was performed using WAKFlow HLA typing kit (Wakunaga, Hiroshima, Japan) and a Luminex Multi-Analyte Profiling system (xMAP; Luminex, Austin, TX), according to manufacturer's instructions.

Statistical Analysis

The association of the SNP in the GWAS and the replication study was tested with the Cochran-Armitage trend test. Combined analysis was performed with the Mantel-Haenszel method. Comparison of HLA allele frequency was assessed by the χ^2 test for an allelic 2 × 2 contingency table, and odds ratio (OR) and 95% confidence interval (CI) were estimated using Woolf's method. Fisher's exact test was performed for the tables with expected cell values <5. Bonferroni correction based on the number of the observed alleles were applied for the analysis of HLA alleles and haplotypes (α = .05). Linkage disequilibrium (LD) index, r^2, among HLA alleles were calculated using Haploview version 4.0.[31] LD structure of HLA alleles were visualized using textile plot, a graphical representation for high-dimensional multivariable data, which may help to understand underlying LD patterns hard to capture by conventional triangular heat map display of LD index.[32] Haplotype frequency was estimated by the expectation-maximization algorithm using the haplo.stats package version 1.4.4.[33] No cutoff threshold of haplotype frequency was assigned in the estimation. Association analysis of the haplotype was performed using haplo.glm function implemented in haplo.stats.[33] The haplotype with the highest frequency was adopted as the base haplotype. In multivariate regression analysis, HLA haplotypes and alleles significantly associated between UC and CD were adopted as independent variables. Associations were assessed using logistic regression model assuming additive effects of the expected dosages of the HLA haplotypes and the genotype counts of the HLA alleles. Proportion of the risk explained by HLA haplotypes and alleles was estimated using population attributable risk[34] based on ORs obtained in the multivariate logistic regression model. Independent association of the SNP was tested with multivariate logistic regression model assuming additive effect with adjustment for the HLA haplotypes and alleles as independent covariates. *R* software version 2.9.0 (http://cran.r-project.org) was used for general statistical analysis.

Results

Comparative GWAS Between UC and CD

In the comparative GWAS, 3 UC cases and 2 CD cases were excluded due to low call rates, and 1 UC case and 2 CD cases were excluded due to close relationships. A principal component analysis plot clearly separated the subjects into 3 clusters as indicated previously (Supplementary Figure 1).[35] Our study population was in concordance with the cluster of East-Asian individuals and no outlier was detected, suggesting homogeneous ancestries of our study population. Finally, 461,368 autosomal SNPs for 372 UC cases and 372 CD cases fulfilled the quality-control criteria.

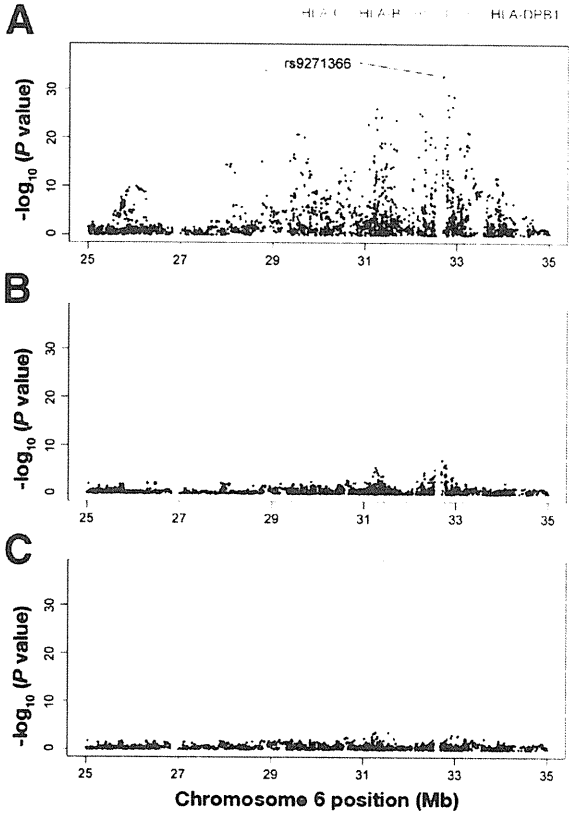

Figure 1. Result of the comparative GWAS using 372 UC cases and 372 CD cases around the MHC region (Chr. 6, 25–35 Mb). Positional plots of $-\log_{10}$ (*P* value) of the SNPs (*A*) before adjustment, (*B*) after adjustment of HLA-Cw*1202-B*5201-DRB1*1502 haplotype, and (*C*) after adjustment of the all associated HLA haplotypes and alleles including HLA-Cw*1202-B*5201-DRB1*1502, HLA-Cw*1402-B*5101, DRB1*0405, DRB1*1501, and DPB1*0501. The *gray horizontal lines* in the plots represent the genome-wide significance threshold of $P = 5.0 \times 10^{-8}$.

We evaluated the associations of the SNPs between UC and CD, and identified significant associations that satisfied the genome-wide significance threshold of $P < 5.0 \times 10^{-8}$ in the MHC region (Figure 1*A*, Supplementary Figure 2). After excluding the SNPs in the MHC region, no remarkable discrepancy from null hypothesis was suggested with the inflation factor, λ_{GC}, being 1.09 (Supplementary Figure 3).

Replication Study

To find additional genetic loci that have distinct role between UC and CD, we selected candidate SNPs that showed $P < 1.0 \times 10^{-4}$ for the replication study. We evaluated the associations for 52 loci and found signifi-

cant associations for rs9271366 and rs2006996 after Bonferroni correction ($P < .05/52$). The combined analysis of the GWAS and the replication study revealed that 2 loci reached genome-wide significance level ($P < 5.0 \times 10^{-8}$) of associations (rs9271366 located close to *HLA-DRB1* at 6p21, $P = 1.6 \times 10^{-70}$, OR = 4.44, 95% CI: 3.74–5.27; rs2006996 in the *TNFSF15* locus at 9q32, $P = 3.7 \times 10^{-13}$, OR = 0.60, 95% CI: 0.52–0.69; Table 2). Statistical power of this study was estimated to be 54.7% under the assumption of the risk variant with OR of 1.5 and allele frequency of 0.3 ($\alpha = 5.0 \times 10^{-8}$).

When the frequencies of the 2 SNPs in UC or CD cases were compared with those of 905 healthy controls, the C allele of rs2006996 in the *TNFSF15* locus showed a significant susceptible effect on CD ($P = 3.7 \times 10^{-16}$, OR = 1.75, 95% CI: 1.53–1.99), but did not show any association with UC ($P = 0.54$, OR = 1.04, 95% CI: 0.91–1.20), which was compatible with the previous report.[4] On the other hand, the C allele of rs9271366 in the MHC region demonstrated a significant susceptible effect on UC ($P = 3.4 \times 10^{-31}$, OR = 2.41, 95% CI: 2.07–2.81), but showed a protective effect on CD ($P = 8.3 \times 10^{-11}$, OR = 0.56, 95% CI: 0.47–0.67; Table 2).

Associations of HLA Alleles

A total of 22 HLA-C alleles, 39 HLA-B alleles, 32 HLA-DRB1 alleles, and 17 HLA-DPB1 alleles were genotyped. In the comparison of the HLA allele frequencies between UC and CD cases, significant associations were observed in 2 HLA-C alleles, 2 HLA-B alleles, 3 HLA-DRB1 alleles, and 2 HLA-DPB1 alleles after Bonferroni correction ($\alpha = .05$, n = 110, $P < .00045$; Table 3 and Supplementary Table 1). Several HLA alleles, namely Cw*1202, B*5201, DRB1*1502, and DPB1*0901, conferred strong associations between UC and CD ($P < 1.0 \times 10^{-23}$). These HLA alleles also showed susceptible effects on UC ($P < 1.0 \times 10^{-12}$, OR ranged from 2.25 to 2.62), but showed protective effects on CD ($P < 5.0 \times 10^{-5}$, OR ranged from 0.40 to 0.52). On the other hand, DRB1*0405 indicated significant association between UC and CD ($P = 2.4 \times 10^{-8}$), with susceptible effect on CD ($P = 3.8 \times 10^{-7}$) and no significant association with UC ($P = .072$). These results were compatible with the previously reported associations of B*5201 and DRB1*1502 with UC,[22–24] or DR4 alleles with CD.[23,24]

LD Structure of HLA Alleles

Because strong and complex LD pattern exists throughout the MHC region,[36,37] we evaluated the LD among the HLA alleles associated between UC and CD. A triangular heat map display of LD index in the controls demonstrated that strong LD existed among Cw*1202, B*5201, and DRB1*1502, and between Cw*1402 and B*5101 ($r^2 > 0.75$) (Figure 2*A*). Visualization of LD structure of these HLA alleles using textile plot clearly showed that Cw*1202, B*5201, and DRB1*1502 composed one long-range haplotype (Figure 2*B*). Moreover, this haplotype was distinctly isolated from other HLA alleles in its vertical po-

Table 2. Significantly Associated SNPs Between UC Cases and CD Cases

rsID	Chr	Position	Gene	Allele 1/2	Study Set	No. of subjects UC	CD	Control	Allele 1 frequency UC	CD	Control	UC vs CD OR (95% CI)	UC vs CD *P* value[a]	UC vs control OR (95% CI)	UC vs control *P* value[a]	CD vs control OR (95% CI)	CD vs control *P* value[a]
rs9271366	6	32,694,832	MHC Region	C/T	GWAS	372	372	—	0.37	0.10	—	5.21 (3.94–6.90)	6.1×10^{-34}	—	—	—	—
					Replication	376	607	—	0.40	0.14	—	3.97 (3.20–4.94)	4.5×10^{-36}	—	—	—	—
					Combined	748	979	905	0.39	0.13	0.21	4.44 (3.74–5.27)	1.6×10^{-70}	2.41 (2.07–2.81)	3.4×10^{-31}	0.56 (0.47–0.67)	8.3×10^{-11}
rs2006996	9	116,632,459	*TNFSF15*	C/T	GWAS	372	372	—	0.55	0.65	—	0.65 (0.53–0.80)	6.2×10^{-5}	—	—	—	—
					Replication	376	607	—	0.53	0.67	—	0.56 (0.46–0.67)	1.0×10^{-9}	—	—	—	—
					Combined	748	979	905	0.54	0.66	0.53	0.60 (0.52–0.69)	3.7×10^{-13}	1.04 (0.91–1.20)	0.54	1.75 (1.53–1.99)	3.7×10^{-16}

[a]Obtained by Cochran–Armitage trend test for the GWAS and the replication study, by Mantel–Haenzel test for the combined study.

Table 3. Significantly Associated HLA-C/B/DRB1/DPB1 Alleles Between UC Cases and CD Cases

HLA allele	Allele frequency			UC vs CD		UC vs control		CD vs control	
	UC (n = 372)	CD (n = 372)	Control (n = 905)	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
HLA-C									
Cw*1202	0.29	0.077	0.14	4.98 (3.64–6.81)	7.4×10^{-27}	2.57 (2.09–3.17)	5.5×10^{-20}	0.52 (0.38–0.70)	1.3×10^{-5}
Cw*1402	0.050	0.11	0.074	0.42 (0.28–0.62)	1.1×10^{-5}	0.65 (0.45–0.95)	.026	1.58 (1.18–2.10)	.0019
HLA-B									
B*5101	0.063	0.13	0.088	0.46 (0.32–0.66)	2.3×10^{-5}	0.69 (0.50–0.97)	.033	1.51 (1.15–1.97)	.0028
B*5201	0.29	0.075	0.14	5.09 (3.72–6.98)	2.3×10^{-27}	2.62 (2.13–3.22)	1.6×10^{-20}	0.51 (0.38–0.70)	1.3×10^{-5}
HLA-DRB1									
DRB1*0405	0.10	0.21	0.13	0.44 (0.33–0.59)	2.4×10^{-8}	0.78 (0.59–1.02)	.072	1.78 (1.42–2.23)	3.8×10^{-7}
DRB1*1501	0.093	0.043	0.070	2.26 (1.46–3.48)	.00016	1.37 (1.01–1.86)	.044	0.61 (0.41–0.90)	.013
DRB1*1502	0.28	0.060	0.14	6.09 (4.31–8.59)	3.2×10^{-29}	2.46 (1.99–3.03)	9.8×10^{-18}	0.40 (0.29–0.56)	4.0×10^{-8}
HLA-DPB1									
DPB1*0501	0.38	0.46	0.39	0.69 (0.56–0.85)	.00044	0.95 (0.80–1.14)	.60	1.38 (1.16–1.64)	.00023
DPB1*0901	0.24	0.057	0.13	5.36 (3.76–7.63)	7.1×10^{-24}	2.25 (1.81–2.80)	1.2×10^{-13}	0.42 (0.30–0.59)	3.1×10^{-7}

NOTE. HLA alleles that satisfied Bonferroni correction based on the number of the observed alleles in the comparisons of UC and CD cases are indicated ($\alpha = .05$, n = 110, $P < .00045$). Results of all the observed HLA alleles are indicated in Supplementary Table 1.
n, number of subjects enrolled in the analysis.

sition, reflecting strong LD within these alleles and weak LD with other alleles. Interestingly, its frequency was high in UC, middle in controls, and low in CD, suggesting its opposite directions of effects on UC and CD.

Haplotype-Based Analysis of HLA Alleles

We then performed haplotype-based association analysis in strong LD (Table 4). HLA-Cw*1202-B*5201-DRB1*1502 haplotype demonstrated significant associations between UC and CD ($P = 1.1 \times 10^{-33}$, OR = 6.58, 95% CI: 4.60–9.42), with a susceptible effect on UC ($P = 4.0 \times 10^{-21}$, OR = 2.65, 95% CI: 2.14–3.29) and a protective effect on CD ($P = 1.1 \times 10^{-7}$, OR = 0.40, 95% CI: 0.28–0.57). Although HLA-DPB1*0901 was in moderate LD with Cw*1202, B*5201, and DRB1*1502 ($r^2 = 0.54–0.66$), we did not include it in the risk haplotype because both HLA-Cw*1202-B*5201-DRB1*1502-DPB1*0901 and HLA-Cw*1202-B*5201-DRB1*1502-non DPB1*0901 haplotypes indicated significant associations between UC and CD ($P < 1.0 \times 10^{-5}$; data not shown). HLA-Cw*1402-B*5101 haplotype also indicated significant associations between UC and CD ($P = 1.8 \times 10^{-5}$), but their associations with UC and CD were suggestive ($P < .05$).

To account for the relative effects among the HLA alleles and confirm that the distinct effects of HLA-Cw*1202-B*5201-DRB1*1502 haplotype on UC and CD were not the reflection of other UC- or CD-specific effect alleles, we performed a multivariate regression analysis including all the associated HLA haplotypes and alleles (HLA-Cw*1202-B*5201-DRB1*1502, HLA-Cw*1402-B*5101, DRB1*0405, DRB1*1501, and DPB1*0501). This analysis demonstrated a significant association of HLA-Cw*1202-B*5201-DRB1*1502 haplotype ($P = 3.0 \times 10^{-22}$) with a susceptible effect on UC ($P = 2.0 \times 10^{-19}$) and a protective effect on CD ($P = 7.2 \times 10^{-5}$), confirming the distinct effects of this haplotype on UC and CD were independent of other HLA alleles. Combination of these HLA haplotypes and alleles explained 41% of the difference of the risks between UC and CD. Among them, HLA-Cw*1202-B*5201-DRB1*1502 haplotype accounted for 63% of the explained genetic risk of HLA haplotypes and alleles.

When CD cases were stratified by colonic (n = 53) and noncolonic (n = 315) phenotypes, frequencies of HLA-Cw*1202-B*5201-DRB1*1502 haplotype were significantly different among UC, colonic, and noncolonic CD (0.27, 0.10, and 0.043, respectively; $P < .01$; Supplementary Table 2). Compared with the frequency of healthy controls (0.12), HLA-Cw*1202-B*5201-DRB1*1502 haplotype had a significant susceptible effect on UC ($P = 4.0 \times 10^{-21}$, OR = 2.65) and a significant protective effect on noncolonic CD ($P = 5.2 \times 10^{-9}$, OR = 0.32), but had no effect on colonic CD ($P = .58$, OR = 0.83).

Associations of SNPs in MHC Region After Adjustment of HLA Alleles

Because the previous studies supposed that the associations of the SNPs in the MHC region are the reflection of the associations of the HLA alleles via long-

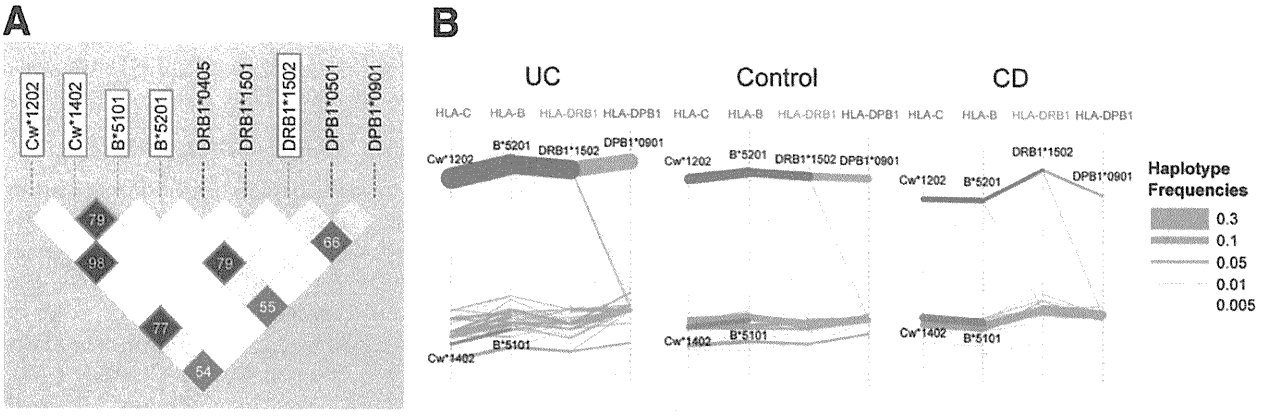

Figure 2. LD map and haplotype structure of HLA alleles. (*A*) Triangular heat map display of LD index, r^2, among the HLA alleles associated between UC and CD. LD map based on the controls is drawn using Haploview version 4.0.[31] r^2 value >0.5 is indicated in the *diamond*. Pairs of the HLA alleles in strong LD ($r^2 > 0.75$) are highlighted with *magenta* or *orange-red*. (*B*) Haplotype structure of HLA alleles represented by textile plot.[32] The *dotted vertical axis* indicates each of the 4 HLA genes, and the queues of the axes correspond to their physical order in the MHC region. A point on an axis indicates an HLA allele, and a segment connects 2 alleles on adjacent genes. The thickness of the segment corresponds to the haplotype frequency between the 2 HLA alleles, relative to thicknesses of lines shown in the legend. The vertical positions of HLA alleles are simultaneously chosen so that all connected segments are aligned as horizontally as possible. Haplotypes consisted of the HLA alleles in strong LD ($r^2 > 0.75$) are highlighted in the same color as in (*A*) along with the names of the alleles. The existence of the haplotype consisting of Cw*1202, B*5201, and DRB1*1502 is clearly shown, although the connection between DRB1*1502 and DPB1*0901 seems relatively weaker. Frequency of the haplotype decays from in order of UC cases, the controls, and CD cases, representing its opposite directions of effects on UC and CD.

range LD with them,[25] we performed the multivariate logistic regression analysis of the SNPs in the MHC region with the adjustment of the identified HLA haplotypes and alleles. After adjusted for HLA-Cw*1202-B*5201-DRB1*1502 haplotype, most of the associations in MHC region were largely weakened (the smallest $P = 1.0 \times 10^{-7}$; Figure 1*B*). When the SNPs were further adjusted for other associated HLA haplotypes and alleles, no significant association was observed (the smallest $P = .00023$; Figure 1*C*). This suggested that HLA-Cw*1202-B*5201-DRB1*1502 accounted for most of the associations in the MHC region observed in our comparative GWAS, and the remaining weak associations could also be attributable to other HLA haplotypes and alleles.

Discussion

Through a comparative GWAS between UC and CD and a follow-up study using high-resolution HLA alleles, we demonstrated that a particular HLA haplotype, HLA-Cw*1202-B*5201-DRB1*1502, independently confers a susceptible effect on UC, but has a protective effect on CD. Although previous studies suggested distinct associations of some HLA-DRB1 alleles with UC and CD,[23,24] their associations were not substantially evaluated.[38] Our study clearly showed that one haplotype extending throughout the MHC class I, III, and II regions confers opposite directions of effects on UC and CD. This haplotype accounted for two thirds of the difference of the genetic risks between UC and CD in the MHC region, suggesting its substantial role in the etiology of IBD. Although recent comparative association studies for IBD mostly identified the risk loci shared between UC and CD,[16,19,21,39] our study is the first to identify the loci with the opposite directions of the effects.

Contrary to our results, the comparative study for IBD in European populations did not demonstrate the distinct effects in the MHC region.[12,16,21] One probable explanation for this discrepancy would be the ethnic differences of haplotype frequencies. According to HapMap populations, frequencies of Cw*1202, B*5201, and DRB1*1502 were relatively high in the Japanese population (0.091 for Cw*1202, 0.091 for B*5201, and 0.102 for DRB1*1502, respectively), but were low in the European population (0.0111 for Cw*1202, 0.0167 for B*5201, and 0.0056 for DRB1*1502, respectively).[36] It would be plausible that the loss of statistical power due to the low haplotype frequency in European populations hampered the detection of the distinct effects on IBD in the MHC region. In addition, our comparative approach by comparing UC and CD directly would have effectively highlighted the distinct effects.

The intestinal immune system is maintained to protect against bacterial infection while avoiding the destructive inflammatory response to normal microbiota. Innate immune cells including dendritic cells (DCs) and macrophages provide the first line of defense against entry of pathogens across the mucosal barrier.[2,40] The entry of pathogenic bacteria activates DCs, and activated DCs present specific MHC class II molecules on its surface. According to this antigen presentation, naïve $CD4^+$ T cells proliferate and differentiate into various effector subsets characterized by the production of distinct cytokines.[41,42] CD have been considered to be a typical Th1 disease that is characterized by overproduction of interferon-γ in the inflamed gut, while UC is referred to as a "Th2-like" or "mixed" phenotype.[3] Several studies showed that immune responses induced by intestinal DCs vary among bacterial pathogens and a distinct differentiation

Table 4. Associations of the Haplotype Consisting of HLA Alleles Associated Between UC Cases and CD Cases

Haplotype			Frequency[a]			UC vs CD		UC vs control		CD vs control	
			UC (n = 372)	CD (n = 372)	Control (n = 905)	OR (95% CI)[b]	P value[b]	OR (95% CI)[b]	P value[b]	OR (95% CI)[b]	P value[b]
Haplotype for Cw*1202, B*5201, DRB1*1502											
Cw*1202	B*5201	DRB1*1502	0.27	0.054	0.12	6.58 (4.60–9.42)	1.1×10^{-33}	2.65 (2.14–3.29)	4.0×10^{-21}	0.40 (0.28–0.57)	1.1×10^{-7}
Cw*1202	B*5201	—	0.025	0.022	0.013	1.51 (0.76–2.97)	.15	2.24 (1.21–4.14)	.0080	1.49 (0.79–2.81)	.21
—	—	DRB1*1502	0.011	0.0056	0.012	2.64 (0.81–8.65)	.030	1.10 (0.49–2.46)	.54	0.42 (0.15–1.19)	.067
—	—	—	0.69	0.92	0.85						
Haplotype for Cw*1402, B*5101											
Cw*1402	B*5101		0.050	0.11	0.072	0.42 (0.28–9.42)	1.8×10^{-5}	0.67 (0.46–0.98)	.039	1.62 (1.21–2.17)	.0010
—	B*5101		0.013	0.016	0.017	0.78 (0.33–1.81)	.57	0.78 (0.38–1.61)	.48	1.01 (0.51–1.98)	.90
—	—		0.94	0.87	0.91						

NOTE. HLA alleles other than Cw*1202, B*5201, DRB1*1502, or Cw*1402, B*5101 are pooled and denoted as "—."
n, Number of subjects enrolled in the analysis.
[a]Haplotype with >0.5% of frequency in the controls are indicated.
[b]Obtained by the comparison of haplotype frequencies between each of the haplotype and the haplotype with the highest frequency.

of Th-cell subsets are induced according to the pathogens.[40,43] These results indicate that the specific pathogens recognized by HLA-Cw*1202-B*5201-DRB1*1502 haplotype will promote the inappropriate proliferation and differentiation of naïve $CD4^+$ T cells and induce the Th1/Th2/Treg imbalance in the intestinal immune response. This imbalance will contribute to the opposite directions of the susceptibility to UC and CD. Further studies to clarify the mechanisms of this HLA haplotype on the homeostasis of the intestinal immune system are needed.

Clinical importance of differential diagnosis of UC and CD has been recognized, and incorporation of genetic markers in the diagnosis is proposed as a promising clue.[44,45] The identified HLA haplotype distinguishes UC and CD with OR of as large as around 6.5, which would have more impacts than the previously evaluated variants.[45] Thus, utilization of the genotype information of the HLA haplotype, or alternatively the SNP(s) in LD with it, might contribute to improvements of diagnostic approaches on UC and CD.

In summary, our study demonstrated that the particular HLA haplotype has the opposite directions of genetic effects on UC and CD. Our findings should shed light on the pathogenesis of IBD.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at doi: 10.1053/j.gastro.2011.05.048.

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Reprint requests

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Conflicts of interest

The authors disclose no conflicts.

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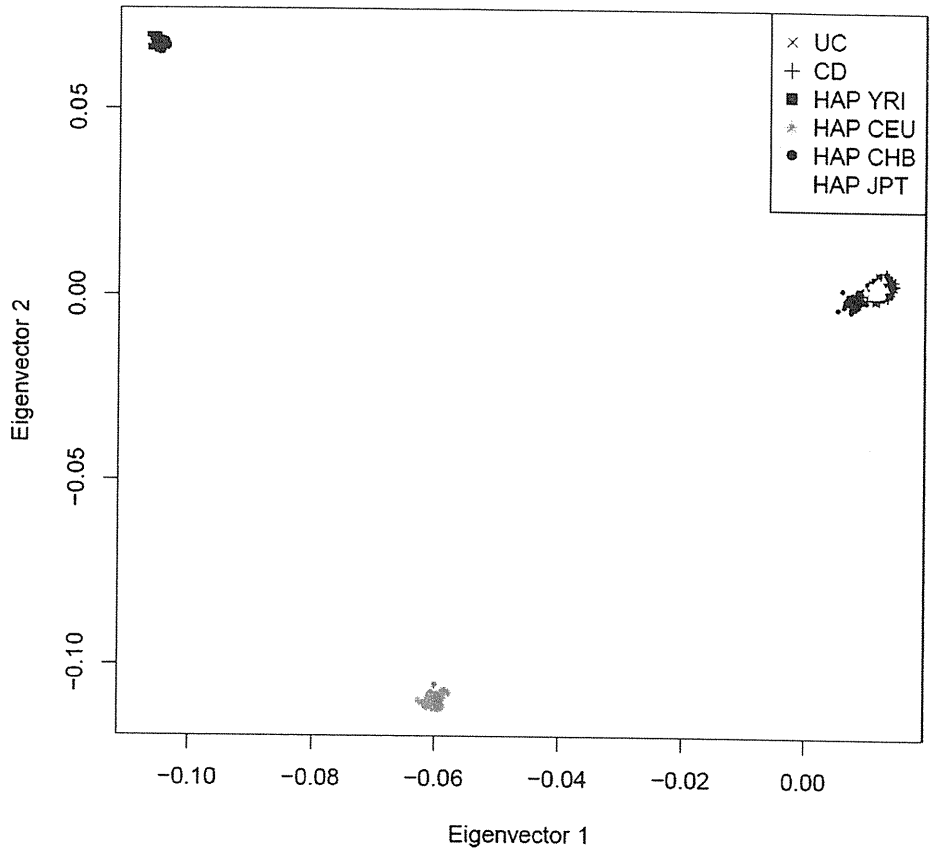
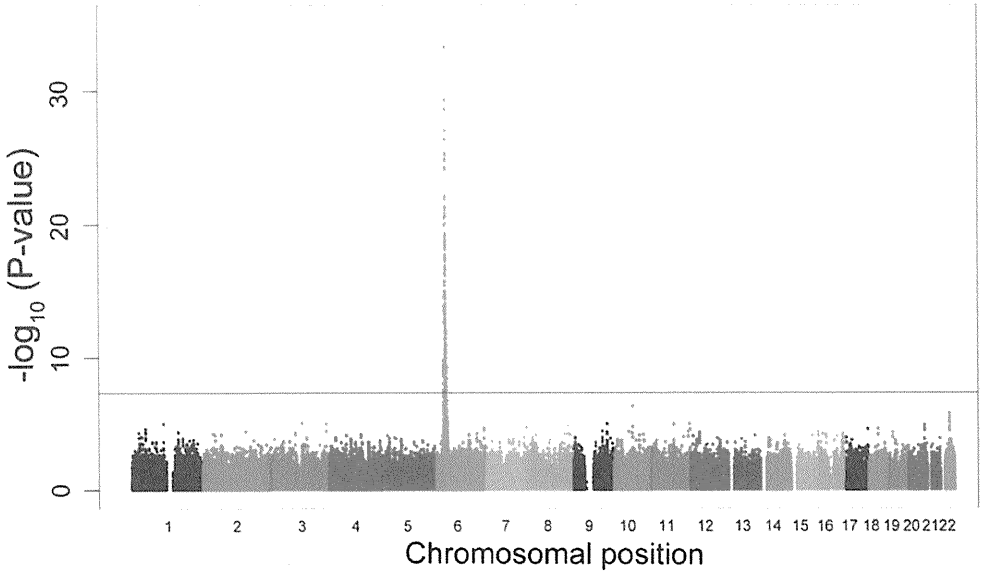
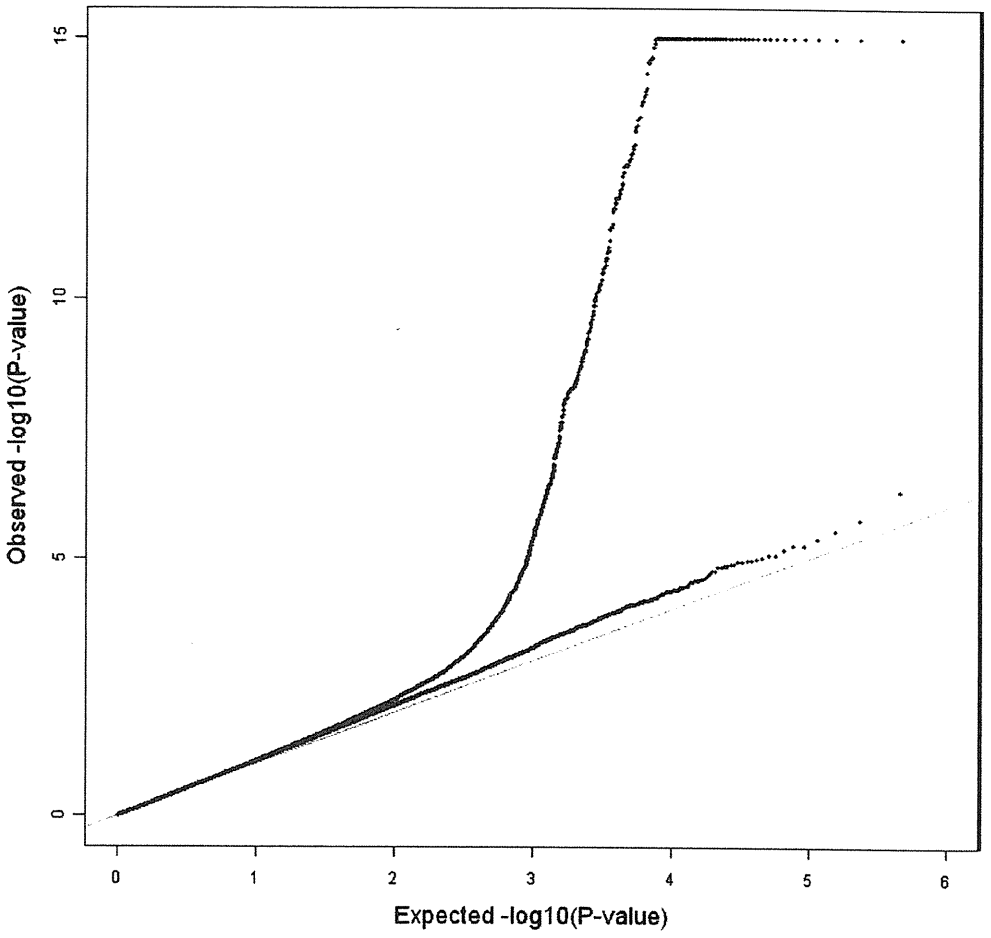


Supplementary Figure 1. Principal component analysis (PCA) plot of the subjects. UC cases and CD cases enrolled in the GWAS are plotted based on eigenvectors 1 and 2 obtained from the PCA using EIGENSTRAT, along with the European (CEU), African (YRI), Japanese (JPT), and Chinese (CHB) individuals obtained from the Phase II HapMap database (release 22).



Supplementary Figure 2. Manhattan plot of the comparative GWAS using 372 UC cases and 372 CD cases. The *gray horizontal lines* in the plots represent the genome-wide significance threshold of $P = 5.0 \times 10^{-8}$.



Supplementary Figure 3. Quantile-Quantile plot (QQ-plot) of *P* values in the GWAS. The QQ-plot of Cochran–Armitage trend test *P* values in the GWAS is on a logarithmic scale. The x-axis represents the expected *P* values under the assumption of a uniform distribution of *P* values, and the y-axis represents the observed *P* values in the GWAS. The QQ-plot for the *P* values of all the SNPs that passed the quality control criteria is indicated in *black*. The QQ-plot for the *P* values after the removal of the SNPs included in the MHC region is indicated in *blue*. The SNPs for which the *P* value was $<1.0 \times 10^{-15}$ are indicated at the upper limit of the plot. The *gray dotted line* indicates y = x.

Supplementary Table 1. Associations of HLA-C/B/DRB1/DPB1 Alleles Between UC Cases and CD Cases

	No. of alleles			Allele frequency			UC vs CD		UC vs control[a]		CD vs control[a]	
HLA allele	UC	CD	Control	UC	CD	Control	OR (95% CI)[b]	*P* value[b]	OR (95% CI)[b]	*P* value[b]	OR (95% CI)[b]	*P* value[b]
HLA-C												
Cw*0102	108	151	333	0.15	0.20	0.18	0.66 (0.51–0.87)	.0030				
Cw*0103	3	5	4	0.004	0.007	0.002	0.60 (0.09–3.08)	.51				
Cw*0302	2	7	17	0.003	0.009	0.009	0.28 (0.03–1.49)	.11				
Cw*0303	73	87	191	0.098	0.117	0.106	0.82 (0.59–1.14)	.23				
Cw*0304	77	81	193	0.10	0.11	0.11	0.94 (0.68–1.31)	.72				
Cw*0323	1	0	0	0.001	0	0	—	1				
Cw*0401	21	24	80	0.028	0.032	0.044	0.87 (0.48–1.57)	.64				
Cw*0403	0	0	1	0	0	0.001	—	1				
Cw*0501	1	4	7	0.001	0.005	0.004	0.25 (0.01–2.52)	.22				
Cw*0520	0	1	0	0	0.001	0	0.00 (0.00–38.9)	.50				
Cw*0602	4	8	20	0.005	0.011	0.011	0.50 (0.15–1.65)	.24				
Cw*0701	0	1	2	0	0.001	0.001	0.00 (0.00–38.9)	.50				
Cw*0702	92	83	216	0.12	0.11	0.12	1.12 (0.82–1.54)	.48				
Cw*0704	3	9	14	0.004	0.012	0.008	0.33 (0.09–1.22)	.081				
Cw*0801	36	54	145	0.048	0.073	0.080	0.65 (0.42–1.00)	.049				
Cw*0803	8	9	20	0.011	0.012	0.011	0.89 (0.34–2.31)	.80				
Cw*1202	218	57	251	0.29	0.077	0.14	4.98 (3.64–6.81)	7.4E–27	2.57 (2.09–3.17)	5.5E–20	0.52 (0.38–0.70)	1.3E–05
Cw*1203	2	2	4	0.003	0.003	0.002	1.00 (0.07–13.8)	1				
Cw*1214	0	0	1	0	0	0.001	—	1				
Cw*1402	37	83	134	0.050	0.11	0.074	0.42 (0.28–0.62)	1.1E–05	0.65 (0.45–0.95)	.026	1.58 (1.18–2.10)	.0019
Cw*1403	38	51	115	0.051	0.069	0.064	0.73 (0.47–1.12)	.15				
Cw*1502	20	25	62	0.027	0.034	0.034	0.79 (0.44–1.44)	.44				
HLA–B												
B*0702	31	21	102	0.042	0.028	0.056	1.50 (0.85–2.63)	.16				
B*1301	8	6	15	0.011	0.008	0.008	1.34 (0.46–3.87)	.59				
B*1302	4	2	2	0.005	0.003	0.001	2.00 (0.29–22.2)	.69				
B*1401	0	1	0	0	0.001	0	0.00 (0.00–39.0)	1				
B*1501	42	38	126	0.056	0.051	0.070	1.11 (0.71–1.75)	.65				
B*1507	1	2	19	0.001	0.003	0.011	0.50 (0.01–9.62)	1				
B*1511	3	7	14	0.004	0.009	0.008	0.43 (0.11–1.65)	.20				
B*1518	8	15	25	0.011	0.020	0.014	0.53 (0.22–1.25)	.14				
B*1527	0	1	1	0	0.001	0.001	0.00 (0.00–39.0)	1				
B*2704	3	1	3	0.004	0.001	0.002	3.01 (0.24–158.0)	.62				
B*2705	1	1	1	0.001	0.001	0.001	1.00 (0.01–78.6)	1				
B*3501	51	57	115	0.069	0.077	0.064	0.89 (0.60–1.31)	.55				
B*3701	0	6	17	0	0.008	0.009	0.00 (0.00–0.85)	.031				
B*3801	1	0	2	0.001	0	0.001	—	1				
B*3802	4	2	2	0.005	0.003	0.001	2.00 (0.29–22.2)	.69				
B*3901	26	32	52	0.035	0.043	0.029	0.81 (0.48–1.37)	.42				
B*3902	1	3	1	0.001	0.004	0.001	0.33 (0.01–4.15)	.62				
B*3904	0	3	3	0	0.004	0.002	0.00 (0.00–2.42)	.25				
B*3923	1	2	0	0.001	0.003	0	0.50 (0.01–9.62)	1				
B*4001	40	49	104	0.054	0.066	0.058	0.81 (0.52–1.24)	.33				
B*4002	45	55	113	0.060	0.074	0.063	0.81 (0.54–1.21)	.30				
B*4003	3	3	13	0.004	0.004	0.007	1.00 (0.13–7.49)	1				
B*4006	27	43	109	0.036	0.058	0.060	0.61 (0.38–1.00)	.050				
B*4402	1	4	7	0.001	0.005	0.004	0.25 (0.01–2.53)	.37				
B*4403	38	51	115	0.051	0.069	0.064	0.73 (0.47–1.13)	.16				
B*4601	30	45	86	0.040	0.060	0.048	0.65 (0.41–1.05)	.075				
B*4701	0	0	1	0	0	0.001	—	1				
B*4801	15	11	43	0.020	0.015	0.024	1.37 (0.63–3.01)	.43				
B*5101	47	95	160	0.063	0.128	0.089	0.46 (0.32–0.66)	2.3E–05	0.69 (0.50–0.97)	.033	1.51 (1.15–1.97)	.0028
B*5102	2	4	4	0.003	0.005	0.002	0.50 (0.04–3.49)	.69				
B*5201	218	56	247	0.293	0.075	0.137	5.09 (3.72–6.98)	2.3E–27	2.62 (2.13–3.22)	1.6E–20	0.51 (0.38–0.70)	1.3E–05
B*5401	47	74	159	0.063	0.099	0.088	0.61 (0.42–0.89)	.010				
B*5502	15	13	44	0.020	0.017	0.024	1.16 (0.55–2.45)	.70				
B*5504	1	1	2	0.001	0.001	0.001	1.00 (0.01–78.6)	1				
B*5601	4	3	21	0.005	0.004	0.012	1.33 (0.22–9.14)	1				
B*5603	0	3	5	0	0.004	0.003	0.00 (0.00–2.42)	.25				
B*5801	2	7	17	0.003	0.009	0.009	0.28 (0.03–1.50)	.18				
B*5901	10	21	37	0.013	0.028	0.020	0.47 (0.22–1.00)	.046				
B*6701	14	6	19	0.019	0.008	0.011	2.36 (0.90–6.17)	.072				
HLA–DRB1												
DRB1*0101	32	15	105	0.043	0.020	0.058	2.17 (1.16–4.04)	.013				
DRB1*0301	1	3	8	0.001	0.004	0.004	0.33 (0.01–4.12)	.37				
DRB1*0401	4	12	18	0.005	0.016	0.010	0.33 (0.10–1.02)	.043				
DRB1*0403	11	7	62	0.015	0.010	0.034	1.57 (0.60–4.06)	.35				
DRB1*0404	2	2	7	0.003	0.003	0.004	0.99 (0.07–13.7)	1				
DRB1*0405	77	154	234	0.104	0.209	0.129	0.44 (0.33–0.59)	2.4E–08	0.78 (0.59–1.02)	.072	1.78 (1.42–2.23)	3.8E–07
DRB1*0406	17	25	54	0.023	0.034	0.030	0.67 (0.36–1.25)	.20				
DRB1*0407	0	0	5	0	0	0.003	—	1				
DRB1*0410	10	29	24	0.013	0.039	0.013	0.33 (0.16–0.69)	.0019				
DRB1*0701	2	3	5	0.003	0.004	0.003	0.66 (0.06–5.78)	.69				
DRB1*0801	0	0	1	0	0	0.001	—	1				
DRB1*0802	29	51	64	0.039	0.069	0.035	0.55 (0.34–0.87)	.010				
DRB1*0803	63	67	163	0.085	0.091	0.090	0.93 (0.65–1.33)	.68				
DRB1*0901	74	98	283	0.100	0.133	0.157	0.72 (0.52–0.99)	.045				
DRB1*1001	0	5	18	0	0.007	0.010	0.00 (0.00–1.08)	.030				