Double-Blind, Placebo-Controlled Trial of Oral Tacrolimus (FK506) in the Management of Hospitalized Patients with Steroid-Refractory Ulcerative Colitis

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Background: We report a multicenter study of oral tacrolimus (FK506) therapy in steroid-refractory ulcerative colitis (UC).

Methods: In a placebo-controlled, double-blind study, 62 patients with steroid-refractory, moderate-to-severe UC were randomized into either a tacrolimus group or a placebo for 2 weeks. Patients were evaluated using the Disease Activity Index (DAI). As an entry criterion, patients had to have a total DAI score of 6 or more as well as a mucosal appearance subscore of 2 or 3. Clinical response was defined as improvement in all DAI subscores. Mucosal healing was defined as mucosal appearance subscore of 0 or 1. Clinical remission was defined as a total DAI score ≤ 2 with an individual subscore of 0 or 1.

Results: The mean total DAI score at study entry was 9.8 $\pm$ 1.61 in the tacrolimus group and 9.1 $\pm$ 1.05 in the placebo group. At week 2 the clinical response rate was 50.0% (16/32) in the tacrolimus group and 13.3% (4/30) in the placebo group ($P = 0.003$). The rate of mucosal healing observed was 43.8% (14/32) in the tacrolimus group and 13.3% (4/30) in the placebo group ($P = 0.012$) and the rate of clinical remission observed was 9.4% (3/32) in the tacrolimus group and 0.0% (0/30) in the placebo group ($P = 0.238$). The therapies in this study were well tolerated, with only minor side effects.

Conclusions: Oral tacrolimus therapy in patients with steroid-refractory UC shortened the acute phase and induced rapid mucosal healing. These results suggest that tacrolimus therapy is useful as an alternative therapy for steroid-refractory UC.

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Key Words: ulcerative colitis, immunosuppressive therapy, tacrolimus

Tacrolimus, a macrolide immunosuppressant produced by *Streptomyces tsukubaensis*, a species of *Actinomyces*, was discovered in 1984 on Mt. Tsukuba in Japan. Fellermann et al[1] reported the results of a study of tacrolimus in patients with steroid-refractory, severe ulcerative colitis (UC). With patients initially treated by continuous intravenous infusion and subsequently transferred to oral adminis-

tration, the study showed improved symptoms in five of six patients, with successful induction of remission and steroid tapering achieved in four patients. A report on oral and injectable formulations of tacrolimus stated, "most importantly, oral tacrolimus therapy appears to be effective and obviates the need for intravenous dosing."[2]

Baumgart et al[3] demonstrated the usefulness of low doses of oral tacrolimus (4–6 ng/mL) and Högenauer et al[4] reported, "Oral tacrolimus might be an effective alternative treatment to intravenous cyclosporine for treatment of steroid-refractory UC."

As no evaluation had yet been made of tacrolimus using a placebo as comparator, we conducted a dose-ranging study to evaluate oral administration over 2 weeks.[5] The study established a placebo group, a group with a target tacrolimus trough concentration of 10–15 ng/mL, and a group with a target tacrolimus trough concentration of 5–10 ng/mL. The results indicated a significant difference in efficacy between the 10–15 ng/mL group and the placebo group over the short 2-week period.

Here we report on a multicenter study which was a double-blind study of oral administration for 2 weeks,

comparing a placebo group with a group having a target tacrolimus trough concentration of 10–15 ng/mL.

MATERIALS AND METHODS

Patient Selection

Patients with moderate-to-severe, active UC were eligible for inclusion in this study. UC was defined according to standard criteria for symptoms and standard radiographic and endoscopic criteria.[6] Before starting treatment, infectious diarrhea was ruled out by stool cultures and *Clostridium difficile* toxin testing. Endoscopies were performed during the week prior to the first dose of the study drug. The extent of colonic involvement was determined by total colonoscopy. All patients in the study had left-sided colitis and pancolitis and all were hospitalized.

Patients with known renal or severe hepatic dysfunction and pregnant women were excluded from the study. Pretreatment assessment included taking a history of the patient, physical examination, complete blood count, chemistry screening panel, and urinalysis.

Patients were classified as steroid-resistant or steroid-dependent. Patients with active UC were defined as steroid-resistant when the disease failed to respond to a systemic daily dose of 1 mg per kg of body weight, or 40 mg or more of prednisolone given over at least 7 days, or the equivalent of a daily dose of prednisolone of 30 mg or more over at least 2 weeks. Steroid-dependent patients were defined as patients with active UC in whom attempts to taper steroids had been unsuccessful. The steroid dosage remained the same from study initiation for 2 weeks, while only those patients in whom a dose of prednisolone of 60 mg/day or more was effective were permitted to decrease the dosage during this period. Efficacy was based on improvement in the frequency of stools and a decreased amount of blood in the stool.

Patients were evaluated using the Disease Activity Index (DAI).[7] The DAI score is a sum of subscores for the following four factors: stool frequency, rectal bleeding, mucosal appearance, and physician's overall assessment, each of which is graded on a scale from 0 to 3. The DAI score ranges from 0 to 12; the higher the score, the more severe the disease activity. As an entry criterion, the patient was required to have a total DAI score of 6 or more, as well as a mucosal appearance subscore of 2 or 3.

Patients who started taking azathioprine within 3 months prior to entering the study were excluded from the study, and patients were permitted to continue taking azathioprine at an unchanged dose over the period beginning 3 months prior to the start of the study, until completion of the study. Patients were permitted to continue taking 5-aminosalicylic acid during the study, as long as the drug dosage was not changed over the period beginning 2 weeks prior to the start of the study, until completion of the study. Receiving cytapheresis within 14 days prior to entry in the study was a reason for exclusion from the study. Patients receiving concomitant nutritional therapy continued to receive the same therapy during the study.

As UC therapy with cyclosporin, biological therapies, 6-mercaptopurine, or other immunosuppressants was not covered by health insurance in Japan, the concomitant use of these drugs was prohibited.

Protocol Review

The study protocol was reviewed and approved by each Institutional Review Board. Each patient read and signed a consent form before enrollment in the study.

Study Design

We conducted a multicenter study of oral tacrolimus treatment, consisting of a 2-week placebo-controlled, double-blind, randomized study in which patients with active UC were given either placebo or tacrolimus at an oral dose sufficient to achieve and maintain target blood concentrations of 10–15 ng/mL.

Open-label Extension

After week 2, patients received conventional treatment or tacrolimus open-label treatment. Data were collected during an open-label extension phase of the study. The effect of continuous treatment in the tacrolimus group was evaluated by comparing the condition of patients in the tacrolimus group at weeks 2 and 12.

Administration and Monitoring of Study Drug

The tacrolimus capsules used (Tacrolimus, Astellas Pharma, Japan) contained 0.5 mg or 1 mg of FK506. In consideration of safety, tacrolimus therapy was initiated at a small dose of 1–2.5 mg per time, twice daily. Dose adjustments were determined using proportional calculations of "blood trough concentration at steady state" and "target trough concentration" as shown in Table 1. To reach the target trough concentration quickly, the first dose adjustment occurred at an early stage. This increase required blood collection at 12 hours (C12h) and 24 hours (C24h) after the initial dose for determination of the trough concentration of tacrolimus in whole blood. Steady-state values were estimated to be 4 times the value at C12h, 2.5 times the value at C24h, or 3 times the mean value of C12h and C24h. The dose was adjusted by proportional calculation using a target concentration of 12.5 ng/mL. These equations were created based on the known pharmacokinetic profile of tacrolimus in healthy volunteers (data not shown).

For the next adjustment, measured values were checked against the target trough concentration. When the measured value was outside the range of 10–15 ng/mL, the dose was readjusted using blood trough concentration at steady state.

The randomization was performed by the Control Center (Bellsystem24, a third-party organization independent of study physicians and sponsor). To preserve blinding, blood trough

TABLE 1. Dose Adjustment of Tacrolimus

Dosage calculation method using trough concentration

Blood trough concentration under the same food intake condition as at administration should be used (fed/fasted condition).

For 2 weeks:

The dose is increased to a target trough concentration of 10-15 ng/mL (target of 12.5 ng/mL).

Initial adjustment (a, b, or c)

Initial dose

Weight (kg)	$30 \leq <50$	$50 \leq <70$	$70 \leq <90$	$90 \leq \leq 100$
Dose per time (mg), twice daily	1	1.5	2	2.5

The blood trough concentration at 12 hours (C12h) and/or 24 hours (C24h) after the initial dose.

a: Initial dose (mg) × target trough concentration (12.5 ng/mL) / (average of C12h & C24h × 3).

b: Initial dose (mg) × target trough concentration (12.5 ng/mL) / (C12h × 4).

c: Initial dose (mg) × target trough concentration (12.5 ng/mL) / (C24h × 2.5).

Next adjustment:

The blood trough concentration (C) was measured at steady-state, after 2 days or more following the prevopus adjustment, to check whether the value was within the range of 10-15 ng/mL.

When the measured value was outside the range of 10-15 ng/mL, the dose was readjusted.

Previous dose × target trough concentration (12.5 ng/mL) / C.

levels were measured by SRL (a third-party organization independent of study physicians and sponsor) and relayed to the Control Center (Bellsystem24). Dosages were calculated at the Control Center based on the trough levels. The clinical sites were informed of the adjusted dosage by 3 days after the blood sample was drawn. Patient doses in the placebo group were pseudo-adjusted to preserve study blinding. The Control Center used the equations shown in Table 1 to carry out dose adjustments.

Symptom Assessment and Study Endpoints

The primary endpoint was clinical response based on the DAI score.[7] Clinical response was defined as a reduction in DAI by at least 4 points and improvements in all categories (stool frequency, rectal bleeding, mucosal appearance, and physician's overall assessment). A worse or unchanged score in any category was considered a treatment failure, even if all other scores improved. Secondary endpoints were mucosal healing and clinical remission.[8] Mucosal healing was defined as mucosal appearance subscore of 0 or 1. Clinical remission was defined as a total DAI score ≤ 2 with individual subscore (stool frequency, rectal bleeding, mucosal appearance, and physician's overall assessment) of 0 or 1. When a patient's symptoms worsened at any time and the investigator decided the study drug could not be continued, the treatment was considered a failure.

Statistical Analysis

Fisher's exact test was used to compare the tacrolimus group with the placebo group for demography, efficacy, and safety. The Wilcoxon signed rank test was used to compare each timepoint with baseline for demography. All statistical tests were two-sided with a significance level of 0.05 unless otherwise specified.

Sample Size

Based on previous results,[5] the clinical response was assumed to be 50% in the tacrolimus group and 10% in the placebo group. We estimated that randomizing 31 patients to each group would be sufficient to show a difference in efficacy between placebo and tacrolimus based on the above assumptions and a two-sided alpha of 0.025 and power of 0.9 using a normal approximation.

RESULTS

Patient Population

This study was performed between August 2006 and February 2008. Sixty-two patients in total were recruited. The mean total DAI score of patients enrolled was 9.8 ± 1.61 in the tacrolimus group and 9.1 ± 1.05 in the placebo group.

Drug Exposure

The mean trough concentrations in the tacrolimus group were 1.4 ± 0.9 ng/mL at 12 hours, 2.2 ± 1.5 ng/mL at 24 hours, 9.6 ± 3.1 ng/mL at day 7, 10.3 ± 3.1 ng/mL at day 8, 11.6 ± 3.4 ng/mL at day 10, and 13.0 ± 4.4 ng/mL at day 14.

Efficacy

Figure 1 shows that a clinical response was observed in 50.0% (16/32) of patients in the tacrolimus group and 13.3% (4/30) of patients in the placebo group. Significantly more patients in the tacrolimus group showed improvements compared with the placebo group ($P = 0.003$).

The observed rate of mucosal healing was 43.8% (14/32) in the tacrolimus group and 13.3% (4/30) in the placebo group ($P = 0.012$) at week 2, and clinical
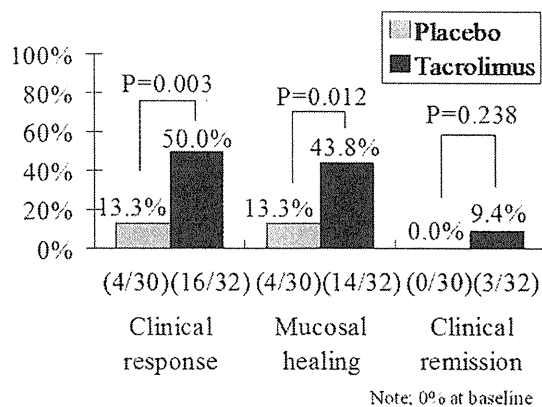
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FIGURE 1. Efficacy result.

remission at week 2 was observed in 9.4% (3/32) of the tacrolimus group compared with 0.0% (0/30) in the placebo group (*P* = 0.238).

Twenty-seven of the 32 patients in the tacrolimus group achieved target trough levels. Among the 27 patients, the observed rate of clinical response, mucosal healing, and clinical remission were 59.3% (16/27), 51.9% (14/27), and 11.1% (3/27), respectively. Among the other five patients who did not achieve target trough levels, clinical response, mucosal healing, and clinical remission were not observed.

The rate of clinical remission was lower than that of mucosal healing. This was supposed to have been associated with the difference in criteria for the former and the latter. While mucosal healing was defined as achieving a mucosal appearance subscore of 0 or 1, clinical remission was more strictly defined as a subscore of 0 or 1 on each of the four factors (stool frequency, rectal bleeding, mucosal appearance, and physician's overall assessment) and a total score of 2 or lower.

Safety

Adverse events and serious adverse events were evaluated in all patients who received at least one dose of the study drug (Table 2). No statistically significant difference in incidence of adverse events was seen between the tacrolimus group (81.3%) and placebo group (70%) (*P* = 0.379).

The most common adverse event seen in patients who received tacrolimus was numbness. All events were mild and did not interfere with the patients' normal functioning. There were no significant adverse events on body temperature, blood pressure, pulse rate, hematologic parameters, electrolytes, renal function, cholesterol levels, and blood glucose levels, and no opportunistic infections were observed. No clinically significant differences in vital signs or laboratory test values were found between the two groups.

The mean values of serum creatinine (mg/dL) in the tacrolimus group and in the placebo group were, respec-

tively, 0.652 and 0.640 at baseline, and 0.633 and 0.672, respectively, at the end of the study. The mean values of BUN (mg/dL) in the tacrolimus group and in the placebo group were, respectively, 9.49 and 9.99 at baseline, and 11.59 and 9.29, respectively, at the end of the study.

Open-label Extension

After 2 weeks the treatment for 20 of the 62 patients in this study was changed to conventional treatment with drugs such as azathioprine. The remaining 42 patients continued to be treated with tacrolimus. Twenty-one of the 42 patients were in the tacrolimus group. The effect of continuous treatment in the tacrolimus group was evaluated by comparing the condition of 21 patients in the tacrolimus group at week 2 and week 12.

The results show an increase in mucosal healing from 66.7% (14/21) to 85.7% (18/21) and in clinical remission from 14.3% (3/21) to 28.6% (6/21) (Fig. 2a).

Seven of the 21 patients had failed azathioprine maintenance over the period beginning 3 months prior to the start of the study. Among the seven patients, the results also show an increase in mucosal healing from 71.4% (5/7) to 85.7% (6/7) and in clinical remission from 28.6% (2/7) to 57.1% (4/7). Among the other 14 patients the results also show an increase in mucosal healing from 64.3% (9/14) to 85.7% (12/14) and in clinical remission from 7.1% (1/14) to 14.3% (2/14).

Furthermore, the mean prednisolone dose was decreased (8.9 mg/day) from that at baseline (24.2 mg/day) (Fig. 2b). One patient was off steroids at week 12 and the total DAI score of this patient was 3. Although the prednisolone doses was not evaluated after week 12, the prednisolone doses in six patients who achieved clinical remission

TABLE 2. Safety Result

No. of Patients (%)	Tacrolimus (*n*=32)	Placebo (*n*=30)
Adverse events	26 (81.3)[a]	21 (70.0)
Related adverse events	19 (59.4)	10 (33.3)
Serious adverse events:	None	None
Related adverse events occurring in > 5% of patients in at least one of the treatment groups		
Nausea	4 (12.5)	3 (10.0)
Headache	4 (12.5)	3 (10.0)
Numbness	4 (12.5)	0 (0.0)
Finger tremor	3 (9.4)	1 (3.3)
Dysmenorrhea	3 (9.4)	1 (3.3)
Hot flushes	2 (6.3)	1 (3.3)
Abdominal pain upper	2 (6.3)	1 (3.3)
Back pain	2 (6.3)	1 (3.3)

[a]Fisher's exact test, *P* = 0.379 vs. placebo.

a) Efficacy result of continuous treatment

Note; 0% at baseline
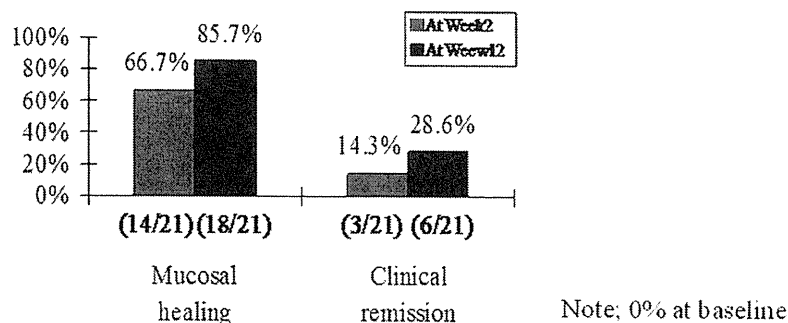
b) Steroid tapering efficacy

The mean steroid dose   Note; Plus-minus values are means± SD
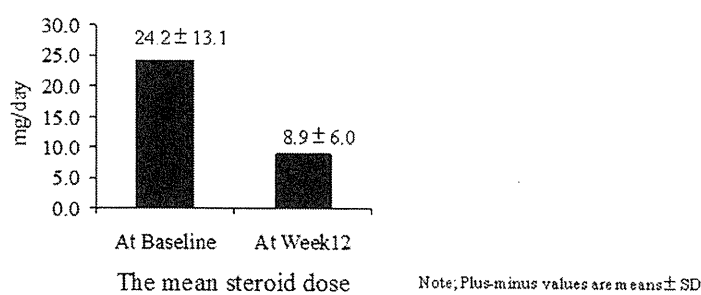
FIGURE 2. Open-label extension.

at week 12 were 10 mg/day, 10 mg/day, 5 mg/day, 5 mg/day, 2.5 mg/day, and 2.5 mg/day, respectively.

A smooth transition to the extension phase was achieved. The mean tacrolimus trough concentrations were 5.5 ± 1.5 ng/mL at week 4, 6.3 ± 1.7 ng/mL at week 8, and 6.7 ± 1.8 ng/mL at week 12.

This open-label extension phase of the study was well tolerated, with only minor side effects and no patients required colectomy.

Compliance

Patients were questioned by the investigator regarding compliance during the study. No cases of noncompliance could be identified.

DISCUSSION

Patients included in this study either had failed treatment with their most recent steroid treatment or were in immediate need of alternative treatment, including operative procedures. Because of these factors, a study design involving administration of placebo for 2 weeks or more was impossible both in terms of ethics and appropriate treatment. Although these results in the short duration of treatment should be treated with caution, it was demonstrated that oral tacrolimus therapy in patients with steroid-refractory,

moderate-to-severe UC shortened the acute phase and induced rapid mucosal healing.

An open-label extension resulted in further improvements and a reduction in steroid dose. Remission induction rates, relapse rates, and surgery rates in patients treated with tacrolimus over the long term are now being investigated in a prospective study.

The efficacy of tacrolimus in severe steroid-refractory UC was also confirmed in another small open-label study, although these results were not published. While intravenous infusion of cyclosporine has been thought to be effective and recognized as an alternative therapy against refractory, severe UC,[9,10] administering oral tacrolimus therapy is more convenient than 24-hour continuous intravenous infusion of cyclosporine. Intravenous infusion imposes a great physical and psychological burden on the patient in hospital. Changing from intravenous injection to oral administration requires prolonged hospitalization to allow for the dose adjustment period; however, oral tacrolimus therapy can eliminate these disadvantages.

With regard to the long-term usefulness of tacrolimus, Baumgart et al[11] and Yamamoto et al[12] have reported the usefulness of long-term administration of tacrolimus for 12 weeks or more as remission maintenance therapy in open-label studies. More recently, Yamamoto et al[13] reported the efficacy of tacrolimus compared with

thiopurines for maintaining remission in patients with refractory UC. They concluded that maintenance therapy with tacrolimus for patients with UC could be considered an alternative to thiopurine therapy.

Naganuma et al[14] summarized how/when we should use tacrolimus in patients with refractory UC. Although our results suggest that tacrolimus therapy is useful as an alternative therapy against steroid-refractory UC, further investigation will be necessary to clarify the clinical usefulness of tacrolimus in comparison with biologics, such as infliximab, as a therapeutic strategy for refractory UC.

ACKNOWLEDGMENT

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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

Table 1. Basic Characteristics of Study Subjects

Set	CD cases		UC cases		Control
	GWAS	Replication	GWAS	Replication	
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 Bead-Chip, 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 ($\alpha = .05$). 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
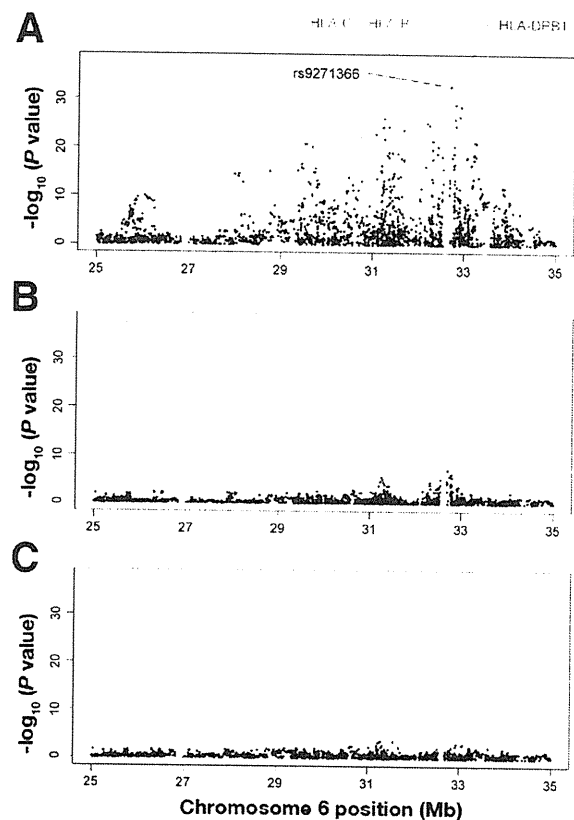
Figure 1. Result of the comparative GWAS using 372 UC cases and 372 CD cases around the MHC region (Chr. 6, 25–35 Mb). Positional plots of $-\log_{10}$ (P value) of the SNPs (A) before adjustment, (B) after adjustment of HLA-Cw*1202-B*5201-DRB1*1502 haplotype, and (C) after adjustment of the all associated 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 $P = 5.0 \times 10^{-8}$.

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.

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 1A, 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 2A). 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 2B). 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			Allele 1 frequency			UC vs CD		UC vs control		CD vs control	
						UC	CD	Control	UC	CD	Control	OR (95% CI)	P value[a]	OR (95% CI)	P value[a]	OR (95% CI)	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}

aObtained 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 (α = .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-
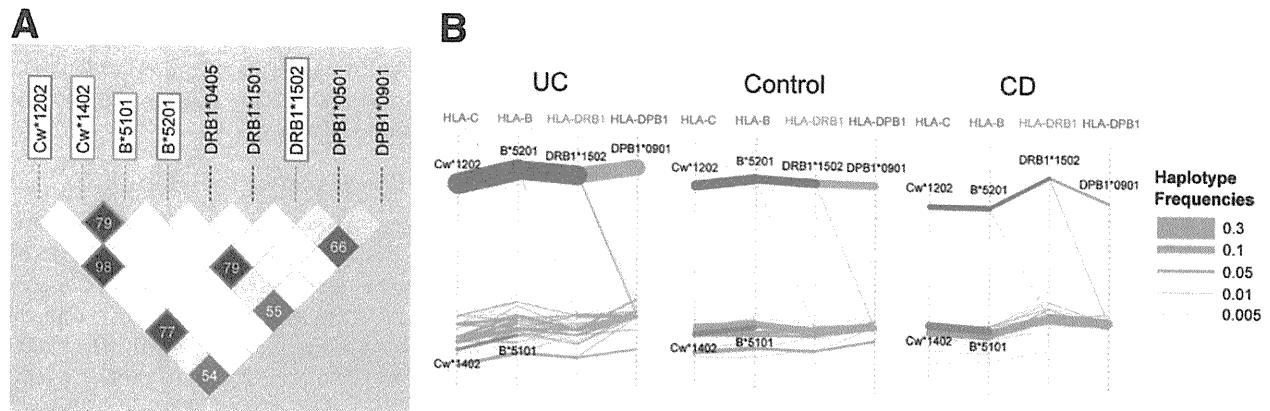
Figure 2. LD map and haplotype structure of HLA alleles. (A) Triangular heat map display of LD index, r^2, among the HLA alleles associated between UC and CD. LD map based on the controls is drawn using Haploview version 4.0.[31] r^2 value >0.5 is indicated in the *diamond*. Pairs of the HLA alleles 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 1B). When the SNPs were further adjusted for other associated HLA haplotypes and alleles, no significant association was observed (the smallest $P = .00023$; Figure 1C). 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 (n = 372)	Frequency[a] CD (n = 372)	Frequency[a] Control (n = 905)	UC vs CD OR (95% CI)[b]	UC vs CD P value[b]	UC vs control OR (95% CI)[b]	UC vs control P value[b]	CD vs control OR (95% CI)[b]	CD vs control 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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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).
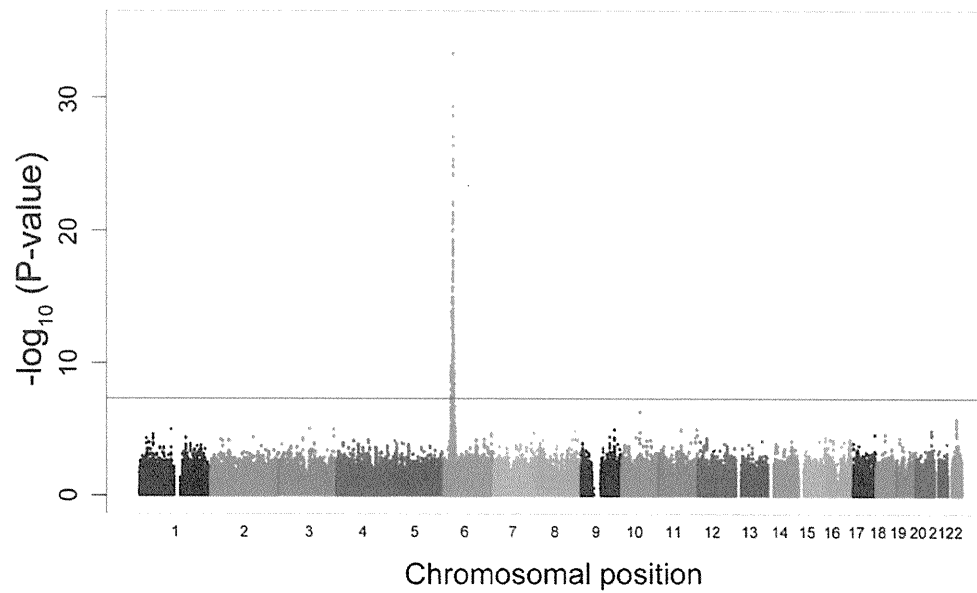
Supplementary Figure 2. Manhattan plot of the comparative GWAS using 372 UC cases and 372 CD cases. The *gray horizontal lines* in the plots represent the genome-wide significance threshold of $P = 5.0 \times 10^{-8}$.
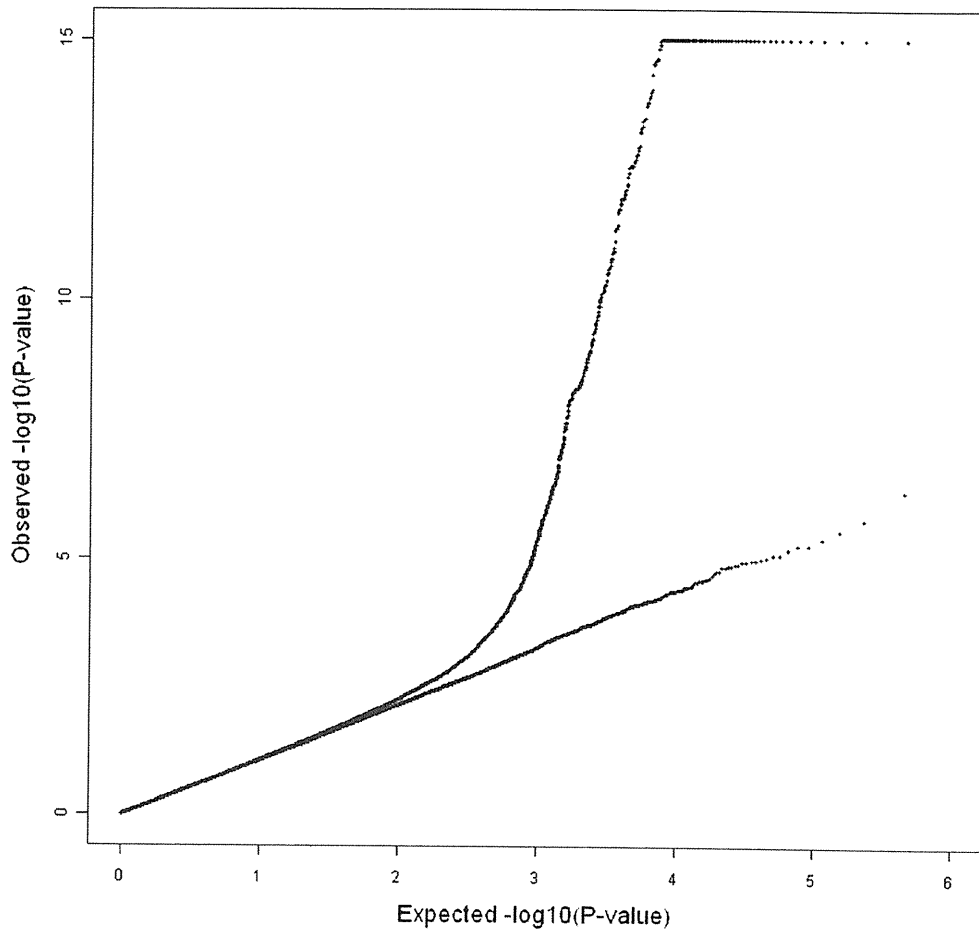
Supplementary Figure 3. Quantile-Quantile plot (QQ-plot) of *P* values in the GWAS. The QQ-plot of Cochran–Armitage trend test *P* values in the GWAS is on a logarithmic scale. The x-axis represents the expected *P* values under the assumption of a uniform distribution of *P* values, and the y-axis represents 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

HLA allele	No. of alleles			Allele frequency			UC vs CD		UC vs control[d]		CD vs control[d]	
	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				

Supplementary Table 1. (Continued)

HLA allele	No. of alleles UC	CD	Control	Allele frequency UC	CD	Control	UC vs CD OR (95% CI)[b]	P value[b]	UC vs control[a] OR (95% CI)[b]	P value[b]	CD vs control[a] OR (95% CI)[b]	P value[b]
DRB1*1101	18	19	41	0.024	0.026	0.023	0.94 (0.49−1.80)	.85				
DRB1*1108	0	0	1	0	0	0.001	—	1				
DRB1*1119	0	1	0	0	0.001	0	0.00 (0.00−38.7)	.50				
DRB1*1123	0	0	1	0	0	0.001	—	1				
DRB1*1201	18	30	71	0.024	0.041	0.039	0.59 (0.32−1.06)	.074				
DRB1*1202	10	7	26	0.013	0.010	0.014	1.42 (0.54−3.76)	.47				
DRB1*1301	2	7	14	0.003	0.010	0.008	0.28 (0.03−1.49)	.11				
DRB1*1302	37	41	97	0.050	0.056	0.054	0.89 (0.56−1.40)	.62				
DRB1*1401	18	37	47	0.024	0.050	0.026	0.47 (0.26−0.83)	.0082				
DRB1*1403	7	12	20	0.009	0.016	0.011	0.57 (0.22−1.47)	.24				
DRB1*1405	15	22	30	0.020	0.030	0.017	0.67 (0.34−1.30)	.23				
DRB1*1406	10	8	21	0.013	0.011	0.012	1.24 (0.49−3.17)	.65				
DRB1*1407	2	2	1	0.003	0.003	0.001	0.99 (0.07−13.7)	1				
DRB1*1429	1	0	1	0.001	0	0.001	—	1				
DRB1*1501	69	32	126	0.093	0.043	0.070	2.26 (1.46−3.48)	1.6E−04	1.37 (1.01−1.86)	.044	0.61 (0.41−0.90)	.013
DRB1*1502	207	44	246	0.279	0.060	0.136	6.09 (4.31−8.59)	3.2E−29	2.46 (1.99−3.03)	9.8E−18	0.40 (0.29−0.56)	4.0E−08
DRB1*1602	6	3	14	0.008	0.004	0.008	1.99 (0.42−12.4)	.51				
HLA−DPB1												
DPB1*0201	114	165	412	0.153	0.222	0.228	0.63 (0.49−0.82)	6.4E−04				
DPB1*0202	28	24	76	0.038	0.032	0.042	1.17 (0.67−2.04)	.58				
DPB1*0301	25	44	62	0.034	0.059	0.034	0.55 (0.33−0.91)	.019				
DPB1*0401	30	30	84	0.040	0.040	0.046	1.00 (0.59−1.67)	.99				
DPB1*0402	53	51	170	0.071	0.069	0.094	1.04 (0.70−1.55)	.85				
DPB1*0501	279	345	698	0.375	0.465	0.386	0.69 (0.56−0.85)	4.4E−04	0.95 (0.80−1.14)	.60	1.38 (1.16−1.64)	.00023
DPB1*0601	5	3	7	0.007	0.004	0.004	1.67 (0.32−10.8)	.73				
DPB1*0901	181	42	226	0.243	0.057	0.125	5.36 (3.76−7.63)	7.1E−24	2.25 (1.81−2.80)	1.2E−13	0.42 (0.30−0.59)	3.1E−07
DPB1*1301	10	14	29	0.013	0.019	0.016	0.71 (0.31−1.61)	.41				
DPB1*1401	11	15	23	0.015	0.020	0.013	0.73 (0.33−1.59)	.42				
DPB1*1701	2	1	2	0.003	0.001	0.001	2.00 (0.10−117.9)	1				
DPB1*1901	6	4	5	0.008	0.005	0.003	1.50 (0.35−7.26)	.75				
DPB1*2501	0	1	0	0	0.001	0	0.00 (0.00−38.9)	.50				
DPB1*3601	0	0	4	0	0	0.002	—	1				
DPB1*3801	0	3	2	0	0.004	0.001	0.00 (0.00−2.41)	.12				
DPB1*4101	0	0	6	0	0	0.003	—	1				
DPB1*4701	0	0	2	0	0	0.001	—	1				

[a]Calculated for the HLA alleles that indicated significant associations between UC cases and CD cases. Based on Bonferroni correction for the number the observed alleles (n = 110), P < .00045 was considered to be significant (α = .05).
[b]Obtained by the comparison of allele frequencies.

Supplementary Table 2. Case-Case and Case-Control Associations of HLA-Cw*1202-B*5201-DRB1*1502 Haplotype Stratified by Colonic and Noncolonic CD

	Analyzed groups	No. subjects (group 1/group 2)	Frequency (group 1/group 2)	OR (95% CI)	P value
Within case analysis	UC vs CD	372/372	0.27/0.054	6.58 (4.60−9.42)	1.1×10^{-33}
	UC vs colonic CD	372/53	0.27/0.10	3.19 (1.67−6.09)	6.4×10^{-5}
	UC vs noncolonic CD	372/315	0.27/0.043	8.36 (5.50−12.72)	8.8×10^{-36}
	Colonic CD vs noncolonic CD	53/315	0.10/0.043	2.62 (1.26−5.48)	.0083
Case-control analysis	UC vs control	372/905	0.27/0.12	2.65 (2.14−3.29)	4.0×10^{-21}
	CD vs control	372/905	0.054/0.12	0.40 (0.28−0.57)	1.1×10^{-7}
	Colonic CD vs control	53/905	0.10/0.12	0.83 (0.44−1.58)	.58
	Noncolonic CD vs control	315/905	0.043/0.12	0.32 (0.21−0.48)	5.2×10^{-9}