- Surveillance of patients with dysplasia was not standardized in detection methods (eg, performed by using standard white light without chromoendoscopy or image enhancement at various intervals) or in endoscopic removal methods.
- Follow-up data did not account for duration of IBD.

- A pooled analysis was thought inappropriate due to significant heterogeneity in patients, definitions, intervention, and outcome.

General descriptive summary

- The majority of studies did not recommend proctocolectomy for patients with endoscopically invisible low-grade dysplasia.
- All studies emphasized the cumulative incidence of cancer and important role of vigilant surveillance.
- Some studies reported increased cumulative incidence of cancer when endoscopically invisible dysplasia is multifocal or when it is located in the distal colon.
- Studies provide insufficient power and/or longitudinal data to report on colorectal cancer incidence and/or mortality.

- For the articles that we identified for this statement, we interpreted the report of "flat dysplasia" as endoscopically invisible dysplasia unless there were clear morphologic features described. We used this approach based on the historic guidelines and literature that have used the term *flat dysplasia* to refer to endoscopically undetectable lesions and the term *raised dysplasia* to refer to endoscopically detectable lesions.
- A recent meta-analysis on the cancer risk of low-grade dysplasia in chronic ulcerative colitis that included 20 surveillance studies totaling 508 flat low grade dysplasia or low grade dysplasia with dysplasia-associated lesions or masses. The studies predate the use of current technology of image enhanced endoscopy or even high resolution endoscopy. The authors reported a 9-fold risk of developing cancer (odds ratio [OR] 9.0, 95% CI, 4.0-20.5) and a 12-fold risk of developing any advanced lesion (OR 11.9, 95% CI, 5.2-27). The absolute risk of cancer in this meta-analysis was 14 (95% CI, 5-34) cancers/100 years patient follow-up. Meta-analysis: cancer risk of low-grade dysplasia in chronic ulcerative colitis. Thomas T, Abrams KA, Robinson RJ, et al. Aliment Pharmacol Ther 2007;25, 657-68.

High-grade dysplasia

- We identified no studies on the natural history of endoscopically invisible high-grade dysplasia followed with surveillance that reported findings during the videoendoscope era (1990 to present).

A systematic review of findings from the fiberoptic era reported a probability of finding cancer in a patient with high-grade dysplasia of 42% (10/24) if colectomy was done immediately and 32% (15/47) if colectomy was done after some follow-up: Bernstein CN, Shanahan F, Weinstein WM. Are we telling patients the truth about surveillance colonoscopy in ulcerative colitis? Lancet 1994;343,71-4. Importantly, interpretation of the data should be with caution due to significant limitations in the sensitivity of the fiberoptic technology to detect dysplasia or cancer at index colonoscopy. Furthermore, surveillance of patients with dysplasia was not standardized (eg, performed without chromoendoscopy or image enhancement at various intervals or in endoscopic removal methods). Thus, the true incidence of synchronous colorectal cancer in the setting of high grade dysplasia as well as the true natural history of endoscopically invisible high grade dysplasia is not known.

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ORIGINAL ARTICLE

Low-Dose Aspirin and Non-steroidal Anti-inflammatory Drugs Increase the Risk of Bleeding in Patients with Gastroduodenal Ulcer

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Abstract
Background Non-steroidal anti-inflammatory drugs (NSAIDs), low-dose aspirin (LDA), non-aspirin antiplatelet medications (APs), and anticoagulant medications (ACs) increase the risk of gastrointestinal bleeding.
Aim To examine whether NSAIDs, LDA, APs, and ACs use is associated with bleeding from gastroduodenal ulcers.
Methods This was a case–control study of patients with endoscopically verified gastroduodenal ulcer diagnosed at our institution from 2004 to 2011. Among 1,611 patients, we identified those who required endoscopic hemostasis for bleeding ulcers as cases. Age-matched, sex-matched, and *Helicobacter pylori* status-matched patients who did not require therapeutic interventions served as controls. Use of NSAIDs, LDA, APs, and ACs within 2 weeks prior to the endoscopy was compared between cases and controls, and effects on ulcer bleeding were calculated.
Results We recruited 341 cases and 668 controls. The site and number of ulcers were not different between groups. Multivariate analyses revealed that LDA and NSAIDs, individually, were associated with the increase in the risk of bleeding (OR 1.80 and 95 % CI 1.18–2.75 for LDA; 1.35 and 1.01–1.80 for NSAIDs). In addition, a combination of LDA and NSAIDs or LDA and APs contributed more profoundly to the bleeding (OR 3.59 and 95 % CI 1.19–10.81 for LDA/NSAIDs; OR 6.70 and 95 % CI 1.83–24.50 for LDA/APs). However, ACs, alone or in combination, were not associated with bleeding ulcers.
Conclusions Both LDA and NSAIDs are risk factors for upper GI bleeding in patients with gastroduodenal ulcer, while ACs are unrelated to the increased risk. The risk of bleeding with LDA may increase with simultaneous use of APs.

Keywords Gastroduodenal ulcer · Gastrointestinal bleeding · Non-steroidal anti-inflammatory drugs · Low-dose aspirin · Antithrombotic medication

Introduction

Helicobacter pylori (*H. pylori*) infection and use of non-steroidal anti-inflammatory drugs (NSAIDs) and low-dose aspirin (LDA) are well-established causes of gastroduodenal ulcer [1]. *H. pylori* and NSAIDs are independent risk factors for upper gastrointestinal (GI) bleeding. In addition, antithrombotic medications, which include non-aspirin antiplatelet medications (APs) and anticoagulants (ACs), are associated with a high incidence of upper GI bleeding [2].

Several case–control studies in the literature have evaluated the contribution of LDA, NSAIDs, and antithrombotic medications to the development of bleeding gastroduodenal ulcers [3–5]. In those studies, however, healthy controls or patients admitted for other diseases were selected as controls; thus, the studies failed to evaluate the relationship between the medications and actual bleeding from gastroduodenal ulcers. In addition, because Japan has the highest worldwide prevalence of *H. pylori* infection, and *H. pylori* is an independent risk factor for upper GI bleeding, it is not clear whether LDA, NSAIDs, APs, and ACs are actually risk factors for bleeding from

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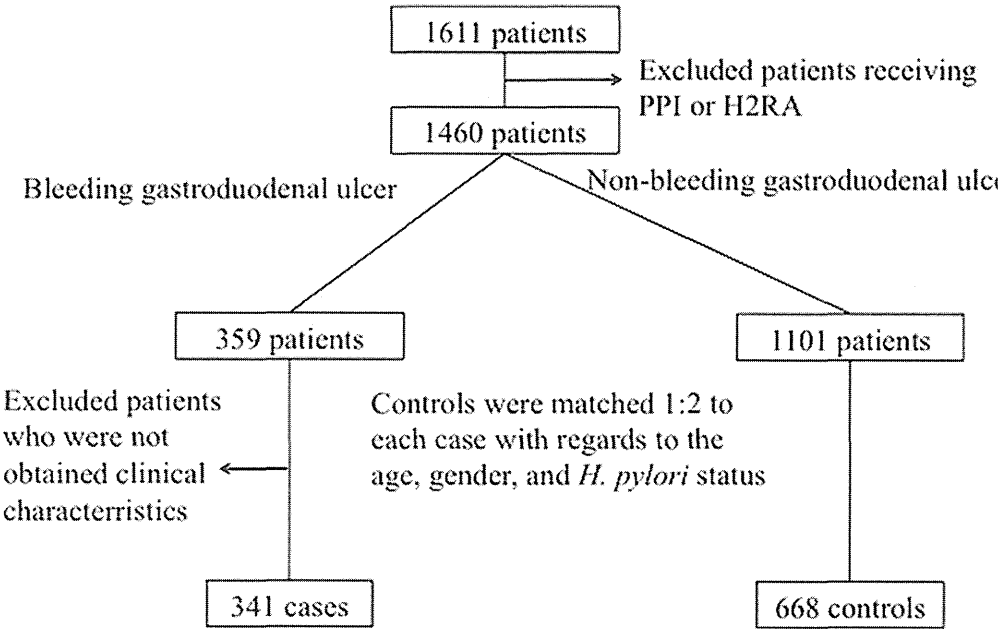

Fig. 1 Selection of cases and controls. Among 1,611 patients with a diagnosis of gastroduodenal ulcer, we first excluded patients who had been receiving treatment with proton pump inhibitors (PPI) or histamine-2 receptor antagonists (H2RA). Among the remaining patients, those who were diagnosed as having a bleeding gastroduodenal ulcer treated with endoscopic hemostasis were regarded as cases. We then identified two controls with non-bleeding gastroduodenal ulcers for each case. Controls were matched to each case with regard to age, sex, and H. pylori status. We enrolled 341 cases and 668 controls

gastroduodenal ulcers when compared to non-bleeding ulcers [6, 7].

To examine whether the use of LDA, NSAIDs, APs, ACs, and any combination of these medications actually contributes to the increase in the risk of bleeding from gastroduodenal ulcers, we undertook a single-center, case–control study of patients with endoscopically verified gastroduodenal ulcers.

Patients and Methods

Study Population

This was a case–control study based on retrospective data collection. We reviewed the endoscopy database at Matsuyama Red Cross Hospital from 2004 to 2011, and identified all of the patients with a diagnosis of active gastroduodenal ulcer under esophagogastroduodenoscopy (EGD). We subsequently identified cases and controls according to the following criteria (Fig. 1). First, we excluded patients who had been receiving treatment with proton pump inhibitors (PPI) or histamine-2 receptor antagonists (H2RA). Among the remaining patients, those who were diagnosed as having a bleeding gastroduodenal ulcer treated with endoscopic hemostasis were regarded as cases. Then, we identified two controls with non-bleeding gastroduodenal ulcers for each case. The controls were matched to each case with regard to age (within a maximal difference of 5 years), sex, and *H. pylori* status as determined by the procedure described below.

The protocol of this case–control study was approved b Institutional Review Board at Matsuyama Red Cro Hospital.

Exposure Definition

Clinical characteristics of the study subjects were invest gated on the basis of chart review. The characteristi included age, sex, *H. pylori* infection, medication at tl time of endoscopic diagnosis of gastroduodenal ulcers, ar endoscopic findings of the ulcers.

The endoscopic characteristics included the site ar number of active ulcers. An ulcer was defined as a mucos defect larger than 5 mm in diameter. Mucosal defects le than 5 mm in size, histologically verified cancer, and ca dial mucosal defects presumably related to Mallory–Wei syndrome were not regarded as gastroduodenal ulce *H. pylori* status was determined by the following thr procedures: (1) Histology of the biopsy specimen, (2) tit of serum IgG antibody to *H. pylori* as measured with a enzyme-linked immunosorbent assay kit using the E pla test (Eiken Kagaku, Tokyo, Japan), and (3) ^{13}C-urea brea test. Subjects were considered to be positive for *H. pylo* infection if they had a positive result on any one of tl three tests and to be negative if all three demonstrate negative results. LDA, NSAID, AP, AC, and steroid u was checked by reviewing the prescriptions at our hospit and the confirmation sheet for medications at the time the endoscopy. The latter was completed and filed b medical staff members for patients who had prescriptio from referring physicians or who were taking comme cially available medications. The use of LDA, NSAID

Table 1 Characteristics of cases and controls

Demographics	Cases ($N = 341$)	Controls ($N = 668$)	p value
Age (mean ± SD)	65.2 ± 15.3	65.1 ± 15.0	NS
Gender			
M (%)	230 (67.4)	446 (66.8)	NS
F (%)	111 (32.6)	222 (33.2)	
H. pylori infection			
+ ve (%)	234 (68.6)	465 (69.6)	NS
− ve (%)	107 (31.4)	203 (30.4)	
Number of active ulcers (mean ± SD)	1.7 ± 1.7	1.7 ± 1.9	NS
Site of ulcer			
Stomach (%)	253 (74.2)	460 (68.9)	NS
Duodenum (%)	88 (25.8)	208 (31.1)	
Steroid user [*n* (%)]	21 (6.2)	42 (6.3)	NS

F female, *M* male, *NS* not significant, *SD* standard deviation

APs, and ACs was regarded to be positive if the patient had taken the agent within 2 weeks prior to the EGD. LDA included both coated and buffered aspirin.

Statistical Analysis

Parametric data are expressed as mean ± SD. Nonparametric data are expressed as numbers and percents. Comparisons between any two groups were performed with the Mann–Whitney test or Chi-squared test where appropriate. Stepwise multivariate logistic regression analysis was used to calculate odds ratios (ORs) and 95 % confidence intervals for bleeding ulcers. Probabilities less than 0.05 were considered to be significant. All statistical computations were performed with JMP version 11 (Statistical Discovery Program, USA).

Results

There were 1,611 patients in the database who had a diagnosis of active gastroduodenal ulcer. After excluding those who did not meet the inclusion and exclusion criteria described above, we enrolled 341 patients as cases and 668 patients as controls. The procedures for endoscopic hemostasis (for cases) were argon plasma coagulation (APC) for 94 cases, injection of alcohol or hypertonic saline–epinephrine solution (HSE) for 28 cases, endo-clip for 14 cases, a combination of HSE and APC for 183 cases, or other combinations for 22 cases. Primary endoscopic hemostasis could be achieved in all of the cases.

Table 1 compares the baseline clinical and endoscopic characteristics between cases and controls. The rate of *H. pylori* positivity was 68.6 % in cases and 69.6 % in controls. There was no difference in the number or location of the ulcers between groups. The incidence of steroid use did not differ between groups.

NSAIDs taken by the cases and controls included loxoprofen, diclofenac, ibuprofen, meloxicam, etodolac, lornoxicam, ketoprofen, mefenamic acid, celecoxib, and others. APs included ticlopidine, clopidogrel, cilostazol, ethyl icosapentate, limaprost alfadex, and others, and ACs included warfarin and heparin sodium. As shown in Table 2, the use of LDA (14.7 vs. 8.1 %, $p = 0.002$), NSAIDs (31.4 vs. 25.3 %, $p = 0.04$) and APs (10.0 vs. 6.1 %, $p = 0.03$) was more frequent in cases than in controls. However, the difference in the use of ACs did not reach statistical significance (8.5 vs. 5.2 %, $p = 0.06$).

Results of multivariate analysis for bleeding ulcers are shown in Table 3. A logistic regression analysis revealed that neither APs nor ACs increased the risk of bleeding (OR 1.44 and 95 % CI 0.88–2.35 for APs; OR 1.52 and 95 % CI 0.90–2.35 for ACs). In contrast, LDA and NSAIDs increased the risk of bleeding (OR 1.80 and 95 % CI 1.18–2.75 for LDA; OR 1.35 and 95 % CI 1.01–1.80 for NSAIDs).

There were six combinations of LDA, NSAIDs, APs, and ACs in the study population. As shown in Table 2, two combinations (LDA plus NSAIDs and LDA plus APs) were more frequently used in cases than in controls. As a consequence, these two combinations increased the risk of bleeding, with an OR of 3.59 for LDA plus NSAIDs and 6.7 for LDA plus APs. Of note, addition of APs to LDA resulted in a 3.7-fold increase in the risk of bleeding. However, a combination of NSAIDs and APs and three combinations including ACs did not significantly increase the risk of bleeding.

To further determine the effect of the medications on ulcer bleeding according *H. pylori* status, we performed a stratified analysis (Table 4). When the subjects were classified into 699 patients (234 cases and 465 controls) positive for *H. pylori* and 310 patients (107 cases and 203 controls) negative for *H. pylori*, multivariate analyses revealed that LDA was marginally but significantly associated with the increase in the risk of ulcer bleeding (OR 1.83 for the *H. pylori*-positive group and 1.95 for the *H. pylori*-negative group).

Discussion

We showed that LDA and NSAIDs are risk factors for bleeding in patients with gastroduodenal ulcer and that a combination of LDA plus NSAIDs or APs increases the risk of bleeding in patients with gastroduodenal ulcer.

Table 2 Results of univariate analysis for the risk of bleeding

Type of medication	Cases ($N = 341$)	Controls ($N = 668$)	p value	Odds ratio (95 % C
LDA	50 (14.7 %)	54 (8.1 %)	0.002	1.95 (1.30–2.94)
NSAIDs	107 (31.4 %)	169 (25.3 %)	0.04	1.35 (1.01–1.80)
APs	34 (10.0 %)	41 (6.1 %)	0.03	1.69 (1.05–2.72)
ACs	29 (8.5 %)	35 (5.2 %)	0.06	1.68 (1.01–2.80)
Combinations				
LDA + NSAIDs	9 (2.6 %)	5 (0.75 %)	0.02	3.59 (1.19–10.81)
LDA + APs	10 (2.9 %)	3 (0.45 %)	0.002	6.70 (1.83–24.50)
LDA + ACs	5 (1.47 %)	6 (0.9 %)	0.52	1.64 (0.50–5.42)
NSAIDs + APs	6 (1.76 %)	9 (1.35 %)	0.59	1.31 (0.46–3.72)
NSAIDs + ACs	8 (2.35 %)	7 (1.05 %)	0.17	2.27 (0.82–6.31)
ACs + APs	1 (0.29 %)	2 (0.30 %)	1	1.00 (0.09–10.84)

CI confidence interval, *LDA* low-dose aspirin, *NSAIDs* non-steroidal anti-inflammatory drugs, *APs* non-aspirin antiplatelet medications, *ACs* anticoagulant medications

Table 3 Results of multivariate analysis for the risk of bleeding

Type of medication	p value	Odds ratio (95 % CI)
LDA	0.006	1.80 (1.18–2.75)
NSAIDs	0.04	1.35 (1.01–1.80)
APs	0.14	1.44 (0.88–2.35)
ACs	0.12	1.52 (0.90–2.35)

CI confidence interval, *LDA* low-dose aspirin, *NSAIDs* non-steroidal anti-inflammatory drugs, *APs* non-aspirin antiplatelet medications, *ACs* anticoagulant medications

Furthermore, ACs did not increase the risk of bleeding, either independently or adjunctively.

It is well established that LDA and NSAIDs increase the risk of upper GI bleeding in the general population. Lanas et al. [4] undertook a prospective case–control study and showed that the use of either LDA or NSAIDs was associated with an increased risk of upper GI bleeding. In that study, the OR of upper GI bleeding for the use of LDA was 3.9 and that for NSAIDs was 5.3. In a Japanese retrospective case–control study by Sakamoto et al. [5], the OR of upper GI bleeding was 5.5 for LDA and 6.1 for NSAIDs. However, it should be noted that in those case–control studies, the controls were selected from patients who were admitted during the same period or from population registries in the same district [3–5, 8–13]. As a consequence, it remains unclear whether LDA and NSAIDs increase the risk of gastroduodenal mucosal lesions, the risk of bleeding from the mucosal lesions, or both. To show to what extent the medications increase the risk of bleeding from the mucosal lesions, we performed the present case–control study. As a consequence, we showed that LDA and NSAIDs are risk factors for bleeding from gastroduodenal ulcers.

In addition to the individual risk incurred by the use of LDA and NSAIDs, combinations of LDA plus NSAIDs and LDA plus APs increased the risk of upper GI bleeding. The increase in the risk of bleeding ulcers with a comb nation of LDA and NSAIDs seems reasonable, becau such a synergistic effect has been confirmed in two lar clinical studies [14, 15]. In a prospective case–contr study, the OR of upper GI bleeding was 3.9 for LDA ar 5.3 for NSAIDs, while it increased up to 12.7 for a con bination of LDA and NSAIDs [4]. However, the synergist effect of LDA and APs on GI bleeding has been shown only a few population-based case–control studies [2, 16]. a study with 1,443 cases of upper GI bleeding and 57,7 controls, the OR of upper GI bleeding was calculated to 1.8 for LDA and 1.1 for clopidogrel, whereas it increase to 7.4 for the combination of LDA and clopidogrel [2]. consideration of the results of our present study, higher ri of GI bleeding with a combination of LDA and APs do not seem to be a consequence of the more severe gastr duodenal mucosal injury, but rather of an increase in t risk of bleeding from preexisting mucosal lesions [17]. contrast, ACs did not increase the risk of bleeding eith independently or synergistically with LDA or NSAIDs our study population, while it has been well establishe that ACs increase the risk of GI bleeding [2]. Although w are not able to explain the discordance in the effect of AI and ACs on the risk of ulcer bleeding, it can be presume that platelet functions, such as adhesion, aggregation, ar formation of the initial plug, may be pivotal for the pr vention of bleeding from gastroduodenal ulcers.

Helicobacter pylori infection plays an important role the pathogenesis of gastroduodenal ulcers. Furthermore, close association between upper GI bleeding and *H. pylo* infection has been shown in a meta-analysis and a retr spective case–control study [1, 5]. *H. pylori* status shou be taken into consideration for the interpretation of o results, because the prevalence of *H. pylori* infection much higher in Japan than in Western countries [6, 7, 1 19]. In the above-mentioned case–control study of

Table 4 Results of multivariate analysis for the risk of bleeding by *H. pylori* status

Type of medication	*H. pylori*-positive				*H. pylori*-negative			
	Cases ($N = 234$)	Controls ($N = 465$)	p value	Odds ratio (95 % CI)	Cases ($N = 107$)	Controls ($N = 203$)	p value	Odds ratio (95 % CI)
LDA	23 (9.8 %)	25 (5.4 %)	0.049	1.83 (1.00–3.34)	27 (25.2 %)	29 (14.3 %)	0.04	1.95 (1.05–3.63)
NSAIDs	50 (21.4 %)	80 (17.2 %)	0.15	1.34 (0.90–1.99)	57 (53.3 %)	89 (43.8 %)	0.08	1.54 (0.95–2.52)
APs	18 (7.7 %)	22 (4.7 %)	0.22	1.51 (0.77–2.91)	16 (15.0 %)	19 (9.4 %)	0.35	1.43 (0.67–3.01)
ACs	13 (5.6 %)	20 (4.3 %)	0.57	1.23 (0.58–2.52)	16 (15.0 %)	15 (7.4 %)	0.08	2.00 (0.93–4.33)

CI confidence interval, *LDA* low-dose aspirin, *NSAIDs* non-steroidal anti-inflammatory drugs, *APs* non-aspirin antiplatelet medications, *ACs* anticoagulant medications

Japanese population, the OR of upper GI bleeding was 4.9 for NSAIDs in patients with negative *H. pylori* status and 5.4 in patients with positive *H. pylori* status without NSAID use. In contrast, the OR increased up to 10.4 with a combination of NSAID use and positive *H. pylori* status [5]. However, the effect of LDA, NSAIDs, APs, and ACs on the OR for upper GI bleeding was not obviously different between our *H. pylori*-positive and *H. pylori*-negative subjects, with only a marginal effect of LDA in both groups. It thus seems possible that the effect of the medications on ulcer bleeding is unrelated to *H. pylori* status.

This study has some limitations. First, its retrospective nature in a single-center protocol seems to have been a source of potential bias. This is especially the case for the uncertainty regarding the severity of underlying diseases, thereby resulting in a provisional overestimation of the role of LDA and APs. To minimize this type of bias, we recruited age-matched and sex-matched controls. Second, we could not take other potential factors, such as smoking and alcohol use, into consideration. However, we believe that there have not been any data showing that alcohol and smoking have a more significant role than LDA, NSAIDs, and APs in the development of bleeding gastroduodenal ulcers. Third, we could not specifically show the risk of each drug classified as an AP or AC, because of the small sample size for each medication.

In conclusion, this study showed that LDA and NSAIDs increase the risk of bleeding in patients with gastroduodenal ulcer. In addition, a combination of LDA and NSAIDs or LDA and APs increases the risk of bleeding in those patients. Thus, it seems to be reasonable to modify therapeutic considerations for patients with gastroduodenal ulcer according to the use of LDA and NSAIDs. This seems to be the case especially for patients taking LDA, because the risk of bleeding seems to be affected by simultaneous use of APs. This issue needs to be investigated prospectively in a large cohort or in a randomized clinical trial.

Conflict of interest None.

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Therapeutic Strategy for Crohn's Disease with a Loss of Response to Infliximab: A Single-Center Retrospective Study

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Key Words
Crohn's disease · Infliximab · Loss of response · Intensified regimen · Adalimumab

Abstract
Background/Aims: Infliximab (IFX) is an effective treatment for maintaining clinical remission in patients with initially moderate-to-severe Crohn's disease (CD). However, a certain number of patients become unresponsive to IFX, subsequently requiring intensified therapy. The aim of this study was to compare the short- and long-term therapeutic efficacy of intensified regimens in CD patients who fail to respond to IFX. ***Methods:*** The clinical courses of 33 CD patients who failed to respond to treatment with IFX were investigated retrospectively. An intensified regimen involving doubling the dose of IFX was chosen in 13 patients (DD group) versus shortening the IFX interval in 13 patients (SI group) and switching to adalimumab (ADA) in 7 patients (SA group). ***Results:*** The clinical response and rate of clinical remission at 4 weeks were 62 and 54% in the DD group, 77 and 62% in the SI group and 57 and 43% in the SA group, respectively ($p = 0.59$ for clinical response, $p = 0.90$ for clinical remission). The rate of sustained remission at 48 weeks was 44% in the DD group, 54% in the SI group and 33% in the SA group ($p = 0.88$). ***Conclusion:*** The short- and long-term efficacy of doubling the dose of IFX, shortening the interval of IFX or switching to ADA is similar for CD patients who no longer respond to IFX.

Introduction

Crohn's disease (CD) is a chronic inflammatory condition involving the gastrointestinal tract. Although the etiology of CD remains unknown, the complex interplay between environmental factors and aberrant immunologic responses in genetically susceptible individuals has been suggested to be fundamental for the development of the disease [1]. In this context, recent genome-wide association studies have clarified several underlying mechanisms of CD [2–4]. However, no curative treatment has been established [5]. Therefore, the current goal of therapy of CD is to improve the underlying course of the disease and restore a normal bowel function [6].

Since the initial report showing a remarkable improvement following treatment with infliximab (IFX) in cases

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of severe CD [7], the therapeutic efficacy of this drug in patients with moderate-to-severe CD has become widely accepted and its beneficial effects in terms of steroid tapering, reducing hospitalization and abdominal surgery, as well as its usefulness in maintaining remission, have been reported [8, 9]. However, nearly one third of patients who achieve clinical remission under treatment with IFX lose their response to this therapy during their clinical course [8, 10, 11], presumably due to declines in the serum IFX level and antibodies to IFX (ATI) [12–15]. In cases involving a loss of response (LOR) to IFX, intensified therapeutic regimens, such as those comprising doubling the IFX dose, shortening the IFX interval or switching to other biologics, are alternative treatments [8, 10, 16–18]. However, it remains unclear which therapeutic regimen is most effective in such patients. We thus compared the therapeutic efficacy of three intensified regimens (doubling the IFX dose, shortening the IFX interval and switching to adalimumab; ADA) in CD patients exhibiting LOR to IFX.

Methods

Subjects

This study was a single-center retrospective study. Among 246 Japanese patients with CD who visited our hospital during the period from October 2004 to May 2014, we initially recruited 164 patients with a history of IFX infusion therapy. Ninety-one patients maintained clinical remission under standard IFX maintenance therapy (every 8 weeks at a dose of 5 mg/kg) until May 2014. Twenty-one patients discontinued IFX therapy (due to adverse events, including infusion reactions in 11 patients, and patient preference or attending physician's judgment in 10 patients) and 19 patients were lost to follow-up (transfer to other hospitals). As a consequence, a total of 33 patients who required treatment with an intensified therapeutic regimen for LOR to standard IFX maintenance therapy were evaluated. These patients showed deterioration of both the CD activity index (CDAI) and serum C-reactive protein (CRP) level within an 8-week interval after the administration of 5 mg/kg of IFX. Active intestinal lesions were confirmed in 11 patients on endoscopic or radiological examinations, while the remaining 22 patients did not undergo imaging procedures. The present study was approved by the ethics committee of Kyushu University Hospital, and all patients provided their written informed consent.

Definitions of Clinical Remission and Clinical Response

Clinical remission was defined as a CDAI score of less than 150. A clinical response was defined as a decrease in the CDAI score of at least 25% or 70 points from baseline, confirmed during the assessment period. As for the short-term therapeutic efficacy of the intensified therapies, the rates of clinical remission and a clinical response at 4 weeks were calculated. The rate of clinical remission at 48 weeks was chosen to assess long-term efficacy.

Statistical Analysis

The χ^2 test was used to compare the clinical outcomes between the three study groups. The decreases in the CDAI scores and serum CRP levels from baseline at 4 and 48 weeks in each group were compared according to the paired t test. p values <0.05 were considered to be statistically significant. The statistical analysis was performed using the JMP statistical package 9.0 (SAS Institute, Cary, N.C., USA).

Results

Patient Demographics

The study patients comprised 23 males and 10 females, with ages at CD onset ranging from 12 to 41 years (mean 23.9). The mean duration of disease was 12.8 years, with a range from 1.3 to 42.1 years. Fifteen patients had previously undergone bowel resection. Standard maintenance IFX therapy had been administered for an average period of 32.4 months, with a range of 8–89 months at a dose of 5 mg/kg given at 8-week intervals until the onset of LOR to IFX.

The intensified therapeutic regimens included doubling the IFX dose in 13 patients (DD group), shortening the IFX interval in 13 patients (SI group) and switching to ADA in 7 patients (SA group) according to the attending physician's judgment. The patients in the DD group received maintenance IFX therapy every 8 weeks at a dose of 10 mg/kg, while those in the SA group received induction and maintenance therapy consisting of ADA (160, 80 and 40 mg on weeks 0, 2 and 4) followed by biweekly maintenance ADA treatment at a dose of 40 mg. In the SI group, 3 patients received maintenance IFX therapy every 7 weeks at a dose of 5 mg/kg, while 9 patients received this therapy every 6 weeks and 1 patient received this therapy every 4 weeks.

Table 1 summarizes the patient demographics in the three groups. The duration of the disease was shorter in the SI group than in the DD and SA groups. None of the patients in the SA group were current smokers. The rate of concomitant use of steroids and immunomodulators was lower in the DD group than in the other two groups. In addition, both the CDAI score and serum CRP level at the start of the intensified regimen were higher in the DD group than in the SI and SA groups. However, this difference was not statistically significant.

Comparison of Short-Term Therapeutic Efficacy among the Groups

Overall, 22 patients (66.7%) achieved a clinical response and 18 patients (54.5%) achieved clinical remis-

Table 1. Clinical characteristics of the three groups at the time of application of the intensified regimens

	DD group (n = 13)	SI group (n = 13)	SA group (n = 7)
Male	11	9	3
Female	2	4	4
Age at onset, years	23.5 (15–34)	25.0 (12–41)	22.9 (18–28)
Duration of the disease, years	13.7 (1.5–42.1)	11.8 (1.3–23.7)	13.5 (5.3–38.1)
Current smoker	4 (30.7)	4 (30.8)	0
Disease location			
Ileitis	4 (30.7)	4 (30.8)	1 (14.3)
Colitis	1 (7.8)	1 (7.7)	2 (28.6)
Ileocolitis	8 (61.5)	8 (61.5)	4 (57.1)
Previous bowel resection(s)	6 (46.2)	5 (38.5)	4 (57.1)
Duration of standard IFX maintenance therapy, months	30 (11–89)	21 (8–59)	40 (9–69)
Concomitant medication			
Enteral nutrition (≥300 kcal/day)	8 (61.5)	7 (53.9)	3 (42.9)
5-Aminosalicylic acid	12 (92.3)	11 (84.6)	5 (71.4)
Steroid	1 (7.8)	4 (30.8)	2 (28.6)
Immunomodulator	2 (15.4)	8 (61.5)	4 (57.1)
CDAI	269.6±90.1	206.5±52.1	211.7±60.8
Serum CRP level, mg/dl	3.86±5.24	2.87±2.61	2.84±1.93

Data are expressed as n with percentage in parentheses, mean with range in parentheses, or mean ± SD.

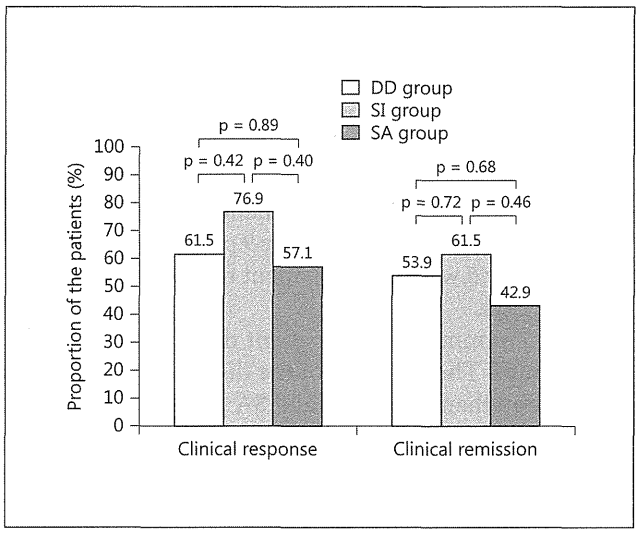

Fig. 1. Clinical response and remission rates at 4 weeks after the application of the intensified regimens in each group.

sion at 4 weeks. Figure 1 shows the rate of short-term efficacy in the three groups. The clinical response rate was 62% in the DD group (8 of 13 patients), 77% in the SI group (10 of 13 patients) and 57% in the SA group (4 of 7 patients). Similarly, the clinical remission rate was 54% (7 of 13 patients), 62% (8 of 13 patients) and 43% (3 of 7 patients), respectively. There were no statistical differences in the rates of either the clinical response or clinical remission between the three groups.

Figure 2 shows the changes in the CDAI scores from week 0 to week 4 in each treatment group (fig. 2). In both the DD and SI groups, the mean CDAI score at week 4 was significantly lower than that observed at baseline (163.9 ± 66.5 vs. 269.6 ± 90.1, p = 0.0005; 138.8 ± 43.0 vs. 206.5 ± 52.1, p = 0.039, respectively). In the SA group, the mean CDAI score at week 4 was lower than that noted at baseline. However, the difference was not statistically significant (156.1 ± 47.5 vs. 211.7 ± 60.8, p = 0.11).

We also compared the serum CRP levels at week 4 with those measured at baseline in each group (fig. 3). Consequently, in the DD group, the mean serum CRP level at week 4 was significantly lower than that observed at baseline (0.85 ± 1.21 vs. 3.86 ± 5.24, p = 0.033). In both the SI and SA groups, while the mean serum CRP level at week 4 was lower than that seen at baseline, the difference did

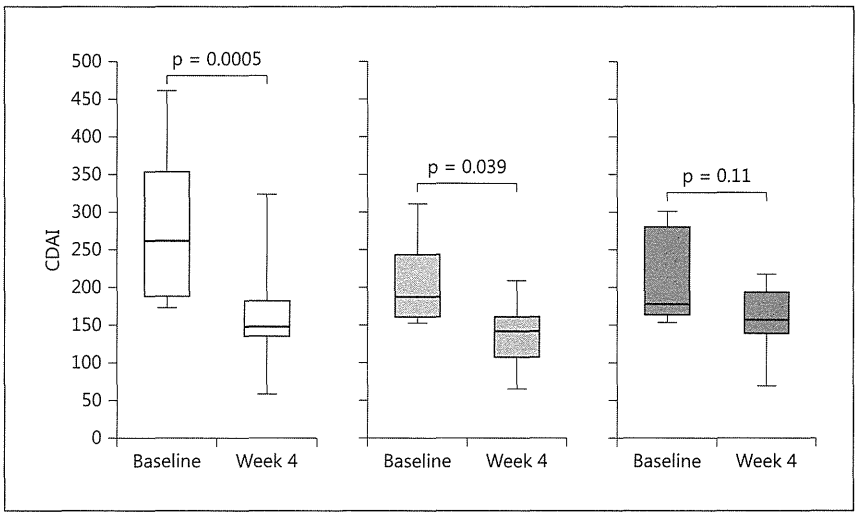

Fig. 2. Comparison of the CDAI scores between baseline and 4 weeks after the initiation of the intensified regimens: DD group (left), SI group (middle) and SA group (right). The box plots illustrate the 95% range (vertical lines), median (horizontal lines) and interquartile range (boxes).

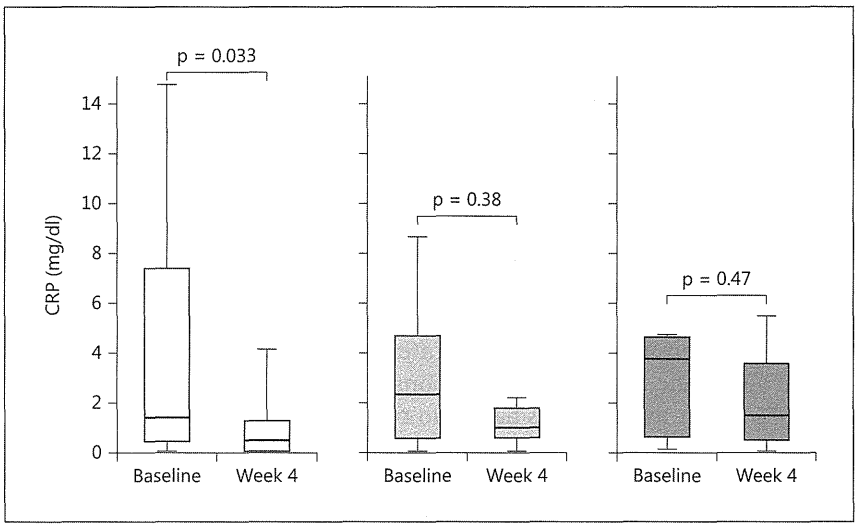

Fig. 3. Comparison of the serum CRP levels between baseline and 4 weeks after the start of the intensified regimens: DD group (left), SI group (middle) and SA group (right). The box plots illustrate the 95% range (vertical lines), median (horizontal lines) and interquartile range (boxes).

not reach a level of statistical significance (1.11 ± 0.70 vs. 2.87 ± 2.61 mg/dl, p = 0.38; 2.02 ± 1.89 vs. 2.84 ± 1.93 mg/dl, p = 0.47).

Comparison of Long-Term Therapeutic Efficacy among the Groups

The long-term efficacy at 48 weeks was analyzed in 28 of 33 patients (9 in the DD group, 13 in the SI group and 6 in the SA group). Thirteen patients (46%) were in clinical remission at week 48. The rate of clinical remission was 44% in the DD group, 54% in the SI group and 33% in the SA group (fig. 4). There were no significant differences in long-term efficacy between the three treatment groups.

Discussion

With the increasing use of IFX therapy for the induction and maintenance of remission in patients with CD, the management of individuals exhibiting LOR to IFX has become an important clinical issue. Intensified therapeutic regimens, including doubling the IFX dose, shortening the IFX interval and switching to ADA, are potential options in such patients [8, 10, 16–18]. However, the therapeutic efficacy of intensified therapies has not been comparatively investigated to date. Therefore, we retrospectively compared the clinical outcomes of patients showing LOR to IFX who subsequently received intensified therapy. As a result, we observed equivalent short- and long-

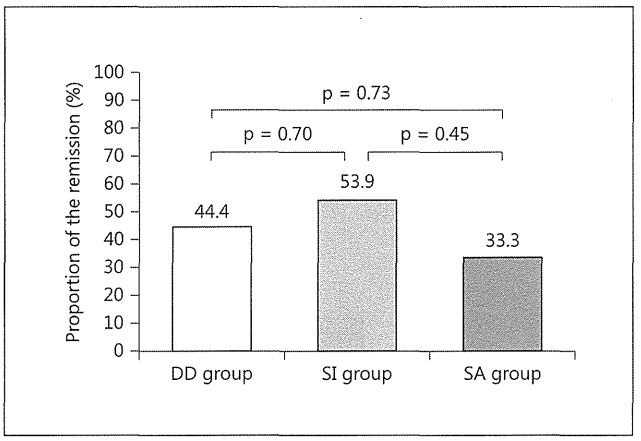

Fig. 4. Rate of clinical remission at 48 weeks after the initiation of the intensified regimens in each group.

term therapeutic efficacy among the DD, SI and SA groups.

The therapeutic efficacy of IFX intensification in CD patients with LOR to IFX, including regimens involving dose escalation and shortening the interval of treatment, has recently been investigated. Chaparro et al. [19] assessed the short-term efficacy of IFX intensification and found clinical response and remission rates of 79 and 34%, respectively, after the first dose intensification step. In that study, the rate of clinical response at 12 months was 69%. Other investigators have also reported equivalent clinical response (65–75.9%) [19–22] and clinical remission (55.6%) [22] rates at approximately 48 weeks after IFX intensification. In the present investigation, since the rates of a clinical response and clinical remission in the DD and SI groups were similar to those of previous reports, it can be assumed that our findings reflect a normal level of therapeutic efficacy for IFX intensification.

IFX has been shown to possess linear pharmacokinetics, with an elimination half-life of 12 days, without plasma accumulation after multiple infusions [23]. Therefore, the use of maintenance therapy with a shortened interval, such as every 6 weeks, is thought to be both pharmacologically acceptable and safe in patients with LOR to IFX. Similar findings were reported by St Clair et al. [24] in terms of pharmacokinetic modeling based on the findings of a cohort of rheumatoid arthritis patients. However, the authors failed to show the efficacy of a 6-week dosing interval. As for CD, Kopylov et al. [25] showed that the early and sustained clinical response to the administration of double-dose IFX maintenance therapy (either every 8 weeks at a dose of 10 mg/kg or every 4 weeks at a dose of 5 mg/kg) did not differ from that of the administration of IFX maintenance therapy every 6 weeks at a dose of 5 mg/kg. Katz et al. [26] further demonstrated that the rates of early and sustained clinical response to IFX dose doubling (every 8 weeks at 10 mg/kg) were not inferior to those of IFX interval halving (every 4 weeks at 5 mg/kg). In the present study, there were no significant differences in the short- or long-term efficacy between the DD and SI groups. Taking these results into consideration, it is likely that strategies involving double dosing or interval shortening of IFX have equivalent therapeutic efficacy in CD patients with LOR to IFX.

According to the results of a systematic review by Ma et al. [27], the rate of short-term clinical response to ADA in CD patients treated with the discontinuation of IFX ranges from 41 to 83%, while the rate of long-term clinical response ranged from 19 to 68%. In Japanese patients with CD, including not only those with LOR to IFX, but also those naïve to IFX, the clinical remission rate at induction and maintenance therapy consisting of ADA was reported as 33.3 and 38.1%, respectively [28]. Since the rate of response and remission in the SA group did not differ to those previous reports, it also can be assumed that our findings reflect a normal level of therapeutic efficacy for ADA. However, to date, no clinical trials have compared the therapeutic efficacy of IFX intensification with that of switching to ADA in patients with LOR to IFX. In the present study, the short- and long-term efficacy did not differ among the DD, SI and SA groups. However, neither CDAI score nor serum CRP level decreased significantly after 4 weeks in the SA group. Considering the modest therapeutic efficacy observed in the SA group, IFX intensification may be preferable to switching to ADA in CD patients exhibiting LOR to IFX. Thus, Ishida et al. [29] showed that combination therapy with ADA and azathioprine might be more effective than ADA monotherapy in CD patients including both those naïve and LOR to IFX. Although the efficacy of combination therapy with ADA and immunomodulators remains unclear, it seems to be preferable to combine immunomodulators when switching to ADA.

Considering the relatively low rates of sustained clinical remission [19, 22, 27], as well as the subsequent onset of LOR even after the administration of an intensified regimen and the considerably high costs of biologics, the application of individualized therapy is imperative. In this regard, measuring the serum drug level and antidrug antibody value is useful for determining the appropriate management strategy for CD. Imaeda et al. [30] reported

that the presence of ATI was related to a low serum IFX trough level and high CDAI score and serum CRP level. They showed similar results with ADA and anti-ADA antibody, and the rate of anti-ADA antibody was significantly higher in patients with LOR to IFX than in patients naïve to IFX [31]. Steenholdt et al. [32], using an algorithm based on the combination of measurements of the serum IFX level and ATI value, showed the cost of individualized therapy to be lower than that of IFX dose intensification (every 4 weeks at a dose of 5 mg/kg). However, because the appropriate cut-off values for the serum drug level and anti-drug antibody value remain debatable, further investigation is needed to validate the clinical usefulness of this technique in determining individualized therapy.

The present study is associated with several limitations. The first limitation is the small sample size, which may be responsible for the lack of statistical significance observed in some variables. The second limitation is the nonrandomized retrospective nature of the study design. Sources of selection bias, such as the selection of the intensified regimen based on the discretion of the attending physician, cannot be excluded. However, considering the lack of data for this type of comparison, we believe that the current results provide considerable information for the management of CD patients exhibiting LOR to IFX.

In conclusion, the present study demonstrated equivalent therapeutic efficacy between doubling the dose of IFX, shortening the IFX interval and switching to ADA in CD patients with LOR to IFX. These preliminary data should be corroborated in a prospective controlled study with a large sample size in the near future.

Disclosure Statement

The authors have no conflict of interest to declare.

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ORIGINAL ARTICLE

Associations of *HLA* class I alleles in Japanese patients with Crohn's disease

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Previous studies have suggested that the human leukocyte antigen (HLA) is involved in the etiology of Crohn's disease (CD); however, few reports are available on the association between HLA class I antigens and CD in Japan. In this study, we performed association analysis of HLA class I antigens in CD using 208 Japanese patients and 384 healthy controls. We identified novel positive associations between CD and *HLA-A*02:01* (odds ratio (OR) = 1.64, $P = 0.016$) and *HLA-A*02:07* (OR = 2.31, $P = 0.0067$) and confirmed previously reported positive associations between CD and *HLA-Cw*14:02* (OR = 2.18, $P = 0.0021$) and *HLA-B*51:01* (OR = 1.70, $P = 0.033$). We also identified novel negative associations between CD and *HLA-A*24:02* (OR = 0.60, $P = 0.0047$) and *HLA-B*07:02* (OR = 0.38, $P = 0.0041$). Although the associations were not significant after full Bonferroni correction, we suggested that HLA class I genes have dual functions, susceptibility and resistance in controlling the development of CD.

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INTRODUCTION

Crohn's disease (CD) and ulcerative colitis, two main subtypes of inflammatory bowel disease, are chronic and relapsing inflammatory disorders of the gastrointestinal tract caused by an aberrant response of the intestinal immune system to commensal bacteria.[1,2] CD affects all regions of the gastrointestinal tract, most commonly the ileum and colon, whereas the inflammation in ulcerative colitis is confined to the colon. The pathological changes in CD are typically discontinuous and often transmural. By contrast, those in ulcerative colitis are continuous and confined to the mucosa and submucosa.[2] Although the prevalence of CD is lower (23.6/100 000 persons) in Japan compared with European populations, it has been increasing continuously over the past several decades in Japan and other Asian countries.[3]

Although their exact etiology remains unclear, genetic factors appear to contribute to the susceptibility to these diseases. The genetic component seems stronger in CD than in ulcerative colitis.[4] A statistically significant association of human leukocyte antigen (HLA) with CD has been widely reported, especially for the *HLA* class II antigens;[5–9] reports of this association include a meta-analysis by Stokkers *et al.*[10] However, the data from these analyses should be interpreted with caution because they include data from both serological and molecular typing of *HLA*, as well as studies on different ethnic groups. There have been a few reports on the association of *HLA* class I alleles with CD in the Japanese population; however, there have been no reports specifically on the association of *HLA-A* alleles with CD in Japan. To elucidate the role of *HLA* class I antigens in the etiology of CD, we investigated the genetic association between the *HLA-A*, *-C* and *-B* alleles and CD in this study.

RESULTS

Association of HLA class I antigens with CD

We analyzed 10 *HLA-A*, 12 *HLA-C* and 18 *HLA-B* alleles with frequencies >1.0%. Compared with the controls, the antigen frequencies of *HLA-A*02:01* (odds ratio (OR) = 1.64, $P = 0.016$), *HLA-A*02:07* (OR = 2.31, $P = 0.0067$), *HLA-Cw*14:02* (OR = 2.18, $P = 0.0021$), *HLA-B*46:01* (OR = 1.88, $P = 0.018$) and *HLA-B*51:01* (OR = 1.70, $P = 0.033$) were increased in patients with CD, whereas the antigen frequencies of *HLA-A*24:02* (OR = 0.60, $P = 0.0047$) and *HLA-B*07:02* (OR = 0.38, $P = 0.0041$) were decreased (Table 1). Thus, we identified three novel susceptibility alleles for CD, *HLA-A*02:01*, *HLA-A*02:07* and *HLA-B*46:01*, and we confirmed two previously reported susceptibility alleles for CD, *HLA-Cw*14:02* and *HLA-B*51:01*.[11] Furthermore, we identified two novel protective alleles for CD, *HLA-A*24:02* and *HLA-B*07:02*. However, it should be noted that these associations were not statistically significant after Bonferroni correction. Therefore, the associations newly found in our study need to be replicated using another sample set in future study.

Among the three novel susceptibility alleles, linkage disequilibrium (LD) is known to occur between *HLA-A*02:07* and *HLA-B*46:01* in the Japanese population. Conditional analyses showed that *HLA-A*02:07* affected the strength of the association between *HLA-B*46:01* and CD and vice versa (Table 2). This observation, along with the fact that the association for *HLA-A*02:07* was more significant than the association for *HLA-B*46:01* in the non-conditional analysis (Table 1), suggests that *HLA-A*02:07* is the primary susceptibility allele for CD.

The novel protective alleles, *HLA-A*24:02* and *HLA-B*07:02*, are also known to be in LD in the Japanese population. However, we observed that the associations between these alleles and CD were still significant even when conditioned on each other (Table 2).

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This observation suggests that *HLA-A*24:02* and *HLA-B*07:02* are independent protective HLA class I alleles for CD. In conclusion, we identified *HLA-A*02:01* and *HLA-A*02:07* as novel *HLA* class I susceptibility alleles for CD and *HLA-A*24:02* and *HLA-B*07:02* as HLA class I protective alleles for CD.

LD structure between *HLA-A*24:02, HLA-Cw*14:02* and *HLA-B*51:01*

Although we found the *HLA-A*24:02* allele to be protective and the *HLA-Cw*14:02* and *HLA-B*51:01* alleles to be associated with susceptibility for CD, these alleles constitute a common HLA haplotype in the Japanese population. Therefore, we investigated the differences in the degree of LD among these alleles between the CD and control groups. As shown in Figure 1, moderate or strong LDs were observed between *HLA-A*24:02* and *HLA-Cw*14:02* ($D' = 0.36$), between *HLA-A*24:02* and *HLA-B*51:01* ($D' = 0.51$) and between *HLA-Cw*14:02* and *HLA-B*51:01* ($D' = 0.97$) in the control group. In contrast, although the LD between *HLA-Cw*14:02* and *HLA-B*51:01* was at the same level ($D' = 0.97$) in the CD group, the LDs between *HLA-A*24:02* and *HLA-Cw*14:02* and those between *HLA-A*24:02* and *HLA-B*51:01* were weaker in the CD group ($D' = 0.13$ and 0.06, respectively) than in the controls. These results suggest that the *HLA-A*24:02-Cw*14:02-B*51:01* haplotype carries unobserved variant adjacent to *HLA-A*24:02*, which contributes to a protective effect on CD.

Table 1. Association of *HLA* class I with CD

HLA allele	*Antigen frequency (count)*		P-*value*	*OR (95% CI)*
	CD (n = 208)	*Controls (n = 384)*		
HLA-A				
*A*02*[a]	0.50 (105)	0.36 (123)	3.8×10^{-4}	1.92 (1.34–2.76)
*A*02:01*	0.29 (60)	0.20 (76)	0.016	1.64 (1.10–2.44)
*A*02:07*	0.12 (25)	0.060 (23)	0.0067	2.31 (1.26–4.24)
*A*24:02*	0.50 (104)	0.62 (239)	0.0047	0.60 (0.43–0.86)
HLA-C				
*Cw*14:02*	0.19 (39)	0.099 (38)	0.0021	2.18 (1.33–3.57)
HLA-B				
*B*07:02*	0.058 (12)	0.14 (53)	0.0041	0.38 (0.20–0.73)
*B*46:01*	0.15 (32)	0.094 (36)	0.018	1.88 (1.11–3.16)
*B*51:01*	0.20 (41)	0.13 (49)	0.033	1.70 (1.07–2.70)

Abbreviations: CA, Crohn's disease, OR, odds ratio CI, confidence interval.
[a]HLA-A*02:01, HLA-A*02:06 and HLA-A*02:07 were combined.

DISCUSSION

In our study, novel positive associations between the *HLA-A*02:01* and *HLA*-A*02:07 alleles and CD were identified. Likewise, a serologically defined *HLA-A2* allele has been previously identified as a significant susceptibility allele for CD in Caucasians.[12] Therefore, we analyzed the total frequency of all *HLA-A*02* subtypes that were increased in CD, which included *HLA-A*02:01, HLA-A*02:06* and *HLA-A*02:07* (OR = 1.92, $P = 3.8 \times 10^{-4}$) (Table 1).

Among the *HLA-A*02* alleles, *HLA-A*02:07* was more strongly associated with CD than was *HLA-A*02:01*, and *HLA-A*02:06* did not exhibit a significant positive association. To explain these differences, we compared the amino acid sequences of each *HLA-A*02* allele subtype. There is a polymorphic change from phenylalanine to tyrosine at amino acid position 9 in the *HLA-A*02:06* allele compared with the susceptibility alleles *HLA-A*02:01* and *HLA-A*02:07*, which is not associated with CD susceptibility,

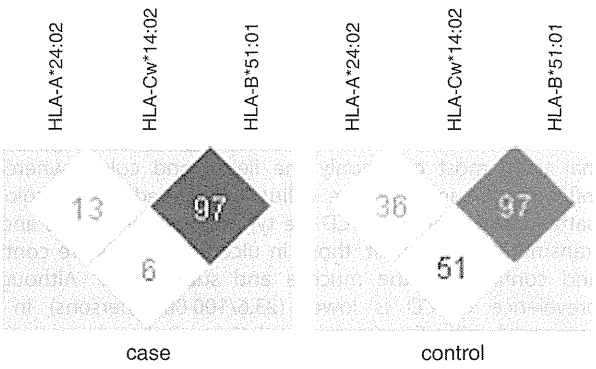

Figure 1. LD between the protective *HLA-A*24:02* allele and the susceptible *HLA-Cw*14:02* and *HLA-B*51:01* alleles for CD in cases and controls. The D' values are indicated in each box.

Table 2. Conditional analyses of the associations between *HLA* class I and CD.

HLA	*Non-conditioned*	*Conditioned with*	
		*A*02:07*	*B*46:01*
Susceptibility allele			
*A*02:01*	0.016 (1.64, 1.10–2.44)	NA	0.012 (1.67, 1.11–2.50)
*A*02:07*	0.0067 (2.31, 1.26–4.24)	NA	0.12 (1.87, 0.85–4.11
*Cw*14:02*	0.0021 (2.18, 1.33–3.57)	0.0011 (2.29, 1.39–3.78)	0.0013 (2.26, 1.37–3.72)
*B*46:01*	0.018 (1.88, 1.11–3.16)	0.41 (1.33, 0.68–2.63	NA
*B*51:01*	0.033 (1.70, 1.07–2.70)	0.018 (1.76, 1.10–2.82)	NA
		*A*24:02*	*B*07:02*
Protective allele			
*A*24:02*	0.0047 (0.60, 0.43–0.86)	NA	0.017 (0.65, 0.46–0.93)
*B*07:02*	0.0041 (0.38, 0.20–0.73)	0.012 (0.42, 0.22–0.83)	NA

The *P*-values (OR, 95% confidence interval) obtained by conditional logistic analysis using HLA-*A*02:07* or *HLA-B*46:01* as the susceptible *HLA* allele and using *HLA-A*24:02* or *HLA-B*07:02* as protective *HLA* alleles in a dominant model are indicated.

Table 3. Polymorphic amino acid changes among *HLA-A*02* alleles.

*HLA-A*02*	*Polymorphic residue positions*				
	9	*99*	*149*	*152*	*156*
*A*02:01*	F	Y	A	V	L
*A*02:06*	**Y**	Y	A	V	L
*A*02:07*	F	**C**	A	V	L

The residues that are different among the three alleles are shown in bold face.

and a change from cysteine to tyrosine at position 99 in *HLA-A*02:01* compared with *HLA-A*02:07* (Table 3).[13]

It has been reported that the amino acids at positions 9 and 99 in HLA-A2 comprise the contact residues of the peptide binding pocket B. In these amino acid changes, the increased hydrophobicity of the residues at positions 9 and 99 has a greater effect on the susceptibility to CD (hydropathy index: phenylalanine 2.8, tyrosine −1.3, cysteine 2.5). The analysis of the amino acid changes among the *HLA-A*02:01*, *HLA-A*02:06* and *HLA-A*02:07* alleles suggests the following: (i) The phenylalanine at position 9 in *HLA-A*02* is critical to determine the susceptibility to CD. (ii) The change from cysteine to tyrosine at position 99 weakens the susceptibility to CD. In addition, *HLA-A*02:01* and *HLA-A*02:07* have been reported to bind peptides with leucine/methionine and leucine residues, respectively, at position 2, which is buried deep within peptide binding pocket B,[14,15] whereas *HLA-A*02:06* accommodates binding to valine/glutamine residues at position 2. These differences may also affect disease susceptibility to CD.

In summary, we identified two novel susceptibility HLA class I alleles, *HLA-A*02:01* and *HLA-A*02:07*, and two protective alleles, *HLA-A*24:02* and *HLA-B*07:02*, for CD in the Japanese population, although the association signals were not significant after full Bonferroni correction. It was suggested that the susceptibility conferred by the *HLA-A*02* alleles might be due to the polymorphic amino acid changes in these alleles that occur within pocket B in the antigen binding groove of the HLA-A2 molecule. All these findings may suggest a direct involvement of the HLA class I molecule in the susceptibility and resistance to CD by regulating the immune response in the gastrointestinal region.

MATERIALS AND METHODS

Subjects

Unrelated Japanese individuals with CD and healthy controls (208 and 384 individuals, respectively) were enrolled in this study. The CD diagnoses were made by gastroenterologists using conventional endoscopic, histologic and clinical criteria. Patients with indeterminate colitis were excluded in advance. Documented informed consent was obtained from each participant according to the Declaration of Helsinki. This study was also approved by the Ethics Committee at Kyushu University, Keio University and Tokyo Medical and Dental University in Japan.

Genotyping of HLA alleles

We determined the *HLA-A*, *HLA-C* and *HLA-B* genotypes (4-digit) using the Luminex assay system (Luminex Corporation, Austin, TX, USA) and HLA typing kits (Wakunaga, Hiroshima, Japan). We observed a total of 79 alleles (*HLA-A*: 21, *HLA-C*: 18 and *HLA-B*: 40 alleles). The *HLA* alleles with a frequency >0.01 (*HLA-A*: 10, *HLA-C*: 12 and *HLA-B*: 18 alleles) were subjected to further analyses.

Statistical analysis

The association between CD and the *HLA* class I antigen was assessed by logistic regression analysis with an allele dominant model adjusted for sex. For the conditional analysis, the indicated *HLA* class I allele was included in the independent variable. The *P*-values and ORs were computed using PLINK version 1.07 software (http://pngu.mgh.harvard.edu/purcell/plink/).[16] The LD among the *HLA* alleles (*HLA-A*24:02, HLA-Cw*14:02* and *HLA-B*51:01*) was calculated using Haploview version 4.2 software (http://www.broadinstitute.org/scientific-community/science/programs/medical-and-population-genetics/haploview/haploview).[17]

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Characteristics of Japanese inflammatory bowel disease susceptibility loci

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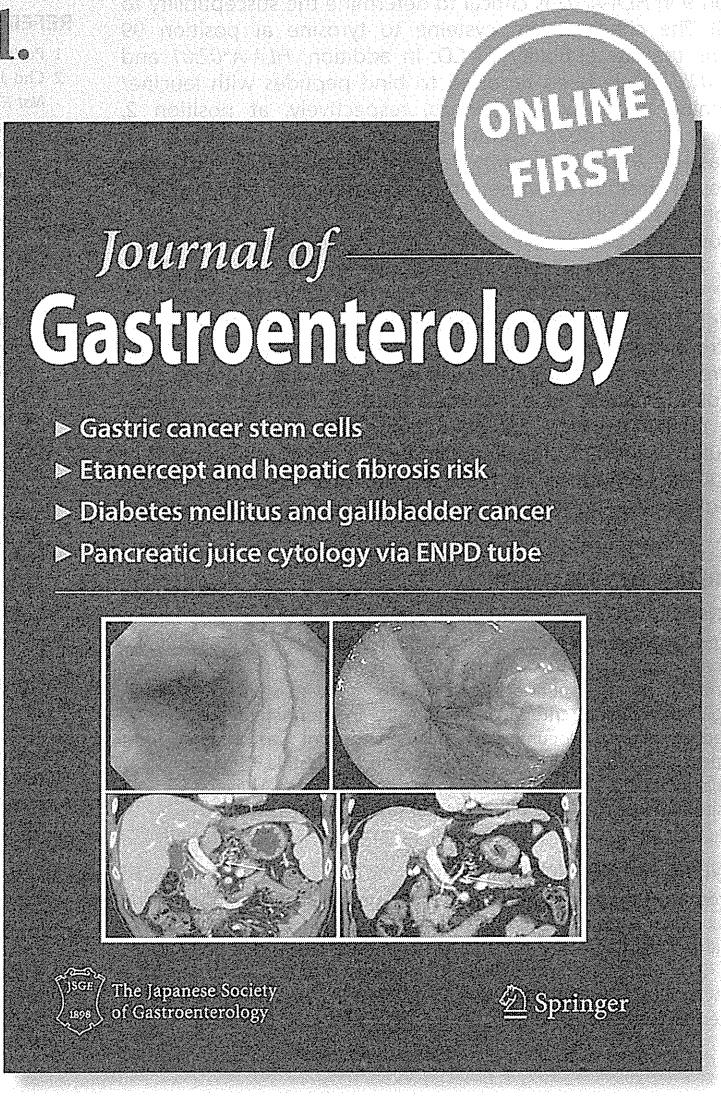

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