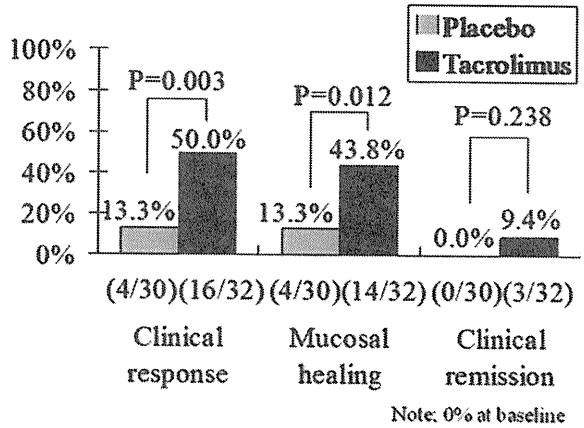

FIGURE 1. Efficacy result.

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, respectively, 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.

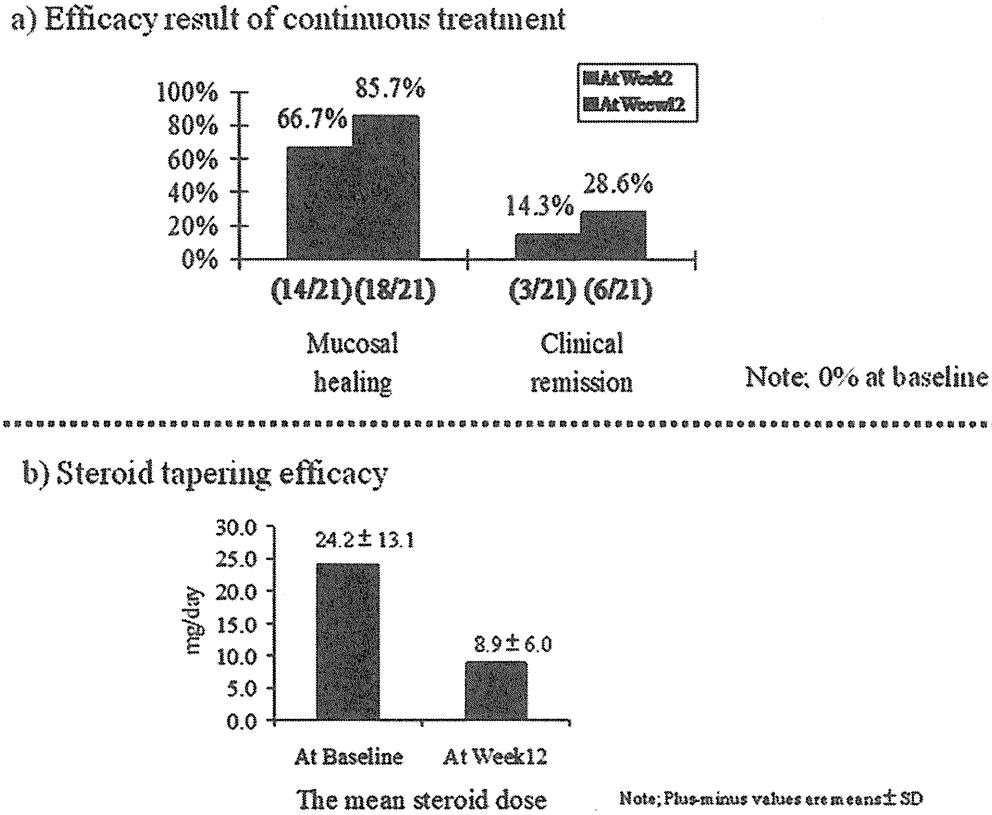

FIGURE 2. Open-label extension.

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.

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Interval of Less Than 5 Years Between the First and Second Operation Is a Risk Factor for a Third Operation for Crohn's Disease

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Background: Previous studies have shown various risk factors for the initial and/or the second operation for Crohn's disease (CD). However, limited data are available with regard to the risk factors for a third operation. We aimed to clarify the risk factors for a third operation for CD.

Methods: A total of 200 CD patients who underwent a second intestinal surgery at 13 institutions were examined. We performed univariate and multivariate analyses to examine the influence of independent variables on the cumulative rate of needing a third operation.

Results: A total of 95 patients underwent a third operation. The overall 5-year and 10-year cumulative rates for the third operation were 42.2% and 71.0%, respectively. In univariate analysis, the interval between the initial and the second operation ($P = 0.0069$), postoperative administration of infliximab ($P = 0.0030$), and the anatomical site of the disease ($P = 0.0132$) were significant risk factors for the third operation. In multivariate analysis, the interval between the initial and the second operation ($P = 0.0287$) and postoperative administration of infliximab ($P = 0.0297$) remained significant risk factors for the third operation. The cumulative 5-year third operation rate was significantly higher in patients with an interval of less than 5 years between the first and second operations than for those with an interval of 5 years or more (47.8% versus 35.2%, $P = 0.0232$).

Conclusions: An interval of less than 5 years between the first and the second operations is a significant risk factor for a third operation in patients with CD.

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Key Words: Crohn's disease, surgery, reoperation, second surgery, risk factor, time trend, time changes

Approximately 50%–80% of patients with Crohn's disease (CD) require surgery at some point during their lifetime.[1–3] Postoperative recurrence is common in CD, and after the initial operation some patients need a second and/or multiple operations. Reoperation rates for recurrence range from 48%–71% at 20 years after the initial surgery.[4] Furthermore, the risk of needing a third operation reaches 40% at 10 years after the second operation.[5,6] Therefore, prevention of recurrence remains one of the major goals in the treatment of CD patients.

In order to prevent recurrence in CD, identification of patients at high risk for future recurrence is important because intensive therapy may be given to such patients to decrease recurrence needing surgical intervention. To identify such high-risk patients, previous studies evaluated various factors that potentially influenced the recurrence rates in CD patients, including age, gender, smoking, steroid use, duration of preoperative history, perforating disease, perianal disease, ileocolic disease, etc.[1,2,7–13] However, these studies have focused on identifying risk factors for the initial or the second surgery. To date, few data have been generated with regard to the risk factors for the third operation except for one study with a comparatively small number of patients.[14] Therefore, in the present study we aimed to evaluate risk factors for a third intestinal operation in a larger number of CD patients. We

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examined a total of 200 CD patients and showed that a shorter interval between the initial and the second operation was a significant risk factor for needing a third operation. To our knowledge, this is the first study that has shown that the interval between the initial operation and the second operation was a significant risk factor for a third operation. To the best of our knowledge, this is also the largest study of patients who underwent a second operation that has focused on the risk of a third intestinal operation for CD.

PATIENTS AND METHODS

Patients and Criteria for Diagnosis

A total of 200 CD patients who underwent initial and second intestinal surgeries were examined. Their onset of disease was between 1963–2003, and the diagnosis of CD was made according to the criteria provided by the Investigation and Research Committee for Intractable Inflammatory Bowel Disease organized by the Japanese Ministry of Public Welfare as described previously.[15] The first and the second operation included intestinal surgery consisting of resection or strictureplasty. Surgeries for perianal disease or other minor surgical procedures without intestinal surgery were excluded from the initial and the second operations included in our study. This study was approved by the local Ethics Committee.

Data Management and Definitions

Case records were collected from 13 institutions which are participating in the Investigation and Research Committee for Intractable Inflammatory Bowel Disease organized by the Japanese Ministry of Public Welfare and scrutinized retrospectively. Data included the patient date of birth, date of onset of symptoms, date of diagnosis, disease localization at diagnosis, type of disease, type of surgery and date of initial/second surgery, and date of final follow-up, which were transferred to a data file (Microsoft Office Excel, Redmond, WA). The indications for surgery included acute abdominal pain, medical intractability, intestinal obstruction, palpable mass/abscess, internal fistulas, colonic dilatation, etc. The disease localization was established at the time of diagnosis and was classified into three groups: 1) small bowel disease (inflammation of the small bowel); 2) ileocolic disease (inflammation involving both the small bowel and the colon); 3) colorectal disease (inflammation confined to the colon or rectum or both). The type of disease was classified into perforating or nonperforating disease, as described previously.[11] Perforating disease included patients who underwent their first operation due to perforating disease, whereas nonperforating disease patients were those who underwent the initial operation due to another cause, such as intestinal obstruction, medical intractability, hemorrhage, etc. Perforating disease was classified as perforating, regardless of the concomitant presence of additional nonperforating disease. The primary outcome measure of this study was the rate of patients needing a third intestinal resection or strictureplasty.

Statistical Analysis

The statistical analysis was performed using the JMP software program (SAS Institute, Cary, NC). The cumulative third operation rate was calculated by the Kaplan–Meier method and compared by log-rank test. Univariate and multivariate analyses were performed by Cox proportional hazards regression models in order to examine the influence of independent variables on the cumulative probability of the third operation. Variables with $P < 0.1$ in univariate analysis were entered into each multivariate analysis. $P \leq 0.05$ was considered statistically significant in all analyses. Probability values and confidence intervals were calculated at the 95% level.

RESULTS

Patient Characteristics

Table 1 shows the characteristics of patients. In the 200 CD patients who underwent a first and second intestinal operation, 95 patients underwent a third intestinal surgery after a median of 3.5 years. The frequency of ileocolic disease or administration of infliximab was significantly higher in patients who underwent the third operation than for those who did not. The overall 5-year and 10-year cumulative rates of needing a third operation were 42.2% and 71.0%, respectively (Fig. 1).

Risk Factors for Reoperation and Cumulative Rate of Reoperation

The impact of possible risk factors that may have influenced the frequency of the third operation was evaluated by univariate and multivariate analyses (Table 2). In an analysis of duration of disease, we evaluated the following three different types of disease duration with respect to the risk of a third operation: first, the period between disease onset and the first operation; second, the period between disease onset and the second operation; and last, the interval between the first and the second operation. In a univariate analysis, significant risk factors for the third operation were the interval between the first and the second operation, the anatomical site of the disease, and postoperative administration of infliximab. The cumulative risk of the third operation was significantly higher in patients whose interval between the first and second operations was less than the median interval (4.7 years). We next examined whether the same trend could be observed when we divided patients according to the interval of either shorter or longer than 5 years between the surgeries. Patients whose interval between the initial and the second operation was 5 years or less also showed a higher risk of requiring a third operation (hazard ratio = 0.617 (95% confidence interval [CI], 0.401–0.935, $P = 0.0226$) compared to the patients whose interval was longer than 5 years. With regard to the anatomical site of the disease, patients with ileocolic disease showed significantly higher risk of

TABLE 1. Patient Characteristics

	Patients Who Received a 3rd operation (n = 95)	Patients Who Underwent Only 2nd operations (n = 105)	P-value	Total Number of Patients (n = 200)
Age at diagnosis				
Median	21.1	22.6	$P = 0.439$	22.4
(Range)	(8–49)	(10–53)		(8–53)
Gender			$P = 0.9417$	
Male	71	78		149
Female	24	27		51
Disease location			$P = 0.0006$	
Ileocolic	69	56		125
Small bowel	26	38		64
Colorectal	0	11		11
Type of disease			$P = 0.3032$	
Non-perforating	43	59		102
Perforating	45	40		85
Others	7	6		13
Postoperative medication				
Immunosuppressants			$P = 0.6132$	
+	17	16		106
−	78	89		94
Infliximab			$P = 0.0170$	
+	30	13		48
−	65	87		152
Elemental diet			$P = 0.6175$	
+	56	63		119
−	39	41		80
Others	0	1		1
Disease duration between onset and the first surgery (yrs)				
Median	3.6	4.0	$P = 0.1494$	4.0
(Range)	(0–22.4)	(0–19.8)		(0–22.4)
Disease duration between onset and the second operation (yrs)				
Median	7.8	11.3	$P = 0.0101$	9.8
(Range)	(1.0–27.0)	(1.0–43.2)		(1.0–43.2)
Interval between the first and the second operations (yrs)				
Median	4.0	5.1	$P = 0.0897$	4.7
(Range)	(0.1–26.0)	(0.5–43.2)		(0.1–43.2)

Duration before surgery: duration between diagnosis and the first surgery.
Reoperation: reoperation after the first surgery.

needing a third operation than patients with either colorectal-only disease or small intestine disease. Other factors such as gender, age at diagnosis, preoperative duration of disease, and type of disease did not show any significant correlation with the third operation rate.

Next, we performed a multivariate analysis among the three risk factors that showed a significant impact on the rate of requiring a third operation by univariate analysis (Table 2). In multivariate analysis, the interval between the first and the second operation, and the use of infliximab remained significant risk factors.

Cumulative Rate of Patients Requiring a Third Operation

Cumulative 5-year and 10-year rates of the need for a third operation were significantly higher in patients whose interval between the first and the second operation was 4.7 years or less (P = 0.0069) (Fig. 2). Also patients

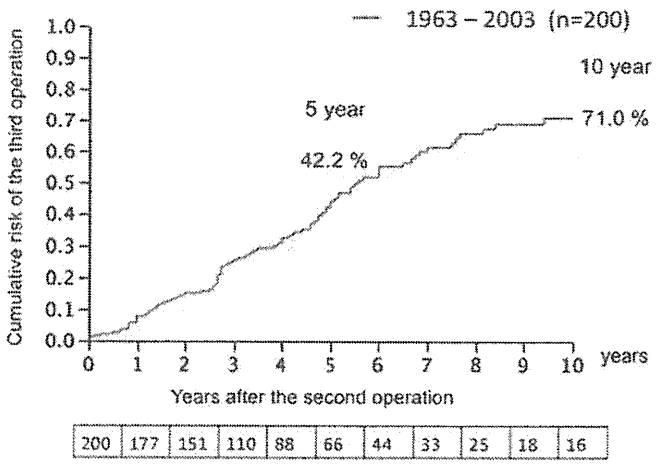


FIGURE 1. The overall 5-year and 10-year cumulative rate of third operations for CD in Japan. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

whose interval was 5.0 years or less showed a higher third operation rate than those with interval more than 5 years (5-year third operation rate; 47.8% versus 35.2%, $P = 0.0232$). Cumulative 5-year and 10-year rates of the need for a third operation were also significantly higher in patients who received infliximab postoperatively than those who did not ($P = 0.0015$) (Fig. 3). With regard to the disease localization, patients with ileocolic disease showed a significantly higher rate of needing a third operation than those with small bowel or colonic disease ($P = 0.0154$) (Fig. 4).

DISCUSSION

The present study showed that the disease interval between the first and the second operation was a significant risk factor for CD patients to need a third operation. A shorter interval was significantly associated with a higher risk of needing a third operation. The 5-year cumulative rate of patients needing the third operation was 47.8% in those with an interval between the first and second surgeries of 5 years or less, while only 35.2% in patients whose interval was more than 5 years ($P = 0.0232$). These results suggest that intensive postoperative adjuvant therapy may be especially important for CD patients who have an interval of 5 years or less between the initial and second surgeries in order to avoid the need for a third operation.

Previous studies demonstrated various risk factors for intestinal operation in CD, however, most of these factors have been focused on predicting the risk of needing the first or second operation.[1,7–13] The risk factors for needing a third operation were unclear. To our knowledge, there has been only one study by Alves et al[14] that evaluated risk factors for a third operation in CD. However, in Alves et al's study, the total number of patients was comparatively small. In their study, there were 28 CD patients who received a second intestinal operation, but since two cases were excluded because of missing data, they only examined a total of 26 CD patients. In the present study, we examined 200 CD patients who underwent a second operation, and to our knowledge, this is the largest number of patients among studies evaluating the risk of needing a third operation for CD. Another difference between Alves et al's study and the present study is the data source. Alves et al's study was based on the data from a single institution. However, a single institution-based study cannot rule out the possibility of patient selection biases. On the other hand, in the present study, we collected data from 13 institutions in Japan and we were able to analyze the risk factors for the third operation based on the multiinstitutional dataset.

Alves et al[14] showed that the third intestinal resection rate was significantly lower in patients treated with immunosuppressive drugs (azathioprine and 6-mercaptopurine, or methotrexate) than in untreated patients (17% versus 58%, $P < 0.02$). However, with regard to the postoperative effect of immunosuppressants in preventing recurrence, previous studies have shown conflicting results.[16–22] For example, Hanauer et al and D'Haens et al[16,18] showed that the postoperative recurrence rate was significantly lower in patients receiving immunosuppressants than in those receiving placebo. A meta-analysis also showed that immunosuppressants are more effective than placebo in preventing both clinical and endoscopic postoperative recurrence in CD.[20] On the other hand, Ardizzone et al[17] reported that there was no difference in the efficacy of immunosuppressants in preventing clinical and surgical relapses after conservative surgery. In the present study, administration of immunosuppressants was not a significant risk factor for needing a third operation. On the other hand, postoperative administration of infliximab was a significant risk factor for the patients needing a third operation in the present study. This is contrary to the results of recent studies, which showed that infliximab is effective for reducing the postoperative recurrence rate.[23–27] In a recent randomized controlled study, Regueiro et al[25] showed that endoscopic (9.1% versus 84.6%, $P = 0.0006$) and histologic (27.3% versus 84.6%, $P = 0.01$) recurrence rates were significantly lower in CD patients who received infliximab after intestinal resective surgery compared to patients who received placebo. One reason for the conflicting results between the present and other studies may be due to a selection bias of the patients who received infliximab. In the present study, patients received infliximab for therapy of recurrent disease. Therefore, there is a possibility that infliximab might have been administered preferably to higher-risk patients for a third operation, while lower-risk patients did not receive these treatments. This patient selection bias may have been responsible for the results indicating infliximab to be a risk factor in the present study.

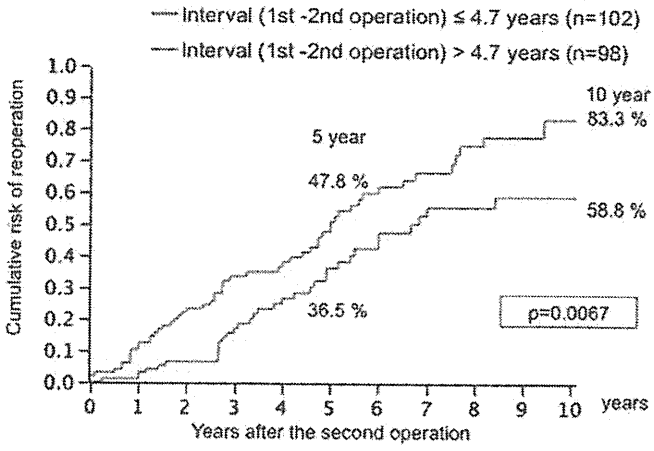
TABLE 2. Results of Univariate and Multivariate Analyses

	Univariate analysis			Multivariate Analysis		
	Hazard Ratio	95% CI	*P*-value	Hazard Ratio	95% CI	*P*-value
Age at diagnosis						
≤22	0.862	0.571–1.295	$P = 0.4747$			
>22						
Gender						
Male	0.772	0.474–1.214	$P = 0.2686$			
Female						
Disease location						
Small bowel, Colorectal	1.749	1.128–2.799	$P = 0.0132$	1.498	0.946–2.413	$P = 0.0859$
Ileocolic						
Type of disease						
Perforating	1.375	0.903–2.098	$P = 0.1370$			
Nonperforating						
Postoperative medication						
Immunosuppressants						
+	1.321	0.752–2.191	$P = 0.3188$			
−						
Infliximab						
+	1.999	1.274–3.068	$P = 0.0030$	1.676	1.054–2.614	$P = 0.0297$
−						
Elemental diet						
+	0.907	0.603–1.377	$P = 0.6413$			
−						
Disease interval between the first and the second operation						
≤4.7	0.566	0.371–0.856	$P = 0.0069$	0.626	0.407–0.953	$P = 0.0287$
>4.7						
Disease duration before the first operation						
≤4.0	1.047	0.691–1.575	$P = 0.8263$			
>4.0						
Disease duration before the second operation						
≤9.8	0.693	0.454–1.045	$P = 0.0802$			
>9.8						

Duration before surgery: duration between diagnosis and the first surgery.
Reoperation: reoperation after the first surgery.

The second reason may be a shorter follow-up period for patients who received infliximab. This is actually one limitation of the present study, because we were unable to evaluate the effect of infliximab with a long enough follow-up period because infliximab did not become available in Japan until 2002. In the present study, more than half of the patients (110 patients) underwent the third operation in or after 2002. Among these patients, the median follow-up period was only 2.8 years. We believe we need to follow patients for a longer period of time to evaluate the true effect of infliximab in the adjuvant setting.

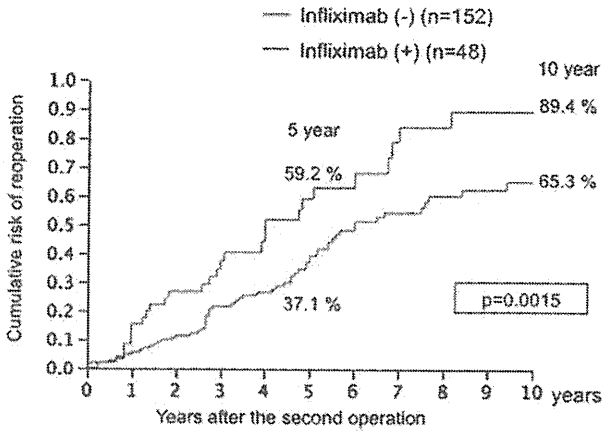
The present study showed that an interval of less than 5 years between the first and the second operation for CD was a significant risk factor for needing a third operation. Previous studies have also shown that there is a correlation between the duration of the disease and a risk of surgery. A number of studies have shown a higher risk of surgery with a shorter history of disease.[28–30] However, these studies examined the relationship between the disease duration before the first operation and this risk of a second operation. None of the previous studies examined the possible role of disease interval on the risk for needing a third operation. We have shown that patients who underwent a second surgery within 5 years of the first operation are at a higher risk of needing a third operation. With regard to the interval between operations, Greenstein et al[11] examined



≤4.7	98	85	72	50	42	33	20	14	10	6	4
>4.7	102	92	79	60	46	33	24	19	15	12	12

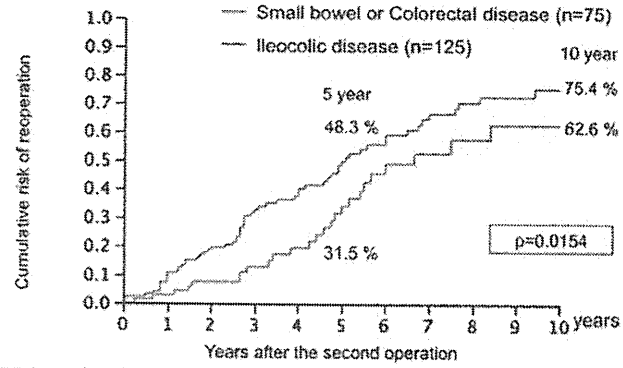
FIGURE 2. The cumulative rate of third operations depends on the interval between the first and the second operations. The cumulative risk of the third operation was significantly higher in patients whose interval between the first and second operations was less than 4.7 years ($P = 0.0067$).

770 patients with CD and reported that third operations occurred faster after second operations than did second operations after the first. Also, in an analysis of CD patients who had undergone multiple operations, Greenstein et al[31] showed that as patients undergo repeated surgical procedures, their postoperative recurrences develop faster after each successive operation. In the present study the mean interval between the second and the third operation (4.4 years) was significantly shorter than that between the



Infliximab (-)	152	137	119	86	72	54	36	28	21	15	13
Infliximab (+)	48	41	33	24	16	12	8	5	3	3	3

FIGURE 3. The cumulative rate of third operations depends on the postoperative administration of infliximab. The cumulative risk of the third operation was significantly higher in patients who received postoperative infliximab ($P = 0.0015$).



Small bowel or Colorectal disease	74	68	59	46	37	28	17	14	9	9	9
Ileocolic disease	125	109	92	64	51	38	27	19	16	11	9

FIGURE 4. The cumulative rate of third operations depends on the localization of the disease. The cumulative risk of the third operation was significantly higher in patients with ileocolic disease ($P = 0.0154$).

first and the second operation (5.8 years) ($P = 0.019$). Our results were in accordance with Greenstein et al's observations.

To our knowledge, the present study was the largest study of CD patients after the second surgery showing the risk of needing a third surgery. These results suggest that patients with a short interval between the first and the second operations need intensive adjuvant therapy postoperatively, such as infliximab. On the other hand, in the present study the disease duration before the first operation was not a significant risk factor for needing a third operation.

Another risk factor for the third operation in the present study was the anatomical site of the disease. Patients who have colorectal involvement will often require a definitive resection with a permanent stoma. In fact, none of the patients with colonic-only disease underwent a third operation in our study. Therefore, we examined patients with small intestine disease and colonic-only disease together against patients with ileocolic disease. By univariate analysis we showed that ileocolic disease was a significantly higher risk factor for needing a third operation, although this did not remain significant by multivariate analysis.

Previously, a number of studies examined the impact of anatomical site of disease on the recurrence, and many studies have demonstrated that the risk of recurrence was highest for ileocolic disease and lowest for colonic-only disease.[32–35] However, most of these studies examined the impact of the anatomical site on the first and/or the second surgery, and therefore, data concerning the need for a third operation with regard to the anatomical site involved is limited. In agreement with previous studies of initial and second surgeries, the present study indicated that there is a significantly higher risk of needing a third operation in

patients with ileocolic disease. The present study shows ileocolic disease to be a risk factor, not only for the first or the second operation, but also for the third operation for CD.

Another unique factor that might affect the risk of needing surgery in Japanese CD patients is the use of the elemental diet (ED) therapy. In Japan, ED, rather than corticosteroid therapy, is considered to be effective in the primary remission-induction therapy for active intestinal inflammation.[36–38] A Japanese randomized controlled trial showed that a "half elemental diet" therapy regimen, in which half of the daily calorie requirement is provided by an elemental diet and the remaining half by a free diet, is effective in reducing the relapse rate compared with patients eating purely a free diet (relapse rate; 34.6% versus 64.0%).[36] However, in the present study ED was not a significant factor for needing a third operation.

One of the limitations of the present study is that we could not examine the association between the third operation and several well-established risk factors including smoking, steroid use, and perianal disease, since they were not available in retrospective review.[7,9,10,12,13] Although these factors are known to be associated with the operation rate, it still remains unclear how these factors affect the risk of the third operation. We believe further studies are necessary to clarify this point. Another important issue is the endpoint of the present study. In the present study we included both intestinal resections and strictureplasties as an intestinal operation. Ideally these two procedures need to be analyzed separately. However, some patients receive both intestinal resections and strictureplasties at the same time, and furthermore the number of each procedure differs between each individual. Therefore, in the present study we did not divide patients according to each procedure. However, we believe that each procedure as well as the number of procedures needs to be evaluated separately. Lastly, although we examined multiple factors in association with the risk of third operation, the number of patients was comparatively small. Therefore, in order to clarify these issues we believe that a prospective study with a large number of patients is necessary.

In conclusion, to our knowledge, the present study is the first to show that a shorter interval between the first and the second operations is a significant risk factor for needing a third operation. Patients whose interval between initial and second surgeries is 5 years or less are at a higher risk of recurrence and, therefore, should receive postoperative adjuvant therapy to prevent the need for a third operation. However, to further confirm this we need to prospectively evaluate CD patients with a longer follow-up period. This is particularly important because the use of infliximab, which is generally thought to reduce disease symptoms and recurrence, was a risk factor for the third operation in our study. However, due to the retrospective nature of the present study, this was considered to be due to a bias, because these drugs might have been administered more frequently to higher-risk patients. Nevertheless, further studies are needed to confirm whether this is indeed the case.

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Meta-analysis of Published Studies Identified Eight Additional Common Susceptibility Loci for Crohn's Disease and Ulcerative Colitis

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Background: Both ulcerative colitis (UC) and Crohn's disease (CD) have a complex etiology involving multiple genetic and environmental factors. Many genome-wide association studies (GWAS) and subsequent replication studies revealed that both diseases share some of the susceptibility loci; however, common genetic factors for both diseases are not fully elucidated. This study is aimed to identify the common genetic factors for CD and UC by a meta-analysis of published studies.

Methods: We first reviewed the 10 GWAS for CD to select candidate single nucleotide polymorphisms (SNPs). Next, we performed a PubMed literature search up to June 30, 2010 and carried out a systemic review of published studies that examined the association of CD susceptibility loci in UC patients. Meta-analysis was carried out using the inverse variance-weighted method or the DerSimonian-Laird method after estimating the heterogeneity among the studies. The data for highly linked SNPs were combined. Finally, we performed a meta-analysis of 43 published studies in 45 SNPs located at 33 loci by using a total of 4852 to 31,125 subjects.

Results: We confirmed the association of 17 reported common susceptibility loci. Moreover, we found associations at eight additional loci: *GCKR*, *ATG16L1*, *CDKAL1*, *ZNF365*, *LRRK2-MUC19*, *C13orf31*, *PTPN2*, and *SBNO2*. The genetic risk of each locus was modest (odds ratios ranged from 1.05–1.22) except *IL23R*.

Conclusions: These results indicate that CD and UC share many susceptibility loci with small genetic effect. Our data provide further understanding of the common pathogenesis between CD and UC.

(Inflamm Bowel Dis 2011;000:000–000)

Key Words: single nucleotide polymorphism, meta-analysis, shared genetic risk, ulcerative colitis, Crohn's disease

Ulcerative colitis (UC) and Crohn's disease (CD), the two most common forms of inflammatory bowel disease (IBD), have a complex etiology involving multiple genetic and environmental factors. Family and twin studies have clearly indicated the involvement of genetic factors in the development of both diseases.[1] Moreover, UC and CD exist in the same family with higher frequency than the co-occurrence by chance alone, suggesting an etiological relationship between the two diseases.[2,3] Since the chronic relapsing intestinal inflammation induced by the dysregulated mucosal immune response to commensal enteric bacteria is one of the common pathogenesis of CD and UC, it is important to understand the shared genetic factors for both diseases.

Recent genome-wide association studies (GWAS) for CD[4–13] have identified more than 30 susceptibility loci and provided new insights into the immunopathogenesis of this disease, implicating an important role of genes of the innate and adaptive immune systems for disease occurrence.[14] Similarly, several GWAS for UC[15–20] have identified more than 10 susceptibility loci. A comparison of the results of these studies and additional association studies has identified 18 common susceptibility loci between CD and UC, including *IL23R*, *JAK2*, *STAT3*, *BSN-MST1*, *CCNY-CREM*, *KIF21B*, *NKX2-3*, *IL12B*, *ORMDL3*, *ICOSLG*, *LOC441108*, *IRGM*, *CCR6*, *TNFSF15*, 5p13, 6p21, 7p12,

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and 21q21.[16,19–25] However, considering the strong heritability of both diseases, several common genetic factors may not have been found yet and meta-analysis of published studies is one approach by which these factors may be identified. Nevertheless, to our knowledge, only a handful of meta-analysis for common susceptibility loci between UC and CD have been performed, most notably for *NOD2*, *PTPN22*, *ATG16L1*, and *IRGM*.[26–31] Therefore, we performed a comprehensive meta-analysis of published studies that examined the association of CD susceptibility loci in UC patients to clarify common genetic factors for both diseases.

MATERIALS AND METHODS

Single Nucleotide Polymorphism (SNP) Selection for a Literature Search

We reviewed the literature of 10 GWAS for CD including meta-analyses[4–13] published before June 30, 2010. Initially, we selected 62 SNPs for the literature search based on the following criteria: 1) SNPs showed a significant level of overall *P*-value less than 5×10^{-7} in an initial GWAS for CD; and 2) located at non-MHC region because of the broad and strong linkage disequilibrium across the MHC region (Supporting Information Table 1).

Literature search strategy and study selection criteria

We performed a PubMed literature search (National Center for Biotechnology Information [NCBI]; http://www.ncbi.nlm.nih.gov/pubmed/) up to June 30, 2010 using the following terms: (ulcerative colitis or inflammatory bowel disease) and (polymorphism* or variant* or loci or locus). References from the selected publications were manually scanned to identify other relevant studies. Studies were included if: 1) they were case–control studies for Caucasian UC; 2) they included at least 100 UC cases; 3) they were published in English; 4) they examined the selected SNPs or the highly linked SNPs with the selected ones ($r^2 \geq 0.95$ in the HapMap Southern Utah residents of European descent [CEU] samples [release #27, build 36]); and 5) they provided enough data to calculate odds ratios (ORs) and 95% confidence intervals (CIs). For publications using overlapping samples, we discarded the smaller dataset (13 studies). The literature search and data extraction were conducted by two authors (K.A. and J.U.). Disagreement over eligibility was resolved by a detailed discussion after review by one additional author (T.M.). Details of this search strategy are shown in Figure 1. Finally, a total of 43 articles[16,19–25,27,29,30,32–63] were included in the meta-analysis (Table 1).

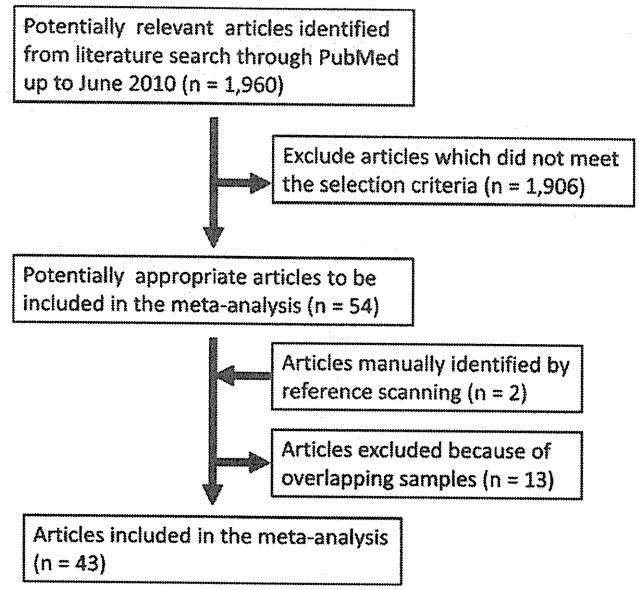

FIGURE 1. Flowchart of search strategy for meta-analysis.

Meta-analysis

We assessed heterogeneity across the studies using Cochran's *Q* test and I^2 statistics. *P*-value > 0.10 and I^2 statistics < 25% indicated a lack of heterogeneity.[64] If there was no heterogeneity among the studies, meta-analysis was carried out using the inverse variance-weighted method. This method is a fixed-effect model based on the assumption that the true OR of all studies is the same and no interstudy variance exist. When heterogeneity was present, we used the DerSimonian-Laird method. This method is a random-effect model which considers interstudy variance to estimate the combined OR. Publication bias was investigated by funnel plot and evaluated using Egger's test.[65] Funnel plot is a scatterplot which displays the OR of each study on the X axis against sample size on the Y axis. If there is no publication bias, OR will be distributed symmetrically and its variation may be smaller in larger studies. The degree of symmetry of funnel plot was estimated by Egger's test. We considered the evidence of significant publication bias as an obvious asymmetry of funnel plot and Egger's *P*-value < 0.05. All statistical analyses were undertaken using R (http://www.r-project.org/).

We basically used reported ORs and 95% CIs of the published studies to perform meta-analysis. Since 15 out of 43 articles did not report OR or 95% CI, we calculated OR and 95% CI of each SNP using genotype data in eight studies,[21,27,33,35,38,39,53,61] sample size and minor allele frequency (MAF) in three studies,[32,34,37] *P*-value and OR in three studies,[16,20,59] and *P*-value and MAF in one study.[48] Among the 62 SNPs initially selected, we excluded seven SNPs (rs10801047 [1q31], rs1002922 [5p13], rs10512734 [5p13], rs1373692 [5p13], rs3810936 [*TNFSF15*], rs7848647 [*TNFSF15*], and rs5743289 [*NOD2/CARD15*]) because these SNPs had not been studied in at least two studies. In addition, the data of SNPs in *ATG16L1* (rs2241880, rs10210302, and rs3828309), *BSN-MSTI* (rs9858542 and rs3197999), 5p13 (rs4613763 and rs17234657), *IRGM* (rs13361189, rs1000113, and rs11747270), *TNFSF15*

TABLE 1. Studies Included in the Meta-analysis

	Study	Reference	Year	Population	Case	Control
1	Ogura	32	2001	USA	182	287
2	Cuthbert	33	2002	UK	566	290
3	Esters	34	2004	Belgium	173	165
4	Büning	35	2005	Hungary	128	208
5	Martín	36	2005	Spain	544	812
6	Waller	37	2006	UK	512	750
7	Oostenbrug	27	2006	Netherlands	207	276
8	Crawford	38	2007	USA	172	104
9	Cucchiara	39	2007	Italy	186	347
10	Tremelling	40	2007	UK and Scotland	975	1345
11	Büning_1	41	2007	Germany and Hungary	296	707
12	Cummings	42	2007	UK	647	1134
13	Glas	43	2007	Germany	456	1381
14	Economou	44	2007	Greece	180	100
15	Büning_2	45	2007	German and Hungary	294	845
16	Roberts	46	2007	New Zealand	466	591
17	Glas	47	2008	Germany	507	1615
18	Lappalainen	48	2008	Finnland	459	292
19	Márquez	49	2008	Spain	363	546
20	Franke	15	2008	Germany	1103	1817
21	Fisher	21	2008	UK	1740	1492
22	Lakatos	50	2008	Hungary	149	149
23	Okazaki	51	2008	Canada	117	310
24	Roberts	52	2008	New Zealand	475	576
25	Fowler	53	2008	Australia	543	1244
26	Weersma_1	54	2009	Netherlands	1120	1350
27	Anderson	23	2009	UK	2527	3028
28	Silverberg	16	2009	USA and Canada	1052	2571
29	Weersma_2	24	2009	Belgium and Netherlands	1442	1045
30	Einarsdottir	55	2009	Sweden	455	280
31	Glas	56	2009	Germany	476	1503
32	Newman	57	2009	Canada	402	1005
33	Palomino-Morales	29	2009	Spain	425	572
34	Márquez_1	30	2009	Spain	368	745
35	Márquez_2	58	2009	Spain	405	800
36	Törkvist	59	2010	Sweden	935	1460
37	Festen	25	2010	Netherlands	1455	1902
38	Sventoraityte	60	2010	Lithuania	123	186
39	Lacher	61	2010	Germany	132	253
40	Cénit	62	2010	Spain	442	1692
41	Franke	19	2010	Germany	1043	1703
42	McGovern_GWAS1	20	2010	USA	723	2880
	McGovern_GWAS2	20	2010	Sweden	948	1408
	McGovern_GWAS3	20	2010	USA and Canada	1022	2503
	McGovern_Replication1	20	2010	Italy	993	826
	McGovern_Replication2	20	2010	Netherlands	1016	754
43	Perdigones	63	2010	Spain	662	1361

(rs6478108 and rs426389), *NKX2-3* (rs11190140 and rs10883365), and *NOD2/CARD15* (rs17221417, rs2066843, and rs2076756) were combined because these SNPs were in high linkage disequilibrium with each other ($r^2 \geq 0.95$) in the HapMap CEU samples. Finally, we performed a meta-analysis for 45 SNPs located at 33 loci by using a total of 4852 to 31,125 subjects. For an easy understanding of the risk direction, we calculated the OR and 95% CI of each SNP according to the risk allele in the GWAS for CD. A *P*-value less than 0.0015 (0.05/33) was considered statistically significant after applying Bonferroni correction.

RESULTS

We found evidence of heterogeneity among the studies for 19 SNPs: rs2476601 (*PTPN22*), rs2274910 (*ITLN1*), rs2241880-rs10210302-rs3828309 (*ATG16L1*), rs4613763-rs17234657 (5p13), rs2188962 (*LOC441108*), rs10077785 (*LOC441108*), rs4958847 (*IRGM*), rs6908425 (*CDKAL1*), rs1456893 (7p12), rs1551398 (8q24), rs6478108-rs4263839 (*TNFSF15*), rs17582416 (*CCNY-CREM*), rs10995271 (*ZNF365*), rs10761659 (*ZNF365*), rs7927894 (*C11orf30*), rs2872507 (*ORMDL3*), rs2542151 (*PTPN2*), rs1736135 (21q21), and rs762421 (*ICOSLG*). Therefore, the pooled ORs and 95% CIs were calculated using a random-effect model in these variants. We found a significant publication bias at rs9292777 on 5p13 locus (Egger's $P = 0.02$) and excluded this SNP from the analysis.

Among the 45 SNPs included in the meta-analysis, 35 SNPs located at 30 loci were investigated by more than five studies. Among the 33 loci examined in this study, we found significant associations with UC in 14 loci and nominal associations ($P < 0.05$) in 11 loci. We confirmed the associations of 17 susceptibility loci which are commonly associated with both CD and UC in the previous study[20]: *IL23R*, *KIF21B*, *BSN-MST1*, 5p13, *LOC441108*, *IRGM*, *IL12B*, *CCR6*, 7p12, *JAK2*, *TNFSF15*, *CCNY-CREM*, *NKX2-3*, *ORMDL3*, *STAT3*, 21q21, and *ICOSLG* (Supporting Information Table 2). Moreover, we found associations with UC in eight additional loci (Table 2): *GCKR* (rs780094, $P = 2.47 \times 10^{-2}$, OR 1.05), *ATG16L1* (rs2241880-rs10210302-rs3828309, $P = 4.70 \times 10^{-2}$, OR 1.05), *CDKAL1* (rs6908425, $P = 7.68 \times 10^{-3}$, OR 1.10), *ZNF365* (rs10761659, $P = 4.67 \times 10^{-4}$, OR 1.14), *LRRK2-MUC19* (rs11175593, $P = 1.54 \times 10^{-2}$, OR 1.21), *C13orf31* (rs3764147, $P = 1.80 \times 10^{-2}$, OR 1.07), *PTPN2* (rs2542151, $P = 2.49 \times 10^{-2}$, OR 1.08), and *SBNO2* (rs4807569, $P = 1.72 \times 10^{-2}$, OR 1.06). For all loci showing association, the directions of risk alleles for UC were all the same as those for CD. The OR of *IL23R* locus was relatively high (rs11209026, OR 1.62, 95% CI: 1.48–1.77), whereas ORs of other loci were modest ranged from 1.05–1.22.

DISCUSSION

We comprehensively reviewed the published studies that examined the CD susceptibility loci in UC patients and performed a meta-analysis to clarify the common genetic factors for both diseases. We found associations at 25 out of 33 candidate loci. Among them, we confirmed the associations in 17 loci reported in the previous GWAS,[20] and this study found an additional eight common susceptibility loci for CD and UC, namely, *GCKR*, *ATG16L1*, *CDKAL1*, *ZNF365*, *LRRK2-MUC19*, *C13orf31*, *PTPN2*, and *SBNO2*. Among these additionally identified loci, *GCKR* and *LRRK2-MUC19* have never shown nominal association with UC in any single studies performed to date. Although the genetic risk of each locus was modest, many genes or loci will contribute to the pathogenesis of both CD and UC.

Previous GWAS identified that the autophagy-related genes are associated with the susceptibility of CD.[6,7,9,10,13] In contrast to the strong association with CD, previous association studies for UC showed inconsistent results in these autophagy-related genes.[16,21–24,29–31,56] Our meta-analysis demonstrated nominal association with *ATG16L1* by using 11,466 cases and 19,659 controls ($P = 4.7 \times 10^{-2}$, OR 1.05, 95% CI: 1.00–1.10). Other autophagy-related genes also showed associations with UC in this study ($P = 1.54 \times 10^{-2}$, OR 1.21, 95% CI: 1.03–1.41 for *LRRK2-MUC19*; $P = 1.18 \times 10^{-3}$, OR 1.14, 95% CI: 1.05–1.24 for *IRGM*). These findings suggest a possibility that autophagy might contribute to the development of both UC and CD, but its effect may be weaker for UC.

There is another possibility that the association of autophagy-related genes are caused by the contamination of colonic CD cases because rs2241880-rs10210302-rs3828309 (*ATG16L1*) and rs4958847 (*IRGM*) showed heterogeneity among the studies. However, we could not find any consistent set of studies that contributed to this heterogeneity. Moreover, when we assume the possibility of this misclassification for *ATG16L1*, colonic CD cases should be included in more than 20% of UC cases based on the assumption of a case–control study of 11,466 cases and 19,659 controls, an allele test model, a risk allele frequency of 0.571 based on the HapMap-CEU population, an allelic OR of colonic CD for 1.25,[13] a statistical power of 0.80, and a *P*-value of 0.05. Since the diagnosis of UC was made by the established guidelines in each study, we think the association of autophagy-related genes in this study might not be caused by the misclassification of colonic CD cases in the previous studies.

Recent genetic studies have revealed shared genetic components of different immune-related diseases.[6,6] For the shared susceptibility genes between CD and UC, previous studies have shown the importance of the common pathogenesis of the IL-23/Th17 signaling pathway, which promotes inflammation in the adaptive immune response.[14]

TABLE 2. Results of Meta-analysis for Eight Additionally Identified Common Susceptibility Loci for CD and UC

	Allele* [1/2]	Study	Number		RAF		OR (95%CI)	Combined		Heterogeneity		Publication Bias
			Case	Control	Case	Control		*P*	OR (95% CI)	*P*	I^2 Statistics	*P*
GCKR												
rs780094	T/C	Anderson (2009)	2464	4002	0.40	0.38	1.07(0.99-1.16)	2.47E-02	1.05(1.00-1.09)	0.48	0	0.40
		Franke (2010)	1043	1703	0.42	0.40	1.10(0.99-1.23)					
		McGovern (2010) GWAS#1	723	2880	—	—	1.03(0.88-1.20)					
		McGovern (2010) GWAS#2	948	1408	—	—	1.00(0.94-1.07)					
		McGovern (2010) GWAS#3	1022	2503	—	—	1.08(0.96-1.21)					
		Total	6200	12496								
ATG16L1												
rs2241880	G/A	Büning_1 (2007)	296	707	0.52	0.51	1.10(0.89-1.35)	4.70E-02	1.05(1.00-1.10)	0.11	0.31	0.43
rs10210302	T/C	Roberts (2007)	466	591	0.51	0.50	1.05(0.87-1.25)					
rs3828309	G/A	Glas (2008)	507	1615	0.55	0.52	1.15(0.98-1.36)					
		Lappalainen (2008)	459	190	0.46	0.47	0.96(0.75-1.23)					
		Franke (2008)	1077	1793	0.55	0.53	1.19(1.01-1.41)					
		Fisher (2008)	1739	1491	0.54	0.52	1.08(0.97-1.20)					
		Lakatos (2008)	149	149	0.54	0.50	1.26(0.91-1.74)					
		Okazaki (2008)	117	310	0.50	0.48	1.02(0.61-1.68)					
		Fowler (2008)	543	1244	0.48	0.51	0.87(0.75-1.01)					
		Newman (2009)	402	1005	—	—	1.19(1.00-1.41)					
		Weersma_1 (2009)	1120	1350	0.55	0.56	0.95(0.84-1.08)					
		Palomino—Morales (2009)	414	666	0.54	0.51	1.10(0.92-1.32)					
		Márquez_1 (2009)	368	745	0.51	0.53	0.93(0.78-1.12)					
		Sventoraityte (2010)	123	186	0.53	0.48	1.26(0.91-1.75)					
		McGovern (2010) GWAS#1	723	2880	—	—	1.08(0.95-1.22)					
		McGovern (2010) GWAS#2	948	1408	—	—	0.91(0.79-1.05)					
		McGovern (2010) GWAS#3	1022	2503	—	—	1.08(0.95-1.22)					
		McGovern (2010) Rep#1	993	826	—	—	1.08(0.94-1.23)					
		Total	11466	19659								
CDKAL1												
rs6908425	C/T	Franke (2008)	1102	1794	0.81	0.79	1.18(1.01-1.39)	7.68E-03	1.10(1.02-1.18)	0.13	0.39	0.33
		Anderson (2009)	2453	4034	0.80	0.77	1.18(1.08-1.29)					
		Weersma_2 (2009)	1442	1045	0.81	0.78	1.18(0.99-1.41)					
		McGovern (2010) GWAS#1	723	2880	—	—	1.05(0.90-1.22)					
		McGovern (2010) GWAS#2	948	1408	—	—	1.03(0.91-1.16)					
		McGovern (2010) GWAS#3	1022	2503	—	—	1.11(0.95-1.30)					
		McGovern (2010) Rep#1	993	826	—	—	0.91(0.76-1.09)					
		Total	8683	14490								
ZNF365												
rs10995271	C/G	Törkvist (2010)	935	1460	—	—	1.03(0.89-1.18)	1.37E-01	1.07(0.97-1.17)	0.02	0.68	0.26
		Franke (2010)	1043	1703	0.44	0.40	1.19(1.07-1.33)					

(*Continued*)

TABLE 2. (*Continued*)

	Allele* [1/2]	Study	Number		RAF			Combined		Heterogeneity		Publication Bias
			Case	Control	Case	Control	OR (95%CI)	*P*	OR (95% CI)	*P*	I^2 Statistics	*P*
		McGovern (2010) Rep#1	993	826	—	—	1.09(0.95-1.24)					
		McGovern (2010) Rep#2	1016	754	—	—	1.00(0.96-1.05)					
		Total	3987	4743								
rs10761659	G/A	Franke (2008)	1088	1775	0.58	0.54	1.10(1.02-1.19)	4.67E-04	1.14(1.05-1.23)	0.24	0.28	NA
		Fisher (2008)	1807	1549	0.57	0.54	1.19(1.07-1.31)					
		Total	2895	3324								
LRRK2—MUC19												
rs11175593	T/C	Anderson (2009)	3026	1132	0.02	0.01	1.31(0.99-1.74)	1.54E-02	1.21(1.03-1.41)	0.70	0	0.08
		Törkvist (2010)	935	1460	—	—	1.11(0.68-1.80)					
		Franke (2010)	1043	1703	0.02	0.02	1.18(0.83-1.70)					
		McGovern (2010) Rep#1	993	826	—	—	1.31(0.97-1.76)					
		McGovern (2010) Rep#2	1016	754	—	—	0.94(0.62-1.43)					
		Total	7013	5875								
C13orf31												
rs3764147	G/A	Anderson (2009)	2424	4017	0.22	0.21	1.07(0.98-1.18)	1.80E-02	1.07(1.01-1.13)	0.39	0.03	0.78
		Törkvist (2010)	935	1460	—	—	1.22(1.04-1.42)					
		Franke (2010)	1043	1703	0.25	0.25	1.02(0.89-1.15)					
		McGovern (2010) Rep#1	993	826	—	—	1.04(0.90-1.20)					
		McGovern (2010) Rep#2	1016	754	—	—	1.02(0.89-1.16)					
		Total	6411	8760								
PTPN2												
rs2542151	G/T	Franke (2008)	1005	1779	0.19	0.15	1.33(1.11-1.59)	2.49E-02	1.08(1.00-1.16)	0.14	0.37	0.06
		Fisher (2008)	1735	1488	0.17	0.17	1.07(0.93-1.22)					
		McGovern (2010) GWAS#1[a]	723	2880	—	—	1.14(0.95-1.36)					
		McGovern (2010) GWAS#2[a]	948	1408	—	—	1.04(0.90-1.20)					
		McGovern (2010) GWAS#3[a]	1022	2503	—	—	1.03(0.85-1.24)					
		McGovern (2010) Rep#1	993	826	—	—	1.00(0.92-1.09)					
		McGovern (2010) Rep#2	1016	754	—	—	1.13(0.94-1.35)					
		Total	7442	11638								
SBNO2												
rs4807569	C/A	Anderson (2009)	2425	4047	0.22	0.20	1.10(1.00-1.20)	1.72E-02	1.06(1.01-1.12)	0.57	0	0.85
		Franke (2010)	1043	1703	0.25	0.24	1.03(0.90-1.17)					
		McGovern (2010) GWAS#1[b]	723	2880	—	—	1.00(0.85-1.18)					
		McGovern (2010) GWAS#2[b]	948	1408	—	—	1.03(0.93-1.13)					
		McGovern (2010) GWAS#3[b]	1022	2503	—	—	1.15(0.99-1.33)					
		Total	6161	12541								

*Allele "1" denotes the reported risk allele.
†OR and 95% CI were calculated using the random-effect model because of the heterogeneity among the studies.
[a]rs1893217 is absolutely linked with rs2542151 ($r^2 = 1.0$).
[b]rs2024092 is absolutely linked with rs4807569 ($r^2 = 1.0$).
RAF, risk allele frequency; OR, odds ratio; CI, confidence interval; NA not applicable.

Many genetic variants including in this pathway such as *IL23R*, *IL12B*, *JAK2*, and *STAT3* are associated with susceptibility for both diseases. Among the eight additionally identified common susceptibility loci for CD and UC, several genes are reported to be associated with various diseases or traits: *C13orf31* is associated with leprosy.[67] *PTPN2* is associated with type 1 diabetes[68,69] and celiac disease.[70] *CDKAL1* is a susceptibility gene for type 2 diabetes.[71–73] *GCKR* is implicated in metabolic traits such as triglyceride,[74–76] fasting glucose,[77] and serum uric acid.[78] However, there is little information how these genes affect the development of CD and UC. Functional analysis of these genes will provide further understanding of the common pathogenesis of CD and UC.

When we compared our results with those of a recent meta-analysis for UC,[20] we could not find a significant association in the 6q21 locus. In the present study we performed a meta-analysis using the data of rs7746082 that showed the strongest association with CD at the 6q21 locus.[13] However, the GWAS meta-analysis estimated the association using the data of rs6938089, best proxy SNP for rs7746082.[20] Although the r^2 value between rs7746082 and rs6938089 is 0.60 for the HapMap CEU population (release #27, build 36), there is a possibility that the hidden causative variant at the 6q21 locus might be different between CD and UC. Further detailed analysis is necessary to clarify the effect of the 6q21 locus on susceptibility to CD and UC.

Significant publication bias was observed at rs9292777 on 5p13 locus. The funnel plot showed that the largest study[21] had the largest OR, whereas the OR of the smaller studies were all shifted to the smaller ones. Based on this asymmetrical distribution of OR, we excluded this SNP in this study.

In conclusion, in addition to the reported common susceptibility loci, we identified eight common susceptibility loci for CD and UC by a meta-analysis of published studies using more than 30,000 subjects. Our data indicate that UC and CD share many genetic factors with small effect. These findings will help to clarify the common pathway involved in the development of both diseases.

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