rate was 47% at 3 years after EBD. It is suggested that this high redilation rate is related to the disease characteristics of CD. As the need for frequent redilation seems to be a problem with EBD, it is desirable to know the factors influencing redilation. However, although we compared patients with and without the need for redilation, we could not identify any factor that influenced the redilation rate.

We are aware of some limitations of the current study. First, its retrospective nature could have resulted in recruitment bias. However, in real clinical practice, we usually attempt EBD in almost all patients with small bowel strictures who meet our indications. Therefore, we believe that the selection bias was relatively low in this study. Second, it is possible that various medical treatments for CD affect small bowel strictures.[29] However, in the present study, the proportion of various concomitant therapies was not significantly different between the EBD successful and unsuccessful cases. Moreover, no factors could be identified that influenced the efficacy of EBD in this study.

The current study confirmed that EBD of small bowel strictures is a safe and effective alternative to surgery in patients with CD. However, the high redilation rate is one of the problems of this procedure. It is necessary to prospectively observe a larger number of patients for a longer period to confirm these results more precisely. Currently, a nationwide, prospective, multicenter study regarding this is ongoing as the framework of a study project undertaken by the Study Group on Intractable Diseases, using Health and Labor Sciences Research Grants from the Ministry of Health, Labor and Welfare of Japan.

CONCLUSION

ENDOSCOPIC BALLOON DILATION using DBE for small bowel strictures in patients with CD provides not only short-term success, but also long-term efficacy in terms of avoiding surgery.

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CONFLICT OF INTERESTS

AUTHORS DECLARE NO conflict of interests for this article.

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Treatment strategy of diminutive colorectal polyp <5 mm in size — Should it be removed and discarded without pathologic assessment?

Development and progression of colorectal cancer based on follow-up analysis

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Elucidating the pathway of colorectal cancer development and progression can lead to identification of lesions that must be treated appropriately. The aim of the present review was to summarize the development and progression of colorectal cancer based on radiological and endoscopic follow-up analyses. These studies revealed several characteristic findings, including that initial morphology with progression to advanced cancer was most commonly 0-Is, followed by 0-IIa. Based on the doubling time, 0-Ip grew slowly in comparison with other morphologies. The observation period from adenomas measuring <10 mm to intramucosal cancers was more than 5 years. This makes it difficult to draw any accurate conclusions about the natural history of colorectal cancer based on follow-up observation alone and it is difficult to exclude the selection bias because of the difficulty of follow up for flat and depressed tumor. However, the only reliable way to elucidate the natural history is to accumulate cases.

Key words: colorectal cancer, development, follow up, natural history, retrospective analysis

INTRODUCTION

IN THE DEVELOPMENT and progression of colorectal cancer, various courses such as the adenoma-carcinoma sequence,[1] de novo carcinoma,[2] the serrated polyp neoplasia pathway,[3] and the dysplasia-carcinoma sequence,[4] are proposed. The adenoma-carcinoma sequence is widely accepted, based on observational studies,[5,6] as a major pathway of the development and progression of colorectal cancer. The removal of colorectal adenomas is the most valid treatment for preventing colorectal cancer and cancer-related deaths.[7,8] However, as a result of limited medical resources, cost, and time, it is difficult to remove diminutive adenomas (<5 mm in diameter) in all cases.

The natural history of colorectal cancer has been examined by comparing sequential morphological changes in patients who can be followed up clinically using radiography or endoscopy. However, in an observational analysis that is primarily retrospective, the histology and depth of invasion of an initial lesion can only be presumed, and it is impossible to determine what phase of tumor development is being observed; for example, whether it is an intramucosal cancer just after a lesion has progressed to cancer, or intramucosal cancer that is continuing to progress to the submucosa. This makes it difficult to draw any accurate conclusions about the natural history of colorectal cancer based on follow-up observation alone. The only reliable way to elucidate the natural history is to accumulate cases.

The aim of the present review was to summarize the development and progression of colorectal cancer based on radiological and endoscopic follow-up analyses.

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INITIAL MORPHOLOGY IN ADVANCED CANCER

IN PREVIOUS RETROSPECTIVE radiological analyses[5,9] in the 1960s and 1970s, it was concluded that sessile tumors were the main route to colorectal cancer. Thereafter, some analyses, including retrospective endoscopic analyses, were reported. Because endoscopy can give a much more detailed picture of small lesions, it is suitable for understanding cancer development arising from a minute or superficial type of cancer.[10] In a meta-analysis of five retrospective radiographic studies[11] of 81 lesions with a final diagnosis of advanced cancer in which the initial lesion was presumed to be early cancer, the initial morphology was 0-Is in 35 lesions (43.2%) and 0-IIa in 18 lesions (22.2%). In an analysis of 35 lesions from 25 retrospective endoscopic reports[10] of 13 lesions with a final diagnosis of advanced cancer, the initial

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morphology was 0-Is in four lesions (30.8%) and 0-Ip and 0-IIa in two lesions each (each 15.4%).

A total of 110 colorectal cancers obtained from retrospective radiological methods, as well as 44 colorectal cancers obtained from retrospective endoscopic methods, at 30 medical facilities in the Kyushu region in Japan were analyzed.[12] Inclusion criteria for this retrospective study were as follows: (i) colonoscopy or barium enema examinations carried out at initial and final examinations; (ii) lesions were judged to be adenomas or up to early carcinoma (pTis–pT1 according to the TNM classification) at initial examination; and (iii) histopathological findings from endoscopic or surgical resection confirmed at the time of final examination. The initial morphology in 26 lesions with progression to advanced cancer (pT2–pT4 according to the TNM classification) in the retrospective endoscopic analysis was most commonly 0-Is (10 lesions, 38.5%), followed by 0-IIa (six lesions, 23.1%). The initial morphology in 73 lesions with progression to advanced cancer in the radiological analysis was most commonly 0-Is (25 lesions, 34.2%), followed by 0-IIa (14 lesions, 19.2%). 0-Is lesions are thus assumed to represent a major initial lesion in advanced cancer. In addition, whereas all of the 0-IIc lesions developed into type 2 advanced cancer, 0-Is and 0-IIa lesions developed with marked configurational changes.

A low rate of superficial depressed-type lesions as initial morphology in advanced cancer was reported, as has been described in previous retrospective endoscopic studies. However, because imaging of small superficial-type lesions is difficult in retrospective studies, many of the lesions that have been followed may have been derived from other than superficial-type lesions. In addition, compared to other lesions, superficial-type lesions, particularly depressed-type lesions, can invade the submucosa when still small, and the grade of malignancy is high.[13,14] If superficial depressed-type lesions are discovered, they may be resected, even if they are diminutive lesions. Therefore, in retrospective studies, the possibility of a small percentage of superficial depressed-type lesions as initial morphology cannot be excluded.

GROWTH SPEED OF CANCER

TO OBJECTIVELY EXPRESS speed of growth, doubling time (DT) was calculated[12,15–17] based on retrospective radiological examination (Table 1). In our multicenter retrospective radiographic studies[12] of 110 cancers, the mean DT was 26.5 months for intramucosal cancer, 25.9 months for submucosal cancer, and 12.3 months for advanced cancer. In a meta-analysis of radiographic studies,[10] the DT ranged from 9.4 to 55.4 months for intramucosal cancer and 4.7 to 12.2 months for advanced cancer. Colorectal cancer grew rather slowly when the cancer was limited to the mucosa. However, when tumors grew down into the submucosa, growth speeds were accelerated.[18]

In our study,[12] DT of advanced colorectal cancer according to the initial morphology was 18.7 months for 0-Ip, 15.7 months for 0-IIa, and 13.2 months for 0-Is. The 0-Ip lesions grew slowly in comparison with other morphologies. However, as traditionally noted, the percentage of 0-Ip lesions among early cancers is relatively high, whereas retrospective studies have not been clear that there is a low rate of 0-Ip lesions as the initial morphology in advanced cancer. The reasons for this include the fact that cancer may remain for a long time within the mucosa. This is also supported by the results of our study; namely, that the DT was longest until advanced cancer.

In contrast, some investigators have proposed the hypothesis that superficial depressed lesions develop more slowly than polypoid tumors. Watari *et al.*[19] prospectively analyzed 75 colorectal adenomas and they reported that minute nonpolypoid adenomas frequently tended to grow slowly, and that tumors with exophytic growth had significantly higher proliferative index/apoptotic index ratios than tumors with

Table 1 Reports on doubling time of colorectal cancer based on retrospective radiological examination

Author	No. cases	Doubling time	
		Early cancer	Advanced cancer
Ushio & Ishikawa[15]	42	55.4 months (*n* = 13)	10.5 months (*n* = 29)
Iwashita *et al.*[16]	37	20.6 months (*n* = 9)	9.7 months (*n* = 28)
Umetani *et al.*[17]	11	9.4 months (*n* = 5)	4.7 months (*n* = 6)
Hisabe *et al.*[12]	110	Intramucosal cancer 26.5 months (*n* = 14) Submucosal cancer 25.9 months (*n* = 23)	12.3 months (*n* = 73)

depressed growth. These results suggest that depressed-type lesions grow slowly because of advanced apoptosis. In our study,[12] the DT of 0-IIc,IIc+IIa was 52.8 months for intramucosal cancer ($n = 3$), 14.7 months for submucosal cancer ($n = 4$), and 6.9 months for advanced cancer ($n = 3$). It has been demonstrated that 0-IIc-type intramucosal cancers have significantly longer DT than other morphologies. However, because the number of patients with depressed lesions who were followed up was small, it is difficult to make accurate comparisons with other gross morphologies. Further accumulation of patients will be needed.

DEVELOPMENT AND PROGRESSION OF COLON ADENOMAS

THERE ARE SOME studies[19–24] on the natural history of colorectal polyps <10 mm in diameter. The observation period ranged from 22 to 43 months, and the cancer development rate ranged from 0 to 2.5%. Although all colorectal adenomas have the ability to become cancers, only a few lesions develop cancer, and most adenomas show no increase in size within a couple of years. On the basis of the results of these follow-up studies, immediate treatment may not be necessary for diminutive colorectal adenomas, and follow-up examination may be sufficient for a couple of years. There is still no consensus regarding the treatment strategies for diminutive colorectal adenomas in Japan.

There are some reports about the characteristics of colorectal adenoma that progressed to cancer. In a retrospective endoscopic analysis of 23 colorectal adenomas diagnosed by biopsy,[12] the mean observation period from six adenomas to intramucosal cancers was 78.5 months, whereas the mean observation periods from four adenomas to submucosal cancers and 13 adenomas to advanced cancers were 41.8 months and 64.5 months, respectively (Fig. 1). The period from adenoma to intramucosal cancer was thus the longest, representing a contradictory result. One factor may have been differences in initial tumor diameter. That is, the initial mean diameter of adenomas that progressed to intramucosal cancer was 6.5 mm, whereas the initial mean diameter of adenomas that progressed to submucosal cancer was 11.3 mm, and the initial mean diameter of adenomas that progressed to advanced cancer was 11.1 mm (Table 2). Furthermore, there was only one small advanced cancer measuring ≤2 cm in 13 colorectal adenomas that progressed to advanced cancer. These data suggest that colorectal adenomas are unlikely to progress to small advanced cancers. The initial morphology of small advanced cancers measuring ≤2 cm was the protruded type in 42.9% of the retrospective radiographic cases and the superficial type in

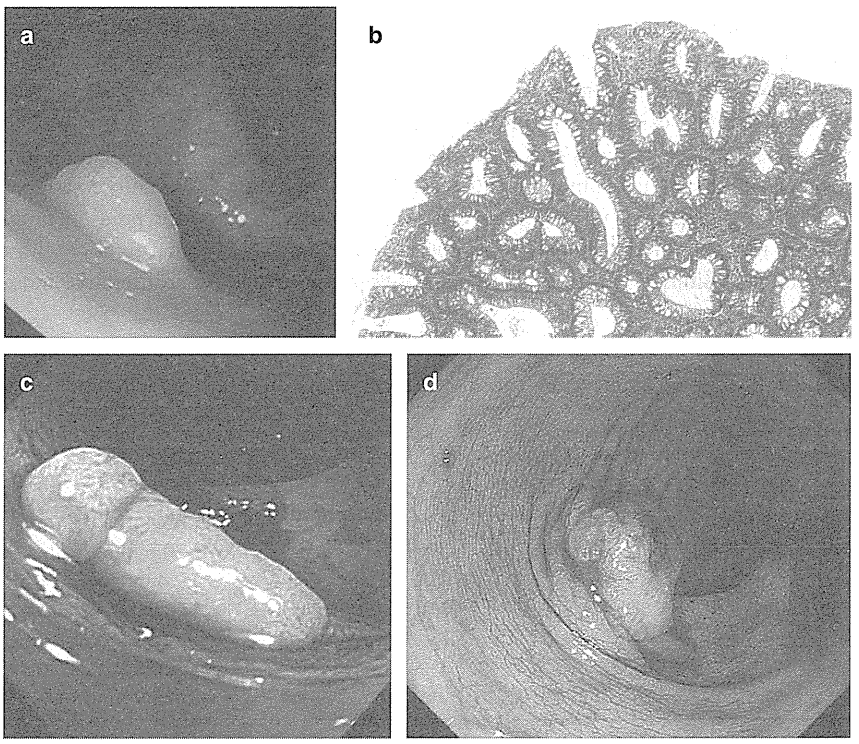

Figure 1 (a) Initial endoscopic finding shows 0-Is type measures 7 mm. (b) Pathological examination showing tubular adenoma with mild to moderate epithelial atypia. (c) The adenoma changed to 0-Is measuring 10 mm at 84 months after initial observation. We had scheduled polypectomy, but the patient did not come to the hospital. (d) The final examination. Type 1 advanced carcinoma (T3, N1, M0) measuring 15 mm at 60 months after the second observation. (Reprinted with permission from reference.[12])

Table 2 Clinical features of colorectal adenomas that developed into cancer

Final depth of tumor invasion	Observation period from adenoma to cancer (mean ± SD), months	Tumor diameter (mean ± SD), mm	
		Initial	Final
Mucosa (n = 6)	78.5 ± 46.7	6.5 ± 2.4	11.5 ± 4.5
Submucosa (n = 4)	41.8 ± 16.6	11.3 ± 3.5	19.5 ± 8.6
Muscularis propria or deeper (n = 13)	64.5 ± 40.6	11.1 ± 4.4	33.2 ± 11.2

57.1%, and among the retrospective endoscopic cases, the protruded type and the superficial type each accounted for 50%. It is likely that the protruded-type lesions participated considerably in the progression to small advanced cancers.

In a previous study[24] on the natural history of 408 protuberant colorectal adenomas <10 mm in diameter, we reported no significant increase in size or other changes such as malignant transformation over a period of at least 5 years, and that the development of small <10 mm protuberant adenomas was slow, with only a small number of adenomas >10 mm developing into cancer. Adenoma size is relevant, considering that cancer development occurs in 1% of <10 mm adenomas, in 10% of adenomas >10 mm and <20 mm, and in 50% of adenomas >20 mm.[6] Furthermore, it is hypothesized that adenomas ≥10 mm with malignant transformation are the lesions showing rapid subsequent development.

CONCLUSION

THESE FOLLOW-UP STUDIES showed several characteristic findings according to the morphology. The initial morphology with progression to advanced cancer was most commonly 0-Is. 0-Ip grew slowly in comparison with other morphologies. The observation period from adenomas measuring <10 mm to intramucosal cancers was more than 5 years. Elucidating the pathway of colorectal cancer development and progression can lead to identification of lesions that should be treated appropriately.

CONFLICT OF INTERESTS

AUTHORS DECLARE NO conflict of interests for this article.

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GASTROENTEROLOGY

Impact of CYP3A5 genetic polymorphisms on the pharmacokinetics and short-term remission in patients with ulcerative colitis treated with tacrolimus

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ABCB1, CYP3A4, CYP3A5, tacrolimus, ulcerative colitis.

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Abstract

Background and Aim: The pharmacokinetics of tacrolimus (Tac) differ among individuals, and genetic polymorphisms of cytochrome P-450 (CYP) 3A4, CYP3A5, and ABCB1 are thought to be involved. The aim of this study was to clarify whether these genetic polymorphisms affect the pharmacokinetics of Tac in patients with ulcerative colitis.

Methods: The subjects in this study were 45 patients with moderate-to-severe ulcerative colitis who were resistant to other therapies and were treated with Tac. The subjects were tested for genetic polymorphisms of CYP3A4, CYP3A5, and ABCB1, and the relationship between Tac pharmacokinetics and the remission rate was investigated.

Results: Of the 45 subjects, 24 (53.3%) were CYP3A5 expressers (Exp), and 21 (46.7%) were non-expressers (Non-Exp). The trough level and the dose-adjusted trough level on days 2–5 were significantly higher in the Non-Exp group than in the Exp group (10.16 ± 5.84 *vs* 4.47 ± 2.50 ng/mL, $P < 0.0001$, 139.36 ± 77.43 *vs* 61.37 ± 41.55 ng/mL per mg/kg/day, $P < 0.0001$). The percentage of patients achieving the optimal trough level on days 2–5 was significantly higher in the Non-Exp group than in the Exp group (40.0% *vs* 4.3%, $P = 0.01$). This trend was also observed on days 7–10. On multivariate analysis, factors associated with achievement of the optimal trough level were food non-intake and Non-Exp of CYP3A5. The remission rate was significantly higher in the Non-Exp group than in the Exp group (47.6% *vs* 16.7%, $P = 0.046$).

Conclusions: CYP3A5 genetic polymorphisms affected the pharmacokinetics of Tac, so that the short-term clinical remission rate was different between Exp and Non-Exp of CYP3A5.

Introduction

In recent years, the calcineurin inhibitor tacrolimus (Tac) has been widely used internationally as an immunosuppressant in organ transplantation patients.[1] In a double-blind trial in Japan, Tac was also shown to be safe and effective in ulcerative colitis (UC) patients with moderate-to-severe activity.[2] In Japan, Tac has been used as remission induction therapy in UC patients since 2009. One characteristic of Tac is that its effect is trough level-dependent.[2,3] Tac metabolism is affected by various factors, including food intake/non-intake, drug metabolism enzymes, and transporters.[4,5] This means that the blood level of Tac varies considerably among individual patients, and therapeutic drug monitoring (TDM) is needed to safely obtain a good therapeutic effect.[6] In terms of drug metabolism enzymes and transporters, Tac is a substrate of cytochrome P-450 (CYP) 3A enzyme and drug transporter ATP-binding cassette sub-family B member 1 (ABCB1).[4] Both CYP3A4 and CYP3A5 are known to be involved in the metabolism of Tac,[7] and there are many reports on the relationship between Tac pharmacokinetics and genetic polymorphisms of CYP3A4, CYP3A5, and ABCB1 in organ transplantation patients.[8–11] However, there has been no investigation of these genetic polymorphisms and Tac pharmacokinetics in inflammatory bowel disease (IBD) patients, and only one report on the response to Tac therapy.[12] Genetic polymorphisms are known to exist in CYP3A4, CYP3A5, and ABCB1, and there are also known to be large differences among ethnic groups.[9–11] In general, CYP3A5 genetic polymorphisms, namely, expressers (Exp) with *1 or non-expressers (Non-Exp) without *1, are thought to have the greatest effect on Tac pharmacokinetics.[13,14] In the present study, CYP3A4, CYP3A5, and ABCB1 genetic polymorphisms and their potential associations with Tac pharmacokinetics and efficacy were analyzed in Japanese IBD patients.

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Methods

Patients and genotyping analysis. In our department, therapy with Tac is indicated for UC patients with moderate-to-severe activity who are resistant to prednisolone (PSL) and other drugs. Many cases are severe, and inpatient therapy is the fundamental approach when starting Tac. As a rule, the initial dose is 0.05 mg/kg twice daily for patients ingesting food and 0.04 mg/kg twice daily for patients who are fasting. To monitor blood levels of Tac, trough levels are normally measured at least on days 2–5 and 7–10 during the early period of therapy. Measurement of Tac blood levels is contracted to SRL, Inc. (Tokyo, Japan), and ELISA is done using the PRO-TRAC II TM FK 506 (Bio-Rad Laboratories, Inc., Los Angeles, CA, USA). Depending on the trough level results on days 2–5 and 7–10 during the remission induction period, the Tac dose is then adjusted to achieve the optimal trough level of 10–15 ng/mL. The equation (previous dose × 12.5 mg/mL/the blood trough level) was used for the dose adjustment of Tac.[2,3] Patients with frequent diarrhea or severe abdominal pain are managed by fasting with total parenteral nutrition for about 2 weeks. Seventy patients with UC were treated by Tac in our department between February 2001 and February 2012. Of these patients, full explanations of the present study were given to 45 patients examined in our hospital between August 2011 and May 2012. There was no special selection; all 45 of these patients undergoing follow-up at our hospital during this period were the subjects of this study. Genotyping analysis of CYP3A5, CYP3A4, and ABCB1 was contracted to SRL, Inc., and gene analysis was done by fluorescence correlation spectroscopy.[15]

CYP3A5 and ABCB1 genotypes and pharmacokinetics of Tac. The initial dose, trough level, and dose-adjusted trough level on days 2–5 and 7–10, and the percentages of patients achieving the optimal trough level on days 2–5 and 7–10 were compared by CYP3A5 and ABCB1 genotypes.

Achieving the optimal trough level. Various factors affecting the achievement of the optimal trough level were compared between the achievement and non-achievement groups. A univariate analysis was done first, followed by a multivariate analysis for items with $P < 0.25$.

Short-term clinical remission. The remission rate after 4 weeks was compared between the Exp and Non-Exp groups.

In our hospital, subjective and objective findings needed for the disease activity index (DAI, also known as the "Sutherland index")[16] or other activity indices are entered in electronic medical records daily for hospitalized UC patients, and blood tests are performed at least once a week. For severe colitis, blood tests are usually checked twice per week. Thus, the DAI score can be calculated accurately even retrospectively.

In this study, the DAI entered in patients' medical records was used to evaluate activity. However, because all patients did not undergo endoscopy 4 weeks after the start of therapy, a partial DAI (pDAI) score excluding the endoscopic subscore was used to define remission. Patients with pDAI ≤ 1 were defined as in remission, and patients with other scores and patients who underwent surgery within 4 weeks after the start of therapy were defined as being in non-remission.

Safety. The frequency and types of adverse effects were investigated. When Tac is used in patients aged 60 years or older in our hospital, cotrimoxazole is administered at the usual dosage with the aim of preventing *Pneumocystis pneumonia* (PCP).

Statistical analysis. An unpaired *t*-test was used to test for differences in mean values, and the chi-square test or Fisher's exact test was used to compare frequencies. Multivariate analysis was used to analyze factors involved in achieving the optimal trough levels. All statistical analyses were done using SPSS ver. 16.0 (SPSS Inc., Chicago, IL, USA).

Ethical Considerations. Written, informed consent was obtained from all subjects. This study was approved by the ethical review board of Fukuoka University.

Results

The subjects in this study were 25 men and 21 women with a mean age of 40.9 ± 13.8 years and a mean duration of disease of 4.9 ± 5.2 years. The DAI score on day 0 of Tac was 10.9 ± 1.2, and the pDAI score was 7.8 ± 1.2.

Pretreatments involved steroid therapy in 35 patients, accounting for 78% of all cases. Of these 35 patients, 25 received intravenous steroids, and the remaining 10 took oral steroids. Other treatments were cytapheresis in six patients, cyclosporine A (CyA) and infliximab in one patient each, and 5-aminosalicylic acid with an immunomodulator in two patients. Fasting management with total parenteral nutrition was selected in 22 patients, which corresponded to about half of the 45 patients in this study. Hydrocortisone was administered intravenously in 20 of these 22 patients, the large majority. The treatments given the subjects prior to starting Tac are summarized in Figure 1.

CYP3A4, CYP3A5, and ABCB1 genotypes and patient profiles. The CYP3A4 genotype was *1*1 in 44 of 45 subjects (97.8%) and *1/*1B in only one subject (2.2%).

The CYP3A5 genotype was *1*1 in four subjects (8.9%), *1*3 in 20 subjects (44.4%), and *3*3 in 21 subjects (46.7%). Thus, 24 of 45 subjects (53.3%) were Exp with *1, and 21 of 45 (46.7%) were Non-Exp without *1. No obvious differences were seen in the baseline characteristics of patients in the Exp group and the Non-Exp group before starting Tac (Table 1).

The genotype of ABCB1 2677G/A/T was G/T in 16 patients (35.6%), G/A in 11 patients (24.4%), G/G in 9 patients (20.0%), T/A in 4 patients (8.9%), and T/T in 5 patients (11.1%). The genotype of ABCB1 3435C/T was C/T in 24 patients (53.3%), C/C in 17 patients (37.8%), and T/T in 4 patients (8.9%).

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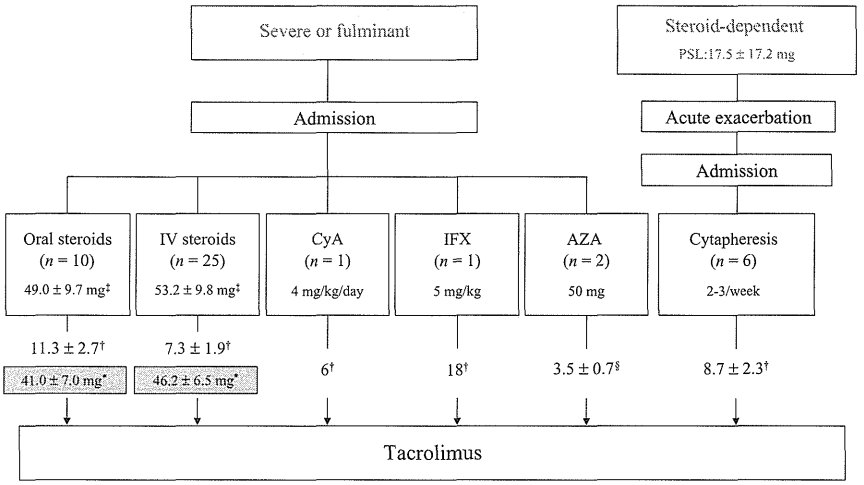

Figure 1 This figure shows the clinical courses and treatments of the subjects prior to starting tacrolimus. Severe or fulminant ulcerative colitis was seen in 39 (86.7%) of 45 subjects. The other six patients (13.3%) showed an acute exacerbation in a chronic steroid-dependent course. *Dose at starting tacrolimus (mg/day, mean ± SD); †period before starting tacrolimus from initial treatment (days, mean ± SD); ‡initial dose (mg/day, mean ± SD); §period before starting tacrolimus from admission (days, mean ± SD). AZA, azathioprine; CyA, cyclosporine A; IFX, infliximab; IV, intravenous; PSL, prednisolone.

Table 1 Comparison of patients baseline characteristics between the CYP3A5 Exp group and the Non-Exp group

	Exp group (n = 24)	Non-Exp group (n = 21)	P value
Sex (M/F)	15/9	11/10	0.49
Age (years, mean ± SD)	39.8 ± 13.7	42.1 ± 14.2	0.58
Disease duration (years, mean ± SD)	5.2 ± 5.6	4.7 ± 4.9	0.63
Disease location			
Left-sided colitis	4	5	0.82
Entire colitis	17	19	
Bowel movements	8.8 ± 2.9	9.2 ± 2.5	0.66
BMI (kg/m^2, mean ± SD)	20.2 ± 3.9	19.4 ± 2.8	0.43
PSL dose (mg, mean ± SD)	34.1 ± 26.8	31.0 ± 25.0	0.69
Food intake (yes/no)	12/12	11/10	0.87
Hb (g/dL, mean ± SD)	10.9 ± 2.2	11.5 ± 2.0	0.43
Alb (g/dL, mean ± SD)	3.0 ± 0.6	2.9 ± 0.7	0.87
CRP (mg/dL, mean ± SD)	5.7 ± 5.1	4.9 ± 6.2	0.62
DAI score (mean ± SD)	11.1 ± 0.8	10.6 ± 1.5	0.13
Partial DAI score (mean ± SD)	8.0 ± 1.1	7.7 ± 1.2	0.35

Exp, expresser (CYP3A5*1*1, CYP3A5*1*3); Non-Exp, non-expresser (CYP3A5*3*3).

Alb, albumin; BMI, body mass index; CRP, C-reactive protein; CYP, cytochrome P-450; DAI, disease activity index; Hb, hemoglobin; PSL, prednisolone.

CYP3A5 and ABCB1 genotypes and pharmacokinetics of Tac. All patients who needed fasting to control their severe symptoms were continued on fasting status until at least day 12 of Tac therapy. Therefore, their fasting status did not affect the analysis of Tac pharmacokinetics on days 2–5 and 7–10.

On days 2–5, there was no difference in the Tac dose between the CYP3A5 Non-Exp group and the Exp group, but the Non-Exp group had a significantly higher trough level (10.16 ± 5.84 *vs* 4.47 ± 2.50 ng/mL, $P < 0.0001$) and dose-adjusted trough level (139.36 ± 77.43 *vs* 61.37 ± 41.55 ng/mL per mg/kg/day, $P < 0.0001$). On days 2–5, the Non-Exp group had a significantly higher percentage of patients achieving the optimal trough level than the Exp group (40.0% *vs* 4.3%, $P = 0.01$). On days 7–10, the Tac dose was significantly higher in the Exp group because of dose adjustment (0.156 ± 0.036 *vs* 0.112 ± 0.044 mg/kg, $P = 0.001$), but the Non-Exp group had significantly higher trough levels (16.81 ± 5.70 *vs* 9.76 ± 2.90 ng/mL, $P < 0.0001$) and dose-adjusted trough levels (185.19 ± 109.55 *vs* 66.52 ± 28.00 ng/mL per mg/kg/day, $P < 0.0001$) than the Exp group. On days 7–10, the Non-Exp group had a significantly higher percentage of patients achieving the optimal trough level than the Exp group (84.2% *vs* 45.5%, $P = 0.04$) (Table 2).

For ABCB1, the trough level and dose-adjusted trough level were compared on days 2–5 and 7–10 between the TT type and all other types in C3435T and between the TT type and all other types in G2677A/T, but no significant differences were seen (data not shown).

Achieving optimal trough level

1 Percentage of patients achieving the optimal trough level on days 2–5 and associated factors

Nine patients (20.9%) achieved the optimal trough levels on the initial measurement. Univariate analysis was done with a total of 28 items, including CYP3A4, ABCB1, CYP3A5 genotype, patient background, pretreatment, activity index, endoscopic severity, and laboratory data (erythrocyte sedimentation rate, white blood cell count, hemoglobin, platelet count, C-reactive protein, albumin) to determine whether an appropriate trough level was achieved (Table 3).

Items with $P < 0.25$ on the univariate analysis (CYP3A5 genotype, food intake/non-intake, BMI<20 kg/m^2, PSL dose at baseline) were taken as explanatory variables on multivariate analysis. Food non-intake (OR 36.7, 95% CI 2.9–471.7) and CYP3A5 Non-Exp (odds ratio [OR] 40.3, 95% confidence interval [CI] 3.2–515.5) were significantly associated with achievement of the optimal trough level on days 2–5 (Table 4).

2 Percentage of patients achieving the optimal trough level on days 7–10 and associated factors

Twenty-six patients achieved high trough levels on the second measurement. As on days 2–5, a univariate analysis was done with a total of 28 items to determine whether an appropriate trough level was achieved (Table 3).

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Table 2 Association between CYP3A5 genotype polymorphism and pharmacokinetics with tacrolimus therapy

	Exp group (n = 24)	Non-Exp group (n = 21)	P value
Days 2–5 of tacrolimus therapy‡			
Dose of tacrolimus (mg/kg, mean ± SD)	0.086 ± 0.019	0.087 ± 0.023	0.79
T0 level† (ng/mL, mean ± SD)	4.47 ± 2.50	10.16 ± 5.84	< 0.0001
Dose-adjusted T0 level (ng/mL per mg/kg/day, mean ± SD)	61.37 ± 41.55	139.36 ± 77.43	< 0.0001
Achievement of optimal trough level (yes/no)	1/22 (4.3%)	8/12 (40.0%)	0.01
Days 7–10 of tacrolimus therapy§			
Dose of tacrolimus (mg/kg, mean ± SD)	0.156 ± 0.036	0.112 ± 0.044	0.001
T0 level† (ng/mL, mean ± SD)	9.76 ± 2.90	16.81 ± 5.70	< 0.0001
Dose-adjusted T0 level (ng/mL per mg/kg/day, mean ± SD)	66.52 ± 28.00	185.19 ± 109.55	< 0.0001
Achievement of optimal trough level (yes/no)	10/11 (45.5%)	16/3 (84.2%)	0.04

†T0 level: blood trough tacrolimus level.
‡One patient of the Exp group and one patient of Non-Exp group were excluded because T0 level had not been measured on days 2–5.
§Three patients of the Exp group were excluded due to surgery within 7 days. Two patients of Non-Exp group were excluded because the T0 level had not been measured on days 7–10.
Exp, expresser; Non-Exp, non-expresser.

Items with $P < 0.25$ on the univariate analysis (CYP3A5 genotype, food intake/non-intake, disease type, duration of disease < 40 months) were taken as explanatory variables on multivariate analysis. Only CYP3A5 Non-Exp (OR 5.9, 95% CI 1.3–26.3) was significantly associated with achievement of the optimal trough level on days 7–10 (Table 4).

Short-term clinical remission. The pDAI score 4 weeks after the start of Tac showed a significant difference between the Exp group and the Non-Exp group (3.9 ± 2.8 *vs* 2.6 ± 1.9, $P = 0.045$). The remission rate was significantly higher in the Non-Exp group (47.6%) than in the Exp group (16.7%) ($P = 0.046$). Four patients required surgery within 4 weeks, all of whom were in the Non-Exp group ($P = 0.078$) (Table 5).

Adverse Effects. Two patients (4.0%) had severe adverse effects that necessitated discontinuation of Tac. One of them had renal dysfunction, and one had PCP. The CYP3A5 gene type was *1*1 in both of these patients. Amelioration of adverse effects with conservative treatment and observation only, without discontinuation of Tac, was seen in 34 of 45 patients (75.6%). These adverse effects included magnesium deficiency in 27 patients, tremor in 18 patients, facial flush in 5 patients, and glucose intolerance in 2 patients. There was no difference in these frequencies between the Exp group and the Non-Exp group (70.8% *vs* 81.0%, $P = 0.66$).

Discussion

To produce a sufficient effect with Tac, the trough level needs to be controlled to a target level, for which TDM is necessary.[6] This target trough level differs depending on the disease being treated. In the case of UC, placebo-controlled, blinded trials have shown that 10–15 ng/mL is the optimal trough level to induce remission.[2,3] Especially in cases of severe UC, control to the optimal trough level as early as possible from the start of therapy is necessary to obtain a therapeutic effect. The trough level is frequently measured in the early period of therapy and the dose is adjusted, but the dose is difficult to estimate because of the large individual differences in Tac blood levels. Many reports have examined the relationships between Tac pharmacokinetics and CYP3A4, CYP3A5, and ABCB1 genetic polymorphisms in the fields of kidney and liver transplantation, and they have concluded that CYP3A5 has a large effect.[13,14,17–19] CYP3A5 genetic polymorphisms are identified prior to transplantation, and setting doses separately for Exp and Non-Exp patients is effective. In a prospective study, Thervet *et al.* divided kidney transplantation patients into groups that did and did not have the initial Tac dose set based on CYP3A5 polymorphism and studied the subsequent pharmacokinetics.[20] The percentage of patients achieving the optimal trough level after six oral administrations, which was the primary end-point, was significantly higher in the group with the initial Tac dose set based on CYP3A5 polymorphism than in the group with the dose not set based on CYP3A5 polymorphism (43.2% *vs* 29.1%). There have been no such studies in the field of IBD; this is the first such report. In the present study, the trough and dose-adjusted trough levels were significantly higher on days 2–5 and 7–10 in the CYP3A5 Non-Exp group than in the CYP3A5 Exp group. Moreover, on both days 2–5 and 7–10, the percentage of patients achieving the optimal trough level was significantly higher in the Non-Exp group than in the Exp group. Higher trough levels were also obtained with low Tac doses in the Non-Exp group than in the Exp group among UC patients, and the optimal trough level was shown to be achieved at an early time.

In an analysis of factors involved in achieving the optimal trough level on days 2–5, CYP3A5 genetic polymorphisms and food intake/non-intake were significant factors on multivariate analysis. Only CYP3A5 genetic polymorphisms were significant on days 7–10. Although the effects of both CYP3A5 and fasting became weak with time, CYP3A5 polymorphism was the only significant factor because of its strong impact. Thus, the results showed that consideration of CYP3A5 genetic polymorphisms is important for early induction of the optimal trough level. ABCB1 is also reported to be associated with Tac pharmacokinetics, although its effect is smaller than that of CYP3A5 genetic polymorphisms.[21] However, involvement of ABCB1 and CYP3A4

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Table 3 Univariate analysis of factors for achievement of optimal blood trough levels on tacrolimus therapy

	Days 2–5 of tacrolimus therapy	Days 7–10 of tacrolimus therapy
Items with $P < 0.25$	CYP3A5 genotype	CYP3A5 genotype
	Food intake/non-intake	Food intake/non-intake
	BMI < 20 kg/m^2	Disease type
	PSL dose at starting of Tac	Duration of disease < 40 months
Items with $0.25 \leq P < 0.5$	Duration of disease < 40 months	BMI < 20 kg/m^2
	Total dose of PSL	PSL at starting of Tac
	Hemoglobin	Total dose of PSL
	CRP	Hemoglobin
	DAI	ESR
	Partial DAI	Albumin
	Bowel movements	Partial DAI
	Platelet count	Bowel movements
	White blood cell count	Platelet count
	Hematocrit	White blood cell count
		Hematocrit
		Cytomegaro virus infection
Items with $0.5 \leq P < 1.0$	Disease type	Age at onset
	Age at onset	Age at starting of Tac
	Age at starting of Tac	DAI
	ESR	CRP
	Albumin	Smoking
	Smoking	CYP3A4 genotype
	CYP3A4 genotype	ABCB1 2677G/A/T genotype
	ABCB1 2677G/A/T genotype	ABCB1 3435C/T genotype
	ABCB1 3435C/T genotype	Gender
	Cytomegaro virus infection	T-chol
	Gender	Triglycerides
	T-chol	Alcohol
	Triglycerides	
	Alcohol	

ABCB1, ATP-binding cassette sub-family B member 1; BMI, body mass index; CRP, C-reactive protein; CYP, cytochrome P-450; DAI, disease activity index; ESR, erythrocyte sedimentation rate; PSL, prednisolone.

Table 4 Multivariate analysis of factors for achievement of optimal blood trough levels on tacrolimus therapy

	Odds ratio	95% CI	*P* value
At days 2–5			
Food intake			
Yes	1.0	Reference	NA
No	36.7	(2.9–471.7)	0.006
CYP3A5			
Expresser	1.0	Reference	NA
Non-Expresser	40.3	(3.2–515.5)	0.004
At days 7–10			
CYP3A5			
Expresser	1	Reference	NA
Non-expresser	5.9	(1.3–26.3)	0.021

NA, not applicable; CI, confidence interval; CYP, cytochrome P-450.

Table 5 Comparison of clinical remission at 4 weeks after the start of tacrolimus therapy between the CYP3A5 expresser (Exp) group and the non-expresser (Non-Exp) group

	Exp group (n = 24)	Non-Exp group (n = 21)	*P* value
Partial DAI score (mean ± SD)†	3.9 ± 2.8	2.6 ± 1.9	0.045
Clinical remission (yes/no)	4/24 (16.7%)	10/21 (47.6%)	0.046
Surgery (yes/no)	4/24 (16.7%)	0/21 (0%)	0.078

†Partial DAI scores of four patients who underwent surgery within 4 weeks were calculated on the day of their surgery.
DAI, disease activity index.

genetic polymorphisms in the trough level was not seen in the present study.

Clinical remission was evaluated 4 weeks after the start of therapy in the present study using the pDAI score, which excluded the endoscopy score. The partial Mayo score has also been used to determine clinical efficacy in recent large-scale studies.[22,23] Judging clinical efficacy with an activity index that excludes the endoscopy score is thought to have a certain level of validity.[24] Because the Mayo score and DAI are essentially the same score, in this study, DAI, which can be determined in a single day, was adopted.[25] The remission rate was significantly higher in the Non-Exp group than in the Exp group. All four patients who underwent surgery within 4 weeks after the start of therapy were in the Exp group. These results are interpreted as showing a higher likelihood of achieving the optimal trough level, resulting in a tendency for a better therapeutic response, in the Non-Exp group. Herrlinger *et al.* reported a relationship between Tac therapy results and CYP3A4, CYP3A5, and ABCB1 genetic polymorphisms in 89 UC patients.[12] In that study, the therapeutic effect was determined 6 weeks after the start of Tac, and it was effective in 75% of cases (61% remission and 14% improvement). It was found that CYP3A4 and CYP3A5 genetic polymorphisms were not associated with efficacy and that the presence or absence of TT type in the 1236C/T, 2677G/T/A, and 3435C/T of ABCB1 was related to the clinical effect. Several differences are thought to be causative factors in this difference from the present study. One major difference is the racial difference in genetic polymorphisms of CYP3A4, CYP3A5, and ABCB1.[9–11] There is a large difference in CYP3A5 Non-Exp in particular at 35–65% in Asians and 85–90% in Caucasians.[9–11] In fact, CYP3A5 Non-Exp accounted for 89.9% in the report by Herrlinger *et al.*,[12] clearly higher than the 46.7% in the present study. Nearly 90% of patients were Non-Exp, and this is thought to be why CYP3A5 genetic polymorphisms did not affect the percentage of patients achieving the optimal trough level and the clinical effect. It may be inferred that the high remission rate of 61% is attributable to the fact that the subjects were Caucasians, a population susceptible to the effects of Tac.

As for adverse effects, the results of the current study were similar to other reports.[3,26] There were no differences in the frequencies of adverse effects between the Exp group and the Non-Exp group.

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A limitation of this study is that the analysis was done with a small number of UC patients in a single institution. However, the results of genetic polymorphisms of CYP3A4, CYP3A5, and ABCB1 were nearly the same as in previously reported analyses of Asian patients.[14,17] The pharmacokinetics and therapeutic effect of Tac were investigated in IBD patients, and interesting new findings were obtained, namely that CYP3A5 Non-Exp is associated with achieving the optimal Tac trough level and short-term clinical remission. These findings suggest that understanding the genetic polymorphisms of CYP3A5 in UC patients is useful in controlling the dosage, such as establishing higher initial dosages in Exp than in Non-Exp and establishing greater increases when changing the dose after confirming the trough level. Thus, it may be possible to implement tailor-made medicine suited to the individual case in the therapy of UC patients. Interestingly, there is some doubt as to a relationship between the pharmacokinetics of cyclosporine, also a calcineurin inhibitor, and CYP3A5 genetic polymorphisms.[27–30] Cyclosporine is also used in treating UC, but unlike Tac, no advantages can be expected from confirming the CYP3A5 genetic polymorphisms.

In conclusion, this study showed that CYP3A5 genetic polymorphisms affect the pharmacokinetics of Tac and short-term clinical remission, at least in Asian patients. Various factors are thought to be related to the individual differences in Tac treatment effect. We conclude that CYP3A5 genetic polymorphisms are important as a major factor in the early stage of treatment. When Tac is used in the therapy of UC patients, it is desirable to confirm the genetic polymorphisms of CYP3A5 in patients if possible. At the very least, it is necessary to understand that the percentage of patients achieving the optimal trough level in the early period of therapy and the short-term clinical outcome will differ in CYP3A5 Exp and Non-Exp cases.

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ORIGINAL ARTICLE—ALIMENTARY TRACT

Possible diagnostic role of antibodies to Crohn's disease peptide (ACP): results of a multicenter study in a Japanese cohort

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Abstract

Background Various noninvasive tests have been studied to screen for patients with Crohn's disease (CD), and were found to have limited accuracy and sensitivity, particularly in Asian populations. The aim of our study was to explore the possible diagnostic utility of antibodies to the CD peptide (ACP) in patients with CD.

Methods In a multicenter study using enzyme-linked immunosorbent assay, serum ACP levels were determined in 196 patients with CD, 210 with ulcerative colitis, 98 with other intestinal diseases, 132 with other inflammatory diseases, and 183 healthy controls. and then examined for correlation to clinical variables. The diagnostic utility of ACP was evaluated by receiver operating characteristics analysis and compared with anti-*Saccharomyces cerevisiae* antibodies (ASCA).

Results ACP levels were significantly elevated in the CD patients, but not in the other groups that included UC, other intestinal diseases, other inflammatory diseases and the healthy controls. Among these other groups, ACP levels were not significantly different. In the CD patients, ACP had a higher sensitivity and specificity (63.3 and 91.0 %, respectively) than ASCA (47.4 and 90.4 %). ACP levels were negatively associated with disease duration, but not with CDAI, disease location, or medical treatment.

Conclusions ACP, a newly proposed serologic marker, was significantly associated with CD and was highly diagnostic. Further investigation is needed across multiple populations of patients and ethnic groups, and more importantly, in prospective studies.

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Keywords Biomarker · Crohn's disease · Ulcerative colitis · Inflammatory bowel disease · Phage display

Abbreviations
BLAST Basic local alignment search tool

Introduction

Crohn's disease (CD) and ulcerative colitis (UC), collectively referred to as inflammatory bowel disease (IBD), are chronic intestinal disorders characterized by frequent flare-ups alternating with periods of remission. While the cause of IBD remains unknown, the current model favors a dysregulated immune system that is triggered by environmental factors, including luminal bacteria and specific antigens, in a genetically susceptible host [1–3]. The diagnosis of IBD is based on endoscopic, histologic, and radiologic criteria. These investigations, however, are sometimes laborious, expensive, invasive, and associated with risks. Therefore, noninvasive screenings are used select the patients in whom these investigations are critical.

Currently, several serologic markers have been suggested to be useful for the diagnosis, differentiation, and better comprehension of the pathogenesis of IBD [4–7]. These markers include the following antibodies; perinuclear antineutrophil cytoplasmic antibodies (pANCA) [8–10], anti-*Saccharomyces cerevisiae* antibodies (ASCA) [8, 11], pancreatic antibodies [12], antibodies to the *Escherichia coli* outer-membrane porin C (OmpC) [13, 14], antibodies to the *Pseudomonas fluorescens*-associated sequence I2 [15–17], and antibodies to bacterial flagellin [18, 19]. However, the sensitivity and specificity of these antibodies are not high enough to differentiate and diagnose IBD, particularly in Asian populations [20–22].

Very recently, using phage display technology [24, 25], we identified a new peptide (TCP-353) that specifically binds to sera from Japanese patients with CD [23]. Interestingly, this peptide has the ability to stimulate pro-inflammatory responses in blood mononuclear cells from CD patients only.

In this multicenter study, we measured serum levels of specific antibodies to TCP-353; in other words, antibodies to the CD peptide, ACP, in patients with IBD, as well as in subjects with other intestinal and inflammatory diseases and in healthy subjects taken as controls. Furthermore, we assessed the diagnostic utility of ACP in the differential diagnosis between CD and non-CD. Finally, we investigated further information on the influence of clinical variables—such as disease activity, location and duration and medical treatment on ACP levels—since this has been omitted from earlier reports.

Patients and methods

Study populations

Between July 1, 2004, and June 30, 2005, we conducted our study in eight medical institutions participating in the Kyushu ACP Study Group: Kurume University Hospital, Ryukyu University Hospital, Fukuoka University Chikushi Hospital, Nagasaki University Hospital, Miyazaki University Hospital, Kagoshima University Hospital, Yame General Hospital, and Social Insurance Tagawa Hospital. Blood samples from patients with CD ($n = 196$), UC ($n = 210$), other intestinal diseases (intestinal controls: $n = 98$), other inflammatory diseases (inflammatory controls: $n = 132$), and healthy controls ($n = 187$) were used. The clinical characteristics of the subjects included in the study are summarized in Table 1. Each patient's diagnosis was confirmed by clinical history, endoscopic and radiologic examination, and histopathology findings. Typical features of CD included skip lesions, cobble stoning, noncaseating epithelioid granulomas, transmural inflammation, deep ulceration, stricturing or fistula formation, and small-bowel or upper gastrointestinal tract involvement. Features of UC included inflammation involving the rectum with or without proximal extension into the colon in a continuous manner without skip lesions and inflammation limited to the mucosal layer. In 196 patients with CD, 137 patients were men and 59 were women, with a mean age of 34.1 years (range 13–70 years) and a mean disease duration of 9.6 years (range 0.0–29.9 years). In 210 patients with UC, there were 105 men and 105 women, with a mean age of 39.6 years (range 13–82 years), and a mean disease duration of 8.1 years (range 0.0–31.0 years). Disease activity was defined according to the score of Crohn's Disease Activity Index (CDAI) in CD [26, 27] and the criteria of Truelove and Witts [28] in UC. For laboratory parameters, we evaluated serum C-reactive protein (CRP), serum albumin, erythrocyte sedimentation rate (ESR) and platelet counts.

One hundred and two cases with non-IBD intestinal diseases (88 with infectious colitis and 14 with irritable bowel syndrome) were used for the intestinal controls, and 132 cases with autoimmune diseases (51 with rheumatoid arthritis, 24 with systemic lupus erythematodes, eight with dermatomyositis and/or polymyositis, 23 with Sjögren syndrome and 24 with unclassified disease) were used for inflammatory controls. For healthy controls, we used 187 age-matched, healthy volunteers. All patients and controls were of Japanese origin.

Table 1 Characteristics of subjects studied

	Crohn's disease	Ulcerative colitis	Intestinal control[a]	Healthy control
No of patients	196	210	98	183
Sex (F/M)	59/137	105/105	49/49	150/33
Age (years)[b]	34.1 ± 11.2	39.6 ± 16.7	41.6 ± 19.6	36.6 ± 10.8
Disease duration (years)[b]	9.6 ± 7.0	8.1 ± 6.6		
Disease location				
Ileal disease	63	–		
Ileocolonic disease	105	–		
Colonic disease	21	210		

[a] Including 88 with infectious colitis and 14 with irritable bowel syndrome

[b] Mean ± SE

Ethical considerations

The ethics committee at each hospital approved the study protocol, and written informed consent was obtained from each participant.

ACP elisa

Serum ACP levels were measured using enzyme-linked immunosorbent assay (ELISA), as previously described [23]. Briefly, serum samples diluted 1/100 were added to wells and incubated for 1 h at room temperature. After washing the wells six times with PBS containing 0.1 % Tween 20 (PBST), horseradish peroxidase (HRP)-conjugated anti-human IgG (Dako Cytomation, Denmark) was added to the wells and incubated for 1 h at room temperature. After washing the wells eight times with PBST, substrate solution (TMB+, Dako) was added and incubated for 30 min at room temperature. Color development was stopped by adding 2 N H_2SO_4. Optical density (OD) was measured at 450–650 nm using a microplate reader. The antibody titer was calculated using a standard curve. This assay had very low intra-assay variation, and the inter-assay coefficient of variation was less than 10 %.

ASCA elisa

ASCA-IgG was measured using a commercially available ELISA kit (Genosis Diagnostics, UK) according to the manufacturer's instructions. Briefly, serum samples were diluted 1/50 and added to a microtiter well pre-coated with purified mannan from *Saccharomyces cerevisiae*. After incubation for 30 min at room temperature, we washed the wells three times and added HRP-conjugated anti-human IgG into each sample. After incubation for 30 min at room temperature, we then washed the wells four times, added substrate solution and incubated for 10 min at room temperature. Color development was stopped and read OD at 450–650 nm. For quantitative assay, a series of dilutions of the positive control, which was provided with the kit, were measured and the titer was calculated using a standard curve.

Statistical analysis

The results were analyzed using SPSS statistical software. We used the Student's t test, Mann–Whitney U test, Wilcoxon signed-rank test, Pearson's correlation test, Spearman's correlation test, χ^2 test, and McNemar's test, as appropriate. All tests were two-sided and p values ≥ 0.05 were considered significant.

Results

ACP levels

The results of ACP measurements in the different subject groups are shown in Fig. 1a. Serum ACP levels found in the CD patients were significantly higher than those in UC, intestinal controls, inflammatory controls, and healthy controls ($p < 0.0001$ for all the other groups). No statistically significant differences were found between the UC patients and the intestinal, inflammatory and healthy controls. Figure 1b shows the results of concomitant ASCA tests. Significantly higher ASCA levels were observed in the CD patients than in all the other groups ($p < 0.0001$ for all the other groups). There was no correlation between the serum levels of ACP and ASCA in the CD patients ($r = 0.048$, $p = 0.2842$).

Diagnostic utility

We next evaluated the diagnostic utility of ACP ELISA and compared it with the ASCA ELISA. Based on differences in antibody levels found among the groups, we calculated the utility of ACP and ASCA in the differential diagnosis between CD and the other groups. Figure 2 shows the receiver operating characteristics (ROC) curves obtained in order to evaluate the sensitivity and specificity of ACP and ASCA in differential diagnosis. The cutoff value was determined by the ROC curves as the point with the best

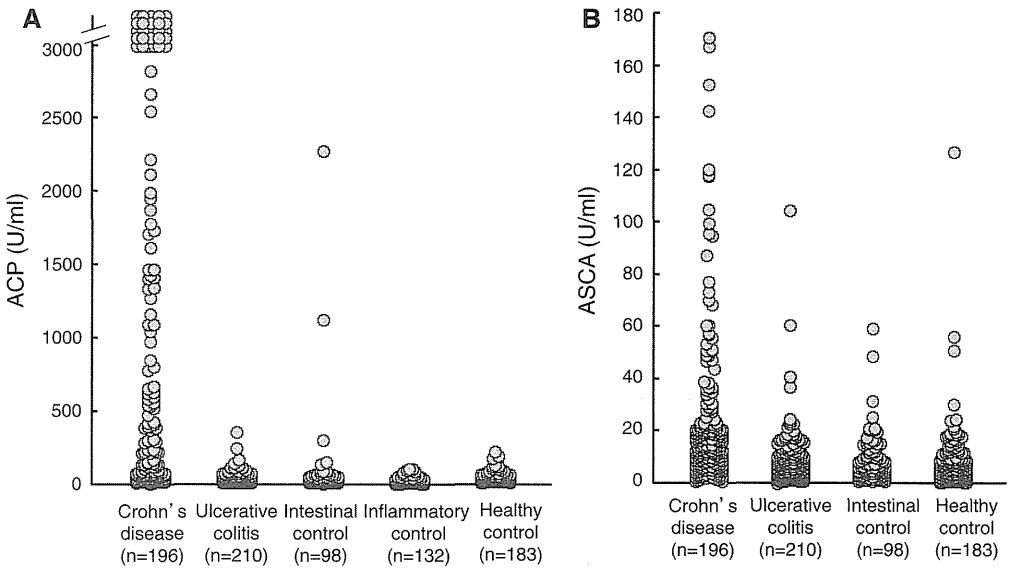

Fig. 1 **a** Serum levels of antibodies to the Crohn's disease peptide (ACP) in patients with Crohn's disease ($n = 196$), ulcerative colitis ($n = 210$), intestinal controls ($n = 98$), inflammatory controls ($n = 132$), and healthy controls ($n = 183$). Patients with intestinal diseases, including 88 with infectious colitis and 14 with irritable bowel syndrome, were represented as intestinal controls, and patients with autoimmune diseases, including 51 with rheumatoid arthritis, 24 with systemic lupus erythematodes, eight with dermatomyositis and/or polymyositis, 23 with Sjögren syndrome and 24 with unclassified disease, were classified as inflammatory controls. **b** Serum levels of anti-*Saccharomyces cerevisiae* antibodies (ASCA) in patients with Crohn's disease ($n = 196$), ulcerative colitis ($n = 210$), intestinal controls ($n = 98$), and healthy controls ($n = 183$)

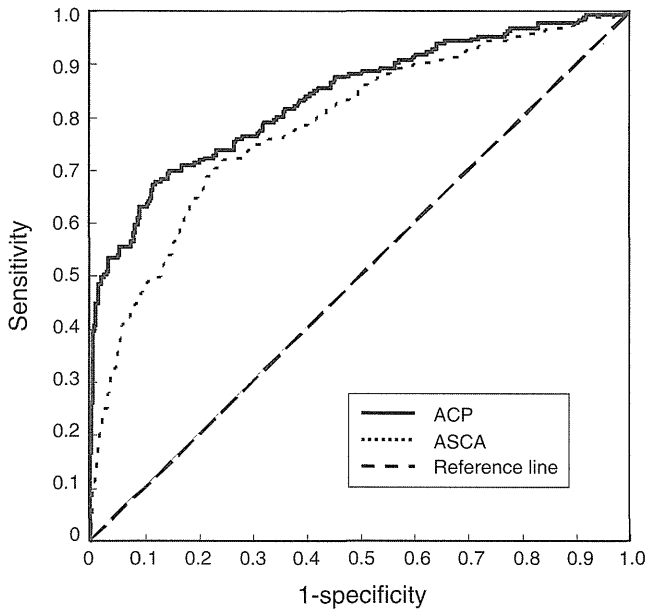

Fig. 2 Receiver operating characteristic (ROC) curves of antibodies to Crohn's disease peptide (ACP) and anti-*Saccharomyces cerevisiae* antibodies (ASCA) for the diagnosis of Crohn's disease. The ROC curves for ACP and ASCA were obtained by plotting sensitivity versus 1-specificity. Sensitivity and specificity were calculated using the results for the 196 Crohn's disease patients and 491 control subjects (210 with ulcerative colitis, 98 intestinal controls and 183 healthy controls) in each ELISA

sensitivity and specificity of more than 90 % in each ELISA; we set the cut-off values for positivity at 68.8 U/mL for ACP and 16.7 U/mL for ASCA. According to the ROC curve analysis, the cutoff values were calculated to consider whether each sample was positive or negative for ACP and ASCA. Based on these cutoff values, we found a 63.3 % ACP-positivity for the CD patients, whereas lower positivity rates were detected for the UC patients as well as the inflammatory and normal controls (12.4, 12.2, and 3.3 %, respectively). As for ASCA, 47.4 % of the CD patients, 9.5 % of the UC patients, 10.2 % of the intestinal controls, and 9.8 % of the healthy controls were positive. Therefore, ACP had a sensitivity of 63.3 %, a specificity of 91.0 %, and a positive predictive value of 73.8 %, while the corresponding values for ASCA were 47.4, 90.4 and 66.0 %, respectively (Table 2). The diagnostic value of CD was significantly different between ACP and ASCA ($p = 0.0034$ by McNemar's test). Thus, ACP had better diagnostic utility than ASCA for the considered differential diagnosis. The combined use of ACP and ASCA resulted in an increase in sensitivity (82.1 %) and a decrease in specificity (81.9 %).

Relation to disease activity

To address whether ACP could be used for evaluating disease activity, we correlated serum ACP levels with the parameter of disease activity (Fig. 3a). There was no statistically significant correlation between the ACP levels and CDAI, though there was the tendency of a positive correlation ($r = 0.149$, $p = 0.0813$). ACP levels also showed no association with laboratory parameters such as ESR, serum CRP, serum albumin and platelet

Table 2 Diagnostic utility of ACP and ASCA ELISA

	ACP-positive	ASCA-positive	Both positive[c]
Crohn's disease (CD)	63.3 % (124/196)	47.4 % (93/196)	82.1 % (161/196)
Ulcerative colitis (UC)	12.4 % (26/210)	9.5 % (20/210)	21.0 % (44/210)
Intestinal control[a]	12.2 % (12/98)	10.2 % (10/98)	22.4 % (22/98)
Healthy control	3.3 % (6/183)	9.8 % (18/183)	12.6 % (23/183)
Sensitivity (CD vs. non-CD)[b]	63.3 %	47.4 %	82.1 %
Specificity (CD vs. non-CD)[b]	91.0 %	90.4 %	81.9 %
Positive predictive value	73.8 %	66.0 %	64.4 %
Negative predictive value	86.1 %	81.1 %	92.0 %

ACP antibodies to Crohn's disease peptide, *ASCA* anti-*Saccharomyces cerevisiae* antibodies

[a] Including patients with infectious colitis and irritable bowel syndrome

[b] Including patients with UC, intestinal controls and healthy controls

[c] Both ACP- and ASCA-positive

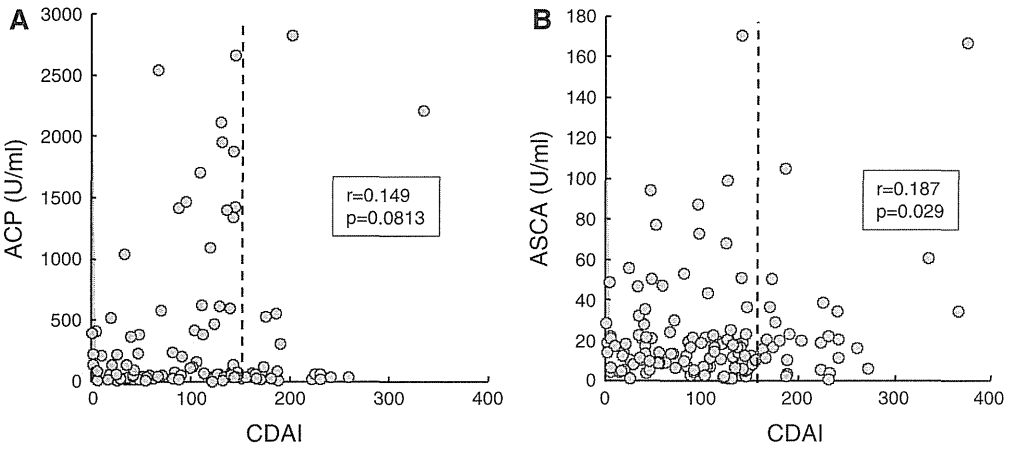

Fig. 3 Serum levels of antibodies to Crohn's disease peptide (ACP) (**a**) and anti-*Saccharomyces cerevisiae* antibodies (ASCA) (**b**) in patients with Crohn's disease in relation to the Crohn's Disease Activity Index (CDAI). The *dotted line* indicates CDAI = 150, and a score of 150 or greater was classified as active disease

counts (Table 3). In contrast, ASCA levels correlated positively with CDAI ($r = 0.187$, $p = 0.0287$) (Fig. 3b) and CRP ($r = 0.168$, $p = 0.0185$) (Table 2). In addition, ACP levels were not related to gender ($p = 0.7389$) and age ($r = -0.062$, $p = 0.3922$) in patients with CD.

Relation to disease location

We next compared serum ACP levels within the CD patients according to the disease location (Fig. 4a). Information was available for 189 CD patients. There was no difference in ACP levels among the ileal, ileocolonic, and colonic diseases. Similarly, no association with disease location was observed in the ASCA levels (Fig. 4b).

Relation to disease duration

We also determined whether serum ACP levels correlated with disease duration (Fig. 5a). Interestingly, there was a significant negative correlation between ACP levels and disease duration ($r = -0.157$, $p = 0.030$), whereas ASCA did not associate with disease duration ($r = -0.022$, $p = 0.7645$) (Fig. 5b).

Table 3 Correlation coefficients between all parameters and corresponding *p* values

	CRP	Albumin	ESR	Platelet
ACP				
r	0.015	−0.108	0.016	−0.025
p	0.8326	0.1327	0.8427	0.7338
n	196	196	152	194
ASCA				
r	0.168	−0.037	0.077	0.009
p	0.0185	0.6045	0.3488	0.9048
n	196	196	152	194

ACP antibodies to Crohn's disease peptide, *ASCA* anti-*Saccharomyces cerevisiae* antibodies, *CRP* C-reactive protein, *ESR* erythrocyte sedimentation rate

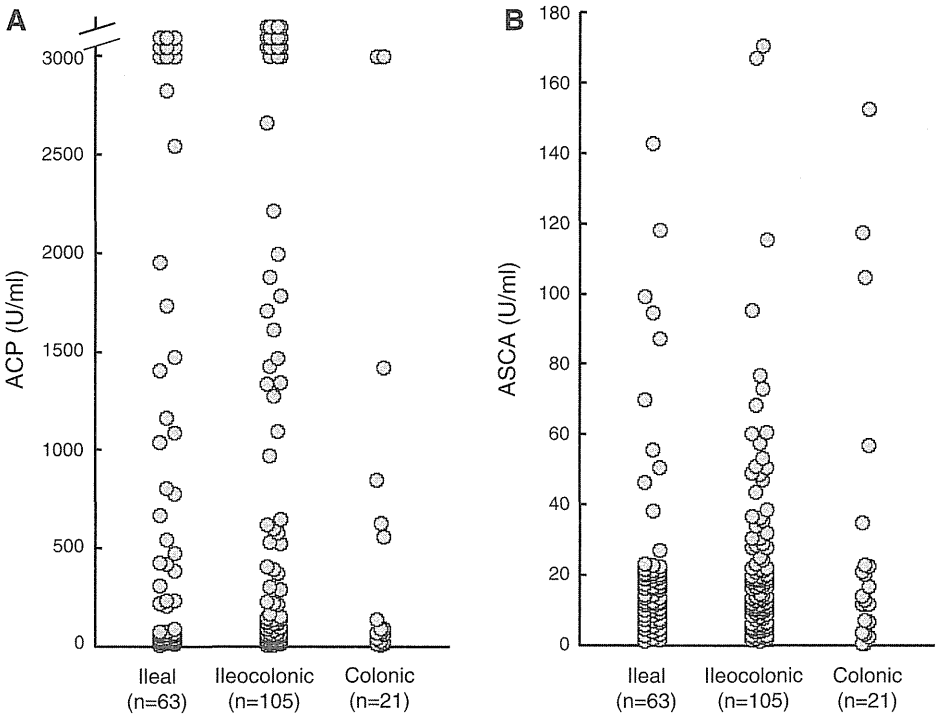

Fig. 4 Serum levels of antibodies to Crohn's disease peptide (ACP) (**a**) and anti-*Saccharomyces cerevisiae* antibodies (ASCA) (**b**) in patients with Crohn's disease according to disease location. Crohn's disease was subdivided into ileal, ileocolonic, and colonic disease according to the area involved

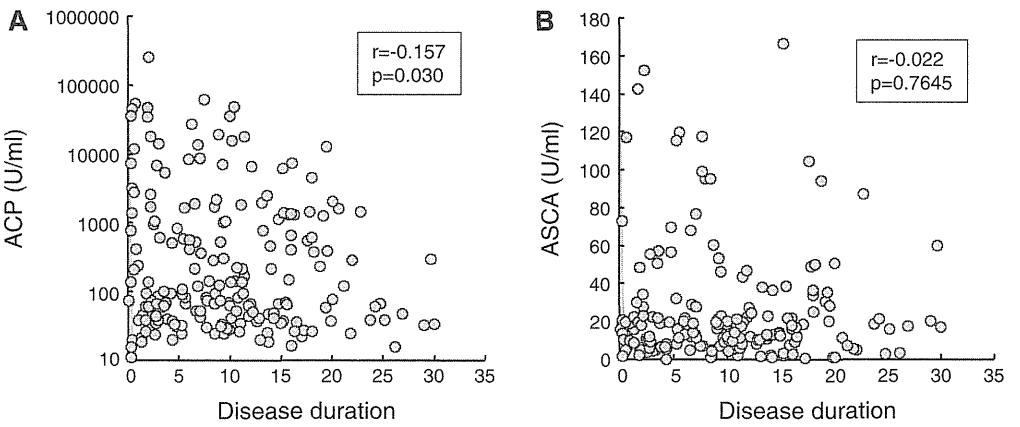

Fig. 5 Serum levels of antibodies to Crohn's disease peptide (ACP) (**a**) and anti-*Saccharomyces cerevisiae antibodies* (ASCA) (**b**) in patients with Crohn's disease, in relation to disease duration

Relation to medical treatment

Another point of interest was the status of a relationship between serum ACP levels and medical treatment. However, no significant association was found for ACP levels, as well as ASCA levels in the cases with or without the use of medical treatment, including 5-aminosalicylic acid, steroids, immunosuppressants, infliximab, elemental diet, and total parenteral nutrition (Fig. 6).

Discussion

Various noninvasive tests have been studied in the past to screen for patients with CD, but were found to have limited diagnostic utility, particularly in Asian populations [20–22]. In the present study, we conducted a multicenter study to examine whether a new proposed serologic marker, ACP, could help us in disease differentiation and stratification in a Japanese cohort of CD.

The present results clearly demonstrated a distinct elevation of serum ACP levels in patients with CD compared with all the other groups, which is in agreement with our earlier preliminary report [23]. With good sensitivity and specificity, serum ACP proved to be a valuable marker that was able to accurately distinguish between patients with CD and those without. According to the ROC curve analysis, a cutoff level of 68.8 U/ml ACP was suggested to be a satisfactory indicator as a tool to screen for CD (sensitivity 63.3 %, specificity 91.0 %). This sensitivity of ACP signifies that a negative test does not rule out CD. However, the high specificity of this antibody strongly indicates that a positive test is useful in screening for CD.