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Small Intestinal Involvement and Genotype-Phenotype Correlation in Familial Adenomatous Polyposis

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Abstract

BACKGROUND AND AIMS: Small intestinal involvement in familial adenomatous polyposis (FAP) remains unclarified. We performed capsule endoscopy (CE) and balloon-assisted (BA) endoscopy to analyze small intestinal polyps and their genotype-phenotype correlations in a large cohort of FAP patients.

METHODS: In this retrospective study, we performed CE in all 149 FAP patients and BA endoscopy in 74 patients with larger polyps. The prevalence of small intestinal adenoma with high-grade dysplasia and cancer was investigated. Correlation between *APC* variant and polyp phenotype was analyzed.

RESULTS: Median patient age was 44 years and 69% were male. Pathogenic germline *APC* variants were found in 117/136 (86.1%). Overall prevalence and median number of small intestinal polyps were 85.2% (127/149) and 17 (IQR 6-36), respectively. A total of 5318 polyps were detected by CE and all 433 polyps histologically examined were confirmed as adenoma. The number of polyps significantly decreased from the proximal-to-distal tracts. Adenoma with high-grade dysplasia was detected in 26.2% (39/149), and its incidence among the polyps was approximately 1.4% (74/5318). Of these, 14 were intramucosal carcinomas with a prevalence of 5.4% (8/149). The germline *APC* variant in codon 1251-1580 was significantly correlated with a high number of small intestinal polyps. Multivariate analysis revealed *APC* variant in codon 1251-1580 and a high number of small intestinal polyps as independent risk factors for adenoma with high-grade dysplasia. Spigelman stage was significantly associated with number of jejunal/ileal polyps and their high-grade dysplasia.

CONCLUSION: Among FAP patients, small intestinal adenoma with high-grade dysplasia was detected in 26.2% and cancer in 5.4%. FAP patients, particularly those with *APC* variant in codon 1251-1580 and/or high Spigelman stage may require surveillance for small intestinal polyps.

Keywords: Multivariate logistic analysis; Spigelman score; Duodenal polyp.

Abbreviations: BA endoscopy, balloon-assisted endoscopy; CE, capsule endoscopy; D1, bulbus and descending portion of duodenum; D2, horizontal and ascending portions of duodenum; EGD, esophagogastroscopy; EMR, endoscopic mucosal resection; FAP, familial adenomatous polyposis; MCR, mutation cluster region; MLPA, multiplex ligation- dependent probe amplification; OR, odds ratio; SBTT, small bowel transit time

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What you need to know

Background

The prevalence of small intestinal adenoma with high-grade dysplasia and cancer, risk factors for these tumors, and their genotype-phenotype correlation in familial adenomatous polyposis (FAP) are unknown.

Findings

The prevalence of small intestinal adenoma with high-grade dysplasia and cancer was 26.2% and 5.4% respectively. The germline APC variant in codon 1251–1580 was a risk factor of these tumors.

Implications for patient care

FAP patients, particularly those with germline APC variants in codon1251–1580 as well as high Spigelman stage may require surveillance for small intestinal polyps.

Introduction

Familial adenomatous polyposis (FAP), caused by pathogenic germline *APC* gene variants, is characterized by numerous colorectal polyps which progress to colorectal cancer in nearly 100% of cases.[1] However, prophylactic colectomy in FAP patients has recently become popular, and consequently the leading causes of death are now desmoid and duodenal cancers.[2]

Recently, small intestinal involvement in patients with FAP has been demonstrated in several studies. However, the prevalence rates of jejunal/ileal polyps range widely from 5% to 83%, attributable to the use of different diagnostic modalities and to the small sizes of the cohorts examined.[3-5] Moreover, there have been no studies on the prevalence of high-grade dysplasia and cancer in jejunum/ileum of FAP patients. Therefore, little is known about the significance and clinical characteristics of jejunal/ileal polyps as precancerous lesions in FAP patients. In addition, no investigations have been reported regarding the clinical relevance of adenoma and cancer in the distal duodenum of FAP patients, an unreachable region with conventional esophagogastroduodenoscopy (EGD).

Capsule endoscopy (CE) is a noninvasive method that appears to be an ideal tool for the detection of small bowel lesions and is comparable to balloon-assisted (BA) endoscopy especially for smaller jejunal/ileal polyps; the reported prevalence of polyps as determined by CE ranges from 5% to 80% in the jejunum and from 10% to 57% in the ileum of FAP patients.[4-6] Considering the high diagnostic yield of CE for small intestinal polyps in polyposis patients,[4] the Canadian Association of Gastroenterology has recommended CE for ongoing surveillance in polyposis patients, including FAP patients, who require small bowel examination.[7] However, specific indication for CE is not described, and the clinical relevance of small intestinal CE in FAP patients remains undetermined.

The APC gene comprises 15 exons encoding 2844 amino acids and is a key regulator of the WNT/β-catenin signaling pathway. Most APC mutations result in a premature stop codon and a truncated protein, which causes accumulation of β-catenin in the cells.[8] The hotspot of the somatic APC mutation is reported to be a mutation cluster region (MCR; codon 1251-1400) in exon 15. Moreover, genotype-phenotype correlations have been reported in FAP between the site of germline APC gene variant within the MCR and the profuse colorectal polyposis.[9] However, the genotype-phenotype correlation for small intestinal tumors is unknown.

Thus, the prevalence rate, characteristics, and malignant potential of small intestinal polyps in FAP patients remain largely unknown. Particularly, information on the prevalence of small intestinal adenoma with high-grade dysplasia and cancer in FAP, and risk factors for these tumors, and their genotype-phenotype correlation, is totally lacking. Accordingly, international guidelines on FAP have not reached a consensus on the surveillance strategy for jejunal/ileal tumors and distal duodenal tumors.[10] Therefore, we first investigated the prevalence and clinical characteristics of small intestinal polyps using CE in a large cohort of FAP patients. We then performed histological examination of polyps using BA endoscopy to evaluate the prevalence of adenoma with high-grade dysplasia and cancer, and assessed risk factors for these tumors. Moreover, we analyzed genotype-phenotype correlation for small intestinal polyps in FAP patients.

Methods

Patients

We performed a retrospective study on small intestinal polyps in patients with FAP. A consecutive series of FAP patients were screened for enrollment, underwent small intestine CE at 3 hospitals, and were analyzed. Patients at least 15 years of age who had an established diagnosis of FAP (and attenuated FAP) were eligible. Patients with FAP who had a history of bowel obstruction, an installed cardiac pacemaker, or other electromedical devices were excluded. This study was approved by the institutional review board of each hospital, and written informed consent was obtained from all patients.

Measurement and Evaluation of Polyps

We defined the small intestinal tract as the duodenum, jejunum, and ileum, and evaluated prevalence of adenoma with high-grade dysplasia and cancer, risk factors for these tumors, and genotype-phenotype correlations. We also classified the polyps morphologically into 4 types consisting of white spot lesion, white plaque lesion, protruded lesion, and broad-based lesion, as defined in the Results section.

All CE videos were sent to Tokushima University Hospital and evaluated by the assessment committee, which consisted of 3 fellows certified by the Japanese Association for Capsule Endoscopy (KT, YS, NM), without any clinical

background information. Prior to the assessment, they were trained in the diagnosis of each type of small intestinal polyp. To avoid misidentifying the same polyp twice, they assessed the videos for polyps in manual mode compared with automatic mode (RAPID workstation), which should automatically eliminate the same images. Because CE allows only an approximate estimation of the size of polyps, the polyps were classified into 3 groups: <3 mm, 3-5 mm, or >5 mm, by using a reference image of open biopsy forceps (5 mm, Boston Scientific Corp.) for calibration and with reference to small bowel morphology. An EGD was also performed within 6 weeks after CE, and biopsy was performed to identify the pathological findings and to stage the degree of duodenal polyposis according to the revised Spigelman stage classification.[11]

For FAP patients with polyps >5 mm detected by CE, we further investigated the polyps using double-balloon endoscopy (DBE; Fujifilm Corp., Tokyo, Japan) or single-balloon endoscopy (SBE, Olympus Inc, Tokyo, Japan) for more detailed observation, biopsy, or removal of the lesion by endoscopic mucosal resection (EMR) or polypectomy, as described previously.[12] In the initial phase of this study, we took biopsies from small white spot and white plaque lesions.

Histological Examination

Specimens obtained by EMR, polypectomy, or biopsy during BA endoscopy or EGD were histologically evaluated. They were pathologically diagnosed as adenomatous polyp (adenoma) with low-grade dysplasia or high-grade dysplasia, depending on the grade of dysplasia, according to World Health Organization (WHO) criteria for tumors of the small intestine.[13] In addition, the polyps were histologically diagnosed according to the criteria of the Japanese Society for Cancer of the Colon and Rectum.[14] The specimens were graded by 2 independent pathologists who were not aware of the subjects' histories. In case of disagreement, the opinion of a third pathologist was requested.

Germline APC Gene Analysis

Genomic DNA was isolated from peripheral blood using a QIAamp DNA Blood Kit (Qiagen, Hilden, Germany). DNA was amplified by PCR using oligonucleotide primers for APC, and the library was then prepared. Samples were pooled for multiplexed sequencing, and sequencing was performed using a MiSeq instrument (Illumina Inc.). Sequenced reads were mapped against reference sequences, and variants were identified using Genomics Workbench Software v7.5 (Qiagen) and confirmed with Omixon Target Software (Omixon Ltd, Budapest, Hungary).

Multiplex ligation-dependent probe amplification (MLPA) analysis was performed using a Salsa P043 kit for the APC gene (MRC-Holland, Amsterdam, the Netherlands), according to the manufacturer's instructions, in patients with no pathogenic variant sequence detected in the sequencing analysis.

Detailed information on the CE and BA endoscopy procedures, germline APC gene analysis and statistical analysis, is provided in Supplementary information.

Results

Patient Characteristics

A total of 164 consecutive FAP patients were screened for enrollment, and 153 patients underwent CE between May 2014 and April 2018 (Supplementary Figure 1). Of these, 4 patients were excluded from the analysis; 2 patients due to poor bowel preparation and 2 due to inability of CE to reach the cecum. Therefore, finally 149 patients were analyzed; all the 149 patients underwent CE. The patient baseline characteristics are shown in Table 1. They comprised 69 males and 80 females, with a median age of 44. Most of the patients (80.5%) had a family history of FAP and 47 patients (31.5%) had a history of prophylactic colectomy. Most patients were at Spigelman stage II (24.2%), followed by stage 0 and I (22.8% for each), and stage III (15.4%). Most patients (79.9%) were intermediate-type of colorectal polyposis.

CE transit time data were described in Supplementary information. No apparent adverse events including capsule retention were observed.

Table 1. Baseline Characteristics of the Patients

Number of Patients	149
Age, years	
Median (range)	44 (16-85)
Sex, *n* (%)	
Male	69 (46.3)
Female	80 (53.7)
Family history of FAP, *n* (%)	120 (80.5)
Post total colectomy, *n* (%)	47 (31.5)
IRA	30 (63.8)
IAA	14 (29.8)
Unknown	3 (6.4)
Spigelman stage, *n* (%)	
0	34 (22.8)
I	34 (22.8)
II	36 (24.2)
III	23 (15.4)
IV	20 (13.4)
V	0 (0)
Unknown	2 (1.3)
Severity of colorectal lesions, *n* (%)	
Profuse	6 (4.0)
Intermediate	119 (79.9)
Attenuated	4 (2.7)
Unknown[a]	20 (13.4)

IAA, ileo-anal anastomosis; IRA, ileo-rectal anastomosis.
[a]No information was available on the colorectal lesions before colectomy.

Appearance and Histologic Features of Small Intestinal Polyps in FAP Patients

A total of 5318 small intestinal polyps were observed in 149 patients with FAP utilizing CE. They were morphologically classified into 4 types, and representative images are shown in Figure 1. Figure 1a shows a diminutive (< 3 mm) whitish nonpolypoid lesion, defined as a "white spot" lesion. Histological examination of the lesion exhibited epithelial cells with spindle or oval nuclei located almost on the basal side, and mild cellular atypia, which are findings compatible with an adenoma with low-grade dysplasia (Figure 1e). Figure 1b shows a small (3-5 mm) whitish, non-polypoid, flat lesion defined as a "white plaque" lesion, which also showed histological findings compatible with adenoma with low-grade dysplasia (Figure 1f). Figure 1c shows polypoid/elevated lesion more than 3 mm in size, defined as a "protruded lesion." Histological examination of this lesion exhibited cylinder epithelia with swollen nuclei and formation of clear glandular structures, findings compatible with adenoma with high-grade dysplasia (Figure 1g). Figure 1d shows a nodular broad-base protrusion with or without slightly depressed lesions greater than 5 mm in diameter, defined as a "broad-based lesion." This lesion also showed findings compatible with adenoma with high-grade adenoma (Figure 1h).

In total, we observed 2950 white spot lesions (55.5%), 1901 white plaque lesions (35.7%), 306 protruded lesions (5.8%), and 161 broad-based lesions (3.0%) in small intestine including the duodenum, jejunum, and ileum.

Eventually, when the number of polyps was compared between CE and BA endoscopy in 12 patients in initial phase of this study, there was no significant difference between the 2 modalities, as revealed by Wilcoxon signed rank test ($P = 0.20$).

Prevalence, Number, and Distribution of Small Intestinal Polyps in FAP Patients

The prevalence rate of small intestinal polyps in the 149 FAP patients was 85.2% (127/149) as determined by CE (Table 2); the prevalence rates of polyps in the duodenum, jejunum, and ileum were 78.5% (117/149), 61.7% (92/149), and 40.3% (60/149), respectively, indicating a stepwise decrement from the proximal-to-distal tract ($P < 0.01$, respectively). The prevalence of nonduodenal (jejunal/ileal) polyps was 75.2% (112/149). The median number of polyps (interquartile range; IQR) was 17 (6-36) in small intestine; 10 (2-20) in duodenum, 2 (0-10) in jejunum, and 0 (0-3) in ileum, similarly indicating a stepwise proximal-to-distal decrement ($P < 0.01$, respectively). When the duodenum was divided into 2 parts—D1 (bulbus and descending portion) and D2 (horizontal and ascending portions)—the prevalence and number of polyps were significantly higher in D1 than in D2 ($P < 0.01$, respectively), also indicating a proximal-to-distal decrement (Supplementary Table 1).

Multivariate Logistic Regression Analysis to Evaluate Possible Factors Associated With High Number of Small Intestinal Polyps in FAP Patients

Multivariate logistic analysis was performed to investigate the correlation between high number of polyps (median 17) and clinicopathological factors including age, sex, colorectal lesion severity, and APC gene status (Table 3). In this analysis, Spigelman stage was excluded because the number of duodenal polyps is a confounding factor for Spigelman stage. The presence of germline APC gene variant was a significant factor associated with a high number of small intestinal polyps (odds ratio, 9.97

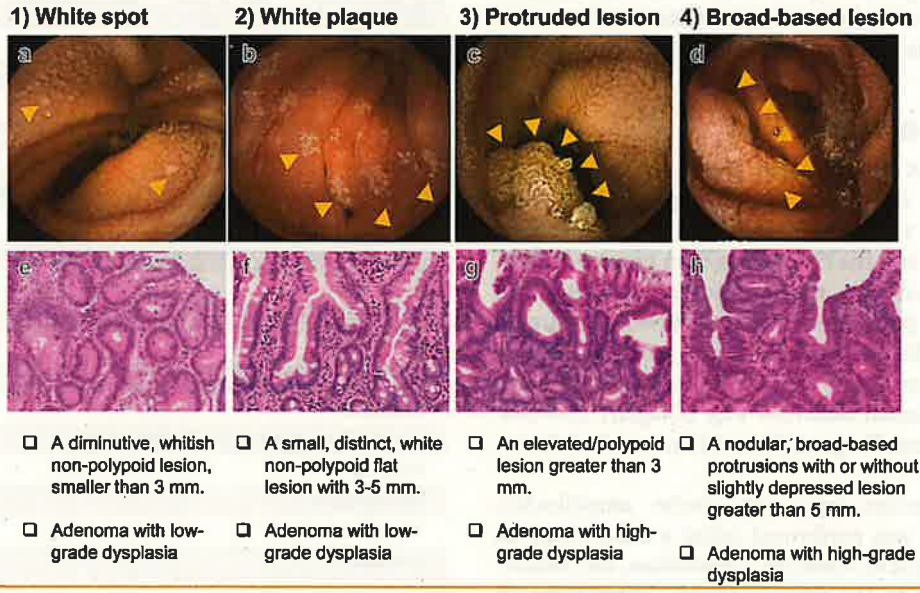

Figure 1. Capsule endoscopy (CE) images and histologic findings of small intestinal lesions in patients with familial adenomatous polyposis (FAP). The 4 representative types of CE images (a-d) and their histology with H&E staining (e-h) are shown. (a,e) "White spot" lesion, (b,f) "White plaque" lesion, (c,g) "Protruded lesion," and (d,h) "Broad-based lesion."

Table 2. Prevalence, Number, and Distribution of Each Type of Small Intestinal Polyps in Patients With FAP Detected by CE

	Total, *n* (%)	Duodenum, *n* (%)	Jejunum, *n* (%)	Ileum, *n* (%)	*P* Value		
					D vs J	D vs I	J vs I
Prevalence rate	127 (85.2)	117 (78.5)	92 (61.7)	60 (40.3)	<0.01[a]	<0.01[a]	<0.01[a]
Median number/person (interquartile range)	17 (6-36)	10 (2-20)	2 (0-10)	0 (0-3)	<0.01[b]	<0.01[b]	<0.01[b]
Type							
White spot	2950 (55.5)	1712 (58.0)	980 (33.2)	258 (8.7)	<0.01[b]	<0.01[b]	<0.01[b]
White plaque	1901 (35.7)	1350 (71.0)	515 (27.1)	36 (1.9)	<0.01[b]	<0.01[b]	<0.01[b]
Protruded lesion	306 (5.8)	129 (42.2)	154 (50.3)	23 (7.5)	1.00[b]	<0.01[b]	<0.01[b]
Broad-based lesion	161 (3.0)	124 (77.0)	37 (23.0)	0 (0.0)	<0.01[b]	<0.01[b]	<0.01[b]

D, duodenum; I, ileum; J, jejunum.
[a]*P* value by Fisher's exact test.
[b]*P* value by Friedman's test.

95% CI 1.16-85.50; $P = 0.04$). No other factors were significantly associated with a high number of small intestinal polyps. Thus, APC gene variant was an independent risk factor for high number of small intestinal polyps. Logistic analysis focusing on jejunal/ileal (nonduodenal) polyps yielded similar results.

APC Variant and Genotype-Phenotype Correlation

A germline APC gene variant was detected in 117/136 (86.0%) of the patients including deletion mutations in 4 patients (2.9%), whereas APC gene variant was not detected in 19 patients (13.9%). Complete APC gene deletion was found in 3 patients, and exon 4 to 6 deletion was detected in 1 patient. The APC variant sites of the remaining 113 patients were classified into 5 APC functional domains/segments as follows; 48 patients (41.0%) in pre-amardillo repeat region (segment A; codon 1-453), 29 (24.8%) in armadillo repeat region (segment B; codon 454-1019), 19 (16.2%) in β-catenin/post β-catenin binding region (segment C; codon 1020-1250), 11 (9.4%) in colorectal/post colorectal MCR region (segment D; codon 1251-1580), and 6 (5.1%) in the remaining region (segment E; codon 1581-2843).

Table 3. Multivariate Logistic Regression Analysis of Possible Factors Associated With High Number of Small Intestinal Polyps

Variable	OR	95% CI	*P*
Age	0.97	0.93-1.00	0.08
Sex			
Female	1.00 (reference)		
Male	0.73	0.30-1.78	0.49
Severity of colorectal lesions			
Not profuse	1.00 (reference)		
Profuse	4.92	0.49-49.90	0.17
Germline *APC* variant			
No	1.00 (reference)		
Yes	9.97	1.16-85.50	0.04

APC, adenomatous polyposis coli; CI, confidence interval; OR, odds ratio.

Since multivariate analysis revealed the *APC* variant as a determinant for a high number of polyps, we examined the relationship between *APC* gene variant site and number of small intestinal polyps in 113 FAP patients (Figure 2a). The number of small intestinal polyps in patients with *APC* variant in segment D was significantly higher than in the other segments ($P < 0.05$). Similarly, the number of duodenal polyps in patients with segment D variant was significantly higher than those with variants in other segments ($P < 0.05$). A similar tendency was observed for the association between the number of jejunal polyps and segment D variant, while almost no correlation was observed between the number of ileal polyps and segment D variant. All the patients with segment D variants (11/11) had a higher number of small intestinal polyps (median 66, range 22-491) beyond the median of 17 in the all-patient groups (Supplementary Figure 2). The baseline characteristics and clinicopathological findings of all 11 patients with segment D variants are listed in Supplementary Table 2. Adenoma with high-grade dysplasia was detected in 6 of 11 (54.5%) patients with segment D variant despite the young patient group (median 31, range 20-49). Importantly, incidence of gastric and duodenal cancers in past histories (45.5%, 5/11) and of extracolonic cancers in family histories (54.5%, 6/11) was very high. Thus, *APC* segment D variant was a strong determinant of the risk for severe small intestinal polyposis, although it is prevalent in only 9.4% of FAP patients.

BA Endoscopy Findings and Histopathology

Of the study cohort of 149 patients, 74 underwent BA endoscopy to further examine larger polyps (>5 mm) in the small intestine. We observed numerous small intestinal polyps and removed 382 lesions by EMR or polypectomy and took biopsies from 51 small lesions to evaluate their histopathology. No invasive cancer was identified.

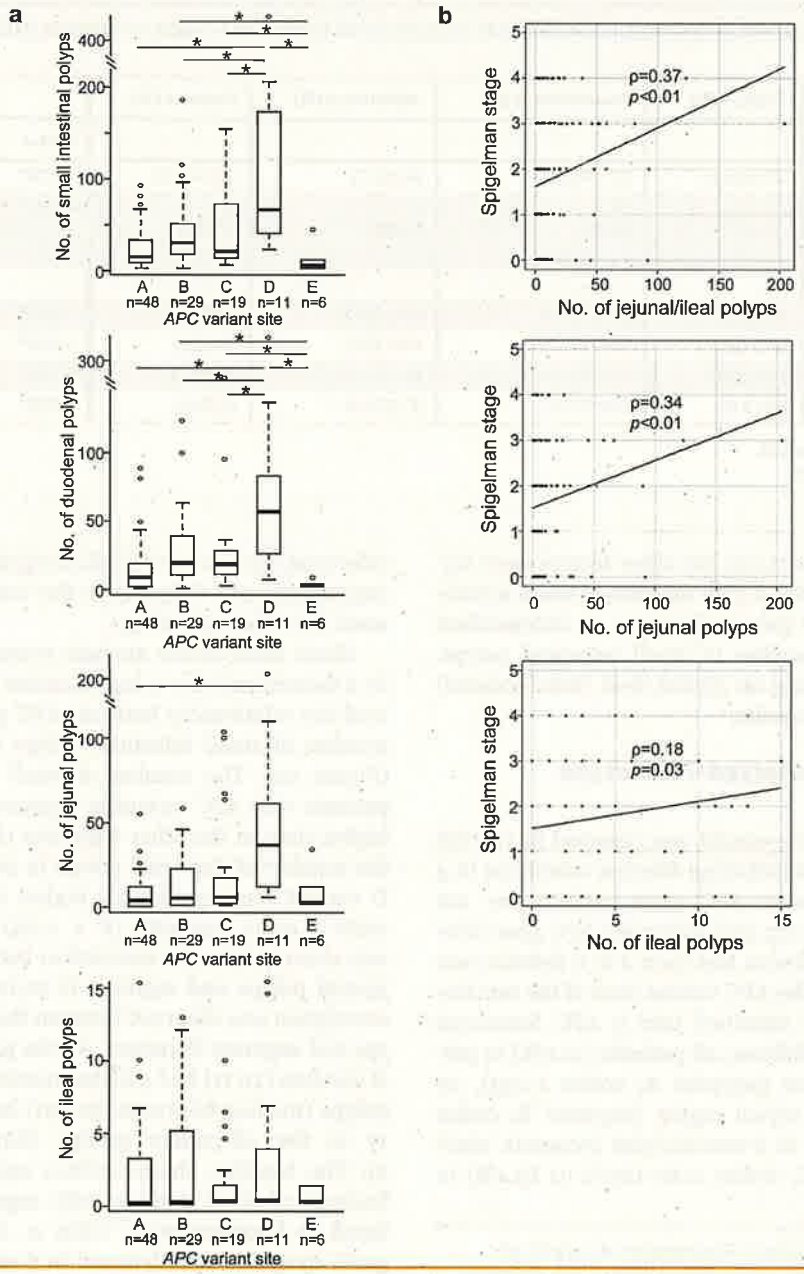

Figure 2. The number of small intestinal polyps in relation to germline *APC* variant site and Spigelman stage. (a) The number of polyps in each group of *APC* variant site was plotted and compared among each group. *$P < 0.05$ by Steel-Dwass test. (b) Association between the number of jejunal/ileal polyps and Spigelman stage was analyzed by Spearman's correlation test. ρ, Spearman's rank correlation coefficient.

Overall, 74 adenomas with high-grade dysplasia were identified in small intestine; 59 (79.7%) in duodenum (43 in D1 and 16 in D2), 14 (18.9%) in jejunum, and 1 (1.4%) in ileum (Supplementary Table 3). The prevalence of adenoma with high-grade dysplasia in small intestine was 26.2% (39/149); 22.8% (34/149) in duodenum, 6.0% (9/149) in jejunum, and 0.7% (1/149) in ileum. The prevalence of adenoma with high-grade dysplasia in patients with polyps >5 mm was 52.7%. The percentage of high-grade dysplasia among the total number of small intestinal polyps was estimated to be 1.4% (74/5318); 1.8% (59/3315) in duodenum, 0.8% (14/1686) in jejunum, and 0.3% (1/317) in ileum. Simultaneous multiple adenomas with high-grade dysplasia in small intestine were observed in 17/39 (43.6%) of patients with high-grade dysplasia.

The incidence of high-grade dysplasia in protruded lesion and broad-based lesion were 16.8% (33/197) and 28.5% (41/144), respectively. No high-grade dysplasia was observed in the white spot (0/32) and white plaque lesions (0/63). All of the adenomas with high-grade dysplasia were endoscopically removed. Accuracy, sensitivity,

and specificity for diagnosis of high-grade dysplasia in protruded lesions were 52.7%, 44.6%, and 54.3%, respectively (Supplementary Table 4). The values for diagnosis of high-grade dysplasia in broad-based lesions were 68.6%, 55.4%, and 71.3%, respectively.

We also made a pathological diagnosis of EMR and polypectomy specimens with intramucosal carcinoma according to the Japanese classification criteria.[14] A total of 14 tumor lesions in 8 patients were diagnosed as intramucosal carcinoma; prevalence was 5.4% (8/149), and incidence was 0.3% (14/5318). All of these cancers were endoscopically removed and diagnosed as intramucosal adenocarcinoma (TisNoMo). Representative CE image and histology of the intramucosal carcinoma in jejunum (case 8) are shown in Supplementary Figure 3. The characteristics of the 8 patients including the number of small intestinal polyps, *APC* variant site, and Spigelman stage are shown in Supplementary Table 5. They consisted of 7 duodenal cancers in 6 patients (4.0%, 6/149) and 7 jejunal cancers in 2 patients (1.3%, 2/149). The patients with jejunal cancer were much younger (median age 32 y) than those with duodenal cancer (65 y) although the number of patients was small. As a characteristic of these cancer patients, the number of small intestinal polyps was much higher than in the other patients group ($P < 0.01$). One patient developed invasive cancer in the duodenum 4 years after CE, resulting in pancreatoduodenectomy, although the follow-up BA endoscopy had not necessarily been properly performed.

Logistic Regression Analysis to Evaluate Risk Factors Associated With High-Grade Adenoma in Small Intestine

To investigate risk factors for small intestinal adenoma with high-grade dysplasia, multivariate logistic regression analysis was performed on possible factors including age, sex, number of small intestinal polyps, and *APC* variant site (Table 4). A greater number of small intestinal polyps was a significant factor for high-grade dysplasia; patients with ≥ 17 (median) polyps exhibited a 2.87-fold higher risk (95% CI 1.46-8.41, $P = 0.04$). Moreover, germline *APC* variant in segment D (codon 1251-1580) was a significant factor for high-grade adenoma in the small intestine (OR 4.48; 95% CI 1.38-20.90; $P = 0.04$). In logistic regression analysis of only patients with jejunal/ileal high-grade dysplasia ($N = 12$), only Spigelman stage was a significant risk factor (OR 2.50; 95% CI 1.25-6.35; $P = 0.04$; Supplementary Table 6).

Association of Spigelman Stage and the Number of Jejunal/Ileal Polyps

Since Spigelman stage is reportedly associated with the development of jejunal polyps and cancer,[15] we further evaluated the correlation between the Spigelman stage and number of jejunal and/or ileal polyps. A significant correlation was observed between Spigelman stage and the number of jejunal/ileal polyps, with a correlation coefficient ρ of 0.37 ($P < 0.01$; Figure 2b). Similarly, correlation analysis for jejunal polyps alone showed a significant correlation between the 2 factors ($\rho = 0.34$; $P < 0.01$). For ileal polyps alone, there was still a significant correlation between the 2 factors despite a lower correlation coefficient ($\rho = 0.18$; $P = 0.03$). These data suggest that Spigelman stage is closely associated with the number of jejunal/ileal polyps, although the correlation gradually weakened from the proximal-to-distal small intestine.

Table 4. Multivariate logistic regression analysis of risk factors associated with high-grade dysplasia in small intestine.

Variable	OR	95%CI	*p*
Age	1.03	0.99-1.06	0.16
Sex			
Female	1.00 (reference)		
Male	0.90	0.34-2.35	0.83
Germline *APC* variant			
A	1.00 (reference)		
B	2.56	0.83-7.89	0.10
C	1.45	0.38-5.57	0.59
D	4.48	1.38-20.90	0.04
E	1.06	0.10-10.90	0.96
No. of polyps			
<17	1.00 (reference)		
≥17	2.87	1.46-8.41	0.04

OR, odds ratio; CI, confidence interval; *APC*, adenomatous polyposis coli.
A, codons 1 to 453 (heptad repeat region/pre-armadillo region).
B, codons 454 to 1019 (armadillo repeat region).
C, codons 1020 to 1250 (β-catenin binding/post β-catenin binding region).
D, codons 1251 to 1580 (colorectal/postcolorectal MCR).
E, codons 1581 to 2843 (basic domain/EB1 and HDLG binding sites).

Discussion

In this study, we demonstrated that most of the small intestinal polyps were diminutive (<5 mm) adenoma with low-grade dysplasia among 149 patients with FAP. However, only a few percent of the polyps were adenomas with high-grade dysplasia (1.4%) and cancer (0.3%), and their prevalence rates were 26.2% and 5.4%, respectively, among FAP patients. Moreover, both the prevalence and number of small intestinal polyps were highest in the duodenum, followed by the jejunum and then the ileum, showing a stepwise proximal-to-distal decrease. To our knowledge, this is the largest-scale study to assess the prevalence, morphological characteristics, pathology, and malignant potential of small intestinal polyps in FAP. In addition, we identified a genotype-phenotype correlation between germline *APC* variant at codon 1251-1580 (segment D) and severe small intestinal polyposis, and also high risk for adenoma with high-grade dysplasia. These data may suggest the necessity of surveying not only the proximal duodenum by EGD but also the distal duodenum

and jejunum/ileum by CE, particularly for FAP patients with germline *APC* variants in codon 1251-1580.

We demonstrated that the prevalence rates of duodenal polyps and jejunal/ileal polyps were 78.5% (117/149) and 75.2% (112/149), respectively, as determined by CE. Our prevalence rates were higher than those in previous studies (all including ≥ 20 patients) which ranged from 17.4% to 52.4% in duodenum and 30.4% to 42.9% in jejunum/ileum.[16,17]This discrepancy may be explained by our use of the newer-generation SB3 CE device in most patients (≥85%), which has higher resolution, whereas previous studies used older-type lower-resolution SB2 or SB1 CE devices. Moreover, our data clearly indicated that the prevalence of adenoma, adenoma with high-grade dysplasia, and intramucosal carcinoma showed a stepwise proximal-to-distal decrement. These results are consistent with a previously reported study in a small cohort of FAP patients ($N = 29$).[18]

It is recognized that CE is less sensitive than EGD in detecting duodenal polyps, and particularly periampullary polyps.[17]In the present study, the prevalence rate of duodenal polyps detected with CE (75.2%, 112/149) was lower than the rate with detection by EGD (77.2%, 115/149), consistent with previous reports.[17]However, we found a total of 3315 polyps in the duodenum by CE; 2266 in D1 and 1049 in D2. Importantly, the number of polyps in D2 (1049) plus jejunum/ileum (2003) was higher than that in D1 (2266). More importantly, the number of adenomas with high-grade dysplasia in D2 (16) plus jejunum/ileum (15) was comparable to that in D1 (43). Of the 74 adenomas with high-grade dysplasia, 14 were intramucosal carcinomas; 6 in D1, 1 in D2, and 7 in jejunum. It is obvious that CE is superior to EGD for the observation of distal duodenal (D2) polyps in addition to jejunal/ileal polyps, which are unreachable with conventional EGD. Therefore, a combination of the 2 modalities should be used to thoroughly examine the duodenum. Thus, this is the first report to evaluate polyps in the distal duodenum (D2) of FAP patients, and our results suggest the importance of surveillance for distal duodenal polyps as well as for jejunal/ileal polyps in FAP patients.

In a study of 30 FAP patients, Matsumoto et al. reported that the prevalence of small-intestinal adenoma was associated with mutations in exon 15 of APC.[18]In the present study, we found that the *APC* variant in codon 1251-1580 (segment D; colorectal/post-colorectal MCR) was significantly associated with a higher number of small intestinal polyps. This is the first report showing a clear genotype-phenotype correlation between the germline APC variant in codon 1251-1580 and severe small intestinal polyposis.

Adenoma with high-grade dysplasia was prevalent in 26.2% (39/149) of the FAP patients in this study. No study investigating the prevalence of adenoma with high-grade dysplasia among small intestinal polyps with CE or BA endoscopy has been reported to date. However, some studies have reported prevalence rates of 5.9% to 25.4% for (proximal) duodenal adenoma with high-grade dysplasia detected by EGD in FAP patients.[19,20]Our data of the prevalence of adenoma with high-grade dysplasia (26.2%) in the small intestine are roughly consistent with previous studies of the proximal duodenum using EGD. Moreover, our study revealed 43 (58.1%) adenoma with high-grade dysplasia in D1, but the remaining 31 (41.9%) were observed in the more distal small intestine including 16 in D2. Additionally, approximately half the patients had multiple adenomas with high-grade dysplasia simultaneously in the small intestine.

Current guidelines for FAP show no consensus on the examination or treatment of distal duodenal and jejunal/ileal polyps, and they do not provide any specific indications for surveillance by CE.[7,10]Based on the analysis of patients with adenoma with high-grade dysplasia in this study, it is possible to identify high-risk patients by using *APC* segment D variant or Spigelman stage III/IV as an indicator. In fact, segment D variant and/or Spigelman stage III/IV accounted for approximately 90% (34/39) of adenoma with high-grade dysplasia patients, but accounted for only 35.8% (19/53) of patients without adenoma with high-grade dysplasia. Thus, the current study suggests that it may be necessary to survey small intestinal polyps by CE in FAP patients, particularly those with *APC* variant in codon 1251-1580 and/or Spigelman stage III/IV. A prospective longitudinal study with a control group of FAP patients would be valuable in the future.

One of the limitations of this study is that we performed BA endoscopy for histological examination only when small intestinal polyps larger than 5 mm were found by CE. Therefore, it is possible that some smaller adenoma with high-grade dysplasia or intramucosal cancer might have been missed without BA endoscopy, and that therefore the prevalence of adenoma with high-grade dysplasia is higher. Another limitation is that the number of colectomy patients and the number of patients with profuse type lesions in our cohort was relatively low. Thus, a further prospective large-cohort study is needed.

In conclusion, although most of the small intestinal polyps in patients with FAP were diminutive adenoma with low-grade dysplasia, a low percentage of polyps were adenoma with high-grade dysplasia (1.4%) and cancer (0.3%), which had a prevalence rate of 26.2% and 5.4% respectively among FAP patients. FAP patients, particularly those with *APC* variants in codon 1251-1580 and/or Spigelman stage III/IV may require surveillance for small intestinal polyps.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.tige.2021.10.001.

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Conflicts of Interest
T.T. received a collaborative research grant from Fujifilm Corp. for the study of sessile serrated lesions. All other authors declare none.

Author Contributions
T.T. and M.M. designed and supervised the study.H.I., K.T., Y.S., and N.O. performed CE. K.T., Y.S., S.T., and N.M. interpreted CE findings. Y.T., K.T., N.O., and Y.M. performed balloon assisted endoscopy. K.T., Y.S., K.O., H. M., and N.M. analyzed and interpreted data. T.S. performed statistical analysis; Y.B. performed pathological assessment. K.T., Y.S., and T.T. wrote the manuscript. All authors have read and approved the manuscript.

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Ethical Statement
The corresponding author, on behalf of all authors, jointly and severally, certifies that their institution has approved the protocol for any investigation involving humans or animals and that all experimentation was conducted in conformity with ethical and humane principles of research.

Supplementary Table 1. Prevalence, number and morphology of proximal and distal duodenal polyps detected by CE.

	Total duodenal polyp, *n* (%)	D1, *n* (%)	D2, *n* (%)	*p* value D1 vs D2
Prevalence rate	117 (78.5)	113 (75.8)	79 (53.0)	<0.01*
Median number/person (inter-quartile range)	10 (2-20)	7 (0-18)	1 (0-6)	<0.01**
Type				
White spot	1712 (51.6)	1243 (54.9)	469 (44.7)	<0.01**
White plaque	1350 (40.7)	893 (39.4)	457 (43.6)	<0.01**
Protruded lesion	129 (3.9)	70 (3.1)	59 (5.6)	0.34**
Broad-based lesion	124 (3.7)	60 (2.6)	64 (6.1)	1.00**

D1, bulbus and descending portion of duodenum; D2, horizontal and ascending portions of duodenum

*p** value by Fisher's exact test

*p*** value by Wilcoxon's signed rank test

Supplementary Table 2. Summary of patients with germline *APC* variant in segment D*.

Case No.	Age (y)	Sex	No. of small intestinal polyps	No. of HGD	*APC* variant	Spigelman stage	Past history	Family history
13	26	F	206	0	c.4348C>T (p.Arg1450X)	III	Gastric cancer#	Great-grandfather, hepatic cancer Granduncle/ grandaunt, gastric cancer
25	44	M	66	0	c.3927_3931del (p.Glu1309Aspfs*4)	III	Duodenal cancer#	Grandmother, uterine cancer
28	44	M	112	2	c.4393_4394del (p.Ser1465Trpfs*3)	IV	N.P.	Grandmother, breast cancer
41	40	M	491	1	c.4710delT (p.Leu1575X)	IV	Gastric cancer#	Grandmother/ uncle, CRC
49	49	M	60	1	c.4192_4193del AG (p.Ser1407X)	IV	N.P.	Father/ grandfather/ uncle, CRC
81	28	F	186	0	c.4348C>T (p.Arg1450X)	III	Gastric cancer#	Great-grandfather, hepatic cancer Granduncle/ grandaunt, gastric cancer
84	30	M	22	0	c.4348C>T (p.Arg1450X)	II	N.P.	Great-grandfather, hepatic cancer Granduncle/ grandaunt, gastric cancer
86	31	F	162	5	c.4308dupT (p.Lys1437X)	IV	N.P.	Grandmother; CRC Grandaunt; breast cancer
88	49	F	45	2	c.3927_3931del AAAGA (p.Glu1312X)	IV	Duodenal cancer#	Father, gastric cancer
94	20	M	25	1	c.4192_4193 del AG (p.Ser1407X)	II	N.P.	Father/ grandfather/ uncle, CRC
116	23	M	35	0	c.4444del (p.Leu1482Phefs*25)	II	N.P.	Mother, CRC

Segment D*, codons 1251 to 1580 (colorectal/postcolorectal MCR); # intramucosal carcinoma.

APC, adenomatous polyposis coli; HGD, high-grade dysplasia; N/A, not applicable; CRC, colorectal cancer; N.P., nothing particular

Supplementary Table 3. Prevalence and number of high-grade adenomas in patients with FAP.

	Total	Duodenum	D1	D2	Jejunum	Ileum
Prevalence (%) (N=149)	39 (26.2)	34 (22.8)	26 (17.4)	8 (5.4)	9 (6.0)	1 (0.7)
No. of adenoma with HGD (%) [Range / person]	74 (100) [0 – 5]	59 (79.7) [0 – 5]	43 (58.1) [0 – 5]	16 (21.6) [0 – 4]	14 (18.9) [0 – 5]	1 (1.4) [0 – 1]

D1, bulbus and descending portion of duodenum; D2, horizontal and ascending portions of duodenum

HGD, high-grade dysplasia

Supplementary Table 4. Accuracy for diagnosis of high-grade dysplasia in protruded and broad-based lesions

	HGD		Accuracy (%)
	+	-	
Protruded lesions			
+	33	164	52.7
-	41	195	
Broad-based lesions			
+	41	103	68.6
-	33	256	

HGD, high-grade dysplasia

Supplementary Table 5. Summary of intramucosal carcinoma according to the criteria of the Japanese Society for Cancer of the Colon and Rectum (JSCCR).

Case No.	Age (y)	Sex	Type	Size (mm)	Location	No. of small intestinal polyps	*APC* variant	Spigelman stage
8	63	F	Broad-based	30	D2	23	Segment C c.3682C>T (p.Gln1228X)	IV
65	70	M	Broad-based	15	D1	28	Segment A c.508_509delGA (p.Asp170X)	IV
89	70	F	Protruded Protruded	8 10	D1	125	All deletion	IV
91	63	F	Broad-based	25	D1	36	Segment B c.1660C>T (p.Arg554X)	IV
95	52	F	Protruded	10	D1	42	Segment B c.2805C>A (p.Tyr935X)	IV
137	67	F	Broad-based	15	D1	80	unknown	IV
3	33	M	Broad-based Broad-based	10 25	Jejunum	185	Segment B c.2086G>T (p.Glu696X)	III
86	31	F	Broad-based Broad-based Broad-based Broad-based Broad-based	8 18 20 20 20	Jejunum	162	Segment D c.4308dupT (p.Lys1437X)	IV

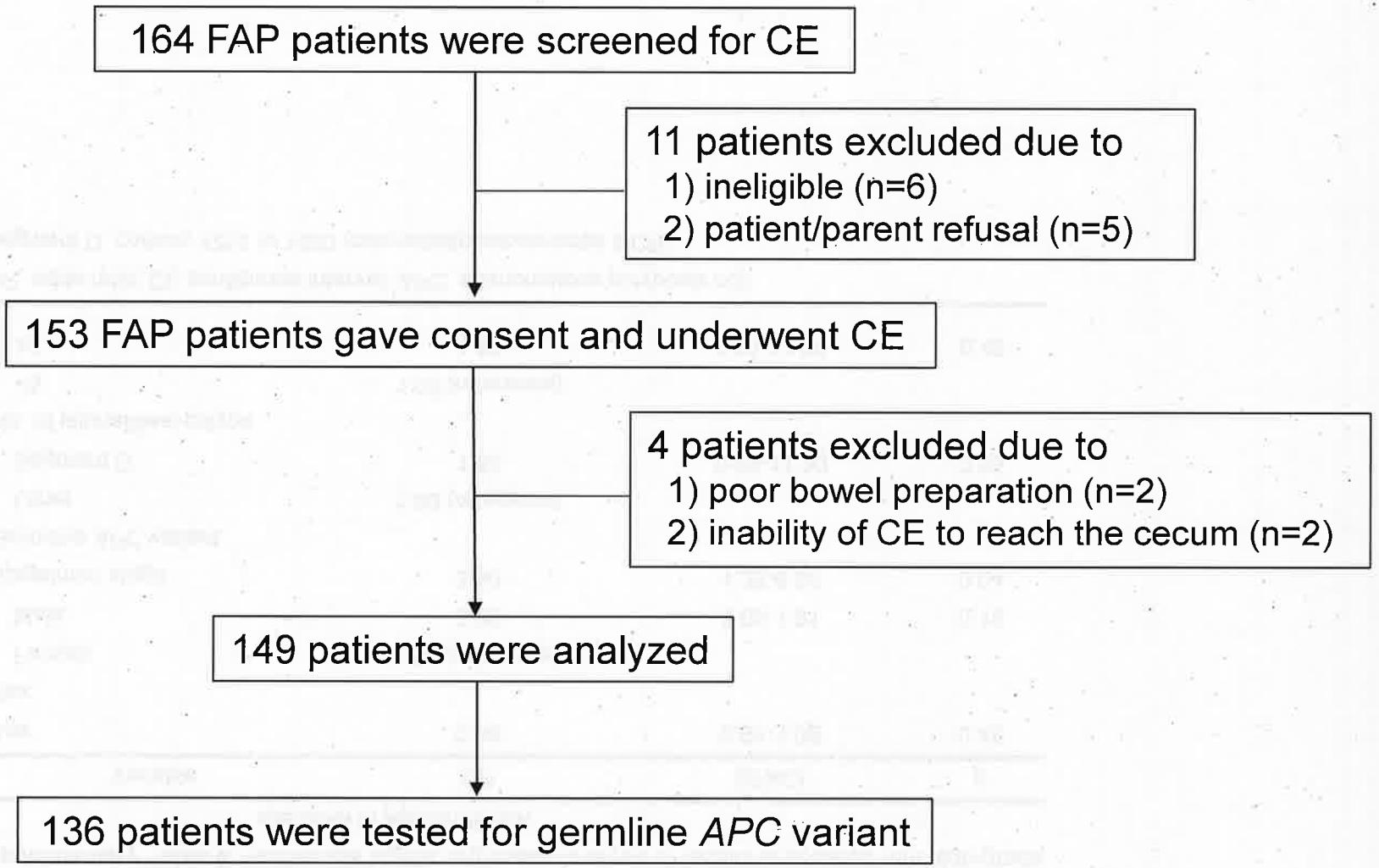
D1, bulbus and descending portion of duodenum; D2, horizontal and ascending portions of duodenum, *APC*, adenomatous polyposis coli

Supplementary Table 6. Multivariate logistic regression analysis of factors associated with high-grade adenoma in jejunum/ileum.

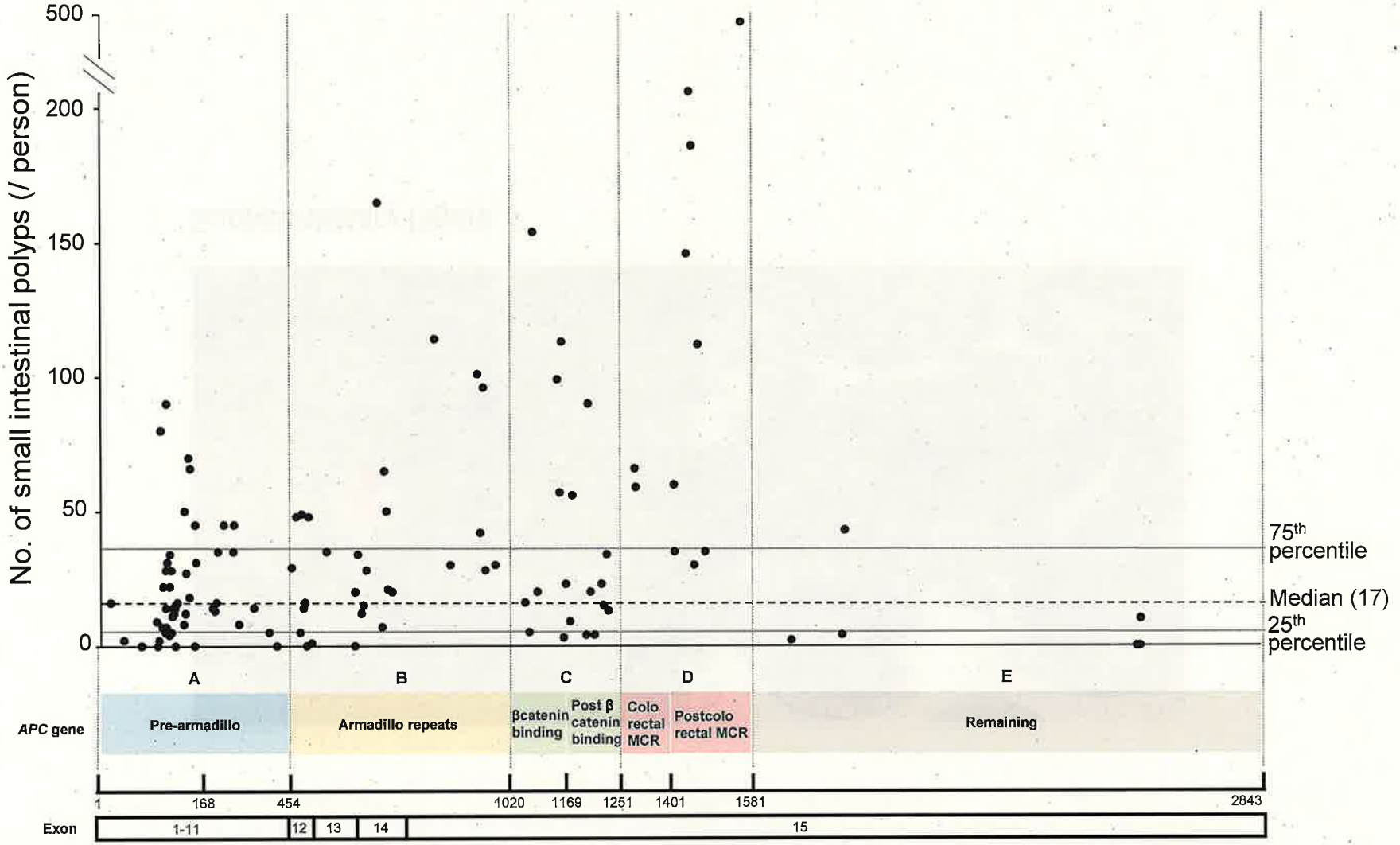
Variable	OR	95%CI	*p*
Age	0.98	0.91-1.05	0.49
Sex			
Female	1.00 (reference)		
Male	0.29	0.05-1.81	0.19
Spigelman stage	2.50	1.25-6.35	0.04
Germline *APC* variant			
Other	1.00 (reference)		
Segment D	1.58	0.22-11.30	0.65
No. of jejunal/ileal polyps			
<5	1.00 (reference)		
≥5	1.90	0.31-11.70	0.49

OR, odds ratio; CI, confidence interval; *APC*, adenomatous polyposis coli

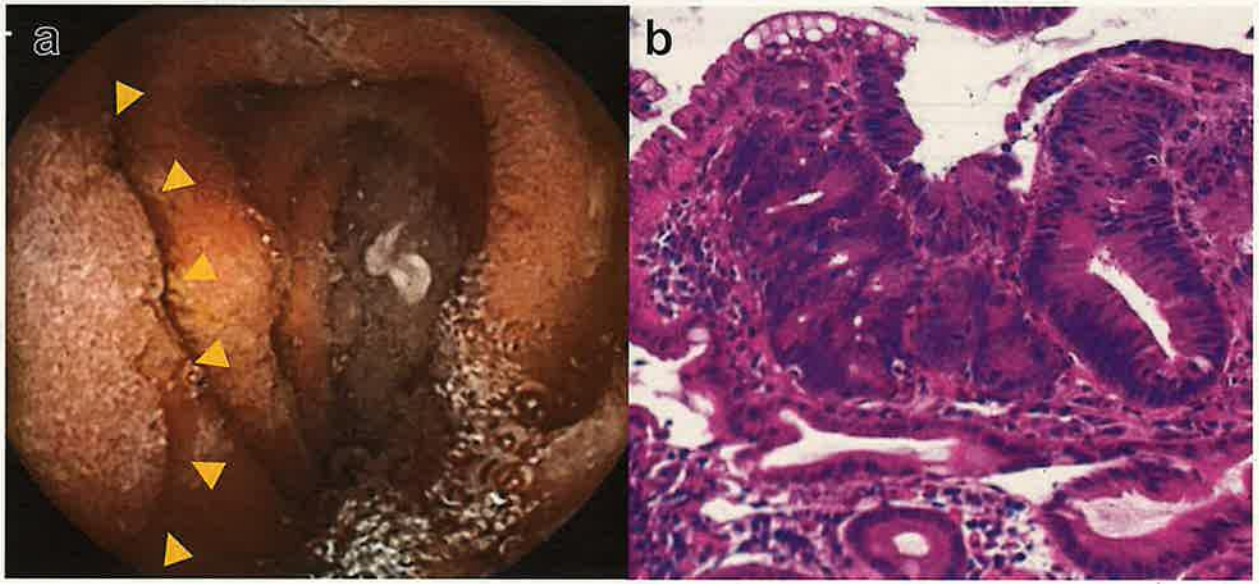
Segment D, codons 1251 to 1580 (colorectal/postcolorectal MCR)



Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3

Supplementary Figure legends

Supplementary Figure 1. A flow diagram for patient enrollment. A total of 164 patients with familial adenomatous polyposis (FAP) were screened for the study of capsule endoscopy (CE) for small intestinal polyps; there were 11 screening failures. A total of 153 patients underwent CE with consent; 149 were evaluable and analyzed, and 136 were tested for germline APC variant.

Supplementary Figure 2. The number of small intestinal polyps by site of germline APC variant in each patient. A, codons 1 to 453, representing preamardillo repeat region; B, codons 454 to 1019, representing armadillo repeat region; C, codons 1020 to 1250, representing β-catenin/post β-catenin binding region; D, codons 1251 to 1580, representing colorectal/post colorectal MCR region; E, codons 1581 to 2843, representing the remaining region. The dashed line represents the median value of 17. The dotted lines represent the 25th and 75th percentile values of 6 and 36 respectively.

Supplementary Figure 3. CE image and histology of intramucosal carcinoma according to the Japanese classification criteria for colorectal cancers. (a) A broad-based lesion was detected in the jejunum (Case No. 86 in Supplementary Table 4) by CE (arrowhead). (b) Balloon-assisted endoscopy was performed and the lesion was removed by endoscopic mucosal resection. H&E staining of the removed lesion exhibited strong nuclear atypia and disorganized glandular structures, leading to the diagnosis of intramucosal carcinoma.

Supplementary information

Methods

CE procedures

CE was performed using a PillCam SB3 (Covidien Japan Inc., Tokyo, Japan) for 138 patients and a PillCam SB2 for 15 patients with analysis carried out using the RAPID workstation and software (Covidien Japan Inc.). All patients included had received 1000 mL of polyethylene glycol-based bowel preparation before the examination and were given dimethicone before swallowing the capsule after an overnight fast.

Polyps located at the proximal side of the ligament of Treitz (identified by tracing CE movement) were considered duodenal polyps, and polyps located from the ligament of Treitz to the ileocecal valve or ileum pouch were considered jejunal and ileal polyps. The transit time of CE from the ligament of Treitz to the ileocecal valve or ileum pouch was dichotomized; the first half and second half were presumed to be the jejunum and ileum, respectively.

Germline APC gene analysis

Genomic DNA was isolated from peripheral blood using a QIAamp DNA Blood Kit (Qiagen, Hilden, Germany). For sequencing, DNA was amplified by PCR using oligonucleotide primers for APC, and the library was then prepared using a Nextera XT System (Illumina Inc., San Diego, CA). Samples were pooled for multiplexed sequencing, and sequencing was performed using a MiSeq instrument (Illumina Inc.) by 150 bp paired-end sequencing with MiSeq Reagent Kit v2 (300 cycles) (Illumina Inc.). Sequenced reads were mapped against reference sequences, and variants were identified using Genomics Workbench Software v7.5 (Qiagen) and confirmed with Omixon Target Software (Omixon

Ltd, Budapest, Hungary). Variants, except for polymorphism, detected by next-generation sequencing were confirmed by Sanger sequencing.

Multiplex ligation-dependent probe amplification (MLPA) analysis was performed using a Salsa P043 kit for the APC gene (MRC-Holland, Amsterdam, Netherlands), according to the manufacturer's instructions, in patients with no pathogenic variant sequence detected in the sequencing analysis. Amplification products were electrophoresed using a ABI 3130xl genetic analyzer (Thermo Fisher Scientific Inc., Waltham, MA), and analyzed for copy number variations using Coffalyser.net software (MRC-Holland).

BA endoscopy procedures

BA endoscopy was performed using double-balloon endoscopy (DBE; Fujifilm Corp., Tokyo, Japan) or single-balloon endoscopy (SBE, Olympus Inc, Tokyo, Japan) for the patients with polyps >5 mm detected by CE.

In patients with an ileal polyp >5 mm detected by CE, we performed both per-oral and per-anal BAE. In the first BAE, we tattooed the terminal point in the jejunum, and in the second BAE, we observed the remaining small intestine up to the tattooed point. In patients with a jejunal polyp >5 mm, we first performed per-oral BAE and looked for the polyp, considering its morphology and size as detected by CE. In most patients, we were able to find the polyp, which was identifiable as the one detected by CE. During per-oral BAE, the number of polyps lessened moving from the proximal-to-distal jejunum, reaching the point of no polyp. In cases when small polyps still existed at the terminal point in the jejunum but the polyp searched for was not found, we then performed per-anal BAE and observed the remaining small intestinal tract to find the polyp in the more distal jejunum.

Statistical analyses and sample size

All statistical analyses were performed with EZR software (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). More precisely, it is a modified version of R commander designed to add statistical functions frequently used in biostatistics.

We performed Fisher's exact test or Friedman test to compare the prevalence, number, size, and type of polyps in each location of small intestine. Multivariate logistic regression analysis was performed to identify factors to affect high number of small intestinal polyps and high-grade dysplasia in small intestine. We performed the Steel-Dwass test for multiple comparisons to compare the number of polyps or Spigelman score in each group of APC variant site, and Spearman's rank correlation test to analyze the correlation between the Spigelman stage and the number of jejunal/ileal polyps. Probabilities <0.05 were considered to be statistically significant.

According to a previous study,[19] the estimated prevalence of adenoma with high-grade dysplasia was roughly 11.8%. Assuming that the prevalence in the small intestine of FAP patients utilizing SB3 capsule endoscopy is 20%, the sample size was calculated to be 149 with 80% power and 5% significance level.

Results

Outcome of CE procedure

The mean gastric transit time and small intestine transit time were 33.5 ± 44.0 and 258.4 ± 95.9 min, respectively. The percentage of CEs reaching the cecum or ileal pouch within the recording period was 98.7% (151/153).