Received: 4 May 2023 | Revised: 3 August 2023 | Accepted: 18 August 2023

DOI: 10.1111/cas.15945

Cancer Science WILEY

ORIGINAL ARTICLE

Genotype–phenotype correlation for extracolonic aggressive phenotypes in patients with familial adenomatous polyposis

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Funding information
Japan Agency for Medical Research and Development, Grant/Award Number: 19ck0106271h0003

Abstract

Familial adenomatous polyposis (FAP) patients develop various life-threatening extracolonic comorbidities that appear individually or within a family. This diversity can be explained by the localization of the *adenomatous polyposis coli* (*APC*) variant, but few reports provide definitive findings about genotype–phenotype correlations. Therefore, we investigated FAP patients and the association between the severe phenotypes and *APC* variants. Of 247 FAP patients, 126 patients from 85 families identified to have *APC* germline variant sites were extracted. These sites were divided into six groups (Regions A to F), and the frequency of severe comorbidities was compared among the patient phenotypes. Of the 126 patients, the proportions of patients with desmoid tumor stage ≥III, number of FGPs ≥1000, multiple gastric neoplasms, gastric neoplasm with high-grade dysplasia, and Spigelman stage ≥III were 3%, 16%, 21%, 12%, and 41%, respectively, while the corresponding rates were 30%, 50%, 70%, 50%, and 80% in patients with Region E (codons 1398–1580) variants. These latter rates were significantly higher than those for patients with variants in other regions.

Abbreviations: *APC*, *adenomatous polyposis coli*; CI, confidence interval; EGD, esophagogastroduodenoscopy; FAP, familial adenomatous polyposis; FGP, fundic gland polyp; *H.pylori*, *Helicobacter pylori*; HGD, high grade dysplasia; IQR, interquartile range; LOH, loss of heterozygosity; MCR, mutation cluster region; OR, odds ratio; PTT, protein truncation test.

Moreover, the proportion of patients with all three indicators (desmoid tumor stage ≥III, number of FGPs ≥1000, and Spigelman stage ≥III) was 20% for those with variants in Region E and 0% for those with variants in other regions. Variants in Region E indicate aggressive phenotypes, and more intensive management is required.

KEYWORDS
adenomatous polyposis coli, desmoid tumor, duodenal neoplasm, familial adenomatous polyposis, gastric lesion, genotype-phenotype correlation

1 | INTRODUCTION

Familial adenomatous polyposis (FAP) is a monogenic disorder of autosomal dominant inheritance caused by a pathogenic germline variant in the *adenomatous polyposis coli* (*APC*) gene. FAP patients develop colorectal cancer if left untreated and also have a high rate of comorbidities in other parts of the gastrointestinal tract and other organs that require appropriate management.[1]

APC genetic testing is not only a definitive diagnosis for FAP but is also expected to be a useful reference for treatment selection and surveillance of comorbidities. Some reports have indicated that the clustering of *APC* gene variants is associated with a severe phenotype in the large intestine,[2,3] and early colectomy may be indicated in children harboring this clustering gene variant.[4,5] Therefore, predicting comorbidities based on the *APC* pathogenic germline variant site could enable the appropriate management of FAP patients. While reports of genotype–phenotype correlations for various extracolonic comorbidities in FAP patients have been published,[6–11] few reports have examined severe extracolonic phenotypes.

The aim of this study was to examine the genotype–phenotype correlation of FAP patients, especially for severe extracolonic phenotypes. We investigated FAP patients with identified *APC* pathogenic germline variant sites. Then, we defined and examined severe phenotypes of desmoid tumor and gastric and duodenal lesions. From this, we elucidated a region within the *APC* gene that, when mutated, can be used as a reference for predicting severe extracolonic phenotypes.

2 | MATERIALS AND METHODS

2.1 | Study design

This work involved a retrospective study of FAP patients who underwent examination of the *APC* pathogenic germline variant site. The manuscript was prepared in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology Statement.[12]

2.2 | Study subjects and extraction of clinical data.

FAP patients who visited Osaka International Cancer Institute or Ishikawa Gastrointestinal Clinic from June 2008 to December 2018 were investigated. At the first visit, the patient, in principle, underwent a consultation on their medical history and family history, as well as an initial screening colonoscopy, followed by an esophagogastroduodenoscopy (EGD) and *Helicobacter pylori* (*H. pylori*) testing. After that, a colonoscopy was performed once within 12 months, and an EGD was performed once every 12 to 24 months. The search for desmoid tumors and thyroid cancer was basically performed by ultrasonography once a year. Then, if necessary, computed tomography or magnetic resonance imaging examination was performed, and pathological examination was added for definitive diagnosis. Genetic information was managed at an external data center, Medical Research Support (Osaka, Japan), which performed data management and monitoring tasks following strict guidelines, and information on FAP patients was extracted. Clinical data were collected from medical and endoscopic records. Age at the time of the last endoscopy, sex, and drinking and smoking history were collected. *H. pylori* status was defined as "infection" if the result was positive for at least one of the following tests: serological test, urea breath test, rapid urease test, and histological analysis. For fundic gland polyps (FGPs), the number and size were examined from EGD images. As for the number of FGPs, in the case of two digits, it was counted in 10s (e.g., 10 to 19 were defined as "10"), while in the case of three digits, it was counted in 100s (e.g., 100 to 199 were defined as "100"). As for the diameter of FGPs, the largest one that was isolated (i.e., not fused to another one) was adopted for the judgment. Because the cases had been histologically evaluated for gastric neoplasm in accordance with the Japanese classification, they were re-evaluated by one pathologist in accordance with the World Health Organization classification of tumors.[13] For the evaluation of histological phenotypes in gastric neoplasm, high-grade dysplasia, intramucosal invasive neoplasia, and adenocarcinoma were defined as the high-grade histological phenotype. Duodenal neoplasms were assessed using the Spigelman classification.[14] The colorectal lesions were classified according to the density of colorectal adenomas as severe, sparse, or attenuated FAP, as defined in a previous report.[15] Regarding comorbidities for extra-gastrointestinal lesions in FAP, we collected each patient's history of desmoid tumors, thyroid cancer, osteomas, adrenal tumors, and hepatoblastoma. In addition, desmoid tumors were staged according to the Cleveland staging system.[6,16]

2.3 | Genetic testing

All patients underwent genetic counseling and provided informed consent for the *APC* genetic testing. It was only performed on patients who had given their consent. Genetic testing was basically performed in accordance with the protocol described below. DNA and RNA were extracted from peripheral blood leukocytes, and the protein truncation test (PTT) was performed as a screen for the presence of pathogenic variants.[17] In the PTT method, a DNA fragment added with a promoter sequence targeting the protein-coding region of the *APC* gene was amplified by PCR. Using the amplified sequence as a template, the *APC* protein was synthesized in the presence of RNA polymerase, and electrophoresis was performed to confirm bands that differed from normal samples. If the presence of a pathogenic variant was suggested, reverse-transcription PCR and the direct sequencing of DNA were carried out on the band with different electrophoretic mobility. Primers used for the sequencing were available upon request. When a pathogenic variant was unknown, analysis of large fragment duplication or deletion and allelic loss was performed using the multiplex ligation-dependent probe amplification assay. We used the SALSA P043-B1 MLPA KIT (MRC-Holland, Amsterdam, The Netherlands) in accordance with the manufacturer's instructions; the probe was set for each exon from the *APC* gene promoter region. The nucleotide and deduced amino acid sequences were compared with reference sequences of the *APC* gene available from the National Center for Biotechnology Information GenBank database using the Basic Local Alignment Search Tool program (http://blast.ncbi.nlm. nih.gov/Blast.cgi).

2.4 | Definition of severe phenotypes and the regionality of the *APC* pathogenic germline variant site.

Definitions were formed to examine the severe phenotypes and the regionality of the *APC* pathogenic germline variant in FAP patients. For desmoid tumors, a severe phenotype was defined as a desmoid tumor stage of ≥III with an increased mortality rate.[16] For gastric lesions, the severe phenotype has not been comprehensively discussed. Therefore, we defined severe phenotypes as follows: for number of FGPs of ≥1000; and for gastric neoplasm, multiple gastric neoplasms and a high-grade histological phenotype. For duodenal and colorectal lesions, Spigelman stage of ≥III and severe colorectal polyposis upon the classification of the density of colorectal adenomas were defined as a severe phenotype. For the regionality of the *APC* pathogenic germline variant site, we referred to previous reports.[18,19] Based on these reports, *APC* genetic sites were divided into six regions. Furthermore, we modified the regions from the results of the severe phenotype in the current study.

2.5 | Statistical analysis

Statistical analysis was performed using Fisher's exact test to compare *APC* pathogenic germline variant sites. We also performed the Steel–Dwass test for multiple comparisons to compare the number of FGPs and the number of gastric neoplasms in each region within the *APC* gene. Statistical significance was set at $p < 0.05$. All statistical analyses were performed with EZR software, version 1.40 (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria).

3 | RESULTS

3.1 | Study subjects

A total of 247 FAP patients were collected at two institutes during the target period, of which 180 were subjected to *APC* germline genetic testing in addition to screening for comorbidities. Among them, the *APC* germline variant site was identified in 143 patients. Nine patients were excluded from examination of the genetic site: seven patients with a large deletion and two with complete loss of an *APC* allele. Furthermore, we excluded eight patients with intron variants to focus on investigating variants in exons. As a result, 126 patients from 85 families were evaluated, and their baseline characteristics are shown in Table 1. The median (interquartile range, IQR) age was 37 (29–48) years, and women constituted 54% of the total. The median follow-up period was 8 (3–9) years. Overall, 24% of the subjects were *H. pylori*-positive. For FGPs, 70% had a number of FGPs ≥100s, while the median (IQR) diameter of the largest FGP was 4 (3–6) mm. The proportion of patients with gastric neoplasm was 36%. The coexistence of duodenal neoplasms was identified in 86% of patients. The proportion of sparse or severe FAP in the classification of the density of colorectal adenomas was 92%, and 72% of colorectal neoplasms were managed by endoscopic intervention alone. Extra-gastrointestinal lesions were desmoid tumors, thyroid cancer, osteomas, adrenal tumors, and hepatoblastoma in 9%, 8%, 2%, 2%, and 1% of patients, respectively.

3.2 | Patient summary of the region showing various severe phenotypes and investigation of regionality of the *APC* gene.

The genotype and phenotype data for all patients in the current study are shown in Table S1. Of the 126 patients, the proportions of patients with desmoid tumor stage ≥III, number of FGPs ≥1000, multiple gastric neoplasms, gastric neoplasm with HGD, Spigelman stage ≥III, and severe colorectal polyposis were 3%, 16%, 21%, 12%, 41%, and 7%, respectively. With reference to previous reports on genotype–phenotype correlations of the *APC* gene,[18,19] patients with an *APC* pathogenic germline variant in codons 1398–1580 showed various severe phenotypes (Table 2). There were 10 FAP patients with a variant within this region. In addition, six *APC* pathogenic germline variant sites were confirmed, of which both c.4192_4193delAG and c.4348C > T were present in three members of the same family. These patients had desmoid tumor stage ≥III,

TABLE 1 Baseline characteristics of 126 patients.

	n = 126 (%)
Age, years, median (IQR)	37 (29–48)
Sex, male/female *n* (%)	58 (46)/68 (54)
Body mass index, kg/m^2, median (IQR)	21 (20–23)
Drinking habit +, *n* (%)	29 (23)
Smoking habit +, *n* (%)	32 (25)
Oral administration, statin/H2 blocker/proton pump inhibitor/NSAIDs, *n* (%)	1 (1)/3 (2)/1 (1)/2 (1)
Follow-up period, years, median (IQR)	8 (3–9)
Helicobacter pylori status, unknown/−/+, n (%)	3 (2)/93 (74)/30 (24)
Number of FGPs, <100s/100s–900s/≥1000, *n* (%)	38 (30)/68 (54)/20 (16)
Diameter of the largest FGP, mm, median (IQR), *n* (%)	4 (3–6)
Histological phenotype of the gastric neoplasm, −/LGD/HGD/IIN/adenocarcinoma, *n* (%)	81 (64)/30 (24)/13 (10)/1 (1)/1 (1)
Multiple gastric neoplasms +, *n* (%)	26 (21)
Duodenal neoplasm +, *n* (%)	108 (86)
Spigelman stage, 0/I/II/III/IV, *n* (%)	18 (14)/20 (16)/36 (29)/32 (25)/20 (16)
Ampullary tumor +, *n* (%)	19 (15)
Classification according to the density of colorectal adenomas, attenuated/sparse/severe, *n* (%)	10 (8)/107 (85)/9 (7)
Colorectal adenocarcinoma +, *n* (%)	8 (6)
Treatment of colorectal neoplasms, endoscopic resection/partial colectomy/IRA/IPAA/TPC, *n* (%)	91 (72)/1 (1)/24 (19)/8 (6)/2 (2)
Extra-gastrointestinal lesion, desmoid tumor/thyroid cancer/osteoma/adrenal tumor/hepatoblastoma, *n* (%)	11 (9)/10 (8)/3 (2)/2 (2)/1 (1)
Desmoid tumor stage, extra-abdominal/I/II/III/IV, *n* (%)	1 (1)/4 (3)/2 (2)/2 (2) /2 (2)

Abbreviations: FGP, fundic gland polyp; H2, histamine receptor type 2; HGD, high-grade dysplasia; IIN, intramucosal invasive neoplasia; IPAA, ileal pouch-anal anastomosis; IQR, interquartile range; IRA, ileorectal anastomosis; LGD, low-grade dysplasia; NSAIDs, nonsteroidal anti-inflammatory drugs; TPC, total proctocolectomy.

number of FGPs ≥1000, multiple gastric neoplasms, gastric neoplasm with HGD, Spigelman stage ≥III, and severe colorectal polyposis at rates of 30%, 50%, 70%, 50%, 80%, and 30%, respectively. Then, we defined the following regionality for the *APC* pathogenic germline variant site: 47 patients (37%) in the pre-armadillo repeat region (Region A; codons 1–453), 32 patients (25%) in the armadillo repeat region (Region B; codons 454–1019), 28 patients (22%) in the β-catenin/post β-catenin binding region (Region C; codons 1020–1249), three patients (2%) in the colorectal mutation cluster region (MCR) (Region D; codons 1250–1397), 10 patients (8%) in the aggressive region (Region E; codons 1398–1580), and six patients (5%) in the remaining region (Region F; codons 1581–2843). Table 3 shows the relationship between severe phenotypes and patients with variants in each defined region. The proportions of patients with desmoid tumor stage ≥III, number of FGPs ≥1000, multiple gastric neoplasms, gastric neoplasm with HGD, and Spigelman stage ≥III were significantly higher for those with variants in Region E. Meanwhile, the proportion of patients with severe colorectal polyposis was significantly higher for those with variants in Region D. In addition, the proportion of patients with three indicators (desmoid tumor stage ≥III, number of FGPs ≥1000, and Spigelman stage ≥III) was examined (Figure 1). Overall, 20% of patients with variants in Region E had all three indicators, while no patients with variants in other regions had all three indicators.

3.3 | Intra-familial phenotypes.

Among the 85 families, there were 15 families with two members, 10 families with three members, and one family with seven members. To examine intra-familial variability, *APC* pathogenic germline variants and severe phenotypes in each of the 10 families with three members were extracted (Table S2). As for the number of cases with FGPs ≥1000, four families had at least one member with FGPs ≥1000. Of these, the proportion of families with at least two members having FGPs ≥1000 was 75%. As for multiple gastric neoplasms, six families included at least one member with multiple gastric neoplasms. Of these, the proportion of families with at least two members having multiple gastric neoplasms was 33%, but in families with one member having multiple gastric neoplasms, these

TABLE 2 Summary of 10 patients with *APC* pathogenic germline variant in Region E*.

ID (family member)	Age, years	Sex	Desmoid tumor stage	Number of FGPs	Number of gastric neoplasms	Histological phenotype of gastric neoplasm	Spigelman stage	Colorectal adenomas	*APC* pathogenic germline variant
78-A	49	Male	–	≥1000	2	LGD	III	Sparse	c.4192_4193delAG
78-B	45	Male	–	800s	2	LGD	III	Severe	c.4192_4193delAG
78-C	20	Male	–	800s	2	HGD	II	Sparse	c.4192_4193delAG
79-A	31	Female	III	≥1000	2	HGD	IV	Severe	c.4308dupT
80-A	29	Male	–	400s	1	LGD	II	Sparse	c.4348C>T
80-B	28	Female	IV	≥1000	16	HGD	III	Sparse	c.4348C>T
80-C	26	Female	II	≥1000	2	HGD	III	Sparse	c.4348C>T
81-A	43	Male	IV	200s	0	–	IV	Severe	c.4393_4394delAG
82-A	21	Male	–	700s	0	–	III	Sparse	c.4661dupA
83-A	39	Male	extra-abdominal	≥1000	5	HGD	III	Sparse	c.4710delA

Note: Region E* is the region of codons 1398 to 1580 in the *APC* gene.

Abbreviations: *APC*, *adenomatous polyposis coli*; FGP, fundic gland polyp; HGD, high-grade dysplasia; LGD, low-grade dysplasia.

members were the oldest in their family. As for duodenal adenomas of Spigelman stage ≥III, seven families had at least one member with Spigelman stage ≥III. Of these, the proportion of families with at least two members having Spigelman stage ≥III was 71%. In the 10 families evaluated, only one patient with desmoid tumor stage ≥III and severe colorectal polyposis and four patients with gastric neoplasm with HGD were found. In this context, it was impossible to investigate the intra-familial variability for genotype–phenotype correlation because of the small number of events in our current study.

3.4 | Relationship of surgical intervention or sex with desmoid tumor

Table S3 shows the relationship considering surgical intervention or sex in patients with abdominal desmoid tumors. Among patients with no previous surgery, desmoid tumors occurred only in patients with germline variants in Region E. In addition, desmoid tumors occurred in male patients with germline variants in Regions D and E. Meanwhile, in patients with previous surgery and who were female, no specific regionality was observed. However, there was no significant regional specificity for either surgical intervention or sex.

3.5 | Relationship of *Helicobacter pylori* infection with gastric lesions.

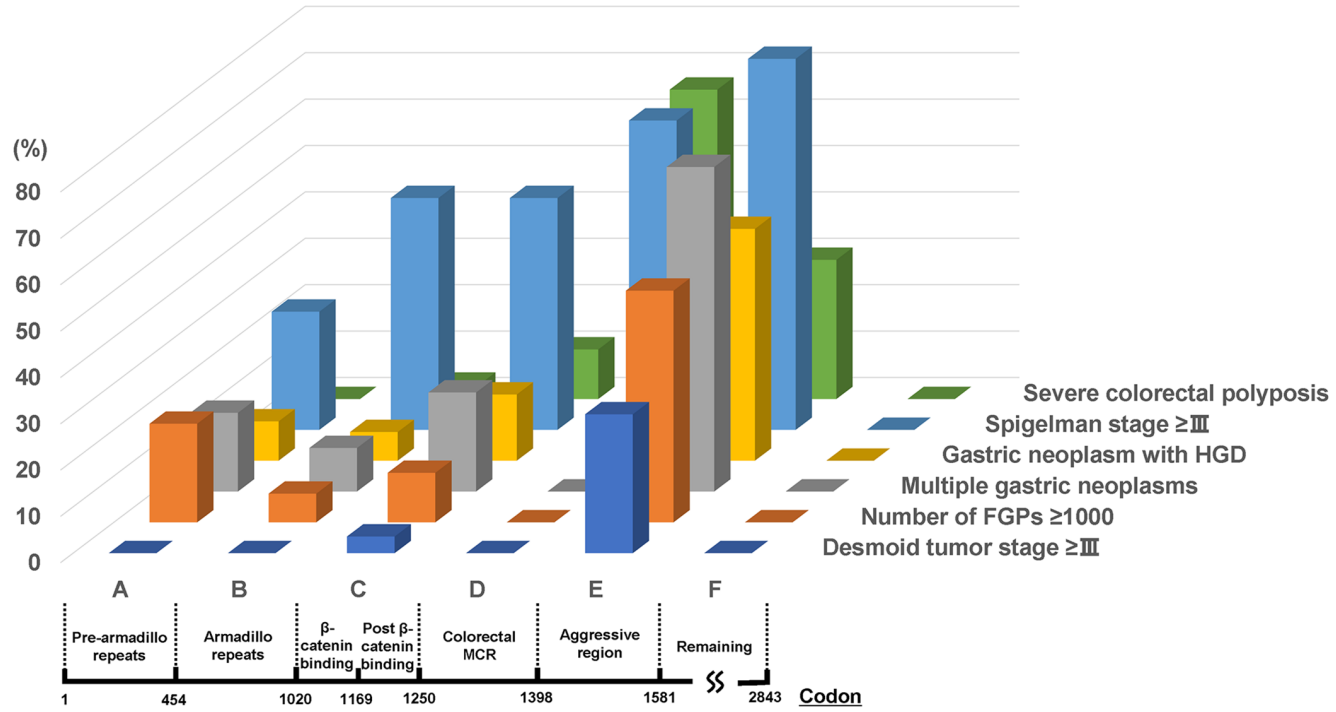
Among 93 *H. pylori*-uninfected patients in the current study, the median (IQR) number of FGPs was the highest in those with a variant in Region E (1000 [800–1000], Figure 2A). Moreover, the median (IQR) number of gastric neoplasms was the highest in those with a variant in Region E (2 [2], Figure 2B). The results of 126 patients in the current study are shown in Figure 2C,D. All results showed no apparent difference compared with the 93 *H. pylori*-uninfected patients. Table S4 shows the results of the comparison between those with a variant in Region E and in other regions among the 93 *H. pylori*-uninfected patients. No differences in trends compared with the results in Table 3 were identified.

4 | DISCUSSION

This study demonstrated a genotype–phenotype correlation for FAP patients with aggressive extracolonic phenotypes. We focused on severe phenotypes of desmoid tumors and gastric and duodenal lesions. We found that they were associated with *APC* pathogenic germline variants in codons 1398–1580. Recognizing that variants in this region are associated with aggressiveness may help predict extracolonic comorbidities in FAP patients.

The penetrance of colorectal cancer is FAP is 100%.[15] Prophylactic colectomy avoids death from colorectal cancer and prolongs survival, but the mortality rate from other comorbidities is increasing.[1]

TABLE 3 Relationship between regions for the *APC* pathogenic germline variant and FAP patients with severe phenotypes.



	A	B	C	D	E	F	
	n = 47	*n* = 32	*n* = 28	*n* = 3	*n* = 10	*n* = 6	*p*-value
Desmoid tumor stage, ≥III[a]	47/0	32/0	27/1	3/0	7/3	6/0	0.003
Number of FGPs, ≥1000[a]	37/10	30/2	25/3	3/0	5/5	6/0	0.031
Multiple gastric neoplasms[a]	37/10	29/3	22/6	3/0	3/7	6/0	0.005
Gastric neoplasm with HGD[a]	43/4	30/2	24/4	3/0	5/5	6/0	0.027
Spigelman stage, ≥III[a]	35/12	16/16	14/14	1/2	2/8	6/0	<0.001
Severe colorectal polyposis[a]	47/0	31/1	25/3	1/2	7/3	6/0	0.002

Abbreviations: FGP, fundic gland polyp; HGD, high-grade dysplasia.
[a]−/+.

Therefore, FAP patients need to undergo routine examinations other than colonoscopy, as recommended in various guidelines.[20–22] However, FAP patients have diverse phenotypes that appear individually or within a family. As an explanation for the diverse phenotypes, the genotype–phenotype correlation has been reported, based on which *APC* pathogenic germline variants in FAP patients are characterized by a majority (95%) of nonsense or frameshift variants in exons, which result in a truncated protein product with abnormal function.[23] Therefore, FAP patients with *APC* pathogenic germline variant sites in neighboring regions are thought to develop similar phenotypes and can be used as a reference for predicting comorbidities. In the current study, we investigated the genotype–phenotype correlation in FAP patients with an *APC* pathogenic germline variant in an exon. FAP patients with *APC* variants of codons 1398–1580 were found to have a high rate of severe phenotypes of desmoid tumor and gastric and duodenal lesions. Hence, patients with variants in this aggressive region (Region E) require more careful extracolonic surveillance planning.

Genotype–phenotype correlation has been investigated to predict diverse phenotypes in FAP patients. However, the published reports are controversial and inconclusive. As for gastric lesions, our study showed severe phenotypes of gastric lesions in patients with variants in codons 1398–1580. This result is close to the relationship between codons 1395–1493 and gastric adenomas reported by Wallis et al.[24] Meanwhile, Oliveira et al. reported that FGP and low-grade gastric adenomas were frequently found in patients with variants in codons 213–1309, and gastric cancer was identified in those with variants in codons 986 and 1166.[25] In addition, Nieuwenhuis et al. noted in their review that some studies suggested that exon 4 and codons 564–1465 seem to be associated with gastric polyps, but variants at the C-terminal to codon 1390 were also suggested to have this association, although no large-scale studies have evaluated upper gastrointestinal polyps.[8] Moreover, whereas the report from a European registry showed that *APC* pathogenic germline variants at the C-terminal to codon 1390 were associated with gastric cancer,[26] in our study, no patient with a variant at the C-terminal region

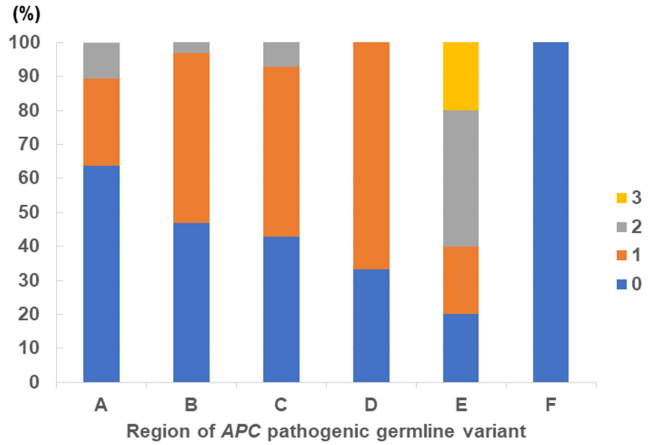


FIGURE 1 Proportions of patients with different numbers of the three indicators (desmoid tumor stage ≥III, number of FGPs ≥1000, and Spigelman stage ≥III) for those with *APC* pathogenic germline variants in each region. "0" indicates patients who did not have all three indicators, "1" or "2" indicates patients who had one or two of the three indicators, and "3" indicated patients who had all three indicators.

of codons 1581–2843 of Region F had gastric lesions with a severe phenotype (Table S1). The above two reports showing genotype–phenotype correlation between gastric lesions and the C-terminal of the *APC* gene both demonstrated a genetic relationship at only one gene site within codons 1581–2843.[10,27] Therefore, although variants at the C-terminus may be related to gastric lesions, it is not possible to confirm that variants in the C-terminal region are positively related to the phenotype of gastric lesions.

As for duodenal lesions, we previously reported that *APC* pathogenic germline variants in codons 1251–1580 were significantly associated with a high number of small bowel polyps, including duodenal ones.[19] In subsequent investigations, there were no desmoid tumor and gastric lesions of severe phenotypes in patients with variants in codons 1250–1397 of Region D (Table S1). Then, in the current study, codons 1398–1580 were targeted for the identification of a genotype–phenotype correlation. The genotype–phenotype correlation between duodenal lesions and variants in codons 1398–1580 is close to the relationship between codons 1395–1493 and duodenal adenomas reported by Wallis et al.,[24] but a report has also described that duodenal polyps are more common in patients with variants in codons 564–1465.[9] Differences in the results of these reports are probably due not only to the number of cases and ethnicity of the subjects but also to the histology of the neoplasms evaluated and the genetic testing methods used in the patients. Therefore, they cannot all be easily compared.

As for severe phenotypes of desmoid tumor, this study was associated with codons 1398–1580, which are similar to those reported in codons 1445–1578 by Caspari et al. and 1444–1581 by Gebert et al.[28,29] Meanwhile, in our report, no patients with variants at the C-terminal to codon 1581 had desmoid tumors with a severe phenotype, although some reports mention such an association with variants at the C-terminus. In a meta-analysis based on 10 studies of desmoid tumors, Sinha et al. reported an association of this tumor with the C-terminal of codon 1399.[7] In addition, Church et al. reported that almost half of patients with variants in the C-terminal of codon 1400 had a desmoid tumor with a severe phenotype.[6] The difference between these reports and our results regarding the effects of variants in the C-terminus may be due to differences in the proportions of patients with a history of abdominal surgery and the sex ratio. Schiessling et al.[30] reported that patients with desmoid tumors were more likely to be associated with surgery and that female patients appeared to have a higher risk of desmoid tumors irrespective of the variant site, whereas in male patients the mutation site appeared to exert more influence. Therefore, we also examined the effects of previous surgery and sex on the development of desmoid tumors, resulting in no change in regionality of codons 1398–1580 (Region E). However, there were no significant differences found in the analysis, possibly due to the small number of cases. However, it is also possible that the small number of patients with a variant in the C-terminal affected the results. In general, the proportion of patients with C-terminal variants is not high,[31–35] with 5% of patients having variants in codons 1581–2843 of Region F in our study. This may be the cause of the statistical variation, and further accumulation of cases is necessary.

APC binds to β-catenin, leading to the latter's degradation, but truncated *APC* proteins can disrupt the Wnt signaling pathway, causing the accumulation of nuclear β-catenin and inappropriate stimulation of downstream genes, leading to tumorigenesis. For binding to β-catenin, seven 20-amino-acid repeats encoded within codons 1262–2033 of the *APC* gene is essential.[35–37] Then, the number of 20-amino-acid repeats in the truncated *APC* protein affects the retention of intact β-catenin.[35] In particular, the truncated *APC* proteins associated with variants close to codon 1300, in which only one of the 20-amino-acid repeats is retained, provide the strongest selective advantage for colorectal adenomas.[38] Meanwhile, truncated *APC* proteins associated with variants in codons 1398–1580 in the current study could have two or three 20-amino-acid repeats. This may support the proposal by Groves et al. that, compared with colorectal adenomas, in cases with upper gastrointestinal polyps and desmoid tumor, β-catenin levels within the cell may be more optimal, if not excessive.[18]

The severe phenotype may be associated with loss of heterozygosity (LOH) of the somatic variants. As for severe colorectal polyposis, patients with *APC* germline variants in the MCR (codons 1250–1407 [1464]) frequently have colorectal adenomas of a severe phenotype.[2,3] In addition, adenomas in patients with the *APC* germline variant in the MCR often have a somatic variant of LOH.[23,38,39] Similarly, Groves et al. evaluated gastric and duodenal lesions and reported that patients with *APC* germline variants after codon 1400 tend to have more severe duodenal polyposis and show allelic loss in their upper gastrointestinal polyps.[18] Lamlum et al. reported that in patients with desmoid tumors and *APC* germline variants in codons 1450 and 1462, the somatic variants in the desmoid tumors involved allelic loss.[38] Furthermore, Miyake et al. reported that in patients with an *APC* germline variant

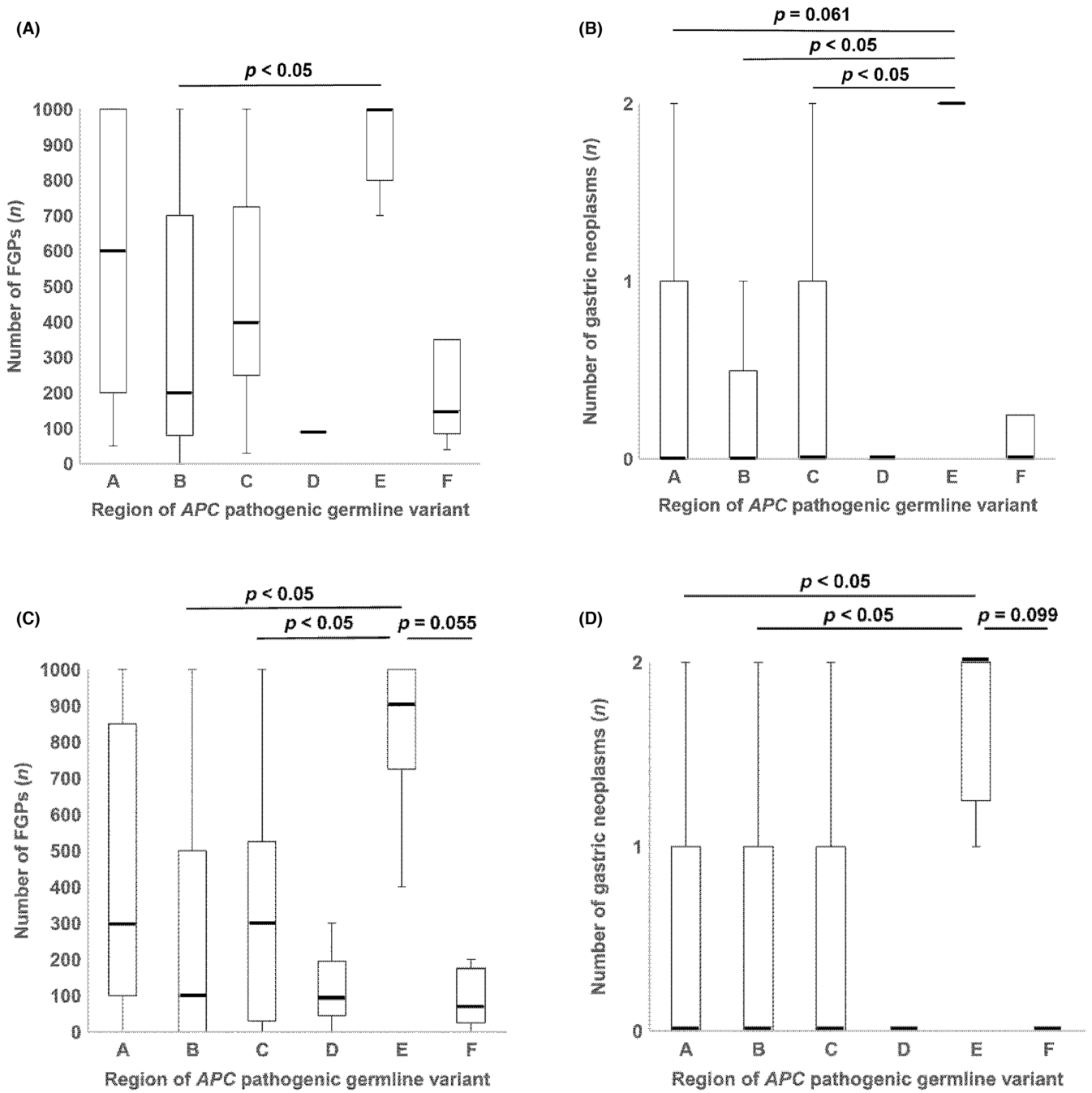


FIGURE 2 Box plots showing comparisons of each region of the *APC* pathogenic germline variant sites for gastric lesions. A total of 93 *H. pylori*-uninfected patients were examined in (A, B), and 126 patients in the current study were examined in (C, D). (A, C) The number of FGPs was plotted and compared in each region. (B, D) The number of gastric neoplasms was plotted and compared in each region.

with deficiency of codons 1462–1465, the desmoid tumor was the largest and had the highest number of recurrences, while the somatic mutation in the desmoid tumor involved allelic loss.[40] Therefore, somatic variants of LOH are suggested to be one of the explanations for the relationship between the regionality of *APC* germline variants and severe phenotypes in FAP patients. In the current study, we showed the relationship between regionality of *APC* germline variants and severe extracolonic phenotypes, which may also be related to somatic variants of LOH. However, desmoid tumors in particular were diagnosed using noninvasive methods in the current study, and few cases had tissue specimens for examination of somatic variants. Therefore, it was difficult to comprehensively evaluate the second hits of the three phenotypes including desmoid tumor. We consider this to be an issue for future study.

Phenotypic differences in FAP patients are not only influenced by the site of the *APC* genetic variant but also vary within a family. Araujo et al. reported high intra-familial diversity of extracolonic manifestations.[41] Meanwhile, in the current study, we investigated whether patients with a severe phenotype had family

members with the same phenotype. The results showed high familial accumulation of an abundance of FGPs and duodenal lesions. Age-dependent penetrance was also suggested. However, few reports have been published on the intra-familial variability in FAP patients, and the methodology for examining intra-familial phenotypes is not unified. It is thus difficult to draw definitive conclusions about the intra-familial variability, and further accumulation of cases is necessary.

Familial adenomatous polyposis and gastric neoplasms were also investigated in the *H. pylori*-uninfected subgroup. *H. pylori* is the main risk factor for sporadic gastric cancer.[42,43] Especially in Japan, where patients have a high risk of developing *H. pylori*-related gastric cancer, the effect of *H. pylori* infection could not be ruled out in this cohort. Furthermore, *H. pylori* infection has been reported to affect FGPs and gastric neoplasms in FAP patients.[44,45] However, in the current study, the results showed that variants in the aggressive region (Region E) were clearly associated with severe phenotypes for both FGPs and gastric neoplasms in *H. pylori*-uninfected patients.

One of the limitations of the current study was that the rate of FAP patients with desmoid tumors was relatively low within the range of reported rates of 8%–20%.[32,34,46,47] Abdominal surgery is generally associated with an increased cumulative risk of desmoid tumors. Meanwhile, as previously reported from our facility,[48,49] we were actively managing colorectal polyposis with endoscopic treatment. Therefore, the small number of surgical cases may have contributed to the decrease in the comorbidity rate of desmoid tumors. However, the rate of patients with desmoid tumors of 9% in the current study was still within the range of rates reported in previous studies, and it was unlikely that there was a significant selection bias. In addition, there were few patients with variants in Region D, a region of MCR that causes severe colorectal polyposis. Patients with *APC* germline variants in MCR were assumed to be difficult to manage with endoscopic intervention, and thus there were fewer referrals to our facility, at which endoscopic management is actively performed. This may have been reflected in the number of patients with variants in Region D. Although institutional bias might have existed, this study was conducted with an "extracolonic" focus, and therefore it was not considered to be a serious bias. Further, the number of cases was limited, although the current study was conducted at two referral institutes. Because FAP is a rare disease, the accumulation of further cases is necessary to improve accuracy and draw definitive conclusions.

In conclusion, in the current study, we investigated the genotype–phenotype correlation in FAP patients. The specific region of the *APC* pathogenic germline variant site would be associated with severe phenotypes of desmoid tumors and gastric and duodenal lesions. This aggressive region (Region E) may predict extracolonic comorbidities.

AUTHOR CONTRIBUTIONS

YS, YT, and HI designed the study. SI, SN, HY, YE, TN, KT, RI, TT, TY, KS, and MM recruited subjects and acquired data. YS and YT analyzed data and wrote the manuscript. All authors have approved the final submitted draft. The work reported in the paper has been performed by the authors, unless clearly specified in the text.

ACKNOWLEDGMENTS

We thank Eri Okuda (Data Manager at the Medical Research Support) for the acquisition of data. We also thank Edanz (https://jp.edanz.com/ac) for editing a draft of this manuscript. This study was supported by the Japan Agency for Medical Research and Development (AMED; 19ck0106271h0003).

FUNDING INFORMATION

This study was funded by the Japan Agency for Medical Research and Development (AMED; 19ck0106271h0003).

CONFLICT OF INTEREST STATEMENT

Yoji Takeuchi has received honoraria from Olympus. The other authors have no actual or potential conflicts of interest to declare. This organization had no role in the design, practice, or analysis of the study reported in this manuscript. Teruhiko Yoshida is a current Editorial Board Member of Cancer Science.

ETHICS STATEMENT

Approval of the research protocol by an Institutional Reviewer Board: Ethical approval was provided by the institutional review board of Osaka International Cancer Institute (approval no. 2018-18,229 and 2019-19,006).

Informed Consent: Informed consent was obtained by enabling the selected FAP patients to opt out of the study by confirming information disclosure via the Internet, in accordance with the Declaration of Helsinki.

Registry and the Registration: N/A.

Animal Studies: N/A.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Shimamoto Y, Takeuchi Y, Ishiguro S, et al. Genotype–phenotype correlation for extracolonic aggressive phenotypes in patients with familial adenomatous polyposis. *Cancer Sci*. 2023;114:4596-4606. doi:10.1111/cas.15945