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Original Article

Using genotype to assist clinical surveillance: a retrospective study of Chinese familial adenomatous polyposis patients

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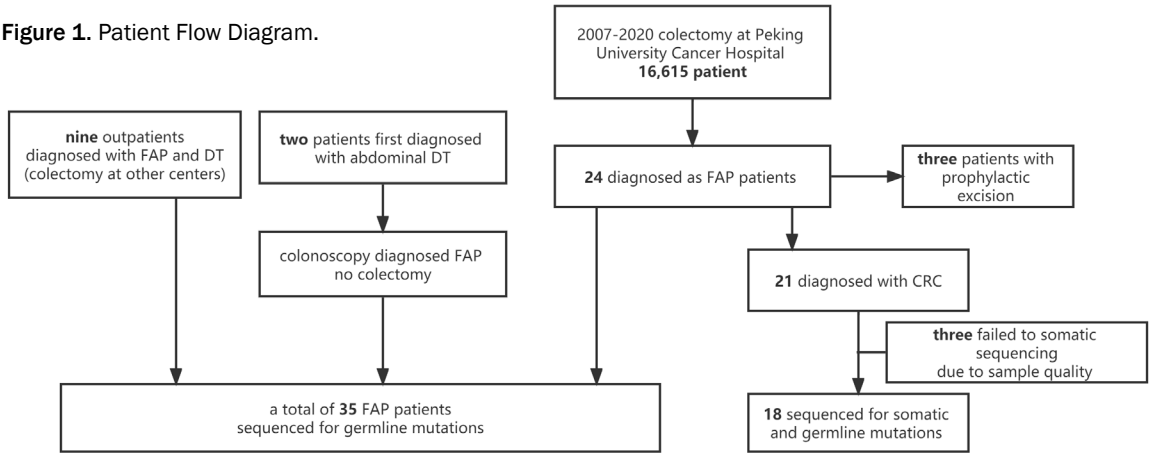
Abstract: Without treatment, familial adenomatous polyposis (FAP) patients will inevitably develop colorectal cancer (CRC) during lifetime. Yet, surgical trauma is a high risk of desmoid tumor (DT), one of the main causes of death in FAP patients. So far, the timing for colectomy is primarily based on the clinician's experience and the patient's preference; most patients undergo surgery at mid-20's. In this study, we analyzed the germline mutation distribution in 35 FAP patients from different families, 16 of them diagnosed with DTs. We also investigated the association between the molecular alterations and the clinicopathological features. Capture-based targeted sequencing using a panel of 520 genes was performed on tumor tissue and paired normal mucosa or white blood cells from 18 FAP probands who were initially diagnosed with CRC. Of all 35 FAP patients, 30 (85.7%) of them harbored germline *APC* mutations scattered from codon 161 to 1578. The mutations in the 16 DT patients scattered from codon 457 to 1578. All three patients with the mutation at the 3' of 1444 codon were diagnosed with DT. The percentage of high-risk DT (stage III or IV) harboring mutations at the 5' of 1062 or 1062-1578 was 14.3% and 77.8%, respectively, and all three patients with 3' of 1399 codon mutation had high risk. In addition, by using public database, we compared 140 FAP patients with DT to all 1880 FAP patients on the Leiden Open Variation Database and found that the odd ratio of DT in codon 159 to 495 was 0.34, while in codon 1310 to 2011 was 2.36. Compared to sporadic CRCs, the somatic spectrum of FAP CRCs was similar to the early onset CRCs, with higher *TP53* (94.1%) and lower somatic *APC* mutations (65.7%), but the *KRAS* mutation rate was the highest (58.5%). One of the 18 FAP CRCs was identified as microsatellite instability-high (MSI-H), with tumor mutation burden (TMB) of 115.65 mut/Mb. Given that no *TP53* mutations were detected in the low- and high-grade adenomas, ctDNA *TP53* sequencing might be used for the close monitoring before FAP colectomy. In conclusion, except mutations at the 5' end of *APC* (5' to 495), all FAP patients need to consider the risk of DT after colectomy. The chance of life-threating DTs was higher in patients with 3' 1062 codon mutation and peaked in patients with 3' 1399 codon mutation. Scheduled monitoring of *TP53* ctDNA is proposed to be a novel tool for optimizing the operation time.

Keywords: Familial adenomatous polyposis, colorectal cancer, desmoid, somatic mutation

Introduction

Colorectal cancer (CRC) is one of the most common malignancies worldwide. Approximately 5-10% of CRCs are caused by hereditary colorectal cancer syndromes, including Lynch syndrome, familial adenomatous polyposis (FAP) diseases, and Peutz-Jeghers syndrome [1]. FAP is an autosomal dominant condition primarily caused by germline *APC* mutations which lead to the truncation of APC and the constitutive activation of the Wnt pathway, as well as the increased mitotic errors and chromosomal instability [1-3]. If FAP is not recognized and treated, almost 100% of FAP cases will develop CRCs, which is the leading cause of death in patients with FAP [4]. As a result, an aggressive early diagnosis and management

Figure 1. Patient Flow Diagram.



strategy is recommended, with the National Comprehensive Cancer Network (NCCN) guideline [1] recommending sigmoidoscopy or colonoscopy every 1 to 2 years beginning at the age of 10 to 12 for classic FAP family members and prophylactic total proctocolectomy during the late-10 s to mid-20 s.

To date, there are no specific guidelines for the timing of prophylactic surgery for FAP patients [5], and the treatment decision mainly relies on the clinician's experience and the patient's preference. Normally, a (procto) colectomy is recommended if the FAP patient develops CRCs, advanced histologic features in polyps, large number of adenomas >5 mm, and an increasing polyp burden beyond endoscopic control [5, 6]. However, this surgical procedure severely compromises patient's quality of life and fertility [7], and more importantly, it may cause 21-31% of FAP patients to have desmoid tumors (DT) as a complication, which is the second cause of death in FAP patients [8, 9]. Other than abdominal surgery, a family history of DT, specific *APC* mutation, and even gender (female), have been reported as high-risk factors in FAP-associated DT. As a result, for patients with high risk, the prophylactic surgery should be performed as late as possible but before cancer development.

With the advancement of chemoprevention and colonoscopy surveillance, young patients with controlled polyp burden could postpone their surgery until there's a compelling reason [10, 11]. In this case, the prediction of DT by genotyping in FAP is important as the susceptibility to DT is correlated with *APC* mutations between codon 1395 and 2000. Other studies also showed a high risk of DT with the mutations at the 3' of codon 1444 [12, 13]. However, when only analyzing intra-abdominal desmoids which are the most life-threatening subtypes, this correlation is no longer statistically significant [14].

Therefore, the aim of this study was to identify patients with a high risk of severe DT phenotype by using next-generation sequencing (NGS) and to identify novel non-invasive methods of CRC monitoring. We first collected the clinical characteristics and genotypic spectrum of 35 Chinese FAP patients, 16 of them pathologically diagnosed with desmoid tumor. Next, we screened both germline and somatic variants of 18 FAP probands who were initially diagnosed as CRC by targeted next-generation sequencing. Furthermore, we analyzed the clinical characteristics and the disease outcomes of these patients and compared the somatic mutations between the sporadic CRCs and the hereditary CRCs with FAP background. The findings from our study will shed light on our understanding of FAP with DT and provide the rationale for choosing the best treatment options and the surgical timing in current clinical practice.

Methods

Patients

Figure 1 shows the patient flow in the present study. All patients under colectomy from January 2007 to June 2020 at Peking University Cancer Hospital (Beijing, China) were screened,

and a total of 24 unrelated probands with a clinically suspected adenomatous polyposis syndrome were enrolled in this study. Patients with hamartomatous polyposis syndromes such as juvenile polyposis syndrome, Peutz-Jegers syndrome and Cowden syndrome were excluded. Detailed family history and medical records, including pathologic, radiologic, and endoscopic examinations, were retrospectively reviewed for all the families. The proportion of FAP patients was significantly lower than expected (0.14%), which might be due to the subject referral bias. As a cancer-specialized hospital, most of our FAP probands were diagnosed initially with CRC, while most FAP patients with prophylactic surgery chose general hospitals where they underwent long-term follow-up.

Nine outpatients from different families diagnosed with FAP and DT who underwent colectomy at other hospitals were also included for germline mutation sequencing (**Figure 1**). In addition, two patients who were initially diagnosed as abdominal DT but were further examined using colonoscopy and identified as FAP, were also enrolled for germline mutation screening. All 35 FAP patients included in this study were probands without chemotherapy prevention or endoscopic polyp treatment before colectomy. This study was performed in compliance with the Declaration of Helsinki and was approved by the ethics committee of the Peking University Cancer Hospital. Written informed consent was obtained from all participants.

Mutation and data analysis

DNA isolation and targeted sequencing were carried out as previously described [15-17] at Burning Rock Biotech, a commercial clinical laboratory accredited by the College of American Pathologists (CAP) and certified by the Clinical Laboratory Improvement Amendments (CLIA). Target capture was performed using a commercial panel consisting of 520 genes (OncoScreen Plus), spanning 1.64 megabases of the human genome. The 520 gene list and 98 cancer susceptibility genes included in the germline mutation analysis were described previously [17]. Briefly, genomic DNA of formalin-fixed, paraffin-embedded (FFPE) tumor tissues and peripheral blood samples were extracted using the QIAamp DNA FFPE tissue kit or QIAamp DNA Blood Mini Kit, respectively, according to the manufacturer's protocol (Qiagen, Hilden, Germany). Indexed samples were sequenced on Nextseq 500 (Illumina, Inc., CA, USA) with paired end reads and an average sequencing depth of 1,000× for tissue samples and 10,000× for liquid biopsy samples.

Tissue samples were compared with their own peripheral blood sample control to identify somatic variants. Loci with depth less than 100 were filtered out. Base calling required at least 8 supporting reads for single nucleotide variations (SNVs) and 2 and 5 supporting reads for insertion-deletion variations (Indels), respectively. Variants with population frequency over 0.1% in the ExAC, 1000 Genomes, dbSNP or ESP6500SI-V2 databases were grouped as single nucleotide polymorphisms (SNPs) and excluded from further analysis. The MSI status was determined based on a read-count-distribution-based method as previously published [18, 19]. The threshold for germline variant detection was set at a variant allele frequency (VAF) of 10%. The TMB per patient was computed as the ratio between the total number of non-synonymous mutations detected and the total coding region size of the panel used by this equation [17]:

$$\text{TMB} = \frac{\text{mutation count (except for CNV, SV, SNPs, and hot mutations)}}{1.003\ \text{Mb}}$$

Online data collection for APC mutation analysis

From the Leiden Open Variation Database (LOVD) [20], we first downloaded the information of 4513 FAP patients with *APC* mutations. Among them, 1880 patients with germline pathogenic *APC* mutations were filtered for further analysis (Supplementary Table 1). Patients with large *APC* deletions or mutations that could not be assigned a codon number were excluded from the analysis.

Statistics

The data were analyzed by SPSS 19.0 software, and *P* values less than 0.05 were considered statistically significant. Fisher's exact test was performed to compare the mutation rate between the reference population and patients with FAP and desmoids tumors. The odds ratio

Table 1. Patient characteristics

Features		Number
Gender	Male	20 (57.1%)
	Female	15 (42.9%)
FAP Family History	Yes	17 (48.6%)
	No (de novo)	18 (51.4%)
Polyps Number	<100	3 (8.6%)
	100-1000	30 (85.7%)
	>1000	2 (5.7%)
CRC	Yes	30 (85.7%)
	No	5 (14.3%)
Median Age	First diagnosed FAP/GI symptoms*	36 (10-63)
	First diagnosed CRC	39 (25-63)
Extraintestinal Manifestations	Desmoid tumors	16 (45.7%)
	Gastric polyps	8 (22.9%)
	Duodenal cancer	1 (2.9%)
	Gastric cancer	2 (5.7%)
	Thyroid Cancer	2 (5.7%)
Germline *APC* Mutations	Yes	30 (85.7%)
	No	5 (14.3%)

*Colonic bleeding or unexplained diarrhea.

and 95% exact confidence interval were determined. The 95% confidence interval of the significant odds ratio did not cross 1.

Results

Patient characteristics

The clinical characteristics of 35 FAP probands were summarized in **Table 1**. After colonoscopy screening of their relatives, including elder relatives, siblings, and cousins, more than 40% of these FAP patients were with *de novo* disease, which was consistent with Chinese FAP patients [21] and presented a much higher rate than reported in published data (usually 25%).

Furthermore, the age of patients when gastrointestinal symptoms first appeared varied from 10 to 63 years (median 36). Among the 35 cases, only 3 (8.6%) had prophylactic surgery, and 30 (85.7%) had therapeutic surgery after the diagnosis of CRC.

APC germline mutations

Among the 35 FAP probands examined, 30 probands (85.7%) harbored germline *APC* mutations scattered from codon 161 to 1578 (**Figure 2**). The other 5 probands were confirmed without large deletion by repeated bioinformatics analysis. As for *APC* mutations, the hotspots were truncating mutation in codon 1062 found in 4 patients (13.3%), E1309 frame shift in 2 patients, and S1068 frame shift in 2 patients. This is consistent with the previous reports that the mutational hotspots in *APC* gene are mostly located in the 5' of exon 15, with codons 1,309 and 1,062 accounting for approximately 17% and 11% of all germline *APC* mutations, respectively [22]. In sum, the mutations in 21 FAP probands (70.0%) were localized in exon 15, from codon 659 to 1578. Only the mutations in 5 FAP probands (16.7%) were localized in the central mutation cluster region (MCR, codons 1250-1464), and 4 of them were diagnosed with DT.

APC germline mutation codons and the risk of desmoid tumors

Patients with desmoid tumors (DT) were found with germline *APC* mutations scattered from codon 457 to codon 1578. In the five patients without germline *APC* mutation, none of them presented DT. All three patients with mutations on the 3' of codon 1444 were pathologically diagnosed with DT, while in the 27 patients with mutations at the 5' of codon 1444, 13 of them (48.1%) developed DT, consistent with the previous reports that the risk of DT in patients with mutation at the 3' of codon 1444 had odds ratio of 3.0 to 7.19 compared with mutations in other sites [14, 23]. Notably, the two patients with rapid DT growth and diagnosed DT before colectomy harbored mutations in the codon 1450 and 1462, respectively. In addition to the incidence, the severity of the desmoid disease in FAP has been reported to be related to genotype as well [24]. We observed a similar result in our study. The clinicopathological features and the genotype of all 16 FAP-associated DTs were summarized in **Table 2**. Specifically, the female to male ratio was 9:7, and only three patients had DT family history (18.8%). The median age of DT diagnosis in females was 36

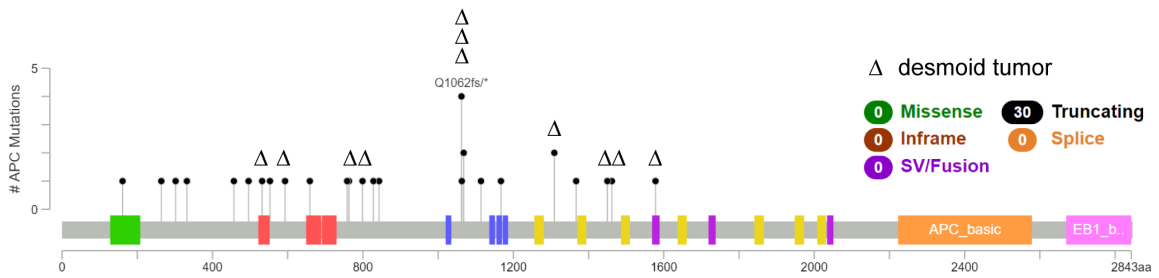


Figure 2. Schematic diagram showing the position, distribution of all *APC* pathogenic germline mutations in 30 Chinese FAP patients (5 patients without gAPC mutation were excluded), all were truncating mutations. Δ showed patients with DT.

years ranging from 30 to 50, while in males was 35 ranging from 28 to 53. The median post-operative time till DT diagnosis was 21 months ranging from 7 to 62 months. Different from sporadic DTs, FAP-associated DTs are mainly intra-abdominal and tend to be treated more aggressively [25]. DTs were staged according to the Cleveland staging system [24, 26], and the percentage of severe DT of stage III and stage IV harboring mutations at the 5' of codon 1062 or 1062-1578 in this study was 14.3% and 77.8% (1/7 and 7/9, P=0.012), respectively. In consistent with the previous report that DTs with mutations in the codon 3' of 1399 exhibited the most aggressive phenotype, our patients with the 3' of 1399 codon mutation had the severe type of DT [24].

Moreover, we observed that among all the FAP patients, only 18.8% of them exhibited mutations at the 3' of codon 1444. We then integrated our results with previous published data [27-30] to calculate the odds ratios for the risk of desmoids tumors in the 8 regions of *APC* gene (**Table 3**). We used LOVD as a reference database to calculate odd ratios. The results showed that FAP patients with DT could have mutations at any spot of *APC* gene. The 5' end region, codon 159 to 495, was a protective factor with an odd ratio of 0.34 (0.20-0.57), while the 3' end region, codon 1310 to 2011, was a risk factor with an odd ratio of 2.36 (1.61-3.46). More than half of the patients carried mutations at the center region between codon 532 to 1309, and the occurrence of DT showed no significant difference from natural distribution.

Somatic mutation spectrum and clinical characteristics of 18 FAP patients with CRC

To investigate the somatic mutation landscape of CRCs with FAP background, we extracted the genomic DNA from 18 FFPE CRC samples and the paired normal mucosa or white blood samples and performed next-generation sequencing for a panel of 520 cancer related genes and MSI determination. Collectively, we identified 279 mutations spanning 162 genes, including 225 single nucleotide variations, 5 insertions or deletions, 31 copy-number amplifications, and translocations (Supplementary Table 2). Notably, the sample from the patient with MSI-H showed 131 mutations and TMB of 115.65 mut/Mb. For all MSS FAP patients, the median TMB was 2.99 ranging from 0-11.96 mut/Mb (Supplementary Table 3). The clinicopathological features of all patients including epidemiology, TNM staging, family history, somatic *APC* mutation, and prognosis were listed in Supplementary Table 3.

For gene mutations, the most common mutations were *TP53* (94%), *APC* (65%) and *KRAS* (59%), whereas all other gene mutations were found in fewer than 2 patients (**Figure 3**). *EPCAM* amplification was detected in 2 stage III CRC patients, which could be caused by the loss of DNA methylation in the *EPCAM* gene as a result of *TP53* disfunction, and was associated with worse survival [31]. Two patients were detected with *GNAS* amplification but without germline *APC* mutation; *GNAS* amplification in ovarian cancer was reported as an independent biomarker for worse prognosis [32]. As for the commonly used targeted biomarkers, one patient had the *KRAS* G12C mutation, while one patient had *ERBB2* amplification, No *BRAF* or *NRAS* mutations were detected in our samples.

We also compared the alteration rate in the genes of interest between early-onset (<40 years-blue) and later-onset (≥50 years-orange) in 9615 MSS CRC patients and 193 MSS CRC

Table 2. Clinicopathological features and genotype of 16 FAP associated DT patients

Mutation site	Gender	Maximum diameter (mm)	DT location (IA, AW)	Behavior[a]	Symptoms	Staging	Colectomy type	DT time after surgery (month)	Age[b]	DT family history
S457*	male	165	IA	rapid	bowel obstruction	IV	Laparoscopic IRA	15	29	no
Q532*	female	16	IA+AW	slow	none	I	Open IPAA	14	50	no
W593*	male	103	IA	slow	none	II[c]	Laparoscopic IPAA	11	35	no
E763*	female	72	IA	slow	none	II	Open IRA	22	31	no
Y799*	male	83	IA+AW	slow	none	II	Open IRA	7	39	no
T828fs	male	83	IA	slow	none	II	Laparoscopic rectal cancer resection	62	53	yes
S843fs	female	46	IA	slow	none	II	Laparoscopic IRA	16	30	no
Q1062fs	female	19	IA	stable	none	I	A. Open IPAA B. Open rectal cancer resection and duodenectomy	144 after A 24 after B	48	no
Q1062*	male	180	IA	slow	none	III	Open IRA	15	37	no
Q1062*	male	206	IA+AW	rapid	none	IV	Laparoscopic IPAA	21	32	no
Q1063fs	female	279	IA	rapid	ureteric obstruction	IV	Open IRA	12	47	no
E1309fs	female	48	IA	slow	ureteric obstruction	III	Open IPAA	36	30	no
Q1367*	female	10	IA	stable	none	I	Laparoscopic IPAA	22	33	no
R1450*	male	192	IA	rapid	life-threatening	IV	NA	NA	28	yes
K1462fs	female	80	IA+AW	rapid	bowel obstruction	IV	NA	NA	36	yes
C1578fs	female	56	IA+AW	slow	ureteric obstruction	III	Open IPAA	21	42	no

IA = intra-abdominal/mesenteric, AW = abdominal wall, NA = not available, IRA = ileorectal anastomosis, IPAA = proctocolectomy with ileal pouch-anal anastomosis, a. Rapid: >50% diameter in 3 months. b. age at diagnoses of DT. c. maximum diameter grew from 9.1 cm to 10.3 cm in 5 months, with completely no symptoms, comprehensively evaluated as stage II. '*' is used to indicate a translation stop codon.

Table 3. Influence of mutation codon on desmoid occurrence

Codon	Number in Published Articles (124) combined with our data (16)	% in Published Population and our data	Number in Reference Database (1613 total)	% in Reference Group	Odds Ratio (95% CI)	*P*-value
159-495	16	11.4	447	27.7	0.34 (0.20-0.57)	<0.0001
532-713	13	9.3	200	12.4	0.72 (0.40-1.30)	0.14
721-972	18	12.9	196	12.2	1.07 (0.64-1.79)	0.40
976-1067	14	10.0	184	11.4	0.86 (0.49-1.53)	0.31
1068-1237	15	10.7	167	10.4	1.04 (0.59-1.82)	0.45
1256-1303	6	4.3	42	2.6	1.67 (0.70-4.01)	0.12
1309	15	10.7	122	7.6	1.47 (0.83-2.58)	0.09
1310-2011	43	30.7	255	15.8	2.36 (1.61-3.46)	<0.0001

patients from the TCGA-COAD dataset [33, 34], as well as our FAP CRC dataset (Supplementary Figure 1). We found a high prevalence of *TP53* loss (94.1%) in all data analyzed, which was even higher in the FAP CRC dataset. The *TP53* mutation frequency has been reported to be different among different age groups, suggesting the involvement of genomic instability in alternative carcinogenesis [35]. We then compared the clinical characteristics between the FAP CRC and the sporadic CRC in TCGA CRC cohorts (Supplementary Figure 2). FAP CRC had a significantly lower age distribution ($P<0.00001$), lower MSI percentage, and left-sided preference.

Theoretically, without close endoscopic intervention for patients with classic FAP, the older the time of initial diagnosis is, the higher depth of tumor invasion will be. In this study, the median age increased progressively with T2 to T4 infiltration (**Figure 4**). The 2 patients diagnosed at T1 stage with good prognosis had gAPC at 5' end (Q161* and R332*), and they were diagnosed as FAP probands at 54 and 63 years old, respectively. However, the third patient with 5' end gAPC mutation (Q264*) was diagnosed as rectum cancer with liver metastasis at the age of 36 (Supplementary Table 3). On the other hand, lymph node or distant metastasis was not correlated with increasing age ($P=0.90$), suggesting that for some patients mid-30s were already too late, while a small group of patients could delay colectomy to their 50 s. For prediction, neither polyp numbers nor 5' end of *APC* mutation were reliable markers; therefore, identifying novel biomarkers for tumor monitoring is an urgent need.

We then integrated our data with the published somatic sequencing results of adenomas and CRCs [36] (Supplementary Figure 3). The somatic mutation of *APC*, *KRAS* and *GNAS* were frequently detected at both low- and high-grade adenomas. *TP53*, which occurs later in the adenoma-carcinoma sequence [37], showed no mutation in low- and high-grade adenomas but 85% in FAP-CRC tumors, suggesting *TP53* as a potential biomarker for CRC early detection.

Discussion

The most effective and the only way to prevent the development of CRC for FAP patients is surgical treatment. The risk of CRC before the age of 20 is less than 1%, and NCCN guidelines recommend *APC* gene testing begins at 10 years old, endoscopies at 10-15 years old, but no prophylactic surgery before 18 [38]. Ideally, patients with FAP should undergo a prophylactic colectomy right before the development of CRC. Currently, the recommended time of prophylactic surgery is before mid-20 s [39]. However, the possibility of sexual dysfunction after rectal resection, the risk of infertility, the need of psychological support, and the higher risk of DT after colectomy should be discussed with these young adults [40]. With the advancement on chemo-prevention [41, 42] and close endoscopic monitoring, whether some of the FAP patients can postpone the operation is under debate [43]. In supporting this, a multi-institutional study indicated that for attenuated FAP patients, the development of CRC might be at the age of 46 [44]. We noticed that even in profuse FAP patients, CRC was not detected in few patients older than 50 who underwent colec-

Figure 3. Somatic mutation landscape of MSS CRC in 17 MSS FAP patients. Heatmap illustrated the top 30 genes identified in our study. Each column represented one patient, while each row represented an alternation. Upper bars represented the tumor mutation burden. The left bars indicated the frequency of mutated genes. A color key was on the right side. The sequence from left to right was based on the clinical staging of CRC. (AFAP = Attenuated FAP, polyp number <100; IFAP = Intermediate FAP, 100< polyp number <1000; PFAP = Profuse FAP, polyp number >1000).

tomy [44], suggesting that not all FAP patients need they surgical intervention at early age. How to stratify this population of patients will be a significant question to answer.

Due to the dysfunction of *APC*, patients with FAP have 800 times more risk of DT than the general population [45, 46]. In clinical practice, it is difficult to differentiate between peritoneal lymph node metastasis of advanced colorectal cancer and DT. The prevalence of DT could reach up to 21-35% in FAP patients [47], leading to poor prognosis. Surgical trauma is a risk factor for DT with 44% of occurrence risk in FAP

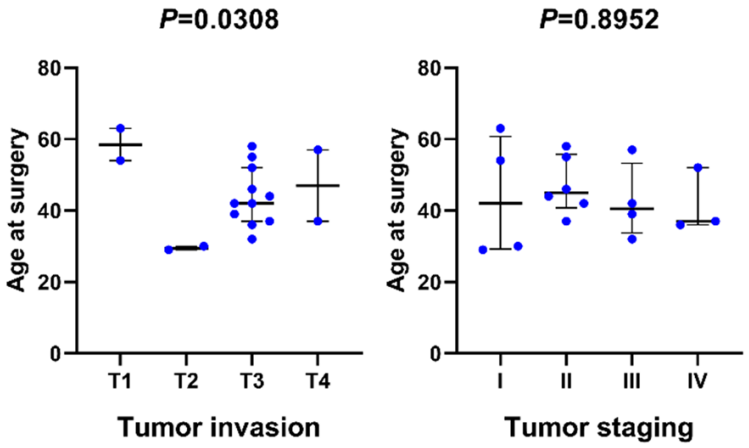


Figure 4. Association of age with depth of tumor invasion (left panel) and tumor staging (right panel) in 17 MSS FAP patients (one-way ANOVA, lines indicate bottom quartile, median and top quartile).

patients [48]. One study from Finland reported that among the 180 FAP patients who underwent prophylactic colectomy before 25, 6 of them developed DT and died at the mean age of 32 [39]. Hence, distinguishing between the attenuated subtype and the high susceptibility of DT is pivotal, and identifying biomarkers that stratify patients who will stay cancer-free at older age is an urgent medical need.

The genotype associated with severe or attenuated FAP was frequently reported [22, 49, 50], and most of studies indicated *APC* mutation at the 3' of 1444 colon was a significant risk factor of DT [12]. Consistent with previous studies [22, 24], we found that mutation at the 3' end of *APC* (codon 1310 to 2011) was associated with higher risk of DT (ORR 2.36, $P<0.01$), while the mutation at the 5' end of *APC* (codon 159 to 495) was associated with lower risk of DT (ORR 0.34, $P<0.01$). Additionally, previously study revealed that the extreme 3' of *APC* is also related to DT [24]. Roughly, *APC* mutations in all the regions account for more than 10% of DT occurrence, indicating that patients harboring *APC* mutation will have a risk of DT after surgery. Another study involving 77 FAP-associated DT patients concluded that in the patients with *APC* mutation at the 5' of codon 400, the desmoids were <10 cm and stable, while in the 43.5% of DTs with mutations at the 3' of 1399 and 29.5% of DTs with mutations in between, the desmoids were >10 cm and with severe symptoms [24]. Similarly, in this study, the percentage of high-risk DT was much higher in patients with mutation in codon 1062 to 1578 than in patients with mutation at the 5' of 1062 ($P=0.012$), and all three patients with mutation at the 3' of 1399 showed severe symptom. Together, the incident of DT in patients with mutation at the 5' end of *APC* (5' to codon 400 or 495) is significantly low, and the symptom is mild. In contrast, patients with mutation at the 3' of 1062 may have DT with aggressive symptom, and mutation at the 3' of 1399 is the most concerned region causing worst symptom. Based on these findings, opting for surveillance rather than immediate prophylactic surgery may be more beneficial for patients with low cancer risk and high severe DT risk.

By exploring the difference between the FAP-associated CRCs and sporadic CRCs, we found that the somatic mutation spectrum was similar to that of early-onset CRCs. In addition, when we compared the data with previous low- and high-grade adenomas [36] (Supplementary Figure 3), our data also indicated *TP53* mutation as the last event in the sequential accumulation of mutations from adenomas to carcinomas [51] since no somatic *TP53* mutations were found in the low- or high-grade adenomas; however, *TP53* mutation was present in 85% of FAP-CRC tumors (17 in 20 patients). Thus, we suggested that for patients who are strongly against prophylactic colectomy or with good medical reasons to postpone the surgery, using ctDNA *TP53* testing to closely monitor the disease progression may be feasible. Indeed, as a promising candidate for blood-based CRC screening, the sensitivity of using *TP53* to predict stage I/II well-differentiated CRC can reach 93% [52]. Importantly, *TP53* pathologic mutations were detected in all our 10 FAP-associated CRCs diagnosed at stage I and II. Therefore, our findings further suggest that *TP53* might serve as a potential biomarker for the detection of early CRC, which offers an alternative non-invasive liquid biopsy method (e.g., plasma circulation tumor DNA).

The detection for MSI status, HER2 expression, and *KRAS* mutation are generally required for the treatment of FAP-associated metastatic CRCs. If patients exhibited with both FAP and

MSI, as 6 out of 45 FAP patients in our study showed dMMR [53], they might benefit from immunotherapy [54].

This study had some limitations. First, it was a retrospective, observational, single-center study with a limited sample size and referral bias. Second, APC mosaicism was not tested in this study, while the somatic results might help in some *de novo* APC mosaicism cases [55]. The panel that we used for targeted sequencing in this study was not designed for LGR (large fragment rearrangement) detection. Thus, the five mutation-negative patients identified in our *APC* germline mutation analysis might harbor LGR. Third, the segmentation of *APC* mutation regions was based primarily on references from largely Caucasian patients, and due to the small sample size, the difference across ethnicities was not examined.

In summary, we postulated that some FAP patients may be able to delay surgery with close endoscopic monitoring depending on the genotype-phenotype of DT incidence and symptom. Additionally, we presented the first somatic mutation spectrum of CRCs associated with FAP and proposed the addition of *TP53* ctDNA as an additional surveillance measure. These findings will help optimize FAP patients' treatment plan and time of surgery in clinical practice.

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Disclosure of conflict of interest

None.

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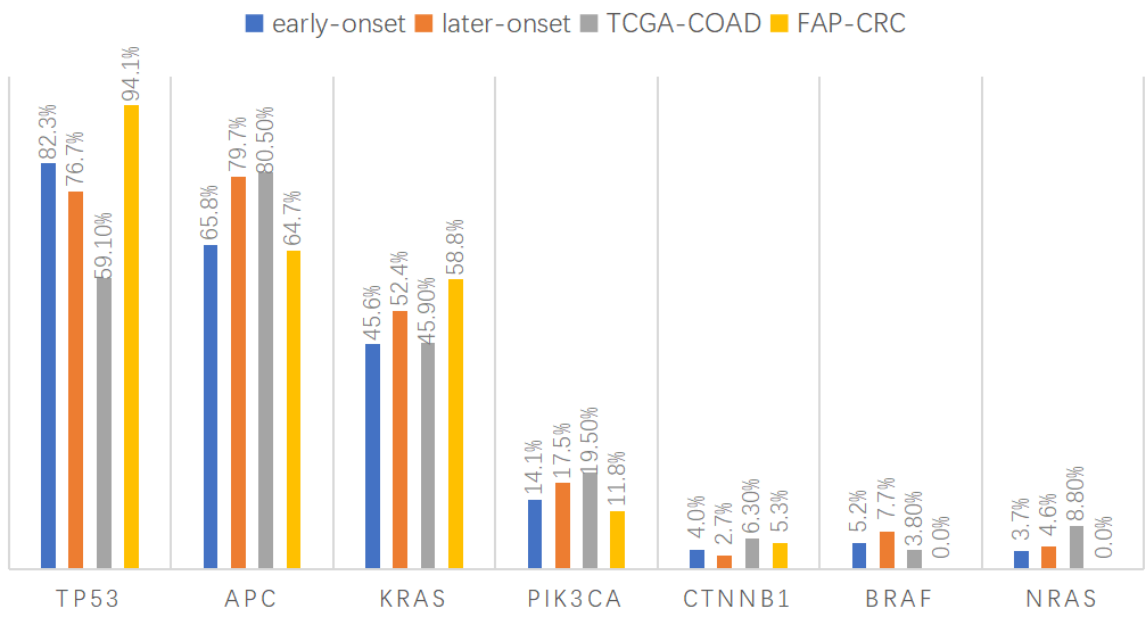
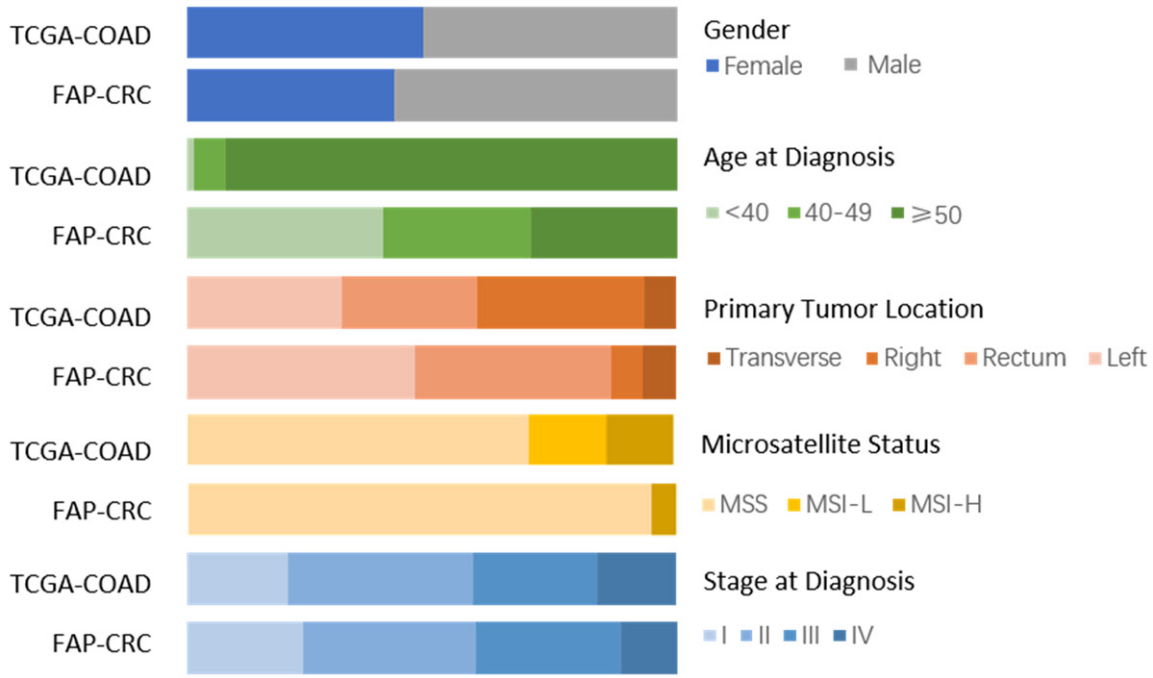
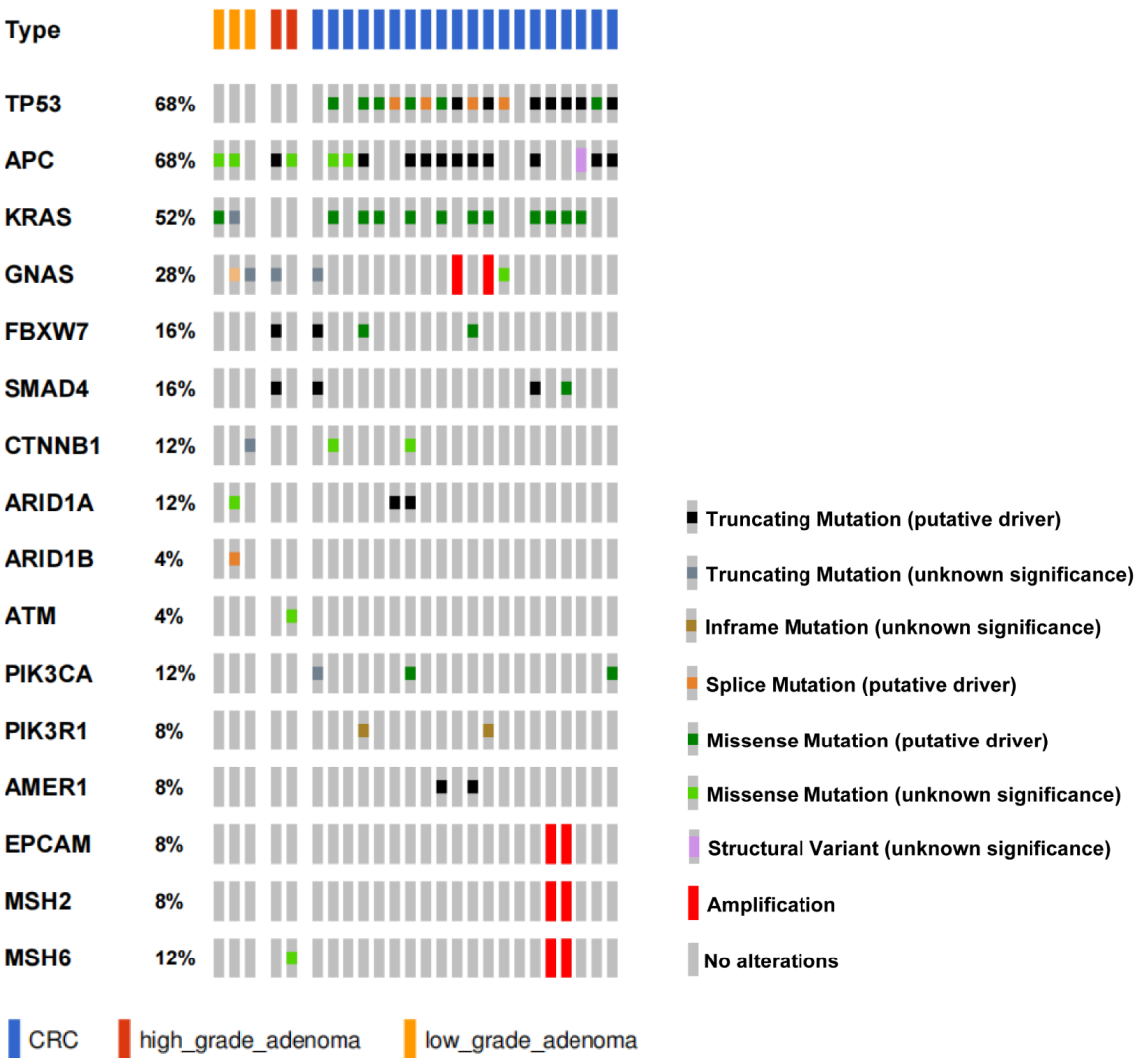


Supplementary Figure 1. Top somatic mutations in MSS patients of different datasets.



Supplementary Figure 2. Comparison of clinical characteristics between the FAP CRC and TCGA CRC cohorts. Comparing to sporadic CRC, the only significant different characteristic is age distribution *(P*<0.00001).



Supplementary Figure 3. Somatic mutations of adenomas and CRCs. Merging analysis of reference {Li, 2020 ref#37} and our 17 MSS FAP CRC patients. In total, the most frequently mutated were *TP53* (68%), *APC* (68%), *KRAS* (52%) and *GNAS* (28%). However, *TP53* is only found in tumor samples which indicate the potential of monitoring *TP53* ctDNA may help decide the timing of surgery.