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Adenomatous Polyposis Coli Gene Mutations in 22 Chinese Pedigrees with Familial Adenomatous Polyposis

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Background: Familial adenomatous polyposis (FAP), which has a very high tendency of progression to colorectal cancer, is mainly caused by mutations of the adenomatous polyposis coli (APC) gene. This study systematically screened the *APC* mutations and observed the correlation of *APC* mutations with clinical manifestations of FAP.

Material/Methods: Eighty subjects (probands and their family members of 22 FAP pedigrees) were enrolled, underwent abdominal ultrasound, computed tomography, and colonoscopic examinations, and were assessed for *APC* mutations between January 2010 and June 2015 at Tianjin Union Medical Center. Peripheral blood was collected from subjects, and DNA was extracted and screened for *APC* mutations using multiplex ligation-dependent probe amplification for large-fragment deletions or PCR-denaturing high-performance liquid chromatography with DNA sequencing for micromutations.

Results: Nineteen of 22 FAP pedigrees were found to have mutations of *APC*, and 17 types *APC* mutations were identified. All the mutations were heterozygosity with autosomal dominant inheritance. *APC* mutations included 8 caused by frameshift, 3 by aberrant splicing, 2 by missense mutation, 2 by nonsense mutation, and 2 by large-fragment deletion. Frameshift mutation was the most common type of *APC* mutation, and Coding DNA Sequence 15 was the most common mutation site. Five novel *APC* mutations, including 1 with large-fragment deletion, were identified.

Conclusions: We systematically screened 17 mutations of *APC* from 22 Chinese pedigrees with FAP. This study will broaden the spectrum of known *APC* germline mutations and help understand the types and distribution of *APC* mutations among Chinese patients with FAP.

MeSH Keywords: Adenomatous Polyposis Coli • Adenomatous Polyps • Mutation • Pedigree

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Background

Familial adenomatous polyposis (FAP), mainly histologically characterized by the appearance of numerous (tens to thousands of) adenomas in the colon and rectum, with an estimated prevalence of 1/11 300–37 600, is a high risk (up to 100% risk without timely intervention) for progression to fatal colorectal cancer before the age of 40 years [1,2]. Effective conservative therapeutic methods are lacking, and the total prophylactic proctocolectomy is essential for prevention of FAP [2].

As an autosomal dominant inherited disease, FAP is mainly caused by mutations of the adenomatous polyposis coli (APC) gene (OMIM #175100) [1]. *APC* is large in size and is located on chromosome 5q21, encoding a 2843-amino acid APC protein [3]. *APC* contains 21 exons with a length of 8535 bp and exon 15 is the largest, covering over 75% of the coding sequence of *APC* [4]. *APC* is a tumor-suppressor gene and encodes a protein with multi-domains and complex functions, participating in regulation of the Wnt-signaling pathway, intercellular adhesion, cell cycle, and apoptosis [4]. APC protein regulates β-catenin, a key component of the Wnt/β-catenin signaling pathway, which plays an important role in colorectal cancer development [5]. *APC* mutation-induced inactivation of APC protein stimulates the Wnt/β-catenin pathway by increasing the nuclear accumulation of β-catenin [6], which induces the expression of various proto-oncogenes such as *c-myc* [2].

Pathogenic germline *APC* mutations are mainly caused by frameshift, nonsense mutation, aberrant splicing, missense mutation, and large-fragment deletion within the *APC* coding sequence [7], which can lead to genome rearrangement, causing mutated or truncated APC protein. In HGMD (*http://www.hgmd.cf.ac.uk/ac*) and LOVD (*http://www.genomed.org/lovd2/home.php?select_db=APC*), more than 1100 types of *APC* germline mutations have been identified, covering the whole *APC* gene. The location and types of the *APC* germline mutations gene are directly associated with the pathological manifestations and onset age of FAP [8].

Occurrence of *APC* mutation types may vary among different patient populations. For example, it was demonstrated that the APC I1307K and E1317Q mutations were not detected in Chinese patients with colorectal cancer [9]. Therefore, it is important to systematically screen the *APC* mutations among Chinese patients with FAP.

APC mutations have been identified in Chinese pedigrees with FAP. Liu et al. [10] reported 3 novel *APC* mutations (c.2510C>G, c.2016_2047del, and c.3180_3184del) from 5 Chinese FAP families, which caused truncated APC protein. Zhang et al. [11] identified 2 novel *APC* pathogenic variants (c.794_795insG/p.Val266SerfsTer11 and c.2142_2143insG/p.His715AlafsTer19) in 5 Chinese families with FAP. We recently identified a splice-acceptor site mutation c.1744-1G>A in intron 14 [12], single-nucleotide deletion-caused frameshift mutations c.3418delC (p.Pro1140Leufs*25) in exon18 [13], c.3992_3993insA (p.Thr1332Asnfs*10) in Coding DNA Sequence (CDS)15 [14], and a large-fragment deletion (exon5-exon16; c.423_8532del) [15] of *APC* in Chinese FAP pedigrees. However, these reports were mainly based on a small number of pedigrees.

In this study, we enrolled 22 FAP pedigrees to screen the APC gene mutations and observe the correlations of characteristics (e.g., distribution and types) of *APC* mutations with the clinical manifestations of FAP. Our results will broaden the spectrum of known *APC* germline mutations and help understand the types and distribution of *APC* mutations among the Chinese patient population. In addition, this study can be referenced for the determination of early resection for FAP, which might be beneficial to prevention and better treatment for FAP-associated colorectal cancer.

Material and Methods

Patients

Eighty subjects (probands and their family members of 22 FAP pedigrees), registered and diagnosed with FAP between January 2010 and June 2015 at the Registration Center for FAP Pedigrees, Tianjin Union Medical Center (Tianjin, China), were enrolled. This study was approved by the Ethics Committee of the hospital. Written consent was signed by each subject. We collected basic medical information (such as disease history), performed abdominal ultrasound, computed tomography, and colonoscopic examinations, and detected the *APC* mutations all probands. In each pedigree, after the diagnosis of FAP and detection of *APC* mutations in the probands, some of their family members were contacted and asked to receive *APC* mutation screening for health guidance, and the positive subjects (with *APC* mutations) older than 12 years then underwent colonoscopic examination for presence or absence of FAP. A peripheral blood sample was taken from each subject and DNA was extracted for gene detection.

Diagnosis

The diagnosis of FAP was based on clinical manifestations (e.g., abdominal pain, diarrhoea, hematochezia, abdominal distension, pernicious vomiting, and abdominal mass), abdominal examination (e.g., abdominal swelling, tenderness, existence and size of mass), colonoscopic observation (e.g., existence, number, size, morphology, site of polyps in the colon), abdominal computed tomography observation (e.g., abdominal mass and desmoids), and histopathological examination.

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Screening of the APC mutations

The *APC* mutations were screened by PCR-denaturing high-performance liquid chromatograph (DHPLC) and DNA direct sequencing for micromutations, or by multiplex ligation-dependent probe amplification (MLPA) for large-fragment deletions.

For micromutations, DHPLC was carried out after PCR of the extracted DNA from the blood sample to monitor the PCR product with specific abnormal elution profile (waveform) using the WAVE DHPLC system (Transgenomic, Omaha, NE, USA), with a single injection volume of 5-8 µL, column temperature of 55–62°C, mobile phase of 0.1 mM N-triethyl acetamide and different concentrations of ethyl cyanide, flow velocity of 0.9 ml/min, and detection wavelength of 260 nm. The PCR product with specific abnormal waveform was then sequenced using an Illumina Hiseq2500 analyzer (Illumina, San Diego, CA, USA). DNA sequence with a total length of 98 480 bp, covering 14 colorectal cancer-associated genes (including *APC, MLH1, MSH2, MSH6, PMS2, AXIN2, BMPR1A, EPCAM, MLH3, MUTYH, PMS1, PTEN, SMAD4, and STK11*), was sequenced, which covered more than 97.84% of *APC*. The mean sequence depth was over 224 x for each base pair, and the sites with mean sequence depth of over 30 x accounted for 95.32%. Data were analyzed using Illumina Pipeline software (version 1.3.4, Illumina). The sequences were aligned with the human reference genome HG19 assembly. The suspected mutations were amplified by PCR and the products then underwent Sanger sequencing using an ABI PRISM 3730 automated sequencer (Applied Biosystems, Foster, CA, USA), which was analyzed by DNASTAR SeqMan (DNASTAR, Madison, Wisconsin, USA). The detected mutations were searched in the databases (NCBI dbSNP, HapMap, 1000 Genomes Project dataset, and database of 100 Chinese healthy adults).

Large-fragment deletions in the *APC* was identified by MLPA technique using an MLPA2P043 detection kit (MRC-Holland, Amsterdam, the Netherlands) which contained various probes to recognize the exons and introns of *APC*. In brief, 100-ng template DNA was denatured at 98°C for 5 min and hybrid captured at 65°C for 24 h. After hybridization, probes were washed and eluted, and the captured DNA was amplified by ligation-mediated polymerase chain reaction (LM-PCR). The PCR reaction system (25 µL) contained 50–100 ng genomic DNA, 0.2 mM dNTP, 0.5 µM upstream primer, 0.5 µM downstream primer, and 2U Tag DNA polymerase. PCR was performed at 95°C for 5 min, 35 cycles of 95°C for 20 s, 55°C for 20 s, 72°C for 20 s, and 72°C for 5 min. PCR products were separated by capillary electrophoresis and detected by ABI StepOne technique using an ABI2100Avant Bioanalyzer sequencer (Applied Biosystems). The copy number of the destined fragments was analyzed with GeneScan 3.1 software (Applied Biosystems).

Results

Between January 2010 and June 2015, probands who were registered and diagnosed at the Registration Center for FAP Pedigrees, Tianjin Union Medical Center, from 22 pedigrees with FAP and totally 80 subjects (including probands and other family members) were enrolled and we detected the *APC* genotypes.

Among the 22 FAP pedigrees, 5 exhibited mild FAP, 12 had intermediate FAP, and 5 had intensive FAP (Table 1). In Pedigrees 7 and 18, the subjects with FAP were assessed with desmoids. Typical images of colonoscopic and histological observation and resected specimens of mild (Pedigree 1#), intermediate (Pedigree 2#), and intensive FAP (Pedigree 18#) are shown (Figure 1). As shown in Table 1, 19 of 22 FAP pedigrees had the mutations of *APC*, and 17 types of *APC* mutations were identified. In Pedigree 1–19, 74 subjects received *APC* mutation examination, and 31 (41.9%) of them were positive for *APC* mutations. In Pedigree 20–22, 6 subjects were negative for *APC* mutations, and 2 subjects in Pedigree 22 were had *MUTYH* mutations.

All the mutations were heterozygous with autosomal dominant inheritance. *APC* mutations included frameshift mutations (c.1285delC (in CDS9), c.1350-1352delinsAC (in CDS10) c.2393_2394insT (in CDS15), c.3418delC (in CDS15), c.1238_1239insA (in CDS10), c.3921_3925delAAAAG (in CDS15), c.3992_3993insA (in CDS15), and c.230_233delTAGA (in CDS3)), splice mutations (c.646-1 G>T (in intron 6), c.1744-1G>A (in intron 14), and c.531+2T>A (in CDS5)), missense mutation (c.289 G>A (in CDS3), and c.474T>G (in CDS4)), nonsense mutation (c.3486T>A (in CDS15), and c.2413C>T (in CDS15)), and large deletional mutations (EX5_16DEL (EX5_16/CDS4_15) and Ex3_16DEl (EX3_16/CDS3_15)). Three mutations were caused by splice, 8 by frameshift, 2 by nonsense mutation, 2 by large-fragment deletion, and 2 by missense mutation. Frameshift was the most common mutation means of *APC*. Two large-fragment deletional mutations were found in 2 cases.

Of the 17 mutations, 2 appeared in introns (intron 6 and 14, respectively), 2 in CDS3, 1 in CDS4, 1 in CDS9, 2 in CDS10, 7 in CDS15, 1 in CDS3_15, and 1 in CDS4_15. CDS15 was the most common mutation site. By searching HGMD and the 1000 Genomes Project dataset, 5 of the identified *APC* mutations – c.646-1G>T (in Pedigree 1#), c.1285delC (in Pedigree 2#), c.1350-1352delinsAC (in Pedigree 3#), EX3_16/CDS3_15 (in Pedigree 15#), and c.230_233delTAGA (in Pedigree 19#) – have not been previously reported. The sequences of these novel *APC* mutations are shown in Figure 2.

Table 1 shows that mild FAP was related to *APC* mutations in CDS3 (nucleotides 233 or 289), CDS5 (nucleotide 531), or intron 6 (nucleotide 646) by splice (in intron 6 or CDS5) or frameshift

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Table 1. *APC* mutations in the pedigrees.

Ped	GN	T/P	*APC* mutation (NM_000038)	Deduced amino acid change	Mutation site	Het	Functional change	Mutation type	FAP type
1#	4	3/2	c.646-1 G>T	--	Intron 6	Het	Splice	Suspected	Mild
2#	3	2/1	c.1285delC	p.Pro429Glnfs*25	CDS9	Het	FS	Suspected	Intermediate
3#	3	2/1	c.1350-1352delinsAC	p.Cys451Leufs*3	CDS10	Het	FS	Suspected	Intensive
4#	3	3/1	c.289 G>A	p.Gly97Arg	CDS3	Het	Missense	Suspected	Mild
5#	3	2/1	c.2393_2394insT	p.Tyr799Leufs*4	CDS15	Het	FS	Suspected	Intermediate
6#	4	4/3	c.3418delC	p.Pro1140Leufs*25	CDS15	Het	FS	Suspected	Intermediate
7#	4	3/2	c.1744-1G>A	--	Intron 14	Het	Splice	Suspected	Intermediate
8#	3	5/1	c.1238_1239insA	p.Arg414Thr fs*5	CDS10	Het	FS	Suspected	Intermediate
9#	4	3/2	c.3921_3925delAAAAG	p.Glu1309Aspfs*4	CDS15	Het	FS	Suspected	Intermediate
10#	3	4/1	c.531+2T>A	--	CDS5	Het	Splice	Suspected	Mild
11#	4	5/3	c.2413C>T	p.Arg805Ter	CDS15	Het	Nonsense	Suspected	Intensive
12#	5	8/4	EX5_16DEL	--	EX5_16/ CDS4_15	Het	Deletion (EX 5-16)	Suspected	Intermediate
13#	4	12/3	c.474T>G	p.Tyr158Ter	CDS4	Het	Missense	Suspected	Intermediate
14#	3	3/1	c.3486T>A	p.Tyr1162Ter	CDS15	Het	Nonsense	Suspected	Intermediate
15#	4	3/1	Ex3_16DEl	--	EX3_16 CDS3_15	Het	Deletion (EX3-16)	Suspected	Intermediate
16#	3	2/1	c.3921_3925delAAAAG	p.Glu1309Aspfs*4	CDS15	Het	FS	Suspected	Intermediate
17#	3	3/1	c.3921_3925delAAAAG	p.Glu1309Aspfs*4	CDS15	Het	FS	Suspected	Intensive
18#	3	4/1	c.3992_3993insA	p.Thr1332Asnfs*10	CDS15	Het	FS	Suspected	Intensive
19#	3	3/1	c.230_233delTAGA	p.Asp78Alafs*7	CDS3	Het	FS	Suspected	Mild
20#	3	3/0	--	--	--	--	--	No	Intensive
21#	3	1/0	--	--	--	--	--	No	Intermediate
22#	3	2/0	--	--	--	--	--	No	Mild

Ped – pedigree; GN – generation number; T/P – total/positive subject number; Het – heterozygosity; AD – autosomal dominant inheritance; FS – frameshift; Suspected – suspected pathogenic mutation; CDS – coding DNA sequence.

(in CDS3). Intermediate FAP was related to mutations in CDS9 (nucleotide 1285), CDS15 (nucleotides 2393, 3418, 3486, or 3921–3925 (c.3921_3925delAAAAG)), CDS10 (c.1238_1239insA), CDS4 (nucleotide 474), intron 14 (nucleotide 1744), EX5_16/CDS4_15 (EX5_16DEL) or EX3_16/CDS4_15 (EX3_16DEL) by frameshift (in CDS9, CDS15, or CDS10), splice (in intron 14), nonsense (in CDS15), missense (in CDS4) or large deletion (in EX5_16/CDS4_15). Intensive FAP was related to mutations in CDS15 (nucleotides 3992–3993 (i.e. c.3992_3993insA), 3921–3925 (c.3921_3925delAAAAG), or 2413) or CDS10 (nucleotide 1350–1352 (c.1350–1352delinsAC)) by frameshift (in CDS15 or CDS10) or nonsense (in CDS15).

Interestingly, patients in Pedigrees 9 and 16, who had a frameshift mutation of c.3921_3925delAAAAG in CDS15, manifested intermediate FAP, while those in Pedigree 17 with the same *APC* mutation were diagnosed with intensive FAP.

Discussion

In HGMD and LOVD databases, over 1100 types of *APC* germline mutations have been identified, including over 200 unique *APC* mutations from Chinese individuals (*http://www.genomed.org/lovd2/home.php?select_db=APC*). In this study, we identified 17 types of *APC* mutations from 19 FAP pedigrees (No 1–19) and

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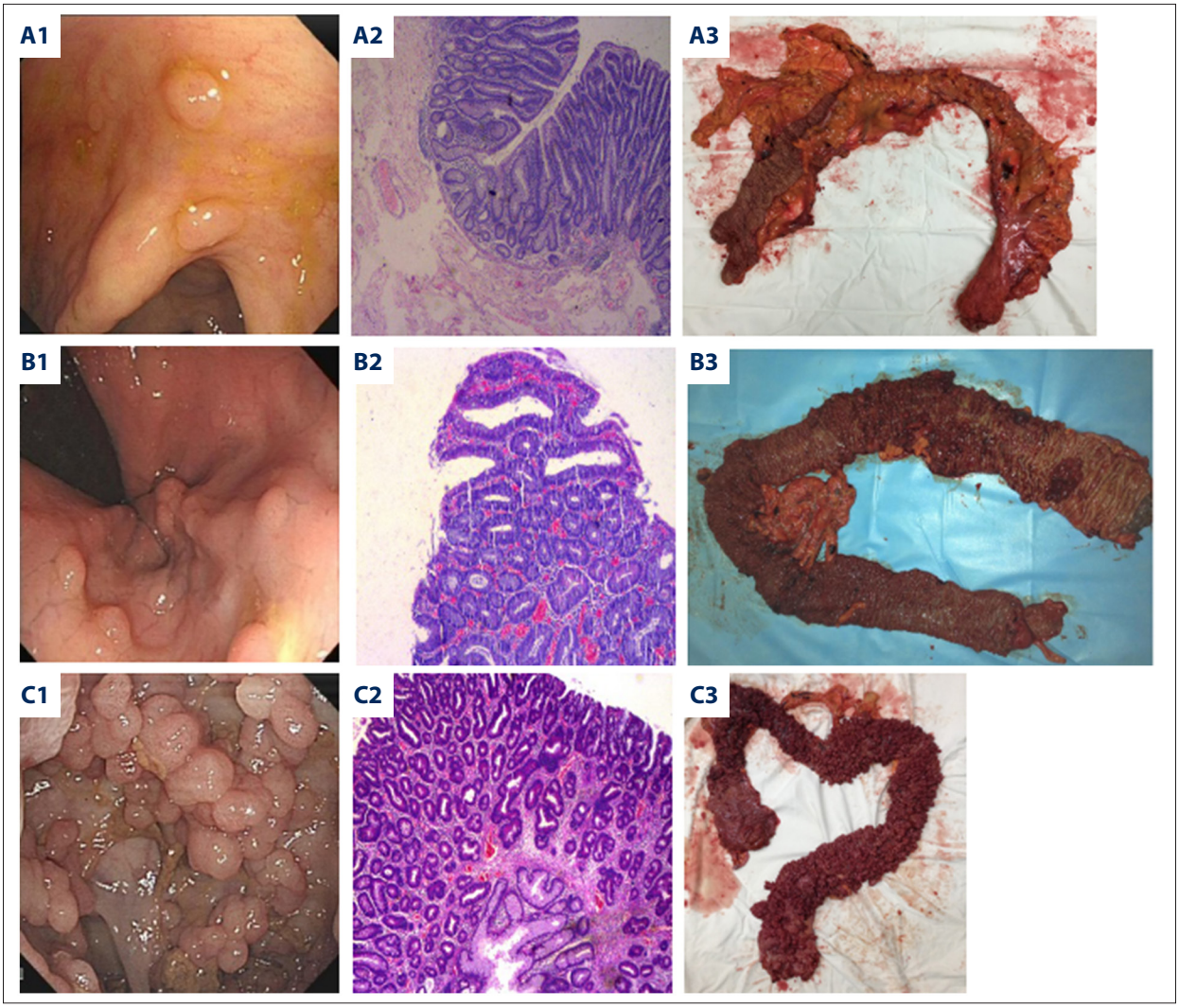

Figure 1. Typical colonoscopic and histological observations and surgically resected specimens of FAP patients. (**A**) Colonoscopic (**A1**) and histological (**A2**) observation and resected specimen (**A3**) of mild FAP (in Pedigree 1) with several polyps. (**B**) Colonoscopic (**B1**) and histological (**B2**) observations and resected specimen (**B3**) of intermediate FAP (in Pedigree 2) with hundreds of polyps. (**C**) Colonoscopic (**C1**) and histological (**C2**) observations and resected specimen (**C3**) of intensive FAP (in Pedigree 3) with thousands of polyps.

no *APC* mutations in the other 3 FAP pedigrees (No 20–22). This suggests that *APC* mutations play an important role in the pathogenesis of FAP, and other pathological factors (such as other pathogenic gene mutations) may also be involved.

Small deletions or insertions (1 bp insertion or 1, 4, or 5 bp deletion) cause frameshift mutations. Small deletional mutations (such as c.3184_3187delCAAA, c.3925_3928delAAAA, c.3926_3930delAAAAG, c.3921_3924delAAAA, c3184_3187delCAAA, and c.3925_3929del AAAAG) frequently occur in Chinese FAP patients, which may thus cause a premature stop codon in *APC* due to frameshift mutation [16,17]. In this study, we identified 8 deletion- or insertion-induced frameshift mutations of *APC*.

In contrast, large-fragment deletional mutations of *APC* are rare, and only a small portion (about 2%) of mutations are caused by large deletions [2]. For example, Jin et al. identified 2 large-fragment deletional mutations (deletion of exons 11 and 10A and that of exon 15 start) of *APC* in Chinese FAP families [18]. In this study, we identified 2 large-fragment deletional mutations (EX3_16/CDS3_15 and EX5_16/CDS4_15 deletions) in Pedigrees 12 and 15, respectively. In addition, we identified 3 splice-site, 2 nonsense, and 2 missense mutations.

Of the 17 identified mutations in this study, 9 were at CDS15 (including 7 in CDS15, 1 in CDS3_15, and 1 in CDS4_15). This indicates that CDS15 may be the most common mutation site, which is consistent with some previous studies [18,19]. This

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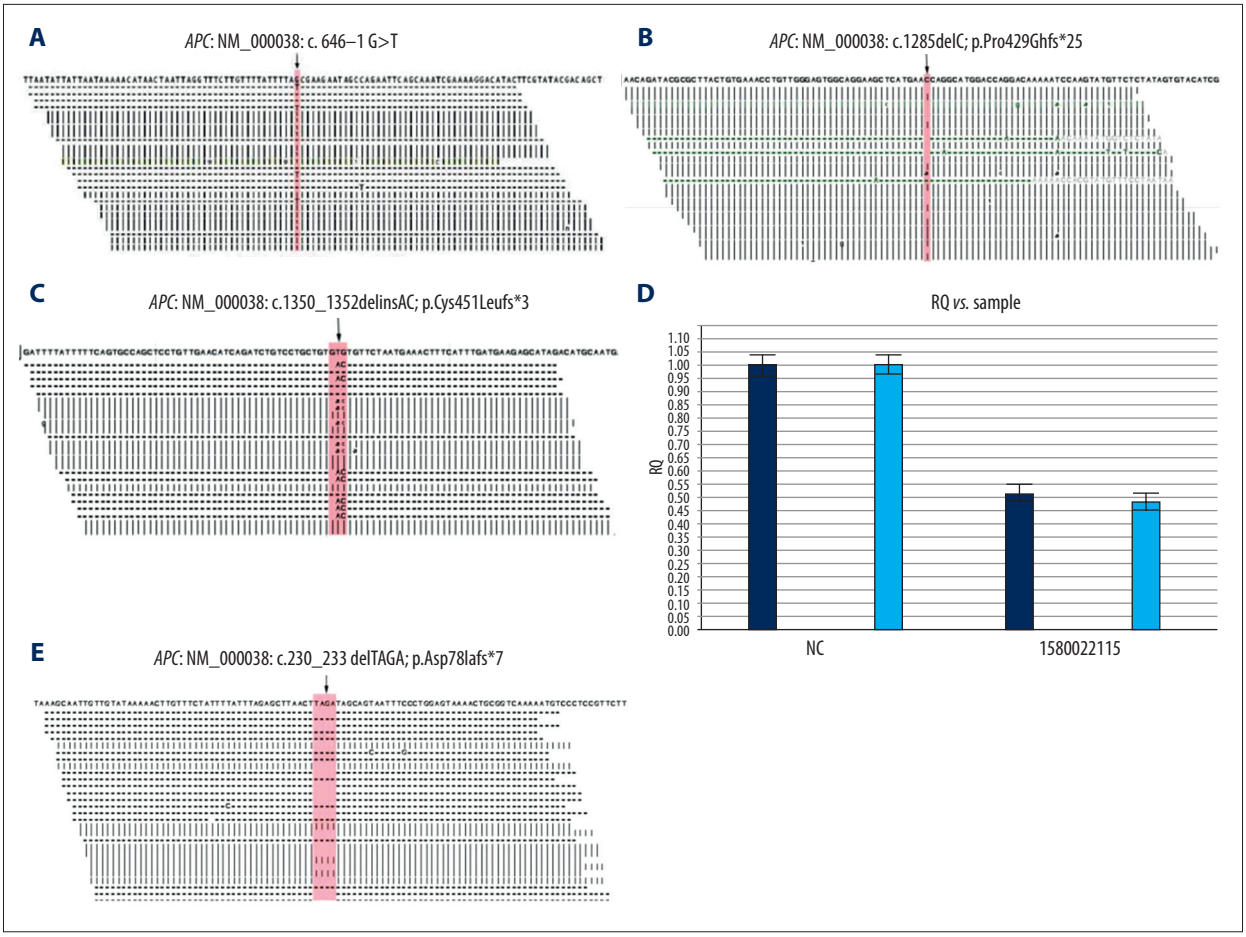

Figure 2. Detection for novel *APC* mutations in pedigrees. (**A**) Next-generation sequencing for c.646-1G>T (in Pedigree 1#). (**B**) Next-generation sequencing for c.1285delC (in Pedigree 2#). (**C**) Next-generation sequencing for c.1350-1352delinsAC (in Pedigree 3#). (**D**) MLPA detection for EX3_16/CDS3_15 (in Pedigree 15#). (**E**) Next-generation sequencing for c.230_233delTAGA (in Pedigree 19#).

might be related to the characteristics of CDS15 covering most of the coding region of *APC*.

Studies have reported *APC* mutations in Chinese patients with FAP, such as 131564T>C (p.1125Val>Ala) [20] in exon 15 of *APC* gene, a large deletional mutation c.1936–2148 del [21] in exon 14–15 and intron 14, a frameshift mutation c.1828_1829insG (p.Asp610GlyfsX23) [22], and c.694C>T [23]. We previously identified mutations c.1744-1G>A in intron 14 [12], c.3418delC (p.Pro1140Leufs*25) in exon18 [13], c.3992_3993insA (p.Thr1332Asnfs*10) in CDS15 [14], and a large-fragment deletion (exon5-exon16; c.423_8532del) [15] of *APC* gene. However, most of these studies just reported *APC* mutation from individual or limited pedigrees. In the present study we systematically screened the *APC* mutations in a relatively large number (22) of Chinese pedigrees with FAP and identified 5 novel mutations not previously reported.

APC mutations occur in over 70% of the Chinese FAP pedigrees, and frameshift mutation is the most common type of *APC* mutation [24–26]. For example, Sheng et al. [25] identified 11 mutations (78.6%) from 14 families with FAP, including 9 micro-mutations and 2 large-fragment deletions. Chiang et al. [24] reported 37 (79%) *APC* mutations, mainly frameshift mutations, in 47 FAP families. In the present study, we showed a higher rate (86.4%) of *APC* mutations (19) in 22 Chinese FAP pedigrees compared with the above results. In addition, frameshift mutations accounted for most cases of *APC* mutations, followed by splice-site mutation and nonsense mutation, which is also consistent with previous reports [24,25].

Codon 1309 is a mutational hotspot for *APC* [8,16,17,26,27]. For example, a large investigation on 680 FAP patients showed 6.9% (47/680) deletional mutation at codon 1309 [26]. Consistently, in the present study we showed that the reported *APC* mutations most commonly occurred at codon 1309, since an *APC* frameshift mutation c.3921_3925delAAAAG (p.Glu1309Aspfs*4)

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at codon 1309 was identified in 3 pedigrees (No. 9, 16 and 17). In addition, *APC* mutations were also identified at codons 1140 (in Pedigree 6), 1132 (in Pedigree 18), and 1162 (in Pedigree 14).

Interestingly, we showed that although some patients with a same *APC* mutation (c.3921_3925delAAAAG in CDS15), different types of FAP were manifested; for example, patients in Pedigrees 9 and 16 with this *APC* mutation were diagnosed with intermediate FAP, while those in Pedigree 17 with the same mutation manifested intensive FAP. This might be related to other pathological factors (e.g., other pathogenic genes of FAP such as *human mutY homolog*) additionally contributing to the progression of FAP.

In this study, *APC* mutations at codons 78 or 97 were detected in mild FAP, those at codons 158, 414, 429, 799, 1140, 1162, or 1309 were detected in intermediate FAP, and those at 451, 805, 1309, or 1332 were detected in intensive FAP. It appears that the *APC* mutations at the front codons are related to mild FAP and those at the back codons are related to intermediate or intensive FAP. In addition, mutation sites might affect the functions of the coded APC protein and the FAP type. We found that mutations at CDS3 (in Pedigrees 4 and 19) or CDS5 (in Pedigree 10) were related to mild FAP, and those at CDS4 (in Pedigree 13), CDS9 (in Pedigree 2), CDS10 (in Pedigrees 3 and 8), or (particularly) at CDS15 (in Pedigrees 5, 6, 9, 11, 14, 16–18) were related to intermediate or even intensive FAP.

Large deletions (in Pedigrees 12 and 15) in this study were related to intermediate FAP, which may be attributable to large-fragment loss in FAP protein. However, large deletions would not necessarily lead to more severe (intensive) FAP, probably because *APC* mutation is not the sole pathogenic genetic factor for FAP. Interestingly, in Pedigrees 1, 7, and 10, although amino acid change of FAP protein was caused by splice-site mutation, mild FAP was diagnosed in Pedigrees 1 and 10 while intermediate FAP was diagnosed in Pedigree 7. Similarly, this might be due to the roles of other pathogenic factors for FAP.

We identified 5 novel *APC* mutations, including c.646-1 G>T (in Pedigree 1), c.1285delC (in Pedigree 2), c.1350_1352delinsAC (in Pedigree 3), c.230_233delTAGA (in Pedigree 19), and Ex3_16DEl (EX3_16/CDS3_15) (in Pedigree 15), with frequency of 0 in the 1000 Genomes Project data set, which have not been previously reported or documented. These mutations might be related to specific gene characteristics of Chinese patients with FAP.

A spice mutation of c.646-1 G>T was found in a proband of Pedigree 1, which may influence the normal splice of mRNA and even the APC function. Mild APC was found in patients, probably because the structure and function of APC is not severely affected by this mutation. A missense mutation of c.289 G>A (p.Gly97Arg) was detected in a proband of Pedigree 4, resulting in a substitution of Glycine at site 97with Arginine. Similarly, only a mild FAP was diagnosed in patients with this mutation, probably because this missense mutation does not strongly influence the structure and function of the FAP protein.

A frameshift mutation of *APC*, c.1350_1352delinsAC (p.Cys451Leufs*3), was found in a proband of Pedigree 3, who was found to have thousands of colorectal polyps (i.e., intensive FAP) by colonoscopic examination. This mutation may lead to a truncated protein with only 452 amino acids, while *APC* normally encodes a 2843-amino acid protein, which has not been previously reported. Supposedly, the frameshift mutation-caused truncation of APC protein may greatly induce structural and functional changes of APC protein, which might contribute to intensive FAP in patients. A frameshift mutation of c.1285delC (p.Pro429Glnfs*25) was detected in a proband of Pedigree 2, which may also cause a truncated 452-amino acid APC protein, as in Pedigree 3. Interestingly, intermediate FAP was observed in patients of Pedigree 2, probably because this mutation can lead to a different truncated APC whose structure and function are not severely influenced.

A frameshift mutation of c.230_233delTAGA (p.Asp78Alafs*7) was detected in Pedigree 19, which can cause a very short 83-amino acid APC. Interestingly, in such mutation-carrying patients, mild FAP was found, perhaps because the pathogenesis and manifestation of FAP result from the collective influence of the *APC* and other pathogenic gene mutations and other pathological factors.

Two large deletional mutations – EX5_16/CDS4_15 with deletion at (EX 5-16) (in Pedigree 12) and EX3_16/CDS3_15 with deletion at (EX3-16) (in Pedigree 15) – may greatly influence the translation of APC, which was unexpectedly related to more severe types of FAP. However, as mentioned above, *APC* mutation is not the sole factor influencing the manifestation of FAP, so it is not surprising that intermediate instead of intensive FAP was diagnosed in Pedigrees 12 and 15.

It is important to determine the correlation of *APC* mutation types and locations with the FAP clinical manifestations. For example, we detected a large-fragment deletion (Ex5-16DEL) in *APC* in a proband (No. 4 in Generation III) of Pedigree 12, suggesting the other family members to should undergo *APC* mutation screening. Three of them (No. 1, 5, and 9 in Generation III) had the same mutation as in the proband, but 1 (No. 2 in Generation III) did not have the mutation. Further, these subjects with *APC* mutations underwent colonoscopic examination and were found to have different extents of polyps; 1 subject was even found to have cancerous polyps. Except for the cancer patient who needed treatment, the other family members with *APC* mutations were advised to have total prophylactic proctocolectomy for colon cancer before 40 years of age.

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A limitation of this study is that although 22 pedigrees were screened, the number of the enrolled subjects from each proband's family was limited. This is related to the fact that in China there is often a lack of necessary knowledge about the progression and prevention of FAP. In the future, education on FAP progression and prevention will be strengthened, and more subjects from each probands' family with FAP will be enrolled to validate these results. In addition, progression of *APC* mutation-carrying subjects usually occurs at a certain age. Due to a limited observational period, some children with *APC* mutations were not yet diagnosed with FAP. In the future, these children will be carefully followed to determine if FAP eventually progresses whether preventive surgical resection is carried out, if necessary.

Conclusions

We identified 17 types of mutations of APC gene from 22 pedigrees, including 5 novel mutations not previously reported. This study will broaden the spectrum of known *APC* pathogenic germline mutations and help understand the types and distribution of *APC* mutations among Chinese patients with FAP. This study will help determine the corresponding early intervention for FAP among Chinese patients, which could assist prevention and treatment for FAP-associated colorectal cancer.

Conflict of interest

None.

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