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Research Article

Study on *APC* Gene Mutation in Chinese Familial Adenomatous Polyposis

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Abstract

Introduction: 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.

Materials/methods: Thirty nine subjects (probands and their family members of 13 FAP pedigrees) were enrolled, underwent abdominal ultrasound, computed tomography, and colonoscopic examinations, and were assessed for *APC* mutations between June 2015 and June 2023 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. This study was approved by the Ethics Committee of the hospital. Written consent was signed by each subject. The data were processed by SPSS 26.0 statistical software.

Results: Eleven of 13 FAP pedigrees were found to have mutations of *APC*, and 11 types of *APC* mutations were identified. All the mutations were heterozygosity with autosomal dominant inheritance. *APC* mutations included 4 caused by frameshift, and 7 by nonsense mutation. Coding DNA Sequence 15 was the most common mutation site.

Conclusions: We systematically screened 11 mutations of *APC* from 13 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.

Keywords: Adenomatous polyposis coli; Family adenomatous polyps; Mutation; Pedigree; DNA sequencing

Abbreviations & Acronyms

FAP: Familial Adenomatous Polyposis; *APC*: Adenomatous Polyposis Coli; DT: Desmoid Tumors; CFAP: Classical Familial Adenomatous Polyposis; AFAP: Attenuated Familial Adenomatous Polyposis; LOVD: Leiden Open Variation Database; MAP: MUTYH-Associated Polyposis; BER: Base Excision Repair; TPC: Total Proctocolectomy; IPAA: Ileal Pouch-Anal Anastomosis; TAC: Total Abdominal Colectomy; IRA: Ileorectal Anastomosis; mTOR: Mammalian Target of Rapamycin; DHPLC; PCR-Denaturing High-Performance Liquid Chromatography; MLPA: Multiplex Ligation-Dependent Probe Amplification

Introduction

Familial Adenomatous Polyposis (FAP) is an autosomal dominantly inherited disease. The main manifestations are abdominal pain, diarrhea, and hundreds of adenomatous polyps in the colon and rectum, of different sizes and in the shape of drops of beads, which could diffuse in any location and gradually grow and multiply, filling the entire intestinal, and presenting with the symptoms of hematochezia, mucinous stool, and anemia. In addition, patients may present with several extra-colonic symptoms, such as Desmoid Tumors (DT) (10% to 15%), gastric/duodenal polyps (80%), hepatoblastoma (3%), thyroid carcinoma (1% to 2%), and so on [1].

It is estimated that FAP has an incidence of between 1 in 7,000 and 1 in 30,000 individuals [2]. The onset age of it varies greatly, and the incidence has no relationship with gender. It has familial hereditary tendencies, and only 10% to 30% of FAP patients are sporadic) [3]. Colorectal polyps often develop in the early teens, and with the increase of age, the number and volume of that also gradually increase, and the clinical symptoms in and out of colorectum will gradually appear and worsen. Without treatment, the risk of colorectal cancer will reach almost 100% by the age of 40. So early diagnosis and intervention are particularly important for the prognosis of FAP patients [1].

According to the difference in the number of whole colorectal polyps and the onset age, the FAP can be divided into Classical Familial Adenomatous Polyposis (CFAP) and Attenuated Familial Adenomatous Polyposis (AFAP). And CFAP could be further divided into intermediate FAPs with the number of polyps ranging from 100 to 1000 and severe FAPs with the number of polyps >1000. CFAP has early onset, early cancerization, and severe symptoms, and the number of polyps was more than 100. The tubular adenoma was more common, and the diameter was generally less than 1 cm. AFAP, on the other hand, usually comes on later with a later onset of cancerization, mild symptoms, and fewer than 100 polyps [4].

APC, a tumor-suppressor gene, is the most frequently mutated gene in colon cancer. It is located on chromosome 5, in the q21 region encodes for 2843 amino acids, which accounts for 21 exons. The main part of the coding sequences is located on exon 15, which occupies a

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large portion of the gene. Different regions of *APC* allow for different functions, with the proximal part responsible for oligomerization, the central part for regulation of WNT signaling pathway and cell cycle, and the C-terminal part for chromosomal segregation, cytoskeletal regulation, and signal transduction. Thus, according to the site in which the *APC* mutation occurs, a different portion of the protein will remain functional, and then various clinical phenotypes will be encountered. There are several mutations of *APC* have been found in patients with FAP, such as nonsense or frameshift mutations, large deletions and duplications, splicing and missense alterations, these years the synonymous SNP [5], Epigenetic regulatory mechanism are also found. But the most common *APC* germline mutations are nonsense or frameshift mutations (95%: about 33%point mutation, 6% small fragment insertions, and 55% small fragment deletions), detected in up to 80% of CFAP cases. Through searching Leiden Open Variation Database (LOVD), we found that more than 1,600 types of *APC* germline mutations have been identified, which covers the whole *APC* gene. The majority of inherited FAP mutations clustered in the 5'-half of the gene. The "mutation cluster region" is located on exon 15, between codon 1285 and codon 1580, and is associated with the most severe disease phenotype, with the presence, among others, of desmoid tumors, hepatoblastoma, and papillary thyroid carcinoma. However, the milder form of the disease, AFAP, is present when *APC* mutations occur in the 3` (codons 1595-2843) or 5` regions (codons 78-167) of the gene [6]. In addition, statistics have found that there are several mutation hotspots in *APC*, that is codon 1061 (5%, 5bp deletions), 213 (3%, C>T substitution), 1068 (2%, 4bp deletions), and 1309 (10%, 5bp deletions) [7].

The most important function of *APC* related to FAP was that it is involved in the regulation of β-catenin in all tissues as part of the WNT signaling pathway. It prevents constant activation of β-catenin that would bring uncontrolled cellular proliferation. Once the mutations of *APC* result in a loss of function, the WNT signaling pathway will be influenced, β-catenin will constantly be activated, and then FAP happened. What`s more, research shows that different functions altered in *APC* mutations may drive and promote CRC genesis, and further additional mutations in other oncogenes or tumor-suppressor genes promote the progression from early adenoma to invasive carcinoma [8]. The research also found that *APC* mutation correlated with poor response to immunotherapy in colon cancer [9].

Inherited biallelic MUTYH mutation is the second most common type of mutations in FAP. FAP caused by it is autosomal recessively inherited. The clinical manifestations of this FAP are similar to AFAP, so some scholars name this FAP "MUTYH-Associated Polyposis (MAP)". It is currently believed that the molecular mechanism mediated by MUTYH mutation is related to the dysfunction of the Base Excision Repair (BER) pathway which leads to the mutations of *APC* won't be repaired [10].

The treatment of familial polyposis is mainly surgery (including laparoscopy and laparotomy). The surgical methods adopted are Total Proctocolectomy (TPC) with ileostomy, Ileal Pouch-Anal Anastomosis (IPAA), Total Abdominal Colectomy (TAC) with Ileorectal Anastomosis (IRA). Among them, TPC+IPAA are considered to be the best operation for FAP. TAC+IRA is recommended for some mild phenotypes of FAP (rectal polyps ≤ 20, colon polyps ≤ 1000) and/or pre-pregnant women, and TPC+IPAA is recommended for FAP patients with multiple rectal polyps who are >25 years of age and/or have an *APC* mutation at codon 1309. What`s more, patients who have undergone IRA find highly atypical hyperplastic adenomas and/or a large number of polyps that cannot be completely removed under colonoscopy, then they can be given an additional IPAA. If cancer develops after the second operation, then an abdominal and perineal resection (Miles) for rectal cancer should be given [11].

In addition, preventing the progression of FAP to colorectal cancer is a key point in the treatment of FAP. The commonly used preventive measures are as follows:

Chemoprevention

NSAID: COX-2 is upregulated in colonic adenoma formation and higher COX-2 expression levels are associated with adenoma features predictive of malignant transformation, so NSAIDS, the irreversible inhibitors of COX, is largely used in the Chemoprevention of FAP in mono- or combination therapy way. The drugs that have been proved to be effective are celecoxib, sulindac/erlotinib (an epidermal growth factor receptor inhibitor) combination therapy.

Other agents: certain free fatty acids have been associated with a reduction in COX2 levels, so some studies used fish oil to control FAP progression, and the results showed a statistically significant reduction in polyp size and polyp count with no adverse events. Vitamin C, or ascorbic acid, has long been associated with antineoplastic properties, studies showed that using Vitamin C, or ascorbic acid in the long term would lead to a significant decrease in polyp area. The Mammalian Target of Rapamycin (mTOR) pathway plays a critical role in epithelial cell growth, when mTOR is inhibited, epithelial proliferation and tumor growth decrease, so some studies used sirolimus in the treatment of FAP, although there was significant toxicity-related adverse events in all patients, there was marked polyp size and number decrease noted [12]. Studies showed that vitamin D could decrease the potential of β-catenin to promote proliferative signaling, and calcium could promote the expression of E-cadherin, so appropriate supplementation of vitamin D and calcium may inhibit the occurrence and development of adenomas to a certain extent [13]. What`s more, several ongoing trials are testing new agents (such as traditional Chinese medicines, cytotoxic drugs, and so on) for FAP.

Endoscopic surveillance

Studies show that endoscopic examination and polypectomy are very important for the prevention, occurrence, and progress of diseases. Long-term endoscopic monitoring and timely polypectomy are needed not only before the occurrence of the disease, but also after the colon resection. Published data indicate that with endoscopic surveillance and polypectomy, long-term rectal preservation without cancer development is possible [14].

As mentioned above, *APC* mutation is the most important cause of FAP. There are so many types of *APC* mutation, and the incidence of *APC* mutation types may be different in different populations: for Westerners, the mutations mostly cluster between codons 1260-1464 of *APC* gene [15]; while in Chinese, mutations are more likely to cluster between codons 849-1376, at the 5' end of *APC* (before codon 500), around codon 1309 of exon 15, and at the 3' end of *APC* (after codon 1580). Therefore, it is important to systematically screen the *APC* mutations among Chinese patients with FAP. In our previous research [16], we have reported the correlations of characteristics (e.g., distribution and types) of *APC* mutations with the clinical manifestations of FAP of 22 FAP pedigrees. Based on that, this study extended the research time and collected more FAP pedigrees to broaden the spectrum of known *APC* germline mutations and help

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understand the types and distribution of *APC* mutations among the Chinese patient population further, then to be referenced for the determination of early resection for FAP, which might be beneficial to prevention and better treatment for FAP-associated colorectal cancer.

Materials and Methods

Patients

Thirty nine subjects (probands and their family members of 13 FAP pedigrees), registered and diagnosed with FAP between June 2015 and June 2023 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 in 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, diarrhea, 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.

Screening of the *APC* mutations

The *APC* mutations were screened by PCR-Denaturing High-Performance Liquid Chromatography (DHPLC) and DNA direct sequencing for micro-mutations, 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 a specific abnormal elution profile (waveform) using the WAVE DHPLC system (Trans-genomic, Omaha, NE, USA), with a single injection volume of 5 μL-8 μL, column temperature of 55°C to 62°C, mobile phase of 0.1 m MN-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 a specific abnormal waveform was then sequenced using an Illumina Hiseq2500 analyzer (Illumina, San Diego, CA, USA). DNA sequence with a total length of 98480 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 a 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 Seq Man (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* were 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 ng-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 20s, 55°C for 20 s, 72°C for 20s, and 72°C for 5 min. PCR products were separated by capillary electrophoresis and detected by ABI Step-One technique using an ABI2100Avant Bioanalyzer sequencer (Applied Biosystems). The copy number of the destined fragments was analyzed with Gene Scan 3.1 software (Applied Biosystems).

Results

Between June 2015 and June 2023, probands who were registered and diagnosed at the Registration Center for FAP Pedigrees, Tianjin Union Medical Center, from 13 pedigrees with FAP and total 39 subjects (including probands and other family members) were enrolled and we detected the *APC* genotypes.

We have studied 22 FAP pedigrees before [16], and now we summarized the results of the two studies: among the 35 FAP pedigrees, 8 exhibited mild FAP, 19 had intermediate FAP, and 8 had intensive FAP (Table 1). In Pedigrees 7, 18 and 28, 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#, 27#) are shown (Figure 1). As shown in Table 1, 30 of 35 FAP pedigrees had the mutations of *APC*, and 28 types of *APC* mutations were identified. In Pedigree 1-19 and 23-33, 111 subjects received *APC* mutation examination, and 52 (46.8%) of them were positive for *APC* mutations. In Pedigree 20-22 and 34-35, 13 subjects were negative for *APC* mutations, and 2 subjects in Pedigree 22 had MUTYH mutations.

All the *APC* mutations in these 13 pedigrees were heterozygous with autosomal dominant inheritance. *APC* mutations included frameshift mutations (c.2376_2379delGCAA (in CDS15), c.3596dupA (in CDS15), c.1682delA (in CDS13), and c.3921_3924delAAAA (in CDS15). Nonsense mutation (c.288T>G (in CDS3), c.637C>T (in CDS5), c.4348C>T (in CDS15), c.1690C>T (in CDS13), c.706C>T (CDS6), c.2935delA (CDS15), and c.3183_3187delACAAA (in CDS1). Four mutations were caused by frameshift and 5 by nonsense mutation. Frameshift was the most common mutation means of *APC*. Of the 11 mutations, 1 appeared in CDS3, 1 in CDS5, 1 in CDS6, 2 in CDS13, 6 in CDS15. CDS15 was the most common mutation site.

Table 1 summarized the results of this study with our previous study, showing that mild FAP was related to *APC* mutations in CDS3 (nucleotides 233 or 289), CDS15 (nucleotide 4348), CDS5 (nucleotide 531), CDS6 (nucleotide 702), or intron 6 (nucleotide 646) by splice, nonsense or frameshift. Intermediate FAP was related to mutations in CDS3 (nucleotide 288), CDS9 (nucleotide 1285), CDS13 (nucleotide 1690), CDS15 (nucleotides 2393, 2935, 3418, 3486, 3596 or 3921-3925

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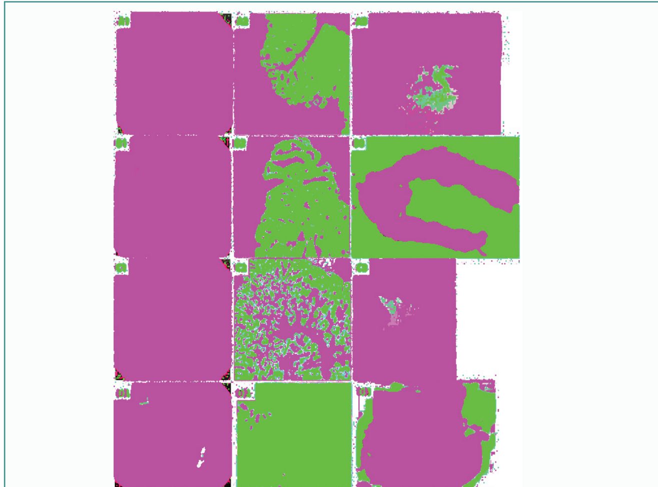

Figure 1: Typical colonoscopic and histological observations and surgically resected specimens of FAP patients. (A) Colonoscopic (A1) and histological (A2) observation and resected specimen (A3) of mild FAP (in Pedigree 1) with several polyps. (B) Colonoscopic (B1) and histological (B2) observations 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 18) with thousands of polyps. (D) Colonoscopic (D1) and histological (D2) observation and resected specimen (D3) of intensive FAP (in Pedigree 27) with thousands of polyps.

(c.3921_3925delAAAAG), 3183_3187 (c.3183_3187delACAAA)), 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, splice, nonsense, missense or large deletion. Intensive FAP was related to mutations in CDS5 (nucleotide 637), CDS15 (nucleotides 2376_2379 (i.e., c.2376_2379delGCAA), 3921_3924 (c.3921_3924delAAAA), 3992-3993 c.3992_3993insA), 3921-3925 (c.3921_3925delAAAAG), or 2413) or CDS10 (nucleotide 1350-1352 (c.1350-1352delinsAC)) by frameshift or nonsense.

Discussion

In HGMD and LOVD databases, over 1600 types of *APC* germline mutations have been identified, including over 200 unique *APC* mutations from Chinese individuals. In this study, we identified 11 types of *APC* mutations from 11 FAP pedigrees (No 23-33) and no *APC* mutations in the other 2 FAP pedigrees (No 34-35). 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 (several bp insertions or deletions) 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 [17], who may thus cause a premature stop codon in *APC* due to frameshift mutation. In this study, we identified 4 deletion- or insertion-induced frameshift mutations of *APC*. Of the 11 identified mutations in this study, 6 were at CDS15. This indicates that CDS15 may be the most common mutation site, which is consistent with some previous studies [17]. This might be related to the characteristics of CDS15 covering most of the coding region of *APC*.

APC mutations occur in over 70% of the Chinese FAP pedigrees, and frameshift mutation is the most common type of *APC* mutation. For example, Sheng et al. [18] identified 11 mutations (78.6%) from 14 families with FAP, including 9 micro-mutations and 2 large-fragment deletions. Chiang et al. [19] reported 37 (79%) *APC* mutations, mainly frameshift mutations, in 47 FAP families. In the present study, we showed a higher rate (84.6%) of *APC* mutations (11) in 13 Chinese FAP pedigrees compared with the above results, but nonsense mutation is the most common mutation, which is not consistent with previous reports. But based on all 35 pedigrees, frameshift mutations accounted for most cases of *APC* mutations, which is consistent with previous reports [18,19].

Combining previous study [20], we found that *APC* mutations at codons 78, 97, 236, 531 or 646 were detected in mild FAP, those at codons 158, 414, 429, 474, 561, 564, 799, 805, 979, 1062, 1140, 1162, 1200, 1309, 1450 or 1744 were detected in intermediate FAP, and those at 213, 451, 793, 805, 1307, 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), CDS5 (in Pedigree 10) CDS6 (in Pedigree 33), or CDS15 (in Pedigree 28) were related to mild FAP, and those at CDS3(in Pedigree 23), CDS4 (in Pedigree 13), CDS5 (in Pedigree 27), CDS9 (in Pedigree 2), CDS10 (in Pedigrees 3 and 8), CDS13 (in Pedigree 26, 29) or (particularly) at CDS15 (in Pedigrees 5, 6, 9, 11, 14, 16-18, 24, 25, 30, 31,33) were related to intermediate or even intensive FAP.

A frameshift mutation of *APC*, c.2376_2379delGCAA (p. Gln793Valfs*26), c.3921_3924delAAAA (p. Ile1307Metfs*13) were found in a proband of Pedigree 24 and 30, which were found to have thousands of colorectal polyps (i.e., intensive FAP) by colonoscopic examination. This mutation may lead to a truncated protein consisting of less than 2, 843 amino acids. Supposedly, the frameshift mutation-caused truncation of *APC* protein may greatly induce structural and functional changes in *APC* protein, which might contribute to intensive FAP in patients. A frameshift mutation of c.3596dupA (p. Ser1200Glufs*8), and c.1682delA (p. Lys561Argfs*9) was detected in a proband of Pedigree 25 and 26, which may also cause a truncated *APC* protein, as in Pedigree 24 and 30. Interestingly, intermediate FAP was observed in patients of Pedigree 25 and 26, 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.

A nonsense mutation of *APC*, c.288T>G (p. Tyr96*), c.637C>T (p. Arg213*), c.4348C>T (p. Arg1450*), c.1690C>T (p. Arg564*), c.706C>T(p.Gln236*), c.2935delA(p.Met979*), c.3183_3187delACAAA (p. Gln1062*) were found in a proband of Pedigree 23, 27-29, and 30. Nonsense mutation introduces termination codon at the mutation site, leading to premature termination of translation, then leading to a truncated *APC* protein. Similar to the above frameshift mutation, nonsense mutation causes the truncated *APC* protein. However, nonsense mutations at different sites cause different truncated *APC*s, so the structure and function of the *APC*

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Table 1: Summary of the results of 35 FAP pedigrees.

Ped	GN	T/P	APC mutation (NM_00038.5)	Deduced amino acid change	Mutation site	Het	Functional change	Mutation type	FAP type
1#	4	03-Feb	c.646-1G>T	--	Intron6	Het	Splice	Suspected	Mild
2#	3	02-Jan	c.1285delC	p.Pro429Glnfs*25	CDS9	Het	FS	Suspected	Intermedia
3#	3	02-Jan	c.1350-1352delinsAC	p.Cys451Leufs*3	CDS10	Het	FS	Suspected	Intensive
4#	3	03-Jan	c.289G>A	p.Gly97Arg	CDS3	Het	Missense	Suspected	Mild
5#	3	02-Jan	c.2393_2394insT	p.Tyr799Leufs*4	CDS15	Het	FS	Suspected	Intermedia
6#	4	04-Mar	c.3418delC	p.Pro1140Leufs*25	CDS15	Het	FS	Suspected	Intermedia
7#	4	03-Feb	c.1744-1G>A	--	Intron14	Het	Splice	Suspected	Intermedia
8#	3	05-Jan	c.1238_1239insA	p.Arg414Thr fs*5	CDS10	Het	FS	Suspected	Intermedia
9#	4	03-Feb	c.3921_3925delAAAAG	p.Glu1309Aspfs*4	CDS15	Het	FS	Suspected	Intermedia
10#	3	04-Jan	c.531+2T>A	--	CDS5	Het	Splice	Suspected	Mild
11#	4	05-Mar	c.2413C>T	p.Arg805Ter	CDS15	Het	Nonsense	Suspected	Intensive
12#	5	08-Apr	EX5_16DEL	--	EX5_16/ CDS4_15	Het	Deletion (EX 5-16)	Suspected	Intermedia
13#	4	12-Mar	c.474T>G	p.Tyr158Ter	CDS4	Het	Missense	Suspected	Intermedia
14#	3	03-Jan	c.3486T>A	p.Tyr1162Ter	CDS15	Het	Nonsense	Suspected	Intermedia
15#	4	03-Jan	Ex3_16DEl	--	EX3_16 CDS3_15	Het	Deletion (EX 3-16)	Suspected	Intermedia
16#	3	02-Jan	c.3921_3925delAAAAG	p.Glu1309Aspfs*4	CDS15	Het	FS	Suspected	Intermedia
17#	3	03-Jan	c.3921_3925delAAAAG	p.Glu1309Aspfs*4	CDS15	Het	FS	Suspected	Intensive
18#	3	04-Jan	c.3992_3993insA	p.Thr1332Asnfs*10	CDS15	Het	FS	Suspected	Intensive
19#	3	03-Jan	c.230_233delTAGA	p.Asp78Alafs*7	CDS3	Het	FS	Suspected	Mild
20#	3	3/0	--	--	--	--	--	No	Intensive
21#	3	1/0	--	--	--	--	--	No	Intermedia
22#	3	2/0	--	--	--	--	--	No	Mild
23#	4	02-Jan	c.288T>G	p.Tyr96*	CDS3	Het	Nonsense	established	Intermedia
24#	3	03-Feb	c.2376_2379delGCAA	p.Gln793Valfs*26	CDS15	Het	FS	established	Intensive
25#	3	03-Feb	c.3596dupA	p.Ser1200Glufs*8	CDS15	Het	FS	established	Intermedia
26#	3	03-Jan	c.1682delA	p.Lys561Argfs*9	CDS13	Het	FS	established	Intermedia
27#	3	04-Mar	c..637C>T	p.Arg213*	CDS5	Het	Nonsense	established	Intensive
28#	3	04-Jan	c.4348C>T	p.Arg1450*	CDS15	Het	Nonsense	established	Intermedia
29#	3	03-Feb	c.1690C>T	p.Arg564*	CDS13	Het	Nonsense	established	Intermedia
30#	3	07-Feb	c.3921_3924delAAAA	p.Ile1307Metfs*13	CDS15	Het	FS	established	Intensive
31#	3	02-Jan	c.3183_3187delACAAA	p.Gln1062*	CDS15	Het	Nonsense	established	Intermedia
32#	3	04-Mar	c.706C>T	p.Gln236*	CDS6	Het	Nonsense	Suspected	Mild
33#	3	03-Jan	c.2935delA	p.Met979*	CDS15	Het	Nonsense	established	Intermedia
34#	3	2/0	--	--	--	--	--	No	Mild
35#	3	4/0	--	--	--	--	--	No	Mild

protein may or may not be significantly changed. What`s more, the pathogenesis and manifestation of FAP result from the collective influence of the *APC* and other pathogenic gene mutations and other pathological factors. Thus, although they are nonsense mutations, Pedigree 27 appeared intensive, whereas Pedigrees 23, 28-29, and 31, 33 appeared intermediate, and Pedigree 32 appeared mild.

It is important to determine the correlation of *APC* mutation types and locations with the FAP clinical manifestations. For example, we detected a frameshift mutation (c.2376_2379delGCAA (p. Gln793Valfs*26)) in *APC* in a proband (No. 2 in Generation II) of Pedigree 24, suggesting the other family members should undergo *APC* mutation screening. Two of them (No. 4 in Generation II and No.1 in Generation III) had the same mutation as in the proband, but 2 (No. 1 and 3 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; the family members with *APC* mutations were advised to have total prophylactic proctocolectomy for colon cancer before 40 years of age.

A limitation of this study is that although 13 pedigrees were screened, the number of 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 proband's family with FAP will be enrolled to validate these results. In addition, the 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 and whether preventive surgical resection is carried out, if necessary.

Conclusions

We identified 11 types of mutations of *APC* gene from 13 pedigrees. Although this study has broadened the spectrum of known *APC* pathogenic germline mutations and helped understand the distribution of *APC* mutations among Chinese patients with FAP to a certain extent, there are many shortcomings in this study due to too few samples, not advanced detection methods, and not comprehensive detection. We hope that more scholars will further study this problem to help determine the corresponding early intervention for FAP, which could assist prevention and treatment of FAP-associated colorectal cancer.

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