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Three Novel Mutations of APC Gene Found in a Chinese Family with Familial Adenomatous Polyposis

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Abstract: *Objective:* To identify the causative adenomatous polyposis coli (APC) gene defects associated with a pedigree of familial adenomatous polyposis (FAP). *Methods:* FAP was diagnosed based on clinical manifestations, family history, as well as endoscopic and pathological examinations. The blood samples of the FAP pedigree members, colonic polyp patients, and normal individuals were collected. Genomic DNA was then extracted from those samples. APC mutation analysis was conducted via direct polymerase chain reaction (PCR) sequencing. *Results:* Three synonymous mutations and a missense mutation were found: c.5034G>A (p.Gly1678Gly), c.5465T>A (p.Val1822Asp), c.5880G>A (p.Pro1960Pro), and c.5274T>G (p.Ser1758Ser). Among them, the homozygous mutation on APC gene c.5034G>A has been reported, while the other three mutations have not been reported in the Chinese Han population. Individuals with c.5465T>A (p.Val1822ASP) missense mutation eventually suffer from colon cancer and have poor prognosis. We found no mutation in patients with simple intestinal polyp and in normal individuals. In addition, there were homozygous and heterozygous mutations in different patients from the same family. *Conclusion:* Three new mutations of APC gene were firstly reported in Han population. The missense mutation of c.5465T>A (p.Val1822Asp) may be the cause of carcinogenesis in this FAP pedigree with poor prognosis.

Keywords: Familial adenomatous polyposis (FAP); Adenomatous polyposis coli (APC); Gene mutation

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1. Introduction

Familial adenomatous polyposis (FAP) (MIM 175100), which was first reported in 1874, is one of the best known and understood autosomal dominant diseases that is classically characterized by the development of hundreds up to thousands of adenomatous polyps of different sizes in the colon and rectum [1]. FAP has an incidence at birth of about 1:8,300 in United Kingdom [2], 1:7,645 in Sweden [3], 1.5:100,000 in China [4], and about 2 to 3:100,000 in several ethnic groups in the 1900s [5,6].

From clinical observation, bowel symptoms such as rectal bleeding, abdominal pain, and diarrhea may occur in the second or third decade of an individual's life, and almost 100% of patients with FAP will develop one or several colorectal cancers at an average age of 40 to 50 if they are not identified and treated at an early stage [7].

In 1991, it was first proposed that the germline mutation of adenomatous colorectal polyposis (APC) gene is the main cause of FAP and autosomal dominant inheritance [8,9]. So far, more than 1,000 different APC mutations have been found in FAP patients. The APC tumor suppressor gene (MIM 611731), which is located on human chromosome 5q21-22 [10], is a large gene spanning about 100 kb of genomic region, giving rise to the majority of FAP. The coding region is divided into 15 exons and encodes a large APC protein (309 kilo-Daltons) with 2,843 amino acids. APC protein is directly involved in the Wnt/β-catenin signaling pathway, which plays a negative regulatory role in signal transduction, but plays an important role in cell-cell adhesion, microtubule stability of cytoskeleton, regulation of cell cycle, and apoptosis [11].

APC gene mutation detection showed that about 95% of APC gene mutations were nonsense mutations or frameshift mutations, resulting in the early termination of APC gene translation and the formation of a truncated protein [12]. Screening for APC gene mutations in FAP family members is useful not only for better understanding the pathogenesis and mutation spectrum of FAP, but also for molecular diagnosis of the precursor phase in high-risk mutation carriers. This significantly reduces the risk of colorectal cancer (CRC) and related mortality in FAP patients.

A FAP family pedigree was found for the study, in which its aim was to carry out genetic analysis on this Chinese FAP pedigree to further elucidate the causative diagnosis.

2. Materials and methods

2.1. Patients

A total of 51 people were divided into three groups. The proband and all her 10 relatives were listed in the FAP group; 20 patients with family history of colon cancer and simple intestinal polyps were assigned to the polyp group; 20 healthy people without colonic polyps were assigned to the control group. They were all diagnosed in Shaanxi Provincial People's Hospital, and they received treatment there. The proband is an 80-year-old female who was first diagnosed by the Department of Gastroentrology when she was 15 years old. Back then, she presented with anemia and blood in the stool, and she underwent total colectomy. She was readmitted in the hospital in view of bleeding and mass prolapse out of the anus upon defecation. The proband has three daughters who are 56, 52, and 30 years old, respectively. The oldest daughter underwent total colectomy 21 years ago after being diagnosed with more than 100 polyps diffused along the colon and a 3 x 3.5 cm colon adenocarcinoma (T2N0M0) in the descending colon. In this study, they all had endoscopic examination of the upper and lower gastrointestinal tract. During the endoscopy, a 2.5 cm diameter disk-shaped mass was found in the proximal rectum of the proband, which was confirmed to be a moderately differentiated adenocarcinoma (T2N1M0) by pathology, and another mass, located in the distal pedicle of the rectum whose surface was flushed due to congestion, was 3 cm in diameter and prolapsible; a full set of polyps was confirmed to be villous adenoma by pathology after endoscopic resection. In the younger daughter, 60 to 80 polyps were found diffused along her colon, whereas 20 to 50 polyps were observed in the second daughter and the third generation. Tubular adenomas and low-grade neoplasia were found in three daughters and grandchildren through parallel endoscopic resection; no polyps in the upper gastrointestinal tract or other tumors or symptoms were observed in this FAP family. The FAP family pedigree was drawn as shown in **Figure 1**. In the polyp group, 1 to 10 polyps were found scattered in the colon, in which most of them were confirmed to be tubular adenomas and inflammatory polyps, but one was found to be high-level neoplasia after pathological diagnosis.

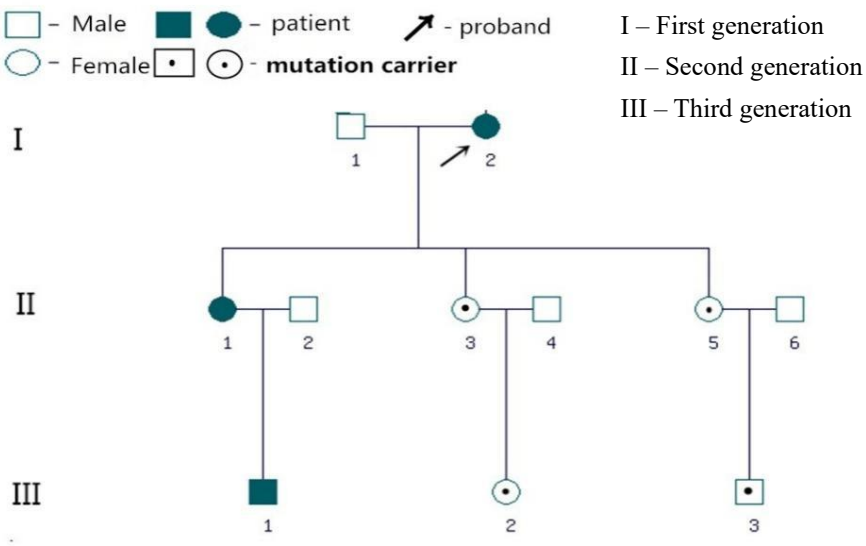

Figure 1. FAP pedigree (I - first generation; II - second generation; III - third generation)

2.2. Genomic DNA extraction

After genetic counseling and obtaining the patient's consent to participate in this study, the first 10ml peripheral blood from the proband was screened for APC gene mutation. Addition blood samples from the FAP family members and other individuals in the polyp and control group were drawn for APC gene sequencing analysis. The genomic DNA samples were all prepared from EDTA peripheral blood samples with TRIzol (TIANGEN blood DNA extraction kit, USA). DNA was successfully extracted by soft gel electrophoresis and stored at -20°C for use.

2.3. Synthesis of PCR primers for target gene

The exon sequences of APC gene and promoter region were obtained from the National Center for Biotechnology Information (NCBI) GenBank (http://www.ncbi.nlm.nih.gov/). Primer Premier 5.0 software was used to design primers based on the principle of primer design [13], as shown in **Table 1**. The primers were synthesized by Beijing Huada Technology Company.

Table 1. Primers and conditions for APC gene (exon 1-15)

Exon	Forward primers (5' → 3')	Referenced primers (5' → 3')	Product size (bp)	Annealing temperature (°C)
1	TTTCTTTAAAAACAAGCAGCCA	CACAGAAAACCTTGCCTCAG	479	56
2	AAGGTGCGTGCTTTGAGAGT	ACCAACACCCAAATCGAGAG	283	54
3	TTACCCTGACCCAAGTGGAC	CTTCAGAATCCCAGGAAACG	556	53
4	GCTCTTCTGCAGTCTTTATTAGCA	CCTAGTTGAACCCTGAGGTCC	385	54
5	CATGCACCATGACTGACGTAT	GTTGCTCAGCAGCCATGAT	370	54
6	TTGAACTGACCCCAATTTGTT	TGAAAGAACATCTATATTTCAAT	343	52
7	TGCTCAAGGGACACACTTC	TGGTACTGAATGCTTCTGG	428	54
8	GACACTTCATTTGGAGTACCTTAAC	GTGCCACCACACTGGCTAAAT	374	58
9	CTGGAAAGGTTTTCCGGTTT	TGCTTTGAAACATGCACTACG	670	53

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Exon	Forward primers (5' → 3')	Referenced primers (5' → 3')	Product size (bp)	Annealing temperature (°C)
10	TTGATCCACTAAAATTCCGTG	ATGCTGGAAACCAGGGTAC	377	54
11	TTCCTAGTATTTAAGTTACC	CGAATGTGAAGCACAGGTTTT	321	54
12	GCTTGGCTTCAAGTTGTCTT	GTGGTGAAACCCCGTCTCT	333	58
13	CAGCCTCCCAAAGTGATAGG	ATGGCTAAAAGAAGGCAGCA	479	58
14	AGGGACGGGCAATAGGATAG	TCCTCCACCTATGGGCTACAC	552	54
15A	AAAGGAGATGTGGAATACTTGG	TGATGAAGAGGAGCTGGGTAAC	666	53
15B	CCCAAGGCATCTCATCGTAG	TAGGTCGGCTGGGTATTGAC	647	55
15C	TGTGACAGATGAGAGAAATGCAC	TCCATGATTAGAACCCACTCG	594	53
15D	CATTTTGGACAGCAGGAATGT	ACTTCGCTCACAGGATCTTC	675	53
15E	GGATGTAATCAGACGACACAGG	AGTACTTTCCGTGGCAAAATGT	606	53
15F	GAGTGAACCATGCAGTGGAAT	TCCCCCGGTGTAAAACTAACAT	665	56
15G	ATGCCAACAAAGTCATCACGT	CGTGTCCTATATTCAGTATTTTGTG	586	56
15H	CCCAAAGGGAAAAGTCACAAG	TTTGGATGACTGGGGAAAAGT	591	55
15I	ACCTCCAACCAACAATCAGC	AGCAGCAGCAGCTTGATGT	641	56
15J	GATGACCTGTTGCAGGAATG	TGATTTCACAGATGGCTTGG	706	54
15K	AATCTCTCGAGGCAGGACAAT	TGATGAAAGTTGACTGGCGTAC	641	56
15L	TCCTCAGGTTCTGGAAAAATG	AGGGATGATGAATGTTTGCTG	574	54
15M	GTCTGTTCAGGCTGGTGGAT	GAGTTCAGGCGATTTTCCAAAC	659	56
15N	GGTAATACTCCCCCGGTGAT	ACCCTCTAACAAGAATCAAACC	603	56

2.4. Mutation analysis

Using the extracted DNA as template, the 15 exons of APC gene were amplified. The reaction system of the PCR was set to 50 ul. PCR was performed with High Fidelity Takara Ex Taq polymerase and "black PCR." The PCR product was preliminarily determined by agarose gel electrophoresis. The PCR products and corresponding primers were then sent to Shanghai Biotech Company for sequencing. According to the APC genome sequence (NM 000038.5) obtained from Genbank database, the sequencing results were analyzed by BLAST in NCBI.

3. Results

A total of 11 families were involved in this study, of which six carried gene mutations. Three patients (I2, II1, III1) were diagnosed with FAP by colonoscopy, pathological biopsy, and definite family history. Among them, the first patient (I2) was diagnosed with colonic polyp (cancerous transformation) at the age of 65 and underwent total colectomy. The postoperative recovery was fair, but rectal cancer was diagnosed 14 years later (79 years old), in which the patient underwent radical resection of the tumor. Three patients (II2, III2, III3) had heterozygous mutation, without signs of the disease. The detailed clinical data of each patient are shown in **Table 2**. The 20 patients with simple adenomatous polyp and the 20 normal controls had no APC gene mutation.

Table 2. Clinical characteristics of the patients

Patient	Age/ gender	Age of onset	Diagnosis	Polyp count	Surgical management
I2	80/F	65Y	CRP, RC	> 100	Total colectomy; ECP; Radical resection of RC (T2N0M0)
II1	56/F	35Y	CRP, CRC	> 100	ECP; Radical resection of CRC (T2N0M0) Total colectomy
II5	52/F	33Y	CRP	> 100	Total colectomy; ECP
III1	30/M	28Y	CRP	> 100	Total colectomy; ECP
III2	25/F	24Y	CRP	> 30	ECP
III3	27/M	N/A	N/A	N/A	N/A

F, female; M, male; Y, year; CRC, colorectal carcinoma; RC, rectal carcinoma; CRP, colorectal polyposis; ECP, electronic colonoscopic polypectomy; N/A, not available

From APC gene screening, we found four mutations in exon 15, including three synonymous mutations and one missense mutation. In addition, we also found that each mutation had different manifestations in different patients from the same family. We found that six patients had 5,465 mutations, of which I2, II1, II5, III1, and III2 were homozygous, while III3 was heterozygous. The specific mutation sites and characteristics are shown in **Table 3** and **Figure 2**.

Table 3. Germline mutation in the APC gene of FAP pedigree

Codon	Exon	Nucleotide change	Amino acid change	Mutation	Figures displaying SNP
5034	15	c.5034G>A	p.Gly1678Gly	Homozygous	**Figure 2A**
5274	15	c.5274T>G	p.Ser1758Ser	Homozygous	**Figure 2B**
5465	15	c.5465T>A	p.Val1822Asp	Heterozygous	**Figure 2C**
5880	15	c.5880G>A	p.Pro1960Pro	Homozygous	**Figure 2D**

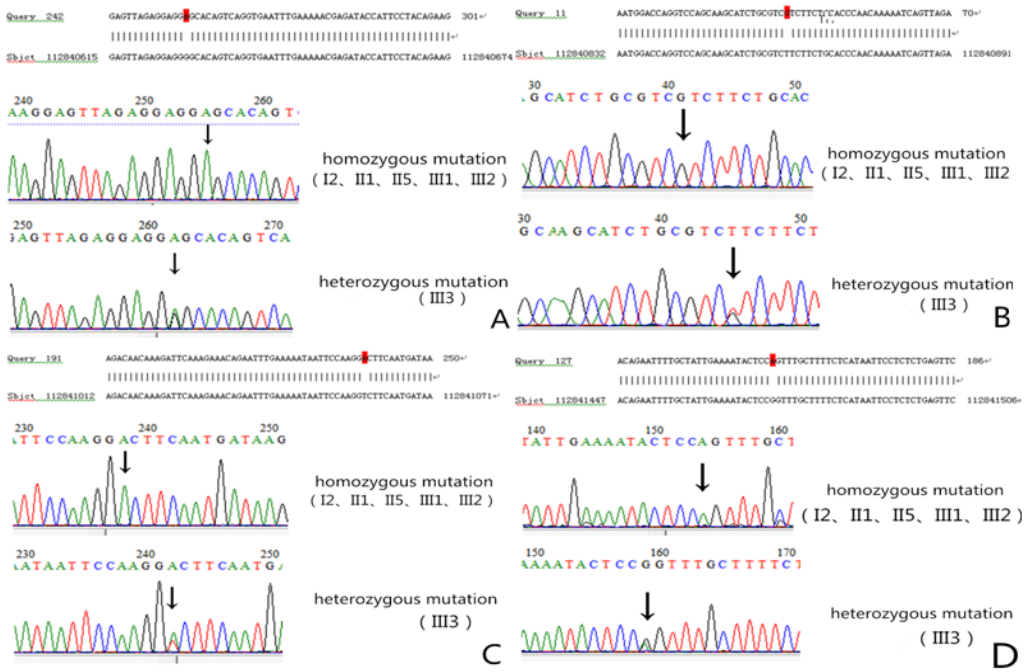

Figure 2. APC mutations detected in the FAP proband; the upper panels show homozygous mutation, while the lower panels show heterozygous mutation

4. Discussion

FAP is an autosomal dominant genetic disease, which is in line with the Mendelian law of inheritance and has more than 90% penetrance [12]. Most patients with FAP are familial genetic cases, but the disease occasionally occurs sporadically in patients without any family history. FAP is caused by mutation of parental germ cells. The predominant clinical manifestation of FAP is the presence of dozens to hundreds, and perhaps, thousands of polyps of various sizes in the large intestine. FAP can be divided into classical familial adenomatous polyposis (CFAP) and attenuated familial adenomatous polyposis (AFAP) based on intestinal manifestations, onset, number of polyps, and the average time of malignant transformation, with the former having more than 100 polyps and an earlier onset, and the latter having less than 100 polyps and a later onset. CFAP usually occurs in adolescence or youth. By the age of 35, 95% of patients with polyps have been identified. Almost 100% of the patients develop colorectal cancer without treatment [13]. The average age of diagnosis of colorectal cancer is about 40 years old, and the average time of malignant transformation from adenoma to cancer is 15 to 20 years [14]. In AFAP, the age of onset of and the average time of malignant transformation are later than those of CFAP. Hence, AFAP can be classified as a mild FAP. In addition to numerous colorectal polyps, more than 70% of cases have extraintestinal manifestations, such as brain tumor, congenital hypertrophy of the retinal pigment epithelium, desmoid tumor, osteoma, tooth deformity, duodenal adenoma, hepatoblastoma, fundic gland polyps, gastric adenoma in the antrum, epidermoid cyst, lipoma, and other malignant tumors, including thyroid cancer [15].

Disclosure statement

The authors declare no conflict of interest.

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