Identification a nonsense mutation of *APC* gene in Chinese patients with familial adenomatous polyposis

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Abstract. Familial adenomatous polyposis (FAP; Mendelian of Inherintance in Man ID, 175100) is a rare autosomal dominant disorder characterized by the development of numerous adenomatous polyps throughout the colon and rectum associated with an increased risk of colorectal cancer. FAP is at time accompanied with certain extraintestinal manifestations such as congenital hypertrophy of the retinal pigment epithelium, dental disorders and desmoid tumors. It is caused by mutations in the adenomatous polyposis coli (*APC*) gene. The present study reported on a Chinese family with FAP. Polymerase chain reaction and direct sequencing of the full coding sequence of the *APC* gene were performed to identify the mutation in this family. A nonsense mutation of the *APC* gene was identified in this pedigree. It is a heterozygous G>T substitution at position 2,971 in exon 15 of the *APC* gene, which formed a premature stop codon at amino acid residue 991 (p.Glu991*). The resulting truncated protein lacked 1,853 amino acids. The present study expanded the database on *APC* gene mutations in FAP and enriched the spectrum of known germline mutations of the *APC* gene. Prophylactic proctocolectomy may be considered as a possible treatment for carriers of the mutation.

Introduction

Familial adenomatous polyposis (FAP; MIM 175100) is a rare autosomal dominant disorder, which is characterized by the development of numerous adenomatous polyps throughout the colon and rectum (1). It is a pre-cancerous disease, which develops into colorectal cancer (CRC) in almost all patients without early diagnosis and colorectal surgery (2). FAP may have extracolonic manifestations, including osteomas, dental abnormalities, congenital hypertrophy of the retinal pigment epithelium (CHRPE) and upper gastrointestinal polyps (3). The incidence of FAP at birth is estimated to be 3-10 per 100,000 individuals (4).

FAP has three phenotypes: Classic FAP (CFAP), attenuated FAP (AFAP) and *MUTYH*-associated polyposis (MAP) (5), with CFAP and AFAP being autosomal dominant disorders. It has been identified that the adenomatous polyposis coli (*APC*) gene on chromosome 5q22.2 is associated with CFAP and AFAP (6). MAP is a recessive dominant disorder caused by mutations in the *MUTYH* gene (7). In the present study, mutations of the *APC* gene were detected in a Chinese family with CFAP by sequencing analysis, and a nonsense mutation was identified.

Materials and methods

Patients. A 40 year-old Chinese male patient was seen at the Department of Emergency of the Second People's Hospital of Heifei (Hefei, China) in August 2011, due to experiencing hematochezia for 1 day. In the past year, he had frequently suffered from moderate diarrhea with scurrying pain around the umbilicus. His medical history was not indicative of colitis and hemorrhoids. The patient was a non-smoker and drank alcohol socially. Colonoscopy findings revealed a huge neoplasm with surface erosion and bleeding 35-38 cm away from the anus (Fig. 1A), as well as congestion, edema and diffused polyps with diameters of 0.3-0.8 cm from the ascending colon to the rectum mucosa (Fig. 1B). The primary diagnosis was FAP. Subsequently, the patient successfully underwent laparoscopic total colectomy and ileal anal anastomosis. Postoperative recovery was good. Pathological findings revealed an abundance of multiple tubular papillary adenoma with low-level intraepithelial neoplasia throughout the entire colon. The family comprised 18 members that spanned three generations, including 3 male (one of which was the proband of the present study) and 2 female individuals affected by FAP (Fig. 2). A similar disease course and abnormalities were found in these patients.

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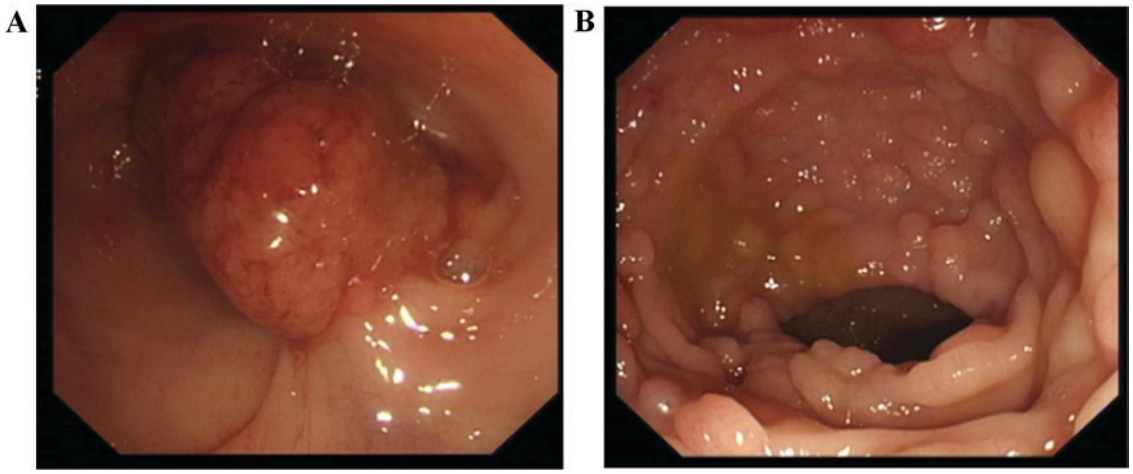

Figure 1. Clinical manifestations of the proband. Endoscopy revealed (A) a large mass in the rectum and (B) innumerable polyps in the rectum and sigmoid colon.

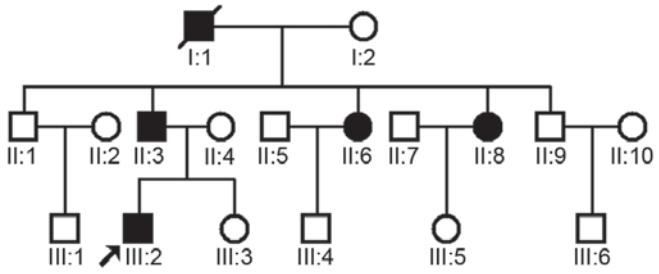

Figure 2. Pedigree of the present study affected by familial adenomatous polyposis. The arrow indicates the proband.

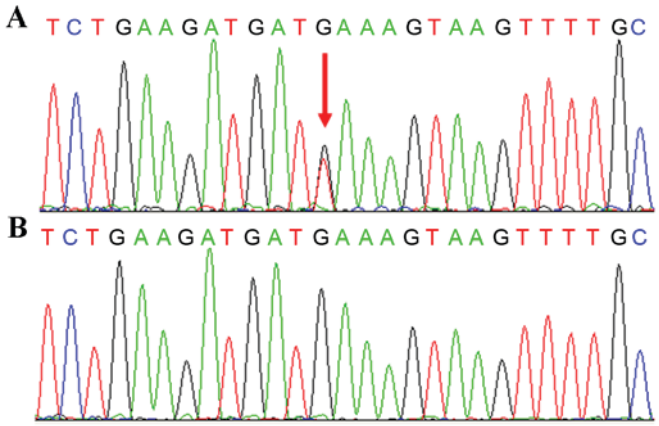

Figure 3. *APC* gene mutation in the proband. (A) Heterozygous nonsense mutation c.2991G>T in exon 15. (B) Sequence of exon 15 of the *APC* gene in a normal subject. The red arrow indicates the mutation. *APC*, adenomatous polyposis coli.

Mutational analysis. The protocol of the present study was approved by the Ethics Committee of the Second People's Hospital of Heifei (Hefei, China) and Zhongshan Hospital (Shanghai, China) and all patients provided written informed consent to be included in the present study. Peripheral blood samples were obtained from the four living patients (II:3, II:6, II:8 and the proband III:2; Fig. 2). In addition, samples from 100 unrelated population-matched controls from Zhongshan Hospital were sequenced for mutations to exclude the possibility that it is a polymorphism in the *APC* gene. DNA was extracted according to standard methods. Primers flanking all 15 coding exons and intron-exon boundaries of the *APC* gene were extracted using the web-based version of the Primer 3.0 program (http://primer3.ut.ee/). The primers used are listed in Table I. The *APC* gene of this family was analyzed by direct sequencing in reaction conditions as previously described (8). Subsequent to amplification, a QIAquick PCR Purification kit (Qiagen, Hilden, Germany) was used to purify the products. The *APC* gene was sequenced using an ABI PRISM® 3730 automated sequencer (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Sequence comparisons and analysis were performed using Phred-Phrap-Consed version 12.0 software (http://www.phrap.org/phredphrap-consed.html). Mutations were identified by comparison with the reported complementary DNA reference sequence (GenBank accession no, NM_000038).

Results

Sequencing results of the proband revealed a nonsense mutation (c.2971G>T, p.Glu991*) located at exon 16 of the *APC* gene (Fig. 3A). This mutation was also verified in the other three patients, but excluded in the unaffected family members and 100 unrelated population-match controls (Fig. 3B). This mutation forms a premature stop codon at amino acid residue 991, which results in a truncated protein short of 1,853 amino acids. This mutation has already been reported a patient with hereditary cancer-predisposing syndrome (https://www.ncbi.nlm.nih.gov/clinvar/15587313/). The present study confirmed this mutation in a Chinese family with CFAP. The result demonstrates that this mutation may be a hotspot mutation in diverse population.

Discussion

FAP is an autosomal dominant disease characterized by the development of hundreds to thousands of adenomas in the colon and rectum, and is at times accompanied with certain extra-intestinal manifestations such as CHRPE, dental

Table I. The primers sequences of APC gene.

Name	Sequence (5'-3')	Product length (bp)
APC-E02_F	CTCTTAGATGCTGCTACTTGA	800
APC-E02_R	GGATAGAACCAGGTACTGAC	
APC-E03_F	ACAGAGACTCCCCATAATCA	587
APC-E03_R	GACTGGCAGAATAGCAACAA	
APC-E04_F	GTTGCTTGAAAATTCCAGTG	642
APC-E04_R	GCTCTAAGTGTTAGCTATCAC	
APC-E05_F	AGCCTTTGGTGAAGTGTAAG	640
APC-E05_R	TTGAACCCTGAGGTCCTCTA	
APC-E06_F	TAACCTCACTCTAACTGGAC	676
APC-E06_R	GAAGACCACCATCTAACTCT	
APC-E07_F	TGATTTGACATAACCCTGAGC	604
APC-E07_R	ACCTTCCCTGGTCTTAATGC	
APC-E08_F	GGATGGCATTCCTGTGAGTC	703
APC-E08_R	GCAAACCTATTCAAGGCAAGC	
APC-E09_F	CTGCAGTTTAATGCTCATATGC	377
APC-E09_R	GCAAAGTAGTCATGGCATTAGT	
APC-E10_F	CAGTTTGTTAGTGAGTATGC	860
APC-E10_R	GCACATAACATTTTCCTTTG	
APC-E11_F	ACTTAGTCAAGGGCAGATGA	468
APC-E11_R	GCTGATAACAGAAGTTGGTG	
APC-E12_F	GGAGAAACTGGCATAAAATGG	578
APC-E12_R	TCACTACTGTGTTCCATCTG	
APC-E13_F	ACTTGTAGGGATCATTTCTGTG	599
APC-E13_R	ATTGCACAACTGCCCTCTAA	
APC-E14_F	CAGTAACCTCAAGCTCCTGG	828
APC-E14_R	CGAGACCAGCCTTACCAACA	
APC-E15_F	AAGTTCTTAATTTACCAGTG	486
APC-E15_R	GTAGTTATCTTTTCACAGTA	
APC-E16-1_F	ATTGGGTCAGAATAGGAAATG	890
APC-E16-1_R	TCTGTTGCTGGATGGTAGTT	
APC-E16-2_F	GTCCCAAGGCATCTCATCGT	667
APC-E16-2_R	GCTGGGTATTGACCATAACTGC	
APC-E16-3_F	ATAGTGTCAGTAGTAGTGATGG	498
APC-E16-3_R	GACACAAAGACTGGCTTACA	
APC-E16-4_F	ATCGAGTGGGTTCTAATCATGG	635
APC-E16-4_R	TGGAACTTCGCTCACAGGAT	
APC-E16-5_F	ATCCAAGTTCTGCACAGAGT	739
APC-E16-5_R	CTCTGAACTGCAGCATTTAC	
APC-E16-6_F	GCTCAAACCAAGCGAGAAGT	750
APC-E16-6_R	TCTGCCTTCTGTAGGAATGG	
APC-E16-7_F	TGCTGGAGAAGGAGTTAGAG	701
APC-E16-7_R	GGTTGGAGGTTAGTTCTGTG	
APC-E16-8_F	GATGATGTTGACCTTTCCAG	574
APC-E16-8_R	CATTATCACCCTTGAGTCTTG	
APC-E16-9_F	ATCAGGCTATGCTCCTAAATCA	824
APC-E16-9_R	TTTCACAGATGGCTTGGCTC	
APC-E16-10_F	GATTCATATTCCAGGAGTTCG	475
APC-E16-10_R	GGCATTCTTGGATAAACCTG	
APC-E16-11_F	TGAGCCAACAGAACCTTACC	777
APC-E16-11_R	AGGAAACGGTCTGAGAAGTAC	
APC-E16-12_F	CTCTATTTCAGGAACCAAAC	878
APC-E16-12_R	CCTCTAACAAGAATCAAACC	

APC, adenomatous polyposis coli; F, forward; R, reverse.

disorders and desmoid tumors (9). *APC* is a tumor suppressor gene located on the long arm of chromosome 5 in band q21, whose mutation is responsible for CFAP and AFAP. The length of the gene is 108,353 bp and it is divided into 15 exons (10). The APC protein has multiple domains that mediate oligomerization as well as binding to a variety of intracellular proteins and has a central role in Wnt signaling by regulating of degradation of proteins associated with this pathway (11).

To date, according to the information available in public databases, such as The Human Gene Mutation Database (http://www.hgmd.cf.ac.uk/ac/index.php), >1,000 different *APC* mutations have been reported, among which >100 cases were contributed by Chinese studies (12-17). While the type of *APC* gene mutation varies, nonsense and frameshift mutations are most frequently seen, and have been predicted to produce truncated proteins, finally leading to the development of diseases (17).

According to certain studies, most FAP patients inherit one *APC* allele mutation from their parents with the other allele being normal. Diseases would not occur until the normal allele undergoes a new mutation (11). It has also been estimated that new germline mutations of *APC* account for one third of FAP patients who have no family history of FAP (18). Certain studies have attempted to explore the correlation between specific *APC* mutations with the clinical phenotype. Certain correlations do exist, for instance, mutations between codons 169 and 1,578 were generally associated with CFAP (19-21). Mutations downstream of codon 1,596 are frequently seen in AFAP (11). Mutations between codons 1,445 and 1,578 were associated with desmoid tumors, whereas those between codons 279 and 1,309 were correlated with the development of duodenal polyposis (22-24). While it appears promising to predict a patient's phenotype by the mutation site of the *APC* gene, this was proven to not be feasible in clinical practice. Considerable variability has been found in the presentation of specific phenotypes in patients with identical mutations (25). This indicates that the phenotype is associated with more factors than genetic mutations (25).

In the present study, the nonsense mutation c.2971G>T (p.E991*) was identified in exon 15 of the *APC* gene. The resulting truncated protein lacked 1,853 amino acids. The wild-type sequence in the affected region of the *APC* gene is highly evolutionarily conserved in different species, including humans, mice, rats, frogs, zebrafish and pufferfish. Through mutation-associated truncation, the APC protein loses its microtubule binding domain, end binding-1 binding domain, β-catenin degradation domain and β-catenin binding domain, which is likely to affect the proliferation and differentiation status of cells and eventually results in colorectal polyps and cancer (26,27). In addition, this nonsense mutation may lead to nonsense-mediated decay of *APC* transcripts. The mutation results in haplo-insufficiency of *APC*, which leads to development of diseases.

While evidence strongly links *APC* gene mutations with FAP, the single factor is not sufficient to explain the etiology of the disease. It is estimated that 10-30 percent of patients with classical FAP do not have any detectable *APC* mutation. A proportion of FAP patients have MAP, an autosomal recessive polyposis syndrome caused by biallelic mutations in the MUTYH gene. Therefore, it is recommended that patients who have a recessive family history of FAP are evaluated for a *MUTYH* mutation (28).

Surgery remains to be the only option to cure the disease, although it remains debatable which surgical option is the golden standard. However, given the substantial risk of rectal cancer developing after colectomy and ileorectal anastomosis, most experts recommend total proctocolectomy for typical FAP patients with multiple rectal adenomas (29). Diet and drugs have been shown to have a role in preventing cancer. Caloric restriction or diet with olive oil, fruits and vegetables significantly reduced the number of polyps in a mouse model of multiple intestinal neoplasia with genetically manipulated APC (30). Randomized trials have shown that celecoxib causes regression of established adenomatous polyps in individuals with FAP. In 2001, the US Food and Drug Administration approved the use of celecoxib in patients with FAP presenting with polyps (31). The proband of the present study successfully underwent laparoscopic total colectomy and ileal anal anastomosis and postoperative recovery was good.

In conclusion, the present study identified a mutation in the *APC* gene in a Chinese family with FAP. The present study added novel variants to the knowledge of *APC* mutations in FAP. Identification of novel mutations will be useful to reveal the correlation between genotypes and phenotypes.

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