APC Gene Mutations in Familial Adenomatous Polyposis:
A Four Family Analysis

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Purpose. APC-associated polyposis conditions include: familial adenomatous polyposis (FAP), attenuated FAP, Gardner syndrome, and Turcot syndrome. FAP is a syndrome with a predisposition for colon cancer, in which hundreds-to-thousands of pre-cancerous colonic polyps develop, with a mean age of onset of 16 years (range, 7-36 years). A colectomy is advised in those affected with classic FAP; however, in those choosing not to undergo a colectomy, colon cancer is inevitable by 39 years of age. We report herein four families with FAP, including a phenotype and genotype analysis.

Methods. This study included five members in four families registered at the ChungHua Christian Hospital. DNA was extracted either from plasma or fresh healthy colonic tissue, and direct DNA sequencing was performed of the mutation cluster region (MCR).

Results. With respect to the phenotype, not all family members expressed polyposis coli. Regarding the genotype, however, three mutations were noted, two of which were novel. All three mutations were heterozygous, one involving codon 1114 (c.3341 insT), one involving codon 1505 (c.4515 delC insAG), and the last involving codon 1556 (c.4669 insA). There were no abnormalities at MCR in one family. There was one polymorphism involving codon 1493 (ACG → ACA) affecting three families.

Conclusion. By using the APC gene MCR testing, we identified three mutation points; nevertheless, taking a detailed family history and conducting a thorough physical examination remain important.

Familial adenomatous polyposis (FAP) is an autosomal dominant disease, which is characterized by high penetrance and a predisposition to the formation of multiple colorectal adenomas and carcinoma of the colorectum. FAP is often accompanied by a variety of extracolonic features, including desmoids, osteomas, epidermoid cysts, congenital hypertrophy of the retinal pigment epithelium (CHRPE), and a variety of other tumors (e.g., brain, thyroid, and liver). FAP arises primarily from a mutation of the adenomatous polyposis coli (APC) gene. APC was cloned in 1991 and was found to be located on chromosome 5q, contained within exon 15. The APC gene has an open reading frame of 8532 bps, with the last exon containing a 6579 bps, uninterrupted
open reading frame. The APC gene is a negative regulator of β-catenin, and is considered to play a role as the gatekeeper in the adenoma-carcinoma sequence. Over 1000 different germ line and somatic mutations in APC have been documented using different techniques. Subsequently, the mutation spectra in the FAP kindred of different countries was established, showing highly variable results (30-80%) and suggesting variation among different ethnic groups. The most common mutations occur from codons 1286-1513, resulting in a truncating protein. A genotype-phenotype correlation has been described for both colonic and extracolonic manifestations, such as CHRPE or desmoid tumors.

In the present study, we report three FAP kindred from the Chang-Hua community in middle Taiwan and conducted a mutation analysis of the APC gene in an effort to facilitate meaningful clinical intervention.

**Patients and Methods**

**Patients**
This study included five members of four families who had registered at the Chang-Hua Christian Hospital. Patients were initially referred based on clinically suspicious findings during colonoscopy.

An initial screening was performed on a single proband from each of the unrelated families. After completion of a thorough genetic counseling session and signing of the informed consent form for participation in the study, a 5 mL blood sample was drawn in EDTA from each individual, including additional family members as available, for the preparation of genomic DNA samples.

**DNA extraction**
DNA in the white blood cell was extracted by using a Puregene® DNA purification kit (Gentra System, USA), according to the manufacturer’s instructions. When fresh colonic tissue was available, normal colonic tissue was used. The DNA was extracted by using a genomic DNA isolation reagent (Geno Marker, Taiwan), according to the manufacturer’s instructions.

**PCR conditions**
The primer used in this study is listed in Table 1, according to the mutation cluster region (MCR) of the APC gene. A polymerase chain reaction (PCR) was performed in a total volume of 50 µL, containing 200 ng genomic DNA, 5 µL of 10x PCR buffer, 25 mM MgCl2, 1.0 µL of 10 mM dNTPs, 1 µL of forward and reverse primers, and 2.5 units of Taq polymerase. The PCR conditions were as follows: initial heat denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 15 s, annealing at 51 °C for 15 s, extension at 72 °C for 15 s, and final extension at 72 °C for 10 min. The PCR products were separated on 2% Agarose gel. The DNA fragment was excised from the gel and purified by using a QIAquick Gel Extraction Kit (QIAGEN Co., USA), then subjected to direct sequencing.

**SNP and published mutations**
To further clarify the identified changes as a polymorphism or mutation, the NCBI dbSNP and HGMD were used as controls.

**Results**
The detailed family pedigree tree is displayed in Fig. 1. There were four families with distinct family histories and no evidence of genetic linkage, including eleven men and five women who were identified by diagnosis. Three of these individuals were diagnosed with polyposis coli only, and thirteen individuals had colorectal cancer. One patient, who had colon cancer was identified to have multiple fibromatosis and hepatocellular carcinoma (HCC). Unfortunately, the year that the diagnosis was made in some family members, especially the proband, was not established with certainty because of the length of time which had elapsed.

There were five members in the four families who were included in the molecular biology study. All study patients were diagnosed with colon cancer. There were three mutation points noted, and all were heterozygous, with two of the mutations deemed novel.
The segments E, G, and H of patients 1 and 2 in family 1 were normal. One polymorphism at codon 1493 in exon 15 (ACG → ACA) was found (Figs. 2a and b). One base-pair deletion with two base-pair insertions at codon 1505 in exon 15 (c.4515 delC insAG) was detected, resulting in a serine-to-arginine substitution (Fig. 3).

The segments E, G, and H of patient 3 in family 2 were normal. The polymorphism was the same as in family 1 (Fig. 2c). One base-pair insertion at codon 1556 in exon 15 (c.4669 insA) was detected, resulting in a threonine-to-asparagine change (Fig. 4). This mutation point was not novel and had been reported by Armstrong.25

The segments G, H, and I of patient 4 in family 3 were normal. The polymorphism was the same as in family 1 (Fig. 2d). One base-pair insertion at codon 1114 in exon 15 (c.3341 insT) was detected, resulting in an arginine-to-leucine switch (Fig. 5).

The MCR of patient 5 in family 4 was normal, thus indicating the mutation site was not detected in this study.

**Discussion**

FAP is an inherited colorectal cancer syndrome and accounts for 1% of all cases of colorectal cancer. It is an autosomal dominant condition and has a prevalence of 1/7000.1 Despite the well-recognized inheritance pattern, approximately 22% of patients have no family history of FAP, revealing a spontaneous mutation.26 A significant breakthrough in the molecular diagnosis of FAP was made with the discovery of the APC gene on chromosome 5. There are two widely used methods for detecting APC germline mutations: the protein truncation test (PTT) and direct DNA sequencing. Generally, the mutation detection rate is up to 80%, and 90% for them.20

In this study, we chose the DNA sequencing direct on MCR for analysis. However, three mutations were noted, and two of them were novel. All three mutations (75%) were heterozygous. Compared with previous studies,9,19,27,28 the mutation rate is similar to Singapore (68%)9 but higher than another two Taiwan report (50%, 44.2%).16,33

The PCR primer used in this study was first reported by Miyaki34 and Fearnhead21.35 It is focused on the MCR design. The most common germline mutation occurs at codons 1061, 1309 in Western country but not in Taiwan or Chinese ethnic groups. Using our study method, the germline mutation in APC gene occurs rather frequently within MCR. However, we cannot detect mutations outside of the region. Otherwise, the detect rate of the four primers are relative acceptable.

Compared with other molecular diagnosis method, it is relative cost effective v.s. PTT, whole APC gene direct sequencing; relative technical feasible v.s. PTT, SSCP, haplotype analysis, heteroduplex analysis, and protein allele-specific expression assay. The DHPLC may be another good choice but machine-limitation should be considered.

After searching the HGMD databases, we found two novel mutations. These two mutations were located at codons 1114 and 1505.

The codon 1114 mutation is located in the 15-amino acid repeats region.21 Those repeats also provided another binding site for β-catenin. Majority of mutation in those region will retain in the APC protein and disrupt subsequent down-regulation of β-catenin. But contrast to other reports, this family with codon 1114 mutation in exon 15 of APC gene did not record CHRPE. CHRPE is mostly present in patients with mutations between codons 457 and 1444.21,29 Pang et al.29 had also mentioned about three FAP with CHRPE patients without any APC mutation be found, thus indicate the CHRPE may involved other mechanism.

The codon 1505 and 1556 were within the seven 20-amino acid repeat (20-AARs) motif region, central of the APC protein, which predicted to be the main binding sites for β-catenin and axin.16,21 The complex promotes GSK3β-mediated phosphorylation. In this way, β-catenin is marked for subsequent degradation. Disruption of it suggested a target for elimination during tumorigenesis.2,21 Because APC is a tumor suppressor gene and consistent with Knudson’s two-hit hypothesis,30 both alleles needed mutated for further tumorigenesis. Loss of heterozygosity
(LOH) phenomena is the most likely second hit in colonic polyps for whose mutation located within the first and second 20-AARs, close codon 1300. Thus the majority of truncated mutant proteins lack all or most of the 20-AARs.

The desmoid tumors in FAP appear to be limited to patients with mutations between codons 1403 and 1578. Desmoid tumor in FAP shows similar LOH association, but different region distal to codon 1400. Most desmoid had two APC hits had one mutated allele with no 20-AARs. Mutations in both the families may be lack of the strong LOH phenomena, and makes the lack of desmoid clinical appearance. In Wei SC study, there is one patient with mutation in codons 1554-1556, but he also did not mentioned cases with desmoid tumor.

Interesting, one patient, with codon 1505 mutation, was noted to have fibromatosis over chew, chest wall, and abdominal wall. This female was also found to have HCC later. It is consisted with most reports that extracolonic manifestations commonly with mutations between codons 1445 and 1578 or between codons 1395 and 1493.

Genetic analysis is the basis for early diagnosis and chemoprevention of FAP. A thorough understanding of the molecular mechanisms will help to classify cases of hereditary colorectal cancer and to design optimal therapies. Individuals should be offered genetic counseling, predictive molecular testing and, when indicated, endoscopic surveillance at appropriate intervals. Likewise, chemoprevention can be prescribed for those with a diagnosis of hereditary colorectal cancer. Clinical surveillance and chemoprevention can be restricted to patients with germ line defects.

Other family members were not included due to their unavailability at the time. Analysis of other family members is needed.

**Conclusion**

A molecular analysis of five members of four families with FAP revealed three mutations, two of which were novel. Such an approach, when combined with a detailed family history and complete physical examination, should improve diagnostic capabilities and optimize treatment plans.

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**References**


31. Lamllum H, Ilyas M, Rowan A, et al. The type of somatic mutation at APC in familial adenomatous polyposis is


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