RESEARCH ARTICLE

Colon Cancer Prevention by Detection of *APC* Gene Mutation in a Family with Attenuated Familial Adenomatous Polyposis

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Abstract

Background: Genetic mutation is a significant factor in colon CA pathogenesis. Familial adenomatous polyposis (FAP) is an autosomal dominant hereditary disease characterized by multiple colorectal adenomatous polyps affecting a number of cases in the family. This report focuses on a family with attenuated familial adenomatous polyposis (AFAP) with exon 4 mutation, c.481C>T p.Q161X of the *APC* gene. Methods: We analyzed 20 members of a family with AFAP. Clinical and endoscopic data were collected for phenotype determination. Genetic analysis was also performed by direct sequencing of the *APC* gene. Result: Five patients with a phenotype of AFAP were found. Endoscopic polyposis was demonstrated among the second generation with genotype mutation of the disease (age > 50 years) consistent with delayed phenotypic adenomatous polyposis in AFAP. *APC* gene mutation was identified in exon 4 of the *APC* gene, with mutation points of c.481C>T p.Q161X. Laparoscopic subtotal colectomy was performed to prevent carcinogenesis. Conclusion: A family with attenuated familial adenomatous polyposis of *APC* related to exon 4 mutation, c.481C>T p.Q161X, was reported and the phenotypic finding was confirmed by endoscopic examination. Genetic mutation analysis might be advantageous in AFAP for long term colon cancer prevention and management due to subtle or asymptomatic phenotype presentation in early adulthood.

Keywords: Colon CA - AFAP - genetic mutation - *APC* gene - cancer prevention

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Introduction

Colorectal cancer (CRC) is one of the most common malignancies worldwide including Thailand (Lohsiriwat et al., 2009). In the Asia Pacific region, the incidence of colorectal cancer has been increasing. It is the second most common cause of death from cancer, responsible for 396,000 deaths annually (Yang et al., 2004). Decreased genome stability, mutation of tumor-suppressor genes and activation of oncogene pathways have been known to contribute to colorectal cancer development (Markowitz and Bertagnolli, 2009). Germ-line *APC* mutations, tumor-suppressor genes, give rise to familial adenomatous polyposis. Familial adenomatous polyposis (FAP) is an autosomal dominant hereditary disease characterized by hundreds of thousands of adenomatous polyps in the large bowel. The standard clinical diagnosis of typical/classical FAP is based on the identification of more than 100 colorectal adenomatous polyps (Vasen et al., 2008). Among patients with a milder polyposis phenotype than classic FAP, the condition is termed attenuated familial adenomatous polyposis (AFAP) (Knudsen et al., 2010). Clinical characteristics of AFAP comprise fewer colorectal adenomas, delayed onset of adenomatosis by up to 20–25 years and delayed onset of colorectal cancer. The clinical diagnosis of AFAP is more difficult. Proposed criteria for diagnosis of AFAP are a dominant mode of inheritance in combination with 3–99 colorectal adenomas at the age of 20 or older (Knudsen et al., 2003).

The *APC* gene located at 5q21-22 contains 15 exons and encodes a multi-domain protein that plays a major role in tumor suppression. Inappropriate activation of protein through loss of *APC* function contributes to cancer progression, as in FAP. Attenuated familial adenomatous polyposis can be caused by inherited mutations in the *APC* gene or autosomal recessive *MYH* gene mutation (van der Luijt et al., 1995), (Sampson et al., 2003). Germ-line *MYH* mutations predispose persons to a recessive phenotype of FAP with negative *APC* gene mutation (Sieber et al., 2003). In AFAP patients, three different regions of *APC* gene mutation have been identified, (1) at the 5' end spanning exons 4 and 5, (2) within exon 9, and (3) at the

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3' distal end of the gene while the phenotype typically demonstrates a predominance of right-sided colorectal adenomas and rectal polyp sparing. (Soravia et al., 1998) The advantage of genetic analysis is early detection of AFAP cases harboring the abnormal gene.

The aim of this study has been early, noninvasive and specific detection of point *APC* gene mutation for the diagnosis of AFAP among members of a family. Genetic mutation analysis of AFAP family members will assist in prophylactic treatment to prevent carcinogenesis especially, in case of delayed on set and mild phenotype expression.

Materials and Methods

This study was conducted on clinical data collected upon conclusion of routine service at Chulalongkorn King Memorial Hospital and presented in this report as anonymous. The patients were informed about the objective of genetic testing for the disease diagnosis and prevention, subsequently gave their written consents.

Subjects

The index case, a 56- year old male was referred to a consultant because his brother aged 51 years had developed colon CA. His mother had succumbed to colon CA at the age of 72 years. Upon endoscopic diagnosis of adenomatous polyposis, all members of the family were invited for investigation and colon CA preventive measures by FAP screening for phenotype and genotype abnormalities. The family comprised 20 subjects, 11 males and 9 females, aged between 16 and 67 years. (Figure1) All were generally healthy individuals. The objective of the study, screening the *APC* gene for mutations in order to facilitate diagnosis of AFAP was explained to the patients and subsequently, written consent was obtained.

Clinical assessment

Initial demographic data, clinical presentation and laboratory data were obtained. Colonoscopy was performed on six members of the second generation. Endoscopic findings of colon polyposis and pathological adenoma were confirmed. Blood samples were obtained from 20 AFAP family members.

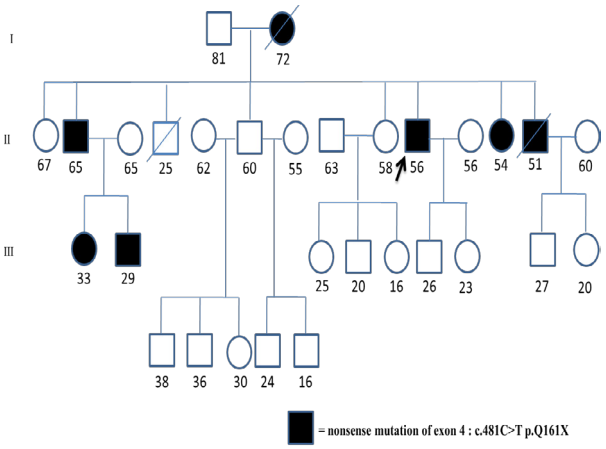

Figure 1. Pedigree of Attenuated Familial Adenomatous Polyposis Family

Detection of APC gene mutation

DNA extraction and PCR amplification: Blood samples were obtained from all family members. Genomic DNA was extracted from 100-µl samples of peripheral blood mononuclear cells incubated with proteinase K in lysis buffer, followed by phenol/chloroform extraction and ethanol precipitation. Finally, the pellet was dissolved in 30 µl sterile water and stored at -20°C until further tested. To determine mutation, specific primer sets within the *APC* gene were designed (primer sequences upon request). Two microli=ters of DNA were used to set up 25-µL PCR reactions by using the Perfect Taq plus MasterMix (5 prime GmbH, Hamburg, Germany). The amplification reaction was carried out under the following conditions; pre-incubation at 95°C for 3 min, followed by 35 cycles comprising denaturation at 95°C for 30s, primer annealing at 55°C for 30s, and extension at 72°C for 1min, and concluded by a final extension at 72°C for 5 min. Upon electrophoresis in 2.0% agarose gel stained with EtBr, the amplification products were visualized under UV light.

Direct DNA sequencing: The PCR products were purified using the Agarose GelExtract mini Kit (5 prime GmbH, Hamburg, Germany) according to the manufacturer's instructions. Direct sequencing was performed by First BASE Laboratories Sdn Bhd (Selangor Darul Ehsan, Malaysia).

APC gene mutation analysis: The nucleotide and deduced amino acid sequences were compared with reference sequences of the *APC* gene available at the NCBI (National Center for Biotechnology Information) GenBank database using the BLAST (Basic Local Alignment Search Tool) program (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Results

A 56-year old male asymptomatic patient with a family history of colon cancer was referred for colon cancer surveillance. His mother (first generation) and brother (second generation) had been diagnosed with colon cancer at the age of 72 and 51 years, respectively. Colonoscopy revealed numerous sessile colon polyps 0.3-1cm in diameter, more predominant at the right side than the left side of the colon (Figure2) sparing the rectum. Histopathology confirmed the diagnosis of adenomatous polyp (Figure3). Endoscopic examination of the entire family showed that two of the remaining five relatives (second generation) also displayed the phenotype of familial adenomatous polyposis. Molecular analysis of the *APC* gene in the proband showed a mutation at exon 4, c.481C>T causing an amino acid alteration from Gln to a stop codon at position 161 (p.Q161X). (Figure4) Furthermore, all second generation family members displaying the phenotype had a point mutation at codon 161 of the *APC* gene and two third generation siblings also carried this mutation (Figure1). The members with the genetic mutation and cases at risk of colon cancer development in the third generation were scheduled for colonoscopy and long term management. All cases with the phenotype of adenomatous polyposis were scheduled

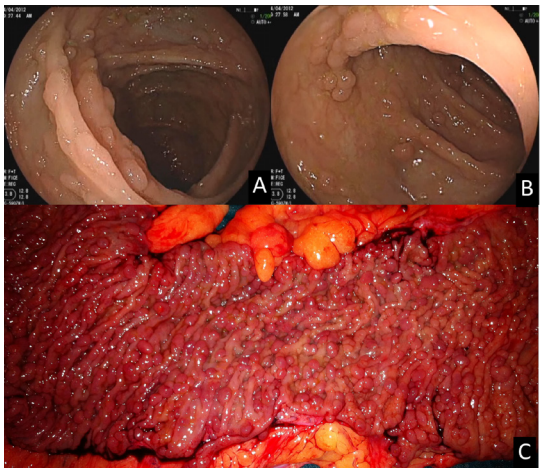

Figure 2. Endoscopy of Adenomatous Colon Polyposis. Numerous Sessile Colon Polyps 0.3-1 cm in Diameter, More Predominant at the Right Side (A) than Left Side of the Colon (B). Surgical Specimen from Subtotal Colectomy. (C) (Endoscopy and Gross Surgical Specimen Picture from Proband)

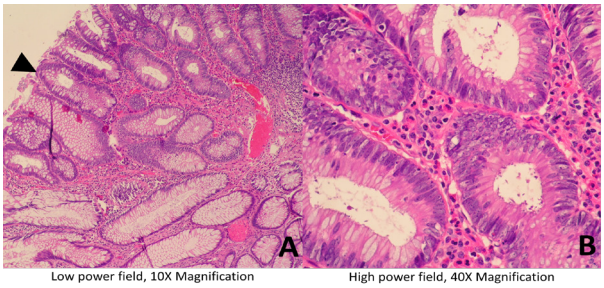

Figure 3. H&E Histopathology of Adenomatous Polyp. (A) Transitional Zone from Normal Mucosa to Adenomatous Change. (Black Arrow), (B) Epithelium Lined with Mostly Low-Grade Dysplastic Colon Epithelium. (Histological Specimen From Proband)

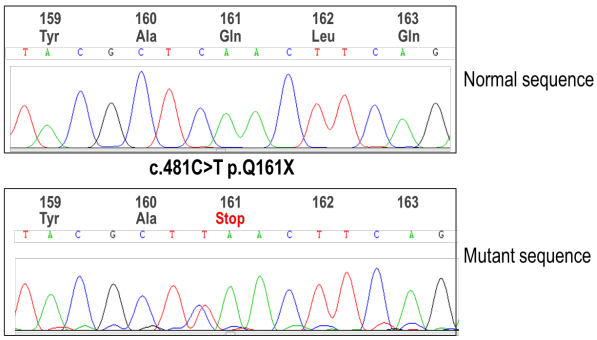

Figure 4. One Allele of the *APC* Gene Exon 4 Mutation at Codon 161 with Amino Acid (Gln; CAA) Alteration to Stop Codon (X; TAA)

for colon cancer prophylaxis management by laparoscopic subtotal colectomy. Regular colonosopic surveillance after subtotal colectomy was also scheduled. Extra-intestinal manifestations including desmoid tumor, osteoma, papillary thyroid carcinoma and hepatoblastoma were not found in any of these family members.

Discussion

Mutations in the *APC* gene cause both classic and attenuated familial adenomatous polyposis. Familial adenomatous polyposis related with *APC* gene mutation is characterized by genetic heterogeneity and different disease severity and diversity. These mutations affect the ability of the cell to maintain normal growth and function. Cell overgrowth results from the mutations (Scott et al., 2001). The *APC* gene encodes a multi-domain protein that plays a major role in tumor suppression by antagonizing the carcinogenesis pathway and also has a role in cell migration, adhesion, chromosome segregation, spindle assembly, apoptosis, and neuronal differentiation (Hanson and Miller, 2005). Mutation of *APC* gene presents as an autosomal dominant disease and the penetration rate of inherited cases is close to 100% at the age of 40 years (Bisgaard et al., 1994).

Among AFAP patients with *APC* gene mutation, the median age of colon cancer development is 58 years with 75% in the proximal colon (Burt et al., 2004). This AFAP family with *APC* gene mutation at codon 161 of exon 4 and phenotypically presenting a predominance of right-sided adenomatous polyposis was diagnosed as typical for AFAP. This point mutation had previously been reported as predicting the AFAP phenotype (Friedl and Aretz, 2005).

The phenotype of AFAP may mimic classic familial adenomatous polyposis and in some cases is difficult to distinguish from sporadic adenomas and colorectal cancer. Hence, genetic mutation analysis might be essential.

Without prophylactic colectomy, the risk of colorectal cancer by the age of 40 years is almost 100% in classical FAP and up to 80% by the age of 80 years in AFAP (Vasenand Moslein, 2008). Due to very high incidence of colon tumorigenesis, patients with adenomatous polyposis have to undergo colectomy (Levin et al., 2008). Despite mutation specific phenotype expression, age at manifestation and course of the disease being rather variable, therapeutic decisions should be based on colonoscopic findings in individual patients rather than on the site of mutation (Friedl et al., 2001). Colectomy should be considered when the patient presents with more than 20 adenomas, with the diameter of the adenoma exceeding 1cm or advanced histology findings (Jasperson et al., 2010). Chemoprevention with medications such as nonsteroidal anti-inflammatory drugs (NSAID) and selective cyclooxygenase-2 (COX-2) inhibitors delay the development of adenomas and prevent recurrence of adenomas in the retained rectum after prophylactic surgery (Kim and Giardiello, 2011). Long term adverse effects of these medications limit their use as medication chemoprophylaxis.

Among patients with FAP, prenatal testing by amniocentesis, chorionic villous sampling (CVS), or pre-implantation genetic diagnosis (PGD) can reveal whether an embryo or fetus is affected. Most patients with FAP are willing to consider prenatal testing to prevent disease transmission to their children (Kastrinos et al., 2007).

We have reported *APC* gene exon 4 mutation in familial cases of attenuated familial adenomatous polyposis in Thailand. Genetic testing is an important tool to identify affected cases and to facilitate more focused examinations of patients at risk. In the future, genetic testing might constitute a promising tool in prophylaxis therapy and preventive measures of any hereditary cancer.

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