Papillary Thyroid Carcinoma Associated with Familial Adenomatous Polyposis: Molecular Analysis of Pathogenesis in a Family and Review of the Literature

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Abstract. We found a case of a papillary thyroid carcinoma that was accompanied by a familial adenomatous polyposis (FAP) in a 29-year-old female. She had hundreds of adenomas inside the entire colon and a congenital hypertrophy of the retinal pigmented epithelium (CHRPE). The patient underwent a total thyroidectomy and a central compartment neck node dissection. Gross examination of the thyroid identified two solid and cystic lesions. The pathological finding of thyroid cancer revealed a mixture of a peculiar nuclear clearing, cribriform, morula formation, trabecular and papillary pattern. The patient’s brother had undergone a total colectomy due to FAP at the age of 25. Genetic analyses of the patient’s family members revealed that she and her brother had the same germline mutation, in which five nucleotides (AAAGA) were deleted from codon 1309 of the adenomatous polyposis coli (APC) gene exon 15. Strong and frequent immunoreactivities of β-catenin and p53 were evident in the tumor tissue. At the time of writing, a preventive colectomy was still under consideration for the patient. Genetic counseling was given to the other family members, who were not attacked by this disease, in order to allay their fears of cancer.

Key words: Papillary thyroid carcinoma, Familial adenomatous polyposis, APC gene, Germline mutation, Genetic counseling

Familial adenomatous polyposis (FAP) is inherited by an autosomal dominant trait and has an incidence of one per 10,000 persons. It is characterized by several hundred or even thousand adenomatous polyps spreading over the colon and the rectum, which can cause a colon and rectal carcinoma if left untreated. FAP is caused by germline mutation of the adenomatous polyposis coli (APC) gene, which is located on the long arms of chromosome 5 (5q21-22), and is known as a tumor suppressor gene [1, 2]. Other possible lesions besides the accompanying colon lesions are an osteoma that develops in the bones, an epidermoid cyst in the skin, a desmoid tumor in the abdominal wall, congenital hypertrophy of the retinal pigment epithelium (CHRPE) in the retina, etc. The polyps are found in the stomach in approximately half of FAP cases, and most of these polyps are the non-tumoral fundic gland polyps that characterize fundic gland hyperplasia and cystic dilatation [3]. Several adenomas can develop in the small intestine as well as in the colon, and a malignant tumor can develop in the duodenum or near the ampulla [4]. Sometimes, the adenomas accompany a thyroid carcinoma [5].

Cases with an accompanying thyroid carcinoma occur in 1–2% of FAP patients, and are found mainly in young women aged 30 years or below at the time of diagnosis. Moreover, 95% of these cases are histologically the papillary type [6, 7], and some show a peculiar cribriform pattern [8]. In Cetta’s study,
codons 1061 and 1309 germline APC mutations were frequent, but this result was not supported in other literature. Cetta et al. suggest that the majority of the mutations was in exon 15 and associated with the CHRPE area (codons 463-1387) [9].

We experienced cases of a germline mutation in a 29-year-old female patient and her brother in which gene analyses showed that five nucleotides were deleted from codon 1309 of the APC gene exon 15. The patient was diagnosed with a papillary thyroid carcinoma and FAP. We report these cases with a molecular analysis and review of the relevant literature.

**Materials and Methods**

**Patients**

A 29-year-old female patient (III-8) was diagnosed with FAP and was recommended for surgery on the basis of a colonoscopic finding of multicentric polyps. However, the patient rejected the operation and was admitted to hospital 2 years later due to a thyroid gland mass that had recently been found by chance.

The patient’s father (II-4) had died of a rectal carcinoma at the age of 42, and the patient’s brother (III-5) had undergone a total colectomy due to FAP at the age of 25 (Fig. 1). The patient’s fixed mass was 2 × 3 cm in size and she did not experience any pain on her neck. Neck ultrasonography revealed a solid mass accompanying a microcalcification and a cystic mass with a clear margin on the right thyroid gland. Computed tomography (CT) of the thyroid gland revealed that the right neck region did not exhibit any lymph node metastasis except for a 2.0 cm by 1.7 cm sized multicentric mass in the thyroid gland. Thyroid scanning revealed two cold nodules in the region of the right thyroid gland.

The hormone examination results were T3 135.96 ng/dl (normal: 80~220 ng/dl), fT4 1.24 ng/dl (normal: 0.73~1.95 ng/dl), TSH 2.44 uIU/ml (normal: 0.34~3.5 uIU/ml), anti-thyroglobulin Ab 10.86 IU/ml (normal: <60 IU/ml), and anti-microsomal Ab 16.61 IU/ml (normal: <60 IU/ml). The tumor marker test showed CEA 0.739 ng/ml (normal: 0~5 ng/ml). However, there were no other specific findings.

A gastroscopy revealed a vast number of polyps in the vestibular region of the stomach and an ulcer in the ampullar region. In addition, colonoscopy identified several hundred colon polyps in the whole colon. Fine needle aspiration biopsy of the thyroid gland showed intranuclear cytoplasmic inclusions consistent with a papillary thyroid carcinoma. All of the colon polyps were tubular adenomas, and the gastric polyps were hyperplastic polyps.

Funduscopia revealed multicentric hyperpigmentation in both fundi, and, in particular, an egg-shaped hyperpigmentation four times larger than that of a disk in the right eye toward the peripheral region. These findings corresponded with the diagnosis of CHRPE.

The patient underwent a total thyroidectomy and a central compartment neck node dissection. At the time of writing, 6 months after initial diagnosis, the patient was still being administered a COX-2 selective antagonist, and a preventive colectomy remained under consideration.

**Methods**

The following gene analyses were performed, with their written informed consent, to confirm whether or not the patient or her family members (III-5, III-6, and III-8) had germline mutations.

**Template preparation**

Genomic DNA was prepared from the patients’ lymphocytes obtained from 10 ml EDTA blood using the protocol described by the QiaAmp DNA blood mini kit (Qiagen; Valencia, CA , USA).
PCR and sequencing

Two overlapping fragments of the APC coding sequence on exon 15 were PCR amplified from genomic DNA (Fig. 2). First 3,141bp fragment underwent PCR in 50 µl reaction mixtures, 300 ng of gDNA, 5 µl 10x PCR buffer, 3 µl 2.5 mM dNTP Mix, 10 pmole of each oligonucleotide primer pair (1960F and 5101R), and 1.5 unit BioTherm DNA polymerase (Genecraft, Munster, Germany) as follows: 94°C for 5 minutes, followed by 35 cycles of 95°C for 5 minute, 50°C for 90 seconds, and 72°C for 2 minutes, and then a final 7-minute extension at 72°C. For PTT test, we performed PCR of Segments I (1,831 bp) and II (2,017 bp) in 50 µl reaction mixtures, 1 µl of PCR-generated DNA templates (3,141 bp), 5 µl 10x PCR buffer, 3 µl 2.5 mM dNTP Mix, 10 pmole of each oligonucleotide primer pair (pAP1F & 3790R and pAP1F & 5101R), and 1.5 unit BioTherm DNA polymerase (Genecraft, Munster, Germany) as follows: 94°C for 5 minutes, followed by 35 cycles of 95°C for 5 minute, 60°C for 90 seconds, and 72°C for 2 minutes, and then a final 7-minute extension at 72°C.

Direct Sequencing of PCR products was performed using a Bigdye terminator cycle sequencing kit on an ABI PRISM® 377 DNA Sequencer (Applied Biosystems, Foster City, CA, USA) according to the manufacturer’s protocol. To detect the deletion of five nucleotides from codon 1309, herein we used the 3714F and 4220R PCR primer pair.

Protein truncation test (PTT)

The essential feature of PTT is a specially designed, tailed sense primer (pAP1F & pAP2F as shown in Fig. 2) which contains five different regions: restriction site sequence (GGATCC), bacteriophage promoter T7 sequence (TAATACGACTCACTATAGG), spacer (AACAGA), eukaryotic translation initiation sequence (CCACCATG), and target sequence.

Transcription and translation of segments I and II were performed with 40 µl TNT® Quick Master Mix (Promega, Madison, WI, USA), 1 µl 1 mM methionine, 2.5–5 µl PCR-generated DNA templates, 1–2 µl Transcend Biotin-Lysyl-tRNA, Nuclease-Free water to a final volume of 50 µl and then the reaction was incubated at 30°C for 60–90 minutes. Four microliters of the translation reaction product was added to 20 µl of SDS sample buffer and heated at 100°C for 2 minutes to denature the protein. The cooled denatured sample was then loaded onto SDS-polyacrylamide gels. Electrophoresis was carried out at a constant current of 15 mA in the stacking gel (5%) and 30 mA in the separation gel (10%). For colorimetric detection, the translation reaction product was blotted from the SDS-PAGE gel to PVDF using standard apparatus and protocol. For analysis of proteins, we used the Transcend™ Non-Radioactive Translation Detection Systems (Promega, Madison, WI, USA) as follows. Fifteen milliliters of TBST was added to PVDF blot membrane and incubated at room temperature for 60
minutes. The TBST was poured off and the diluted
6 μl of Streptavidin-AP solution was added to the
blot and agitated gently for 45–60 minutes. The
Streptavidin-AP was poured off and washed 2 times
for 1 minute each with 15 ml TBST and then 2 more
times with 15 ml distilled water for 1 minute.
When the color was developed to the desired inten-
sity in Western Blue Stabilized Substrate for Alkaline
Phosphatase, the reaction was stopped by washing the
membrane in deionized water for several minutes and
then air-drying it.

Immunohistochemistry

Immunostainings for p53 and β-catenin were per-
formed using mouse monoclonal IgG antibodies of
P53 (Novocasta, Newcastle, UK) and β-catenin
(Zymed, San Francisco, CA, USA), respectively. Four
micrometer-thick tissue sections were heated by micro-
wave with citrate buffer pH 6.0 for 10 minutes. After
blocking endogenous peroxidase and nonspecific bind-
ing, the primary antibody at a dilution of 1 : 100 was
incubated at room temperature for 2 hrs, followed by
detection with the ultrastreptavidin system.

Results

Pathologic findings of the tumor

Gross examination of the thyroid identified two
solid and cystic lesions. The thyroid specimen showed
an ill defined nodule with papillary configurations
containing nuclear inclusions, nuclear grooves and
clearing. In addition, the specimen was combined with a
cribiform, trabecular and morular pattern (Fig. 3).

Protein truncation test and direct sequencing

The patient and her brother exhibited regions of a
truncation mutation on the Protein Truncation Test
(PTT) (Fig. 4). Moreover, DNA sequencing for the
regions found a germline mutation, which produced a
premature stop codon by the deletion of five nucleo-
tides (AAAGA) from codon 1309 of the APC gene
exon 15 (3085-5101 nucleotides) (Fig. 5). However,
the patient’s sister (III-6) showed neither a truncation
mutation finding on PTT nor a germline mutation on
DNA sequencing. As a result of DNA sequencing,
the patient’s brother was evaluated for a thyroid car-
cinoma by ultrasonography, but there were no specific
findings.

Immunohistochemistry

Strong and frequent immunoreactivities of β-catenin
and p53 were evident in the tumor tissue (Fig. 6).

Discussion

Since Crail first published a case of a thyroid carci-
noma in a patient with FAP in 1949 [10], Cetta et al.
reported 112 cases of FAP patients with an accompa-
nying thyroid carcinoma [9]. As a result of their inves-
tigation, the average age at diagnosis was 27.65 years
old, and women were mainly affected with a female to
male ratio of 17 : 1. Moreover, a thyroid carcinoma and
FAP were diagnosed simultaneously in one third
of the cases, and the thyroid carcinoma was diagnosed
first in one third of the cases and later in the other one
third [9]. Consequently, the histological findings re-
vealed papillary thyroid carcinomas in over 95% of the
cases, and particularly revealed many cribriform types,
which are quite unusual, in the thyroid carcinoma
associated with FAP [8]. These findings suggest that
when a patient, who undergoes surgery due to simple
thyroid carcinoma, shows the cribriform type in the
histological finding, the possibility of an accompany-
ing FAP should be investigated, since there is a strong
likelihood the patient will require a constructive exam-
ination such as a colonoscopy. The current case also
showed the cribriform-morular variant in the histo-
logical examination.

FAP is inherited by the autosomal dominant trait
and is accompanied by colorectal multicentric ade-
nomatous polyps and other lesions besides the colon
lesion. The cause of FAP is a germline mutation of
the APC gene, which is located on the long arms of
chromosome 5 (5q21-22), and is known to be a tumor
suppressor gene [1, 2]. The APC gene comprises 15
exons, and shows various expression patterns accord-
ing to the location of the APC gene mutation, based
on a study of the genotype-phenotype correlations.
Classic FAP is shown between codons 1255 and 1467
[11], and attenuated APC is shown between both ends
of the APC gene, i.e. between the proximal part of the
5'-portion and the distal part of the 3'-portion [12].
Fig. 3. Pathological findings of the right thyroid gland. A, B, papillary cancer with nuclear inclusion (thick arrow), mixture of cribriform, trabecular and papillary pattern; H&E stain, ×40, ×400, respectively. C, D, scattered morular areas (thin arrow) and abundant eosinophilic cytoplasms; H&E stain, ×100, ×400, respectively.

Fig. 4. This patient (proband, III-8) and her brother (III-5) exhibited regions (asterix) of a truncation mutation on the Protein Truncation Test (PTT). However, the patient’s sister (III-6) showed no truncation mutation finding.

Fig. 5. DNA sequencing. A, this patient (III-8) had a germline mutation, in which five nucleotides (AAAGA) were deleted from codon 1309 of the APC gene exon 15. B, the patient’s brother (III-5) also had the same germline mutation. C, the patient’s sister with no mutation of the APC gene.

Fig. 6. Immunohistochemistry of the right thyroid gland. A, immunostaining with β-catenin antibodies shows intracytoplasmic staining of the tumor cells, ×400. B, immunostaining with p53 antibodies shows nuclear staining of the tumor cells, ×400.
CHRPE generally appears on the distal part of exon 9 (from codons 463 to 1387) [13], and the desmoid tumor comes from somewhere between codons 1403 and 1578 [14]. The thyroid carcinoma is known to appear in the mutation of the portions usually below codon 1220. Cetta et al. reported that the location of the germline mutation of the APC gene was exon 15 in 11 out of 13 FAP patients with an accompanying thyroid carcinoma. In addition, a germline mutation was observed at codon 1061 in 6 out of 11 patients, at codon 1309 in 2 patients, and near codon 1220 in most patients [9].

The APC gene mutation generates the tumor by activating β-catenin, which is a downstream, signal-transducing substance of the Wnt signal transduction. This is supported by the data reported by Xu et al. [15] who suggested that the accumulation of the mutant β-catenin contributes to the development of the cribriform-morular variant of a papillary thyroid carcinoma. The finding of cytoplasmic β-catenin overexpression in the present patient by immunohistochemistry suggests the accumulation of the β-catenin unbound to APC protein, but the possibility of the mutation of β-catenin gene (CTNNB1) cannot be eliminated.

Soravia et al. reported that a thyroid tumor is generated if a somatic mutation or loss of heterozygosity of the APC gene occurs in addition to the germline mutation of the APC gene and the appearance of a biallelic inactivation. In addition, they mentioned that an interaction between the APC gene and the ret/PTC and p53 genes plays an important role in the occurrence of a thyroid carcinoma in FAP patients [16]. During the ret/PTC rearrangement, ret/PTC 1 and ret/PTC 3 are often found in the thyroid carcinoma accompanying FAP. Moreover, the p53 gene is related with the late occurrence of a thyroid carcinoma and its manifestation is also associated with recurrence [16]. p53 was highly stained in the tumor cells of the present patient. FAP occurs in the colon before and after adolescence. Therefore, it is important that the family members in a direct line with the member attacked by FAP undergo a periodical check up for colon cancer. Consequently, a sigmoidoscopy will be required every year from the age of 10 or 12 [17], and an esophagogastroduodenoscopy every one to three years from approximately 25 years old or at the latest when the first intestinal polyp is found [18]. In the absence of any fixed guidelines for managing a thyroid carcinoma associated with FAP, active management such as physical examination or thyroid ultrasonography needs to be performed [19]. Moreover, because it has been verified that FAP is induced by a mutation of the APC gene, gene analysis is essential for patients with this disease as well as for the unaffected family members. In the current case, because the patient and her brother demonstrated APC gene mutations, they were informed of the possibility of this disease with its associated morbidity and of the necessity of a periodical check up and screening test. Gene analysis confirmed that the patient’s sister (III-6) was a non-gene carrier for FAP. Consequently, she was informed that she was unlikely to contract the disease and was released from the anxiety regarding the onset of FAP and annual colonic screening.

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References


