World Journal of Surgery

Published Online: 17 April 2006

World J Surg (2006) 30: 775–779
DOI: 10.1007/s00268-005-0368-3

Mutational Analysis of the *APC* Gene in Cribriform-Morula Variant of Papillary Thyroid Carcinoma

Shinya Uchino, MD, PhD,[1] Shiro Noguchi, MD, PhD,[1] Hiroto Yamashita, MD, PhD,[1] Hiroyuki Yamashita, MD, PhD,[1] Shin Watanabe, MD,[1] Takahiro Ogawa, MD, PhD,[1] Akiko Tsuno, PhD,[1] Akiko Murakami, PhD,[1] Akira Miyauchi, MD[2]

[1]*Noguchi Thyroid Clinic and Hospital Foundation, Noguchi Naka-machi 6-33, Beppu, Oita, 874-0932 Japan*
[2]*Kuma Hospital, 8-2-35 Shimoyamate-dori, Chuo-ku, Kobe 650-0011, Japan*

Abstract

Introduction: Familial adenomatous polyposis (FAP) is an inherited autosomal dominant syndrome caused by germline mutations in the adenomatous polyposis coli (*APC)* gene. Papillary thyroid cancer is one of the extracolonic manifestations of FAP. A characteristic histologic feature of this type of thyroid tumor is the cribriform-morula variant of papillary thyroid carcinoma (CMVPTC).

Methods: To investigate roles of the *APC* and *β-catenin* genes in the development of CMVPTC, we examined germline and somatic mutations of these genes in a female patient with CMVPTC and FAP. The patient had undergone total colectomy at the age of 19 years and total thyroidectomy at age 25 years.

Results: Numerous tumors were disseminated in both lobes of the thyroid gland, and histopathologic examination revealed typical CMVPTC. DNA was extracted from peripheral blood leukocytes and 12 CMVPTC tumors, and exons 1–15 of the *APC* gene and exon 3 of the *β-catenin* gene were examined. A germline mutation was detected in exon 13 of the *APC* gene, and this mutation generated a premature stop codon. Six somatic mutations (922delC, 1602delA, 1821delT, 1920delG, 2706del20, 2804insA) were found in the CMVPTC specimens. All mutations were truncating mutations in the N-terminus of the APC protein. Loss of heterozygosity was not observed in the remaining tumor tissues without somatic *APC* mutations. There were no mutations of the *β-catenin* gene in peripheral blood leukocytes or 12 CMVPTC specimens.

Conclusions: These results suggest that *APC* mutations play an important role in the development of CMVPTC and occur predominantly in the *5′ side of the APC* gene between codons 308 and 935.

Familial adenomatous polyposis (FAP) is an autosomal dominant syndrome characterized by hundreds to thousands of adenomatous colonic polyps that develop until early adulthood. The prevalence of colorectal carcinoma is approximately 50% of FAP patients at the age of 40 years and approximately 90% of FAP patients at the age of 60 years.[1] The causative gene, *APC*, is located on chromosome 5q21 and is mutated in approximately 70% of FAP patients.[2,3] Extracolonic manifestations include congenital hypertrophy of the retinal pigment epithelium (CHRPE), desmoid tumors, gastric and upper intestinal adenomas and carcinomas, hepatoblastomas, osseous tumors, soft tissue tumors, and other organ tumors.

Correspondence to: Shinya Uchino, MD, PhD, San Francisco Comprehensive Cancer Center, University of California, San Francisco, USA, e-mail: kebebewe@surgery.ucsf.edu

Thyroid tumor is another extracolonic manifestation of FAP and was first described by Crail in 1949.[4] A study of patients in the St. Mark's Hospital Polyposis Registry revealed an association between FAP and thyroid carcinoma.[5] Young women with FAP are at particular risk of developing thyroid cancer, and their chance of being affected is approximately 160 times higher than that of normal individuals. Peculiar nuclear clearing of papillary thyroid carcinoma with FAP was first reported in 1992,[6] and Harach *et al.*[7] described the characteristic histopathology of the cribriform-morula variant of papillary thyroid cancer (CMVPTC) in patients with FAP in 1994. Cetta *et al.*[8] reported that most germline *APC* mutations found in patients with FAP-associated CMVPTC are located in the CHRPE region (codons 457–1444). Mutations in the *APC* gene typically lead to a truncated APC protein that lacks the β-catenin-binding site and therefore cannot degrade β-catenin. APC forms a macromolecular complex with glycogen synthetase kinase-3β (GSK-3β), β-catenin, and Axin and is involved in the Wnt signal transduction pathway. Binding of β-catenin by APC requires phosphorylation of β-catenin by GSK-3β at specific serine and threonine residues. Mutations of *β-catenin* may prevent phosphorylation of serine/threonine residues, resulting in accumulation of this protein in cytoplasm.[9,10] We found frequent somatic *β-catenin* gene mutations without *APC* germline mutations in CMVPTC tumors of FAP patients.[11]

In the present study, we investigated germline and somatic *APC* mutations in a CMVPTC patient with FAP and found mutations in the N-terminus of APC.

MATERIALS AND METHODS

Patients

A 25-year-old woman presented with anterior neck swelling. At the age of 19 years, she was diagnosed with FAP and underwent total colectomy and ileorectal anastomosis. Her father had died of colon cancer at the age of 39 years, and her aunt had been diagnosed with FAP and underwent total colectomy. Neck ultrasonography of the patient revealed at least five tumors in both lobes; the maximum tumor diameter was 23 mm. Tumors were solid and cystic, and aspiration biopsy cytology showed that the tumors were benign. On the basis of the ultrasonography and cytology results, the tumors were diagnosed preoperatively as adenomatous nodules. However, we believed that these tumors comprised CMVPTC on the basis of the family and patient's history.

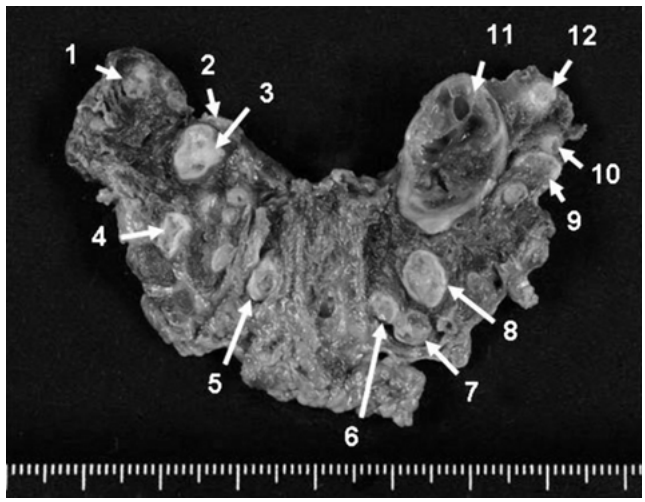

Figure 1. Resected thyroid specimen from a 25-year-old woman with familial adenomatous polyposis. More than 30 nodules were seen in both lobes. Histopathologically, all tumors were diagnosed as cribriform-morular variant of papillary thyroid carcinoma. Twelve tumors (1–12) were selected, and DNA was extracted for gene analysis. Somatic *APC* mutations were found in tumors 1, 3, 5, 6, 8, and 10.

Total thyroidectomy with central node dissection was performed at Noguchi Thyroid Clinic and Hospital Foundation. No tumor invasion of the surrounding tissues was found. The resected thyroid gland is shown in Figure 1. Histopathologic diagnosis revealed all tumors to be CMVPTCs; lymph node metastases were not found.

The ethics committee of Noguchi Thyroid Clinic and Hospital Foundation approved this research, and the patient who participated in germline and somatic screening gave written informed consent for participation in the study.

DNA Extraction and Sequencing

Germline and somatic mutations of the *APC* and *β-catenin* genes were examined in peripheral blood leukocytes and 12 CMVPTC tumors from the patient (Fig. 1). DNA was isolated from fresh frozen thyroid tissues and peripheral blood leukocytes by standard procedures. Oligonucleotide primers for exons 1–15 of *APC* and exon 3 of *β-catenin* were synthesized as reported previously.[2,11,12] The polymerase chain reaction (PCR) was carried out in a 50-μl mixture containing 100 ng template DNA, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 5 pmol each of sense and antisense primers, and 1 U of AmpliTaq Gold (Applied Biosystems, Foster City, CA, USA) with a progene programmable thermal cycler (Techne, Cambridge, U.K.). After initial denaturation at 94°C for 12 minutes, amplification conditions consisted of 45 cycles

Table 1.
Somatic and germline mutations of the *APC* and *b-catenin* genes in a CMVPTC patient

	APC gene mutation			*b-catenin* gene mutation	
Tumor no.	Germline	Somatic	LOH	Germline	Somatic
1	R554X	922delC	–	–	–
2	R554X	–	–	–	–
3	R554X	2706del20	–	–	–
4	R554X	–	–	–	–
5	R554X	1821delT	–	–	–
6	R554X	1920delG	–	–	–
7	R554X	–	–	–	–
8	R554X	2804insA	–	–	–
9	R554X	–	–	–	–
10	R554X	1602delA	–	–	–
11	R554X	–	–	–	–
12	R554X	–	–	–	–

CMVPTC: cribriform-morula variant of papillary thyroid carcinoma.

of denaturation for 1 minute at 94°C, annealing for 1 minute at 48° to 55°C, and extension for 1 minute at 72°C, followed by a 7-minute final extension at 72°C.

For nonisotopic cycle sequencing, DNA products were purified with a QIAquick PCR Purification Kit (Qiagen, Hilden, Germany). Purified products were further subjected to 25 PCR cycles with sense or antisense primer by fluorescence-based dideoxy terminator cycle sequencing (Applied Biosystems). These products were then eluted through a Centri-sep spin column (Applied Biosystems) and subjected to capillary gel electrophoresis. Data collection and analysis were performed on an automated DNA sequencer (model 310; Applied Biosystems). All PCR reactions and sequencing were repeated, and we confirmed the presence or absence of *APC* and *β-catenin* mutations. Loss of heterozygosity (LOH) was judged when a sequencing band was absent or less than 50% of the peak of the heterozygous bands at polymorphic sites.

RESULTS

The germline and somatic mutations detected in the present study are summarized in Table 1. A germline mutation was found in exon 13 (codon 554, CGA[Arg] to TGA[Stop]) in *APC*. In 12 CMVPTC tissues (nos. 1, 3, 5, 6, 8, 10), six (50%) somatic mutations—922delC (exon 8, codon 308), 1602delA (exon 12, codon 534), 1821delT (exon 14, codon 607), 1920delG (exon 14, codon 640), 2706del20 (exon 15, codon 902), 2804insA (exon 15, codon 935)—were found. LOH was not detected based on the analysis of polymorphic sites of *APC*, including codons 1678, 1756, 1960, and 2072. The presence or absence of *APC* mutations was not correlated with the size of the CMVPTC. There were no germline or somatic mutations of *β-catenin* in peripheral blood leukocytes or tumor tissues.

DISCUSSION

We detected the R554X germline mutation and six somatic mutations in the *APC* gene in peripheral blood leukocytes or CMVPTC tissues from a patient with FAP. Detection of different somatic mutations suggests that each focus of CMVPTC developed independently in this FAP patient. These mutations all result in APC protein truncated between residues 308 and 935. Correlations between some extracolonic manifestations and *APC* germline mutations in FAP patients have been reported. CHRPE is one of the features associated with FAP, and it occurs in patients with mutations between codons 457 and 1444 of *APC*.[13,14] Desmoid tumors also occur most frequently in FAP patients with mutations between codons 1403 and 1578 of *APC*.[15,16] Most somatic *APC* mutations in sporadic colorectal carcinoma occur between codons 1286 and 1513, a region called the mutation cluster region (MCR).[17]

Germline or somatic mutations of CMVPTC tumor tissues in patients with FAP have also been described by other researchers. Miyaki *et al.*[18] reported that five of seven CMVPTC tumors from two patients had somatic mutations between codons 857 and 1554, and three of these mutations (codons 857, 886, 1061) occurred upstream of the MCR. Germline mutations at codons 175

and 1110 were found in two CMVPTC patients with FAP in their series. Soravia *et al.*[19] reported a 205-bp somatic deletion and concomitant insertion of 160 bp between nucleotides 4366 and 4571 (codons 1455–1523) in a patient with an aggressive FAP-associated CMVPTC tumor that had a germline mutation in codon 698. Cameselle-Teijeiro *et al.*[20] identified a somatic *APC* mutation in codon 1309 in a CMVPTC sample from a patient without FAP. Cetta *et al.*[8] reported that 12 of 14 CMVPTC patients with FAP had germline mutations in the genomic area between codons 463 and 387 associated with CHRPE. One germline and five of six somatic mutations found in the present study also occurred outside the MCR. All of the mutations detected in the present study occurred in the region associated with CHRPE, and specifically in the 5′ end of this region.

Concomitant germline and somatic mutations were found in 6 of 12 (50%) FAP-associated CMVPTC tissues. Although allele-specific analysis has not yet been performed, it is likely that these mutations reside in each allele and may cause biallelic inactivation of the *APC* gene. In the remaining six CMVPTC tumors without somatic *APC* mutation, LOH was not observed. Although the absence of LOH suggests a lack of biallelic inactivation of *APC* in these specimens, other mechanisms of allelic *APC* inactivation, such as methylation, may occur in these tumor tissues.

Mutations in exon 3 of the *β-catenin* gene affect phosphorylation of the protein at serine and threonine residues and inactivate degradation of the protein. We reported that a somatic mutation in exon 3 of *β-catenin* occurred in seven CMVPTC tumors from two FAP-associated patients and three patients with sporadic CMVPTC; and this mutant β-catenin showed aberrant cytoplasmic and nuclear accumulation.[11] Five of these seven tumors had no *APC* mutations, and therefore the abnormalities in Wnt signal transduction may be due primarily to the abnormalities in β-catenin in these tumors. In contrast, in the present study, no mutations were found in *β-catenin* in peripheral blood leukocytes or CMVPTC tissues. We believe that the tumor tissues we analyzed had germline mutations with or without somatic mutations of the *APC* gene and that abnormalities in Wnt signal transduction were caused by abnormalities in *APC* and not *β-catenin*.

CONCLUSIONS

Our data suggest that a combination of germline and somatic mutations in the *APC* gene between codons 308 and 935, which is in the 5′ area of the CHRPE region, may play an important role in the development of FAP-associated CMVPTC.

REFERENCES

1. Iwama T, Mishima Y, Utsunomiya J. The impact of familial adenomatous polyposis on the tumorigenesis and mortality at several organs: its rational treatment. Ann Surg 1993;217:101–108.
2. Groden J, Thliveris A, Samowitz W, *et al.* Identification and characterization of the familial adenomatous polyposis coli gene. Cell 1991;66:589–600.
3. Kinzler KW, Nilbert MC, Su LK, *et al.* Identification of FAP locus genes from chromosome 5q21. Science 1991;253:661–665.
4. Crail HW. Multiple primary malignancies arising in rectum, brain and thyroid: report of case. US Naval Med Bull 1949; 49:123–128.
5. Plail RO, Bussey HJ, Glazer G, *et al.* Adenomatous polyposis: an association with carcinoma of the thyroid. Br J Surg 1987;74:377–380.
6. Yamashita T, Hosoda Y, Kameyama K, *et al.* Peculiar nuclear clearing composed of microfilaments in papillary carcinoma of the thyroid. Cancer 1992;70: 2923–2928.
7. Harach HR, Williams GT, Williams ED. Familial adenomatous polyposis associated thyroid carcinoma: a distinct type of follicular cell neoplasm. Histopathology 1994;25:549–561.
8. Cetta F, Montalto G, Gori M, *et al.* Germline mutations of the APC gene in patients with familial adenomatous polyposis-associated thyroid carcinoma: results from a European cooperative study. J Clin Endocrinol Metab 2000;85:286–292.
9. Morin PJ, Sparks AB, Korinek V, *et al.* Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. Science 1997;275:1787–1790.
10. Munemitsu S, Albert I, Souza B, *et al.* Regulation of intracellular beta-catenin levels by the adenomatous polyposis coli (APC) tumor-suppressor protein. Proc Natl Acad Sci USA 1995;92:3046–3050.
11. Xu B, Yoshimoto K, Miyauchi A, *et al.* Cribriform-morula variant of papillary thyroid carcinoma: a pathological and molecular genetic study with evidence of frequent somatic mutations in exon 3 of the β-catenin gene. J Pathol 2003;199:58–67.
12. Miyoshi Y, Ando H, Nagase H, *et al.* Germ-line mutations of the APC gene in 53 familial adenomatous polyposis patients. Proc Natl Acad Sci USA 1992;89: 4452–4456.
13. Wallis YL, Macdonald F, Hulten M, *et al.* Genotype-phenotype correlation between position of constitutional APC

gene mutation and CHRPE expression in familial adenomatous polyposis. Hum Genet 1994;94:543–548.
14. Olschwang S, Tiret A, Laurent-Puig P, *et al.* Restriction of ocular fundus lesions to a specific subgroup of APC mutations in adenomatous polyposis coli patients. Cell 1993;75:959–968.
15. Caspari R, Olschwang S, Friedl W, *et al.* Familial adenomatous polyposis: desmoid tumours and lack of ophthalmic lesions (CHRPE) associated with APC mutations beyond codon 1444. Hum Mol Genet 1995;4: 337–340.
16. Davies DR, Armstrong JG, Thakker N, *et al.* Severe Gardner syndrome in families with mutations restricted to a specific region of the APC gene. Am J Hum Genet 1995;57:1151–1158.
17. Miyoshi Y, Nagase H, Ando H, *et al.* Somatic mutations of the APC gene in colorectal tumors: mutation cluster region in the APC gene. Hum Mol Genet 1992;1:229–233.
18. Miyaki M, Iijima T, Ishii R, *et al.* Molecular evidence for multicentric development of thyroid carcinomas in patients with familial adenomatous polyposis. Am J Pathol 2000;157:1825–1827.
19. Soravia C, Sugg SL, Berk T, *et al.* Familial adenomatous polyposis-associated thyroid cancer: a clinical, pathological, and molecular genetics study. Am J Pathol 1999;154:127–135.
20. Cameselle-Teijeiro J, Ruiz-Ponte C, Loidi L, *et al.* Somatic but not germline mutation of the APC gene in a case of cribriform-morular variant of papillary thyroid carcinoma. Am J Clin Pathol 2001;115:486–493.