RAPID COMMUNICATION Jpn. J. Cancer Res. **90**, 372–376, April 1999

Somatic Mutation of the *APC* Gene in Thyroid Carcinoma Associated with Familial Adenomatous Polyposis

Takeo Iwama,[1] Motoko Konishi,[2] Takeru Iijima,[2,6] Keigo Yoshinaga,[5] Takeshi Tominaga,[3] Morio Koike[4] and Michiko Miyaki[2,6,7]

[1]Department of Surgery, Kyoundo Hospital, Sasaki Institute, 1-8 Kanda-Surugadai, Chiyoda-ku, Tokyo 101-0062, [2]Hereditary Tumor Research Project, [3]Department of Surgery, [4]Department of Pathology, Tokyo Metropolitan Komagome Hospital, 3-18-22 Honkomagome, Bunkyo-ku, Tokyo 113-8677, [5]2nd Department of Surgery, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-0034 and [6]Institute of Molecular Oncology, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555

We report the existence of both germline and somatic mutations of the *APC* gene in thyroid carcinomas from familial adenomatous polyposis (FAP) patients. One papillary thyroid carcinoma from a 20-year-old woman, with germline mutation of the *APC* gene (TCA to TGA at codon 1110), showed a somatic mutation of AAAAC deletion between codons 1060 and 1063. Another somatic mutation of CAG to TAG at codon 886 was also found in one of multiple thyroid carcinomas from a 26-year-old woman with attenuated FAP and germline mutation at codon 175 (C deletion). This is the first evidence that total absence of the normal function of the *APC* gene is involved in development of thyroid carcinomas in FAP.

Key words: Familial adenomatous polyposis — Thyroid carcinoma — Somatic mutation of *APC* gene — Histology of thyroid carcinoma

Familial adenomatous polyposis (FAP; MIM 175100) is an inherited condition caused by germline mutation of the *APC* gene.[1,2] It is an accepted theory that complete inactivation of the *APC* gene by 2 mutations is involved in the development of colonic adenoma of the general population and FAP.[3–5] We have previously demonstrated that gastric and duodenal tumors from FAP patients have somatic mutations of the *APC* gene,[6] as do other FAP extracolonic tumors, such as desmoids.[7] Adrenal tumors from FAP patients have also been demonstrated to have somatic alterations of this gene.[8,9] Although thyroid carcinoma is a common tumor in young women with FAP, its genetic changes have yet to be clarified.

In the present study, we examined two cases of thyroid carcinoma associated with FAP. With informed consent, DNA was extracted from the tumor and corresponding peripheral blood, and analyzed for the *APC* mutation using polymerase chain reaction (PCR)-single strand conformation polymorphism (SSCP) and direct sequencing methods, as previously described.[5]

One FAP patient (PLK294), a 20-year-old woman, had multiple thyroid tumors, of which the largest was 3.5×2.5 cm. Examination of the colon revealed scattered small adenomas without carcinoma. Congenital hypertropic retinal pigment epithelium was detected in the left eye. Subtotal thyroidectomy followed by histological examination revealed multiple papillary carcinoma of columnar cell type with cribriform and solid areas (Fig. 1A). A germline mutation of the *APC* gene was detected at codon 1110 (TCA to TGA, stop). The second largest tumor (PLK294-TCa2) exhibited mutant bands in PCR-SSCP, and direct sequencing of these mutant bands revealed a 5-bp deletion (AAAAC deletion) between codons 1060 and 1063, resulting in a stop signal at codon 1063 (Fig. 2, Table I). The second case (PLK29), a 26-year-old woman, underwent resection of the rectum, and excision of a metastatic tumor of the liver. The number of colonic polyps was approximately 25, and there were multiple thyroid carcinomas, the largest of which was 4 cm in diameter. Histopathological diagnosis of these thyroid carcinomas was papillary carcinoma with solid areas (Fig. 1B). This patient was recognized as FAP after a germline mutation of the *APC* gene was detected at codon 175 (ACT to AT, C deletion), resulting in a truncation at codon 184. In one of the thyroid carcinomas (PLK29-TCa1), a somatic mutation was found at codon 886 (CAG to TAG, stop) (Table I).

In the case of PLK294-TCa2, to make detection of mutation easier and clearer by means of PCR-SSCP and direct sequencing methods, we used primer sets, which separately amplified two short regions that include codons 1060–1063 and 1110, as shown in Fig. 2. To clarify whether germline and somatic mutations of PLK294-

[7] To whom correspondence should be addressed.
Email: mmiyaki@opal.famille.ne.jp

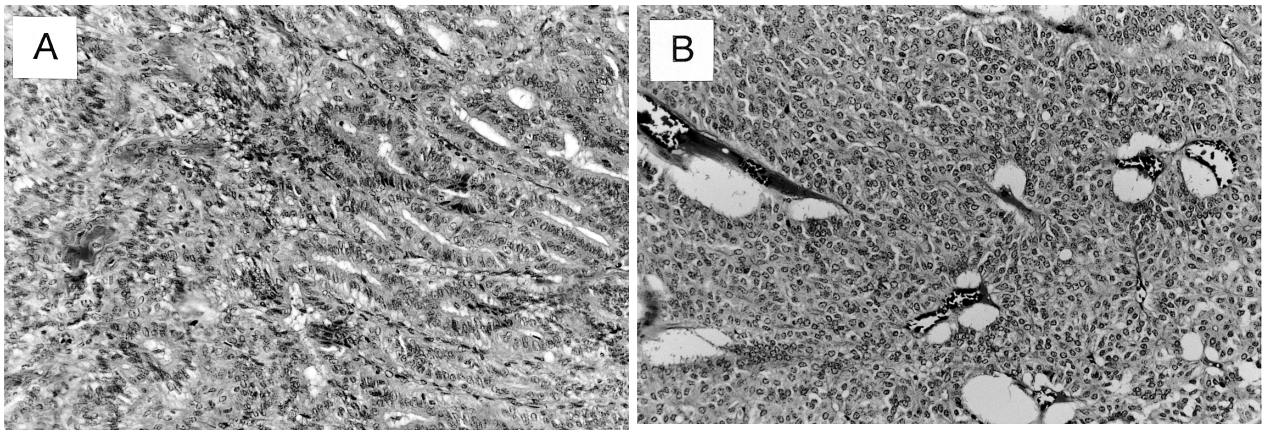

Fig. 1. Histological features of thyroid carcinomas from FAP patients. A, Columnar cell type of papillary carcinoma (PLK294-TCa2). B, Solid area of the tumor (PLK29-TCa1).

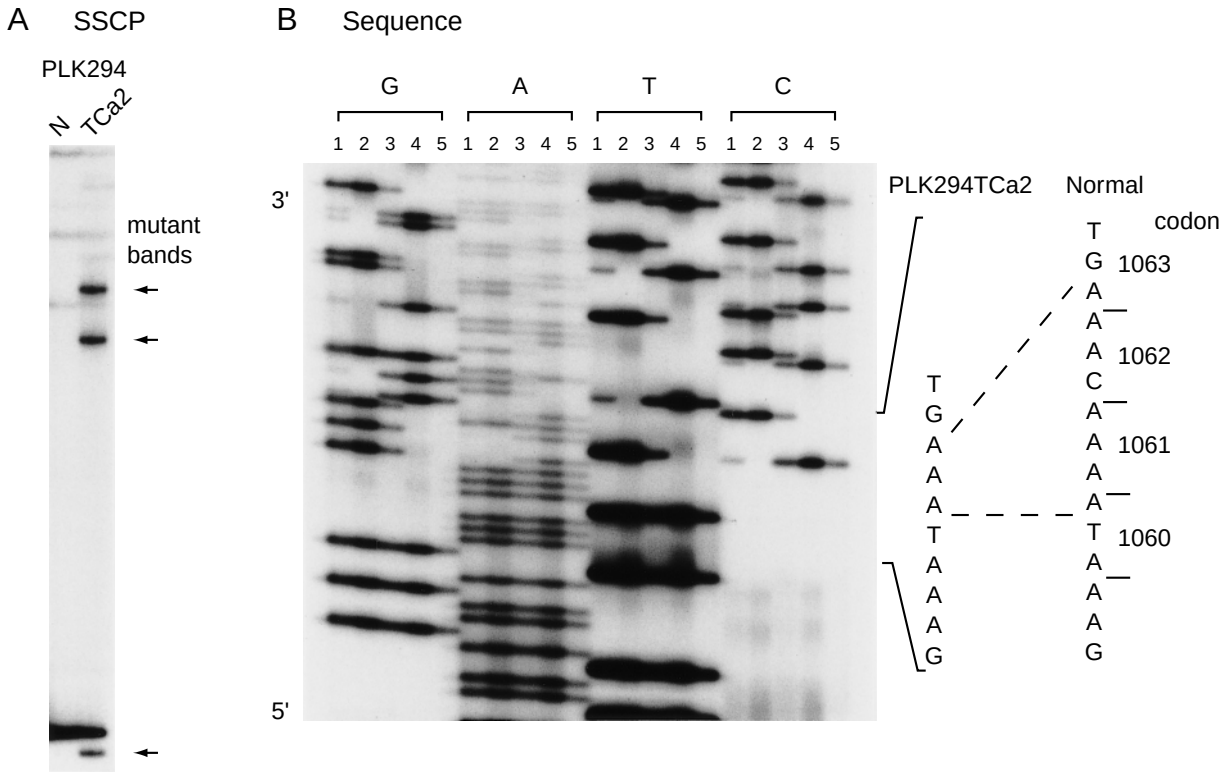

Fig. 2. PCR-SSCP and direct sequencing of somatic mutation in thyroid carcinoma from an FAP patient (PLK294). A, SSCP patterns were obtained after PCR using a primer set which amplifies only the DNA region including codons 1060–1063, but not codon 1110. B, DNA fragments were extracted from the SSCP gel and were subjected to direct sequencing. Lanes 1, 2, and 3 are sequences for 3 different mutant bands in SSCP, and lanes 4 and 5 are sequences for normal bands. Lanes 1 and 3 are contaminated with normal bands, and the extent of the contamination is higher in lane 3 than lane 1. In lane 2 there is no contamination with the normal sequence.

TCa2 existed on different alleles of the *APC* gene, we also performed PCR-SSCP analysis using primers which amplify the region including both germline (at codon 1110) and somatic (at codons 1060–1063) mutations. The SSCP pattern of PLK294-TCa2 showed multiple mutant bands, and direct sequencing of DNA fragments from

Table I. Somatic and Germline Mutations of the *APC* Gene in Thyroid Tumors from FAP Patients

Tumor	Somatic mutation		Germline mutation	
	Codon	Mutation	Codon	Mutation
PLK294-TCa2	1060–1063	AAAAC deletion	1110	TCA→TGA
PLK29-TCa1	886	CAG→TAG	175	C deletion

these bands revealed no mutant band having these two mutations, that is, all the mutant bands analyzed showed only one of the two mutations (data not shown). These results supported the idea that germline and somatic mutations occurred in different alleles of the *APC* gene, resulting in complete inactivation of this gene in PLK294-TCa2. It remains to be confirmed whether this is also the case in PLK29-TCa1.

Thyroid carcinoma is not rare in female patients with FAP,[10, 11] but the cause of thyroid carcinoma in FAP patients has not been clarified. Newly detected somatic mutations in our cases resulted in a stop signal in the *APC* gene. This is the first study to suggest that total absence of the normal *APC* function is a cause of thyroid carcinoma of FAP patients. The histological features of FAP-associated thyroid carcinoma have been described as multi-focal occurrence, and papillary carcinoma with a cribriform pattern, solid areas with spindle cell components, and sometimes, tall cell (columnar) epithelium.[12] Although the prognosis of thyroid carcinoma associated with FAP is as good as that of sporadic papillary thyroid carcinoma,[10, 11] the morphology of these carcinomas is slightly different.[13] The contribution of *APC* mutation may result in different morphology of thyroid carcinoma between FAP patients and sporadic cases, since no *APC* mutations have been reported in sporadic thyroid tumors: somatic mutation of the *APC* gene has been examined in 16 sporadic papillary thyroid carcinomas, but no evidence of mutation has been detected between codons 1289 and 1513.[14] *APC* mutation has also been analyzed in 26 sporadic thyroid tumors for approximately 35% of the coding region, but no significant alteration has been found.[15] These authors have concluded that *APC* abnormality does not play a pathogenic role in thyroid tumorigenesis in patients not affected by FAP. There may be other factors that contribute to the difference between FAP and non-FAP thyroid carcinomas. By using immunochemical methods, enhanced expression of the *RET-PTC* oncogene has been detected in thyroid carcinoma in 2 of 3 patients with FAP in a kindred, and it was suggested that such activation is unique to sporadic papillary thyroid tumors.[16, 17] It is possible that inactivation of the *APC* gene through germline and somatic mutations brings about increased expression of cancer-related genes, such as *RET*, in cells of thyroid origin, although the previous study[17] did not analyze somatic *APC* mutation in thyroid tumors which showed no loss of heterozygosity of the *APC* gene.

With respect to genotype-phenotype relationship, several studies have reported a correlation between the location of the germline mutation in the *APC* gene and the number of colorectal polyps in FAP patients.[18–20] Examples of phenotypic heterogeneity caused by the mutation site or type have also been described,[21, 22] but it seems to be difficult to predict the severity of disease from the position of germline mutation.[23] Previously, germline mutations in FAP patients with thyroid carcinoma have been reported at codon 1061, 1309, or 848,[16, 24] and our recent cases with thyroid carcinoma had germline mutation at codon 1105 or 1061. However, germline mutations of the present 2 cases were not at these spots, suggesting that the development of thyroid carcinoma does not depend on whether the germline mutation is at the 5′ attenuated FAP phenotype region,[19, 25] at codon 175 in our case, or in the severe phenotype area (codon 1309).[18] Regarding the site of somatic mutation, approximately 94% of somatic mutations in gastrointestinal tumors were clustered between codons 1280 and 1500 (MCR).[3, 5, 6, 26] Although in our 2 cases, mutation occurred outside of the MCR, analyses of many thyroid tumors will be needed to establish whether the mutation pattern in thyroid tumor is consistently different from that of gastrointestinal tumor.

In FAP patients, as in the general population, thyroid carcinoma predominantly occurs in women,[10] which suggests that tumor formation in FAP patients requires not only total loss of function of the *APC* gene, but also some other factors, such as hormonal environment,[12] ileal-pouch formation,[27] inflammatory factor,[28] or surgical injury,[29] for specific tumors. Further study of genetic changes is also needed to clarify the cause of the histological differences between FAP and non-FAP thyroid carcinomas.

(Received January 6, 1999/Revised February 16, 1999/Accepted February 24, 1999)

REFERENCES

1) Groden, J., Thliveris, A., Samowitz, W., Carson, M., Gilbert, L., Albersten, H., Joslyn, G., Stevens, J., Spirio, L., Robertson, M., Sargeant, L., Krapcho, K., Wolff, E., Burt, R., Hughes, J. P., Warrington, J., McPherson, J., Wasmuth, J., Le Paslier, D., Abderrahim, H., Cohen, D., Leppert, M. and White, R. Identification and characterization of the familial adenomatous polyposis coli gene. *Cell*, **66**, 589–600 (1991).
2) Nishisho, I., Nakamura, Y., Miyoshi, Y., Miki, Y., Ando, H., Horii, A., Koyama, K., Utsunomiya, J., Baba, S., Hedge, P., Markham, A., Krush, A. J., Petersen, G., Hamilton, S. R., Nilbert, M. C., Levy, D. B., Bryan, T. M., Preisinger, A. C., Smith, K. J., Su, L.-K., Kinzler, K. W. and Vogelstein, B. Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients. *Science*, **253**, 665–669 (1991).
3) Miyoshi, Y., Nagase, H., Ando, H., Horii, A., Ichii, S., Nakatsuru, S., Aoki, T., Miki, Y., Mori, T. and Nakamura, Y. Somatic mutations of the APC gene in colorectal tumors: mutation cluster region in the APC gene. *Hum. Mol. Genet.*, **1**, 229–233 (1992).
4) Ichii, S., Horii, A., Nakatsuru, S., Furuyama, J., Utsunomiya, J. and Nakamura, Y. Inactivation of both APC alleles in an early stage of colon adenomas in a patient with familial adenomatous polyposis (FAP). *Hum. Mol. Genet.*, **1**, 387–390 (1992).
5) Miyaki, M., Konishi, M., Kikuchi-Yanoshita, R., Enomoto, M., Igari, T., Tanaka, K., Muraoka, M., Takahashi, H., Amada, Y., Fukayama, M., Maeda, Y., Iwama, T., Mishima, Y., Mori, T. and Miyaki, M. Characteristics of somatic mutation of the adenomatous polyposis coli gene in colorectal tumors. *Cancer Res.*, **54**, 3011–3020 (1994).
6) Toyooka, M., Konishi, M., Kikuchi-Yanoshita, R., Iwama, T. and Miyaki, M. Somatic mutations of the adenomatous polyposis coli gene in gastroduodenal tumors from patients with familial adenomatous polyposis. *Cancer Res.*, **55**, 3165–3170 (1995).
7) Miyaki, M., Konishi, M., Kikuchi-Yanoshita, R., Enomoto, M., Tanaka, K., Takahashi, H., Muraoka, M., Mori, T., Konishi, F. and Iwama, T. Coexistence of somatic and germ-line mutations of APC gene in desmoid tumors from patients with familial adenomatous polyposis. *Cancer Res.*, **53**, 5079–5082 (1993).
8) Seki, M., Tanaka, K., Kikuchi-Yanoshita, R., Konishi, M., Fukunari, H., Iwama, T. and Miyaki, M. Loss of normal allele of the APC gene in an adrenocortical carcinoma from a patient with familial adenomatous polyposis. *Hum. Genet.*, **98**, 298–300 (1992).
9) Wakatsuki, S., Sasano, H., Matsui, T., Nagashima, K., Toyota, T. and Horii, A. Adrenocortical tumor in a patient with familial adenomatous polyposis. A case associated with a complete inactivating mutation of the APC gene and unusual histopathological features. *Hum. Pathol.*, **29**, 302–306 (1998).
10) Bulow, S., Holm, N. V. and Mellemgaard, A. Papillary thyroid carcinoma in Danish patients with familial adenomatous polyposis. *Int. J. Colorectal Dis.*, **3**, 29–31 (1988).
11) Iwama, T., Mishima, Y. and Utsunomiya, J. The impact of familial adenomatous polyposis on the tumorigenesis and mortality at the several organs. *Ann. Surg.*, **217**, 101–108 (1993).
12) Harach, H. R., Williams, G. T. and Williams, E. D. Familial adenomatous polyposis associated thyroid carcinoma: a distinct type of follicular cell neoplasm. *Histopathology*, **25**, 549–561 (1994).
13) Weing, B. M., Thompson, D. R., Adair, C. F., Shmooklwe, B. and Heffes, C. S. Thyroid papillary carcinoma of columnar cell type: a clinicopathological study of 16 cases. *Cancer*, **83**, 740–753 (1998).
14) Curtis, L., Wyllie, A. H., Shaw, J. J., Williams, G. T., Radulescu, A., DeMicco, C., Haugen, D. R., Varhaug, J. E., Lillehaug, J. R. and Wynford-Thomas, D. Evidence against involvement of APC mutation in papillary thyroid carcinoma. *Eur. J. Cancer*, **30A**, 984–987 (1994).
15) Colletta, G., Sciacchitano, S., Palmirotta, R., Ranieri, A., Zanella, E., Cama, A., Costantini, R. M., Battista, P. and Pontecovi, A. Analysis of adenomatous polyposis coli gene in thyroid tumors. *Br. J. Cancer*, **70**, 1085–1088 (1994).
16) Cetta, F., Toti, P., Petracci, M., Montalto, G., Disanto, A., Lore, F. and Fusco, A. Thyroid carcinoma associated with familial adenomatous polyposis. *Histopathology*, **31**, 231–236 (1997).
17) Cetta, F., Olschwang, S., Petracci, M., Montalto, G., Baldi, C., Zuckermann, M., Costantini, R. M. and Fusco, A. Genetic alterations in thyroid carcinoma associated with familial adenomatous polyposis: clinical implications and suggestions for early detection. *World J. Surg.*, **22**, 1231–1236 (1998).
18) Nagase, H., Miyoshi, Y., Horii, A., Aoki, T., Ogawa, M., Utsunomiya, J., Baba, S., Sasazuki, T. and Nakamura, Y. Correlation between the location of germ-line mutations in the APC gene and the number of colorectal polyps in familial adenomatous polyposis. *Cancer Res.*, **52**, 4055–4057 (1992).
19) Spirio, L., Olschwang, S., Groden, J., Robertson, M., Samowitz, W., Joslyn, G., Gelbert, L., Thliveris, A., Carlson, M., Otterud, B., Lynch, H., Watson, P., Lynch, P., Laurent-Puig, P., Burt, R., Hughes, J. P., Thomas, G., Leppert, M. and White, R. Alleles of the APC gene: an attenuated form of familial polyposis. *Cell*, **75**, 951–957 (1993).
20) Van der Luijt, R. B., Meer Kahn, P., Vasen, H. F. A., Breukel, C., Tops, C. M. J. and Fodde, R. Germline mutations in the 3′ part of APC exon 15 do not result in truncated proteins and are associated with attenuated adenomatous polyposis coli. *Hum. Genet.*, **98**, 727–734 (1996).

21) Curia, M. C., Esposito, D. L., Aceto, G., Palmirotta, R., Crognale, S., Valanzano, R., Ficari, F., Tonelli, F., Battisa, P., Mariani-Costantini, R. and Cama, A. Transcript dosage effect in familial adenomatous polyposis—model offered by two kindreds with exon 9 APC gene mutations. *Hum. Mutat.*, **11**, 197–201 (1998).
22) Gyfe, R., Di Nicola, N. and Redson, M. Somatic instability of the APC I1307K allele in colorectal neoplasia. *Cancer Res.*, **58**, 4040–4043 (1998).
23) Walon, C., Kartheuser, A., Michils, G., Smaels, M., Lannoy, N., Nogounou, P., Mertens, G. and Verellen-Dumoulin, C. Novel germline mutations in the APC gene and their phenotypic spectrum in familial adenomatous polyposis kindreds. *Hum. Genet.*, **100**, 601–605 (1997).
24) Kashiwagi, H., Konishi, F., Kanazawa, K. and Miyaki, M. Sisters with familial adenomatous polyposis affected with thyroid carcinoma, desmoid tumour and duodenal polyposis. *Br. J. Surg.*, **83**, 228 (1996).
25) Giardiello, F. M., Breisinger, J. D., Luce, M. C., Petersen, G. M., Cayoutte, M. C., Krush, A. J., Bacon, J. A., Booker, S. V., Buffil, J. A. and Hamilton, S. R. Phenotypic expression of disease in families that have mutations in the 5′ region of the adenomatous polyposis coli gene. *Ann. Intern. Med.*, **126**, 514–519 (1997).
26) Nakatsuru, S., Yanagisawa, A., Ichii, S., Tahara, E., Kato, Y., Nakamura, Y. and Horii, A. Somatic mutation of the APC gene in gastric cancer: frequent mutations in very well differentiated adenocarcinoma and signet-ring cell carcinoma. *Hum. Mol. Genet.*, **1**, 559–563 (1992).
27) Hoehner, J. C. and Metcalf, A. M. Development of invasive adenocarcinoma following colectomy with ileoanal anatomosis for familial polyposis coli: report of a case. *Dis. Colon Rectum*, **37**, 824–828 (1994).
28) Oshima, M., Dinchuk, J. E., Kargman, S. L., Oshima, H., Hancock, B., Kwong, E., Trzaskos, J. M., Ecans, J. F. and Taketo, M. M. Suppression of intestinal polyposis in APC knockout mice by inhibition of cyclooxygenase 2 (cox-2). *Cell*, **87**, 803–809 (1996).
29) McAdam, W. A. F. and Goligher, J. C. The occurrence of desmoids in patients with familial polyposis coli. *Br. J. Surg.*, **57**, 618–631 (1970).