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# Germline and somatic mutations of the *APC* gene in papillary thyroid carcinoma associated with familial adenomatous polyposis: Analysis of three cases and a review of the literature

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**Abstract.** Patients with familial adenomatous polyposis (FAP), which is caused by the dysfunction of the adenomatous polyposis coli (APC) protein, have the possibility of developing extracolonic manifestations, including thyroid cancer (TC), congenital hypertrophy of the retinal pigment epithelium, desmoid tumors, and gastric and duodenal adenomas. The pathogenesis of these disorders associated with FAP is considered to be affected by the site of the germline mutation on the *APC* gene as a genotype-phenotype correlation. Moreover, β-catenin binding sites consist of 20-amino acid repeats (20-AARs) in the APC protein, and they are essential for the development of colorectal adenomas and certain other extracolonic manifestations. The present study retrospectively analyzed the germline and somatic mutations of the *APC* gene in three papillary TC patients with FAP to analyze the association between the remaining number of 20-AARs and the development of TC. The mutation sites of two TCs did not include 20-AARs in each allele. In one patient, the remaining number of 20-AARs was two in the germline mutation and zero in the somatic mutation. Together with the data on 13 FAP-associated thyroid cancerous lesions in 3 FAP patients reported previously, the majority of the remaining numbers of 20-AARs was zero in the TC patients with FAP (13/16; 81.3%). Consequently, the APC/β-catenin signaling pathway may be strongly involved with the pathogenesis of TC with FAP. Further accumulation of FAP patients with TC will be required to confirm the molecular pathogenesis of TC.

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## Introduction

Thyroid cancer (TC) in a patient with familial adenomatous polyposis (FAP) was first described in 1949 by Crail (1). The association between FAP and TC was subsequently reported in 1968 (2). Approximately 1-2% of FAP patients develop TC within their lifetime (3,4). A previous study from Japan (3) estimated the risk of TC in women with FAP to be ~23-fold higher than that of women without FAP, while the risk of developing TC in young women under the age of 35 years old with FAP has been estimated to be ~160 times that of normal individuals according to the St Mark's Hospital Polyposis Registry (5).

It is well known that dysfunction of the adenomatous poliposis coli (APC) protein due to germline mutations causes development of hundreds and thousands of colorectal polyps, a number of which may progress to colorectal cancer for the majority of patients, unless a prophylactic colectomy is performed. The clinical phenotype, including profuse and attenuated types, appears to be associated with the site of germline mutations on the *APC* gene (6,7). Moreover, the pathogenesis of extracolonic manifestations, including congenital hypertrophy of the retinal pigment epithelium, desmoid tumors, and gastric and duodenal adenomas, is considered to be associated with the site of the germline mutation on the *APC* gene. This association is termed the 'Genotype-Phenotype Correlation' (6,7). However, a somatic mutation that occurs on the other allele may affect the clinical features of FAP patients. The somatic mutation, termed the 'second hit' (8), is usually determined by the site of the germline mutation. This mechanism is widely accepted as the 'just right' model (9) and the 'loose fit' model (10). Normal APC functions include the degradation of β-catenin following the binding to the APC protein at one of the 7 sites, consisting of 20-amino acid repeats (20-AARs), between codons 1265 and 2034 (11). The remaining number of 20-AAR sites on each allele is counted through the sites of the germline mutation and somatic mutation. A previous study (10,12) demonstrated

that the remaining number of 20-AARs in cases of colorectal adenomas range between one and three. The remaining two or three 20-AARs on each allele is associated with gastric and duodenal adenomas, and desmoid tumors (12). Little is known with regard to this in TC patients with FAP. The present study aimed to investigate the remaining number of 20-AARs in TC developed in FAP patients. The study reports the cases of three TC patients with FAP who underwent a resection of the TC in Saitama Medical Center (Saitama, Japan) to investigate the association between the remaining number of 20-AARs by analyzing the germline and somatic mutations on the *APC* gene. The study then discusses the results together with previously published data.

## Patients and methods

*Ethics and consent*. This study was performed in accordance with ethical guidelines for clinical research with the approval of the institutional ethics committee of Saitama Medical Center, Saitama Medical University. Based upon a presumptive clinical diagnosis of FAP, patients received genetic counseling at the Department of Digestive Tract and General Surgery in Saitama Medical Center, and written informed consent for further genetic analyses was obtained from the individuals included in the study.

*Patients*. Genetic analysis of the following three patients with FAP-associated TC was performed.

*Patient 1*. The patient was a 25-year-old female who had been diagnosed with FAP at 20 years old. At this time, a prophylactic colectomy had been performed. A thyroid tumor, 10 mm in diameter, was detected in the right lobe in September 2005. The resected specimen was pathologically diagnosed as cribriform-morula variant of papillary thyroid carcinoma (CMPTC).

*Patient 2*. A 32-year-old female, whose mother and younger sister had been treated for FAP, was diagnosed with FAP plus early colon cancer and a thyroid tumor, 10 mm in diameter, in the left lobe in April 2008. The patient underwent a total colectomy following the diagnosis of sigmoid cancer. A subtotal thyroidectomy was performed two months after the total colectomy. The pathological diagnosis was of CMPTC.

*Patient 3*. In February 2008, a 38-year-old female underwent a total colectomy and ileorectal anastomosis for stage IV transverse colon cancer following a complete response to oxaliplatin-based (mFOLFOX6) chemotherapy for multiple liver metastases. The mFOLFOX6 regimen comprised of intravenous infusions of oxaliplatin (85 mg/m$^2$) and leucovorin (200 mg/m$^2$) for 2 h, followed by rapid intravenous bolus infusions of 5-fluorouracil (5-FU; 400 mg/m$^2$) for 5 min and continuous intravenous infusion of 5-FU (2,400 mg/m$^2$) for 46 h. This regimen was repeated twelve times every 2 or 3 weeks. Two years later, the patient underwent a total thyroidectomy for thyroid tumors that were 10 mm, 8 mm and 3 mm in diameter. A pathological examination revealed CMPTC. The patient has remained free from colorectal and thyroid cancer recurrence for 36 months since the thyroidectomy.

*Germline mutation analysis*. For analysis of the germline mutations, peripheral blood samples were collected from each patient. Genomic DNA extraction was performed using a QIAamp DNA Blood Mini kit (Qiagen, Hilden, Germany). A small piece of thyroid cancer tissue and corresponding normal tissue were taken from the surgically resected specimens to identify any germline and somatic mutations. Part of the genetic analysis of these samples was commissioned to Falco Biosystems Ltd., (Kyoto, Japan). Exon-by-exon polymerase chain reaction (PCR) of the 1st-15th *APC* exons and their boundary intronic sequences was performed on ~600 ng DNA using AmpliTaq Gold DNA polymerase (Applied Biosystems Life Technologies, Foster City, CA, USA). The PCR conditions were as follows: Initial denaturation at 94˚C for 10 min, followed by 35 cycles of 94˚C for 20 sec, 62˚C for 30 sec and 72˚C 1 min, with a final extention of 72˚C for 1 min. PCR products were directly sequenced using BigDye Terminator Ready Reaction Mix (version 1.1; Applied Biosystems Life Technologies) and an ABI 3100 Genetic Analyzer with SeqScape Software (version 2.6; Applied Biosystems Life Technologies).

## Results

The results of the genetic analysis on the germline and somatic mutations for the three patients are summarized in Table I.

In patient 1, the genetic analysis of the *APC* gene from a blood sample and the thyroid cancer tissue identified a germ-line mutation (T deletion at codon 917) and a somatic mutation (A deletion at codon 728), which were each considered to form stop codons, resulting in truncated products of the *APC* gene. These results suggested that none of the 20-AARs were remaining on the *APC* gene in the TC cells.

The germline mutation of patient 2, which was analyzed using peripheral blood collected from the patient, was a 7-bp deletion (TATAGTTTA→TA) at codons 1179-1181. The somatic mutation was a deletion of T at codon 607. These results indicate that there is no 20-AAR in the TC cells by the germline and somatic mutations on the *APC* gene.

In patient 3, the genetic analysis of the *APC* gene from the blood sample and the thyroid cancer tissue identified a germline mutation (C deletion at codon 1483) and a somatic mutation (G deletion at codon 295), which were each considered to form stop codons, resulting in truncated products of the *APC* genes. These results suggest that two 20-AARs (two 20-AARs on the germline mutation and zero 20-AARs on the somatic mutation) existed on the *APC* gene in the TC cells of the patient.

## Discussion

The remaining number of 20-AARs following germline and somatic mutations on the *APC* gene were analyzed in the TC cells developed in the patients with FAP. As shown in Table I, the remaining number of 20-AARs following germline and somatic mutations was zero in two cases (patients 1 and 2), while two 20-AARs remained in one case (patient 3) due to the site of the germline mutation. Mutation analyses of three FAP cases (patients 4-6) with a total of 13 TC lesions have previously been reported in Japan (13,14) and are listed in Table I. Of the 13 cancerous lesions, the remaining number of 20-AARs was zero in 11 lesions. Together with the present cases, the distribution of the number of 20-AARs retained in 16 thyroid cancerous lesions is summarized in Table II. These

Table I. Analysis of association between germline/somatic mutation sites and the remaining number of 20 amino acid repeats in reported cases.

| | | Germline mutation | | Somatic mutation | | |
|---|---|---|---|---|---|---|
| Patient no. | Cancerous lesion no. | Mutation type | Remaining numbers of 20-AARs | Mutation type | Remaining numbers of 20-AARs | First author, year (ref.) |
| 1 | 1 | Codon 917 T deletion | 0 | Codon 728 AAT-AT; A deletion | 0 | Present study |
| 2 | 2 | Codon 1179-11817 bp deletion | 0 | Codon 607 T deletion | 0 | |
| 3 | 3 | Codon 1483 C deletion | 2 | Codon 295 G deletion | 0 | |
| 4 | 4 | Codon 554 CGA→TGA | 0 | Codon 308 deletion C | 0 | Uchino *et al*, 2006 (14) |
| | 5 | | | Codon 534 1602 deletion A | 0 | |
| | 6 | | | Codon 607 1821 deletion T | 0 | |
| | 7 | | | Codon 640 1920 deletion G | 0 | |
| | 8 | | | Codon 902 2706 deletion 20 | 0 | |
| | 9 | | | Codon 935 2804 insertion A | 0 | |
| 5 | 10 | Codon 175 C deletion | 0 | Codon 886, CAG→TAG | 0 | Miyaki *et al*, 2000 (13) |
| | 11 | | | LOH | 0 | |
| | 12 | | | Codon 1536 GAA→TAA | 3 | |
| | 13 | | | LOH | 0 | |
| | 14 | | | Codon 1554-1556 A insertion | 3 | |
| 6 | 15 | Codon 1110 TCA→TGA | 0 | Codon 857 GGA→TGA | 0 | |
| | 16 | | | Codon 1060-1063 AAAAC deletion | 0 | |

20-AARs, 20-amino acid repeats; LOH, loss of heterozygosity.

Table II. Analysis of association between the number of 20-AARs retained and TC (n=16).

| | Number of 20-AARs retained in germline/somatic mutations | | | | | | |
|---|---|---|---|---|---|---|---|
| Cancer type | 0/0 | 0/1 or 1/0 | 0/2 or 2/0 | 0/3 or 3/0 | 1/2 or 2/1 | 1/3 or 3/1 | 2/3 or 3/2 |
| TC | 13 | 0 | 1 | 2 | 0 | 0 | 0 |

TC, thyroid cancer; 20-AARs, 20-amino acid repeats.

results indicated that the majority of the remaining number of 20-AARs was zero in the TC patients with FAP (13/16; 81.3%).

The location of the *APC* mutation may dictate the phenotype of FAP. Genotype-phenotype correlation with FAP refers to the correlation between the germline mutation on the *APC* gene and the clinical phenotype, including extracolonic manifestations (6,7). With regard to the extracolonic manifestations of FAP, the majority of the patients with TC showed *APC* germline mutations at the 5' to the mutation cluster region (codons 1286-1513) (15); when two groups of patients were created, FAP patients with TC and without TC, and their germline mutations prior to and following codon 1220 were assessed, significantly more patients with TC exhibited the germline mutation prior to 1220 (15). A recent study (16) demonstrated that *APC* mutations at codon 1061 and those proximal to codon 512 increased the risk for TC in individuals with FAP. According to the aforementioned data, the sites of the majority of the germline mutations in FAP-associated TC are located proximal to codon 1265, from which 20-AARs begin, resulting in the lack of the 20-AARs in the germline-mutated APC protein. Although somatic mutations of the *APC* gene for FAP-associated TC have not been fully analyzed in comparison with their germline mutations, the results of the present study and previous reports have demonstrated that the majority of the somatic and germline mutations of *APC* in FAP patients with TC lead to the total absence of 20-AARs in the TC cells, while few patients have three or less 20-AARs.

The current results showing that the remaining number of 20-AARs in FAP-associated TC was nearly zero were completely different from those for colorectal adenomas and other extracolonic manifestations, the majority of which retain two or three 20-AARs (10,12). In the analysis of desmoid tumors derived from FAP patients, the majority of desmoid cases retained two 20-AARs in the germline mutation, while the majority of desmoids with two *APC* hits had one somatically-mutated allele with no 20-AARs (12,17). The current consensus is that 20-AARs are associated with the binding to β-catenin through the Wnt signaling pathway (9). When *APC* that is associated with the Wnt signaling pathway functions normally in endothelial cells, β-catenin is constantly degraded by proteasome following the binding to 20-AARs on the APC protein, leading to the suppression of the expression of genes associated with development and carcinogenesis. The remaining number of 20-AARs is associated with the regulation of the amount of β-catenin. A 'just right' model has been proposed to predict the pattern of the second hit following the first hit, including those retaining a few 20-AARs (9).

It appears that the presence of a certain number of 20-AARs is essential for the development of colorectal adenomas and certain other extracolonic manifestations, although two or less 20-AARs have been suggested to be defective with regard to the β-catenin degradation. Based on that mechanism, we suggest that the APC/β-catenin signaling pathway, via the binding to 20-AARs, is strongly involved with the development of TC in patients with FAP, since there were few 20-AARs remaining following the germline and somatic mutations in the present and previous studies.

It has been reported that somatic mutations or loss of heterozygosity of the *APC* gene are extremely rare in FAP-associated TC (18). Previous studies (19-21) have described that the *APC* mutation is extremely rare in sporadic TC. Regarding sporadic colorectal cancer, 60% of all patients have somatic mutations of the *APC* gene (22), leading to the development of carcinogenesis.

Frequent absence of the second-hit somatic *APC* mutation may suggest that the Wnt/β-catenin pathway does not have a major role in the pathogenesis of not only sporadic TC, but also TC associated with FAP, unlike colorectal cancer. Although the germline and somatic *APC* mutations may be associated with the genetic susceptibility to thyroid carcinogenesis, the alternative pathogenesis may be important for the development of TC with FAP. Previously, several studies demonstrated that *RET*/*PTC* rearrangements were frequently activated in FAP-associated TC (23,24). In fact, this hypothesis would be based on the pathogenesis of sporadic TC (25). Furthermore, somatic $BRAF^{V600E}$ mutations were shown to frequently occur in sporadic TC (25). These representative genetic mutations in sporadic TC could cause the pathogenesis of FAP-associated TC, although it will not be a specific mechanism of the onset of FAP-associated TC.

With regard to other factors associated with the pathogenesis of FAP-associated TC, it is particularly worthy of note that the epidemiological findings suggest that FAP-associated TC is a female-dominated manifestation in FAP patients. We propose that sex hormones, such as estrogen and progesterone, may also be important in the development of FAP-associated TC.

In the present study, it was found that the remaining number of 20-AARs on the *APC* gene by the germline and somatic mutations was zero in the majority of the FAP-associated TC cells, indicating that the Wnt signaling pathway is dominantly involved with the pathogenesis of TC in patients with FAP. Our future studies will clarify the molecular mechanism behind the pathogenesis of FAP-associated TC, including the involvement of *RAS*, *BRAF* and *RET*/*PTC*, once more FAP-associated TC samples have been collected.

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