DATA REPORT

Attenuated familial adenomatous polyposis with desmoids caused by an *APC* mutation

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We present here a case of attenuated familial adenomatous polyposis (AFAP) with a family history of desmoids and thyroid tumors. This patient had no colonic polyps but did have multiple desmoids. Genetic analysis identified a 4-bp deletion in codon 2644 (c.7932_7935delTTAT: p.Tyr2645LysfsX14) of the adenomatous polyposis coli (*APC*) gene. In cases with limited numbers of colonic polyps and desmoids, AFAP may be caused by a mutation in the 3′ region of *APC*.

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Familial adenomatous polyposis (FAP) is an inherited disorder characterized by the early onset of multiple adenomatous polyps in the colon and rectum. Unless the colon is removed, most patients with this syndrome develop colorectal cancer by the age of 35–40 years. Gardner's syndrome is a variant of FAP characterized by colonic polyposis, osteomas and soft tissue tumors, such as desmoids. Desmoid tumors are benign, non-inflammatory fibroblastic neoplasms that tend to be locally invasive but are not metastatic. However, they have a high potential for local recurrence following surgical resection. Upper gastrointestinal neoplasms are also typical extracolonic manifestations in FAP. Histologically, the majority of duodenal polyps are adenomas, whereas the majority of gastric polyps are fundic gland polyps. Similar to other hereditary cancer syndromes, there is an increased risk of additional malignancies in FAP, including thyroid cancer.

Germline mutations of the adenomatous polyposis coli (*APC*) gene that is responsible for FAP may also cause Gardner's syndrome. Correlations have been observed between mutation in *APC* and the FAP phenotype. Mutations located between codons 1250 and 1464, especially those at codon 1309, have been reported to be correlated with the development of profuse-type polyposis (>5,000 colorectal polyps).[1,2] This region is closely associated with the mutation cluster region (codons 1286–1513), where more than 60% of somatic mutations occurring in colon cancer patients are clustered.[3] Attenuated FAP (AFAP) is a variant of FAP that is characterized by the presence of less than 100 polyps with a more proximal colonic location in addition to a delayed age of colorectal cancer onset. Mutations in AFAP patients have been reported to be located in three distinct regions of the *APC* gene, including the 5′ end spanning exons 3 to 5, exon 9 and the 3′ distal end.[4] Previous studies of desmoid tumor patients have revealed that patients with mutations beyond codon 1444, especially between codons 1445 and 1578, develop desmoid tumors more frequently than those with mutations in other regions of the *APC* gene.[5,6]

Here, we report a case of a desmoid tumor patient with a family history of desmoid tumors, thyroid cancer and colonic polyposis. Using a next-generation sequencing method, we identified a 4-bp deletion in the carboxyl terminus of the *APC* gene (c.7932_7935delTTAT: p.Tyr2645LysfsX14). Our findings provide evidence that the deletion of the 3′ distal end of the APC protein impairs its function of suppressing desmoid tumors and thyroid cancer, thereby deepening the current understanding of the correlation between the phenotype and genotype of FAP.

A 27-year-old female with desmoid tumors in front of the sternum and on the left elbow visited our hospital for genetic counseling. Evaluation of her family history revealed that her father underwent total colectomy for multiple colon polyps, resection of two abdominal desmoid tumors at the age of 50 years and resection of thyroid and prostate cancer at 52 years of age (Figure 1a). A paternal aunt also underwent resection of several desmoid tumors on the back and developed thyroid cancer in her forties (Figure 1a). Furthermore, the paternal grandfather suffered from colon cancer with multiple colon polyps and cancer of the duodenal papilla (Figure 1a). Colonoscopy showed that the patient had no colorectal polyps. However, gastroscopy revealed numerous fundic gland polyps in her stomach. Considering these findings, we diagnosed her with AFAP.

After obtaining written informed consent from the patient, genomic DNA was extracted from her peripheral blood leucocytes. We first performed the direct sequencing of *APC* to examine the 5′ half of the coding region, which is where most *APC* mutations occur. However, no pathogenic mutations were detected by this initial examination. Although examinations of other regions, including the remaining 3′ region and the promoter region, structural analysis of *APC* by multiplex ligation-dependent probe amplification and the sequencing of other polyposis-related genes, such as *MUTYH*, *POLD* and *POLE,* by the Sanger method, were possible for the second screening, we conducted whole-genome sequencing to examine the utility of next-generation sequencing in clinical testing. A library of approximately 550 bp constructed from 200 ng of genomic DNA was prepared and subsequently sequenced using HiSeq 2500 platforms according to the manufacturer's instructions (Illumina, San Diego, CA, USA).

As a result, seven variants were detected in the coding region of *APC*. Of note, six of the seven variants were detected in the initial

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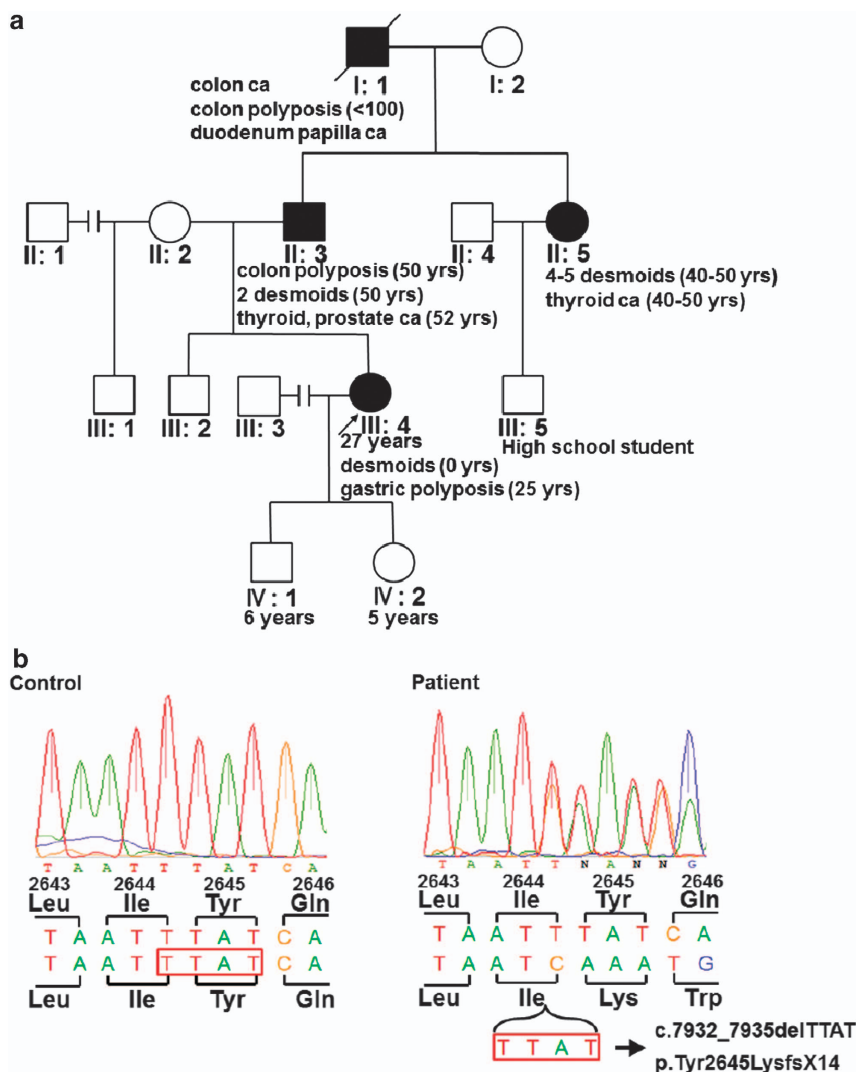Correspondence: Y Furukawa (furukawa@ims.u-tokyo.ac.jp)

Figure 1. Identification of an *APC* mutation in a pedigree of AFAP with desmoid tumors. (**a**) The pedigree of the family assessed in this study. Open square: normal male individual; closed square: affected male individual; open circle: normal female individual; closed circle: affected female individual; slash: deceased individual; and arrow: proband. (**b**) Validation of the 4-bp deletion in *APC*. Direct sequencing of the PCR products of DNA from a healthy volunteer and a patient revealed a deletion of TTAT at codon 2644 of the *APC* gene. The amino acid code and position are shown. The red square indicates the four nucleotides deleted in the patient's DNA. AFAP, attenuated familial adenomatous polyposis; APC, adenomatous polyposis coli.

screening by direct sequencing using the Sanger method, and no other variants were identified. Among the six variants, five were synonymous single nucleotide variants and one was a nonsynonymous single nucleotide variant (Table 1), as determined by evaluations using public databases, including dbSNP (in NCBI),[7] the HapMap project[8] and the Human Genetic Variation Browser.[9] The remaining variant detected by whole-genome sequencing was a 4-bp deletion of nucleotides 7932 to 7935, c.7932_7935delTTAT: p.Tyr2645LysfsX14 (Table 1, Figure 1b), located outside of the region that we had initially screened. This variant causes a frame shift, leading to a premature stop codon near the 3' end of APC. With regard to other polyposis-related genes, including *MUTYH*, *POLD1* and *POLE*, no pathogenic alterations and two single nucleotide polymorphisms (rs3219489 and rs5744751) were detected in these three genes.

APC mutations associated with the incidence of desmoids reportedly accumulate within codons 1445–1578.[5,6] In contrast, the mutation identified here was located at codon 2644, within the 3' region of *APC*. An identical mutation has been deposited in the International Society for Gastrointestinal Hereditary Tumours

(InSiGHT) Variant Databases and has been reported on two different pedigrees.[10,11] Although no detailed clinical information was supplied for one of the two patients with this mutation,[11] the family history of the other showed that four of six affected family members developed desmoid tumors.[10] The patient evaluated in this study also had a family history of desmoid tumors. Notably, the father and aunt in our case developed thyroid cancer, although histological data of their tumors are not available. These findings suggest that this mutation impairs the functioning of the APC product that is responsible for the suppression of desmoids and thyroid cancer.

The *APC* gene product possesses several domains that are critical for its function. The most prominent function of APC is its ability to regulate Wnt/β-catenin-mediated gene transcription. Mutations in the mutation cluster region lead to the loss of the ability to suppress β-catenin-mediated signaling, which plays a crucial role in the development of colorectal adenoma. The mutant APC protein identified in this study is assumed to have a domain that interacts with β-catenin, which may account for her lack of adenomatous polyps. Reportedly, other mutations located

Table 1. Summary of variations in the APC gene detected by NGS

Type	Mutation: protein alteration	dbSNP ID[a]	Ref[b]	Obs[c]
synonymous SNV	c.T5268G: p.S1756S	rs866006	T	G
synonymous SNV	c.G5880A: p.P1960P	rs465899	G	A
synonymous SNV	c.G5034A: p.G1678G	rs42427	G	A
synonymous SNV	c.G4479A: p.T1493T	rs41115	G	A
synonymous SNV	c.G1635A: p.A545A	rs351771	G	A
nonsynonymous SNV	c.T5465A: p.V1822D	rs459552	T	A
deletion	c.7932_7935del: p.Y2645KfsX14	—	TTAT	—

Abbreviations: APC, adenomatous polyposis coli; NGS, next-generation sequencing; dbSNP, database for single nucleotide polymorphism; SNV, single nucleotide variant. [a]The number registered in dbSNP human Build 142 at http://www.ncbi.nlm.nih.gov/snp/. [b]Reference sequence in human genome sequence (GRCh38). [c]Observed sequence in this study.

within the 3′ region of *APC* may also lead to the development of desmoid tumors.[12,13] Interestingly, the accumulation of β-catenin has been observed in desmoid tumors of a patient with a mutation at the 3′ end of *APC*.[14] Thus, a second hit in the wild-type *APC* allele may suppress the function of the truncated APC protein containing the β-catenin-interacting domain. Because the mutation in this case leads to the truncation of approximately 200 C-terminal amino acids, the mutant protein likely lost domains for interacting with EB1 and human homolog of *Drosophila* discs large (hDLG).[15,16] The APC-EB1 and APC-hDLG complexes are reportedly important for the regulation of chromosome segregation and the cell cycle, respectively.[17,18] Therefore, the loss of interaction of this APC product with EB1 and hDLG might cause the deregulation of cell division and proliferation.

With regard to thyroid cancer in FAP, the genotype–phenotype correlation is controversial. Recently, Septer *et al.*[19] have reported an increased risk of thyroid cancer in FAP patients with a mutation at the 5′ end (proximal to codon 528) and at codon 1061 of *APC*. Of note, this group has also reported two patients with an *APC* mutation at codon 2092 who developed thyroid cancer. Therefore, mutations at the 3′ end may also increase the risk of thyroid cancer. As more data are collected, these will help to elucidate the genotype–phenotype correlation of thyroid cancer in FAP.

In the family evaluated in this report, neither of the two females with desmoids had colonic polyps, but both of the affected males had colonic polyposis. This intrafamilial phenotypic variation might be attributed to influences of modifier genes or environmental factors.

In conclusion, we identified a rare *APC* mutation in a desmoid tumor patient with a family history of AFAP with desmoids and thyroid cancer. This mutation results in the production of an APC protein with a truncation at the C-terminal distal end, which renders it incapable of suppressing the development of desmoids and thyroid cancer. In the diagnosis of AFAP patients with desmoids, mutation screening of not only the 5′ region but also of the 3′ region of the *APC* gene is necessary.

HGV DATABASE

The relevant data from this Data Report are hosted at the Human Genome Variation Database at http://dx.doi.org/10.6084/m9.figshare.hgv.578.

COMPETING INTERESTS

The authors declare no conflict of interest.

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