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Familial Adenomatous Polyposis-Associated Thyroid Cancer

A Clinical, Pathological, and Molecular Genetics Study

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We report two familial adenomatous polyposis (FAP) kindreds with thyroid cancer, harboring two apparently novel germline APC mutations. The clinical phenotype in the first kindred was typical of classical adenomatous polyposis, whereas the second kindred exhibited an attenuated adenomatous polyposis phenotype. There was a female predominance with a mean age of 34 years (range, 23–49) at cancer diagnosis. Multiple sections of four thyroid tumors from three FAP patients were analyzed in detail. Histological examination of thyroid tumors showed a range of morphological features. Some tumors exhibited typical papillary architecture and were associated with multifocal carcinoma; in others, there were unusual areas of cribriform morphology, and spindle-cell components with whorled architecture. Immunoreactivity for thyroglobulin and high molecular weight keratins was strong. Somatic APC mutation analysis revealed an insertion of a novel long interspersed nuclear element-1-like sequence in one tumor sample, suggesting disruption of APC. In three FAP patients, *ret*/PTC-1 and *ret*/PTC-3 were expressed in thyroid cancers. No positivity was observed for *ret*/PTC-2. p53 immunohistochemistry was positive in only one section of a recurrent thyroid tumor sample. Our data suggest that genetic alterations in FAP-associated thyroid cancer involve loss of function of APC along with the gain of function of *ret*/PTC, while alterations of p53 do not appear to be an early event in thyroid tumorigenesis. (Am J Pathol 1999, 154:127–135)

Familial adenomatous polyposis (FAP) is an inherited, autosomal dominant syndrome typically characterized by the development of hundreds of colorectal adenomas.¹

FAP is caused by germline mutations of the adenomatous polyposis coli (APC) gene.^{2,3} Many FAP kindreds also manifest various extracolonic features and this entity was previously described as the Gardner syndrome.^{4,5} Benign FAP-associated extracolonic lesions include upper gastrointestinal adenomas, congenital hypertrophic retinal pigment epithelial (CHRPE) lesions, desmoid tumors, osteomas, epidermoid cysts, and dental abnormalities. In addition to duodenal and periampullary neoplasms, extraintestinal cancers have been reported that include cancers of the thyroid gland,⁶ adrenal gland,⁷ and brain.⁸ The majority of germline mutations occur in the first half of the APC gene. Specific genotype-phenotype correlations have been established with regard to an attenuated adenomatous polyposis coli (AAPC) phenotype (<100 polyps),⁹ sparse polyposis phenotype (1000–2000 polyps),¹⁰ diffuse polyposis phenotype (>5000 polyps),¹¹ CHRPE,¹² and desmoid tumors.¹³

The Leeds Castle Polyposis Group has reported an incidence of 1.2% of thyroid carcinoma in FAP patients.¹⁴ Although the relative risk of thyroid cancer has been estimated to be 7.6% (95% CL 2.5–17.7) in FAP,¹⁵ the absolute risk of developing thyroid cancer is only approximately 2%¹⁶ and hence routine screening is not recommended. FAP-associated thyroid cancer is typically characterized by female predominance (94%), age at tumor diagnosis < 30 years (78%), papillary differentiation (89%), and multifocal development.¹⁷ Most reports on FAP-associated thyroid cancer are case studies without molecular genetic investigations and/or specific genotype-phenotype correlations.^{6,17–21} However, three recent reports have described APC germline mutations at codons 848 and 1061.^{18–20}

The molecular pathogenesis of sporadic thyroid carcinoma is still a subject of investigation. Rearrangements involving the *ret* proto-oncogene have been specifically implicated in the development of papillary thyroid cancer.²² *ret*/PTC-1 and *ret*/PTC-3 are formed by paracentric inversions of the long arm of chromosome 10 fusing the tyrosine kinase domain of *ret* to H4 and *ele-1*, respectively. *ret*/PTC-2 is formed by the fusion of the tyrosine

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Table 1. Clinical Data of Kindred #1

Patient	Sex	Age at Diagnosis (Death)	CRC	Location of CRC	Adenomas #, Location	Surgery (2nd surgery)	ECM/other (age)
I-1	F	35 (63)	Y	nr	—	colostomy	—
II-2	F	42 (51)	Y	R	—	R colectomy	—
			Y	rectum	—	—	—
IL-5	M	48	Y	rectum	—	PC	osteomas
IL-6	M	37 (39)	Y	R, L, rectum	—	PC	—
II-8	M	25 (28)	Y	L	—	IRA	cancer recurrence death
II-9	M	50 (50)	Y	rectum	—	APR	cancer recurrence death
III-1	F	38 (55)	N	—	multiple, R=L	IRA (IPAA)	thyroid cancer (38) endometrial cancer (54) thyroid cancer recurrence (55)
III-2	M	23	Y	rectum	multiple, R=L	IRA (APR)	UGI polyposis dental abnormalities UGI polyposis epidermoid cysts
III-3	M	31	Y	L	60, R=L	IRA (APR)	—
III-4	M	23	N	—	multiple, R=L	IRA	mandible osteoma UGI polyposis desmoid tumor
III-5	M	35	N	—	multiple, R=L	IRA (APR)	desmoid tumor
III-6	F	31	N	—	multiple, R=L	IRA	desmoid tumor
III-7	M	26 (46)	N	—	multiple, R=L	IRA (APR)	accidental death
III-9	M	32 (35)	Y	rectum	multiple, R=L	PC	cancer death
III-11	F	24	N	—	multiple, R=L	IRA (IPAA)	thyroid cancer (24) thyroid cancer recurrence (41)
III-12	M	24	N	—	multiple, R=L	IRA	R maxilla osteoma thyroid cancer (23)
III-16	M	21	N	—	multiple, R>L	IRA	skull osteoma UGI polyposis celiac disease
III-17	M	24	N	—	>100, R=L	IRA	—
III-26	F	22	N	—	multiple, R=L	IRA (APR)	—
III-29	F	27	Y	rectum	1, L	LAR (IRA)	—
III-30	M	25	N	—	multiple, R=L	IRA	—
IV-3	M	27	N	—	multiple, R=L	IRA	—
IV-6	M	17	N	—	multiple, R=L	IRA	—
IV-8	F	20	N	—	multiple, R=L	IRA	—
IV-9	M	20	N	—	multiple, R=L	IRA	—
IV-10	M	17	N	—	multiple, R=L	none	—
IV-12	M	22	N	—	multiple, R=L	IRA	—
IV-13	M	23	N	—	multiple, R=L	IRA	desmoid tumor
IV-23	F		N	—	4, L	none	celiac disease epidermoid cysts
IV-28	F	13	N	—	several, R=L	IRA	—
IV-29	F	9	N	—	none	none	2 normal colonoscopies
IV-30	M	9	N	—	several, R>L	none	—
IV-44	F	10	N	—	several, R>L	none	—
IV-46	M	13	N	—	several, R>L	none	—

CRC, colorectal carcinoma; ECM, extracolonic manifestations; IPAA, restorative proctocolectomy with ileal pouch-anal anastomosis; APR, abdomino-perineal resection; PC, proctocolectomy with terminal end ileostomy; UGI, upper gastrointestinal; LAR, low anterior resection.

kinase domain of *ret* to 5'-terminal sequences derived from the regulatory subunit R1a of cAMP-dependent protein kinase A.²² These *ret*/PTC rearrangements appear to be an early event in thyroid tumorigenesis.²³ Alterations of *p53* have been implicated as a late event that correlates with dedifferentiation. However, the factors that determine progression of intermediate forms of differentiation in thyroid cancer are not clear. Tumor suppressor genes like *APC* do not appear to be involved in the progression of sporadic thyroid cancer.²⁴⁻²⁷ However, interactions between *ret*/PTC1 activation and *APC* mutations have been postulated in the development of thyroid cancer in FAP patients.²⁸

In the present study, we report two FAP kindreds harboring two distinct germline *APC* mutations with a variable expression of adenomatous polyposis and thyroid

cancer. Further, we have investigated the respective roles of *APC*, *ret*/PTC, and *p53* genes in the development of thyroid cancer in these FAP patients.

Patients and Methods

Selection of Patients

Two FAP kindreds with associated thyroid cancer were identified at the Familial Gastrointestinal Cancer Registry at Mount Sinai Hospital, Toronto, Canada. Clinical, endoscopic, operative, histological, and follow-up data are summarized in Tables 1 and 2. Molecular genetic testing was offered with pretest and posttest genetic counseling.²⁹ Patient accrual, blood samples, tissue specimen

Table 2. Clinical Data of Kindred #2

Patient	Sex	Age at Diagnosis	CRC	Location of CRC	Adenomas #, Location	Surgery	ECM/other (age)
II-2	F	68	Y	nr	nr	IRA	—
II-10	F	nr	Y	R	nr	nr	—
III-1	F	51	no	—	20, R=L	none	thyroid cancer (49)
III-2	F	47	no	—	34, R>L	IRA	thyroid cancer (36) cancer in situ endometrium (35)
III-3	M	42	no	—	4, L	none	—
III-5	M	37	no	—	55, R>L	none	—
IV-3	M	27	no	—	9, R>L	none	—
IV-4	M	25	no	—	multiple, R>L	none	—

CRC, colorectal cancer; ECM, extracolonic manifestations; IRA, total colectomy with ileo-rectal anastomosis; nr, not reported.

accrual, and predictive genetic testing were carried out according to protocols approved by the Human Ethics Committee of the University of Toronto.

Molecular Genetic Analysis of Germline APC Mutations

APC molecular screening was done using the protein truncation test (PTT) as previously reported.³⁰ RNA and DNA extraction was performed using TRIzol and DNAzol according to the manufacturer's protocol (Life Technologies, Burlington, Ontario). Reverse-transcriptase polymerase chain reaction (RT-PCR) was carried out using standard techniques. Briefly, cDNA was generated from total RNA (2–5 µg) using random hexamers, 1× first-strand buffer, 0.5 mmol/L deoxynucleotide triphosphates, 10 mmol/L DTT, and 200 U Superscript II reverse transcriptase (Bethesda Research Laboratories, Burlington, Ontario). cDNA and genomic DNA were amplified under the following conditions: initial denaturation at 95°C for 2 minutes; 35–40 cycles, each consisting of denaturation (95°C, 30 seconds), annealing (63–65°C, 1 minute 30 seconds), and extension (70°C, 2 minutes), and a final extension (70°C, 5 minutes). Five-milliliter aliquots of PCR products were electrophoresed on a 1.5% agarose gel to confirm amplification.

The *in vitro*-synthesized PTT assay was performed using a commercial kit TNT T7 Quick Coupled Transcription/Translation System (Promega, Madison, WI) according to the manufacturer's protocol. Briefly, APC exon 15 was amplified in four overlapping segments as previously described.³⁰ Exons 1 to 14 were also amplified in two overlapping segments, 1A (exons 1–9) and 1B (exons 8–14).⁹ The 5' end of each forward primer had a T7 promoter sequence and a translation initiation site for coupled *in vitro* transcription and translation and these products were separated on 12.5% polyacrylamide gels. Positive PTT assays were confirmed by two independent PCR reactions.

For sequence determination, PCR products generated from the putative positive PTT samples were purified using the QIAquick Gel Extraction Kit (Qiagen, Chatsworth, CA). The dideoxy-mediated chain-termination method was used for DNA sequencing.³¹ For this purpose, we used α-³²P dideoxynucleotide and the ThermoSequenase radiolabeled terminator sequencing kit (Am-

ersham Life Science, Oakville, Ontario). Sequencing was carried out according to the manufacturer's protocol. Finally, the gel was autoradiographed using a BioMax MR film (Kodak) for 24–48 hours at room temperature.

Molecular Genetic Analysis of ret/PTC and Somatic APC Mutations in Thyroid Cancers

Tissue sections of 20 µm thickness were deparaffinized from archival specimens in 1 ml xylene at room temperature for 20 minutes and washed once with 100% ethanol. After centrifugation, the tissue pellet was air-dried and resuspended in 200 µl of solution containing 6 mg/ml proteinase K (Sigma Canada Ltd., Oakville, Ontario), 1 mol/L guanidinium isothiocyanate, 25 mmol/L β-mercaptoethanol, 0.5% Sarcosyl, and 20 mmol/L Tris (pH 7.5) and incubated at 45°C for 6 hours. One equivalent volume of 70% phenol/30% chloroform was added and phase separation was carried out at 4°C for 20 minutes followed by centrifugation at 14,000 × g. Overnight precipitation at –20°C followed the addition of one volume of isopropanol and 2 µg of glycogen to the aqueous supernatant. The pellet formed after centrifugation at 14,000 × g was washed with 70% ethanol, air-dried, and resuspended in 10 µl of DEPC water containing RNase inhibitor.

RT was performed on one-fifth of the paraffin-extracted RNA samples. The reaction mixture contained 5 mmol/L MgCl₂, 1 mmol/L dNTP, 2.5 mmol/L respective antisense primer, 1 U/µl ribonuclease inhibitor, and 2.5 U/µl Moloney leukemia virus reverse transcriptase (Perkin-Elmer, Branchburg, NJ). RT was performed under following conditions: 15 minutes at 42°C, followed by 5 minutes of denaturation at 99°C and cooled for 5 minutes at 5°C. The integrity of the RNA and efficiency of the RT reaction in each sample was confirmed by PCR for the housekeeping gene *PGK-1*.²² Each reaction mixture contained 1 µmol/L sense and 0.5 µmol/L antisense primers, 0.3 mmol/L dNTPs, 2 mmol/L MgCl₂, and 5 U *Taq* polymerase (Perkin-Elmer). After an initial denaturation at 94°C for 2 minutes, amplification was performed over 35 cycles consisting of 94°C for 30 seconds, 57°C (PGK-1) or 55°C for 2 minutes (*ret/PTC-1, -2, and -3*), 72°C for 2 minutes, and a final extension at 72°C for 4 minutes. The products were resolved on a 1.2% agarose gel containing ethidium bromide.

DNA extracted from sections of archival thyroid tumor specimens were screened for somatic APC mutations in the mutation cluster region (MCR, APC codons 1286–1513) of the gene.³² The MCR was divided into 8 overlapping segments and screened for altered conformants by SSCP analysis.³³ PCR conditions were as follows: initial denaturation at 94°C for 4 minutes, followed by 30 cycles, each consisting of denaturation (94°C, 1 minute), annealing (55–57°C, 1 minute), and extension (72°C, 1 minute) as previously reported.³³

Southern Hybridization

PCR products were transferred to nylon membranes (Boehringer Mannheim, Laval, PQ) by upward capillary action and fixed by UV cross-linking. Digoxigenin labeled probes for *ret*/PTC-1 and -3 were generated by RT-PCR of thyroid tumors known to harbor *ret*/PTC rearrangements.²² A cDNA probe for *ret*/PTC-2 was kindly provided by Dr. Jhiang (Columbus, Ohio). The primers for each probe were identical to those used for RT-PCR (Boehringer Mannheim). Labeling, hybridization, and detection were performed according to manufacturer's protocol.

Histology and Immunohistochemistry

Paraffin blocks of thyroid tumor specimens were sectioned at 5 μ m thickness for histological and immunohistochemical evaluation of *ret*/PTC.²³ Immunostaining was performed using a rabbit polyclonal IgG antibody to the carboxyl terminus of *ret* (Santa Cruz Biotechnology, Santa Cruz, CA). Tissue sections were mounted on sialinized slides and pretreated with 45% formic acid for 15 minutes at room temperature. Endogenous peroxidase was blocked with 3% hydrogen peroxide and nonspecific binding was prevented by incubation with a protein blocker reagent (Signet, Dedham, MA). The primary antibody (1:1000 dilution) was incubated at room temperature overnight, followed by detection with the ultrastreptavidin system (Signet). Immunohistochemical stains for p53 protein were performed on paraffin sections (5 μ m) of thyroid tumor specimens, using a streptavidin-biotin technique and a monoclonal antibody (DO-7, dilution 1/100, Novocastra Laboratories Ltd., Newcastle-On-Tyne, UK).²⁶

Results

Molecular Genetic Testing and Characterization of Germline APC Mutations

Two apparently novel APC germline mutations were found in these two FAP kindreds.^{34,35} In kindred #1, PTT assay demonstrated a truncated mutant protein band of ~40 kd in APC segment 1B (Figure 1A). In kindred #2, a truncated band of ~50 kd in size was detected in APC segment 1A (Figure 1B). DNA sequence analysis revealed the truncation in kindred #1 to be caused by a

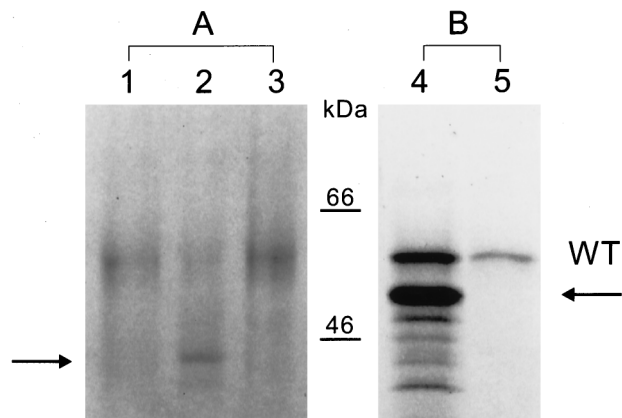


Figure 1. Representative PTT analysis for APC segment 1A (codons 1–379) (Lanes 4 and 5) and APC segment 1B (codons 296–812) (Lanes 1–3). The size of the protein marker is shown. The arrows indicate truncated mutant proteins. Lanes 1, 3, and 5: non-FAP controls. Lane 2: individual III-1 (kindred #1). Lane 4: individual III-1 (kindred #2). WT, wild-type.

transversion T → G, resulting in a substitution of leucine by a stop codon (TTA → TGA) at nucleotide 2092 (codon 698 in exon 15). In kindred #2, the APC mutation occurred at nucleotides 937–938 (deletion GA) within exon 9 (codon 313), resulting in a frameshift leading to a stop codon (TGA) at nucleotides 975–977.

Histopathology of Thyroid Tumors

In individual III-1 (kindred #1), the right lobe of thyroid contained a mass measuring 3.5 × 2.3 cm. The left lobe contained multiple lesions; the dominant nodule in the lower lobe measured 2.1 cm, and multiple microcarcinomas measured from 5 to 9 mm. The tumors had unusual morphological features. The predominant architectural pattern was that of a solid spindle-cell lesion with whorls and squamoid nests (Figure 2a). This was punctuated by tubular structures and papillae lined by tall cells with stratified nuclei (Figure 2b). The nuclear morphology throughout was characterized by irregular nuclear contours, occasional clearing of nucleoplasm, prominent nucleoli, and frequent nuclear grooves. Rare psammoma bodies were identified. The large lesions were surrounded by thick fibrous capsules but there was evidence of capsular and vascular invasion. The tumor cells were immunoreactive for low and high molecular weight cytokeratins and displayed weak but unequivocal staining for thyroglobulin. There was focal nuclear positivity for p53.

In individual III-11 (kindred #1), the first thyroid resection involved the left lobe and contained a solitary nodule that measured 2 cm in diameter. The completion thyroidectomy specimen contained a second nodule that measured 3.5 cm in maximum dimension. Both lesions had predominant macrofollicular architecture with focal papilla formation but there were solid nests of tumor cells scattered throughout both lesions (Figure 2c). The crowded tumor cell nuclei exhibited clearing, grooves, and cytoplasmic inclusions that are characteristic of pap-

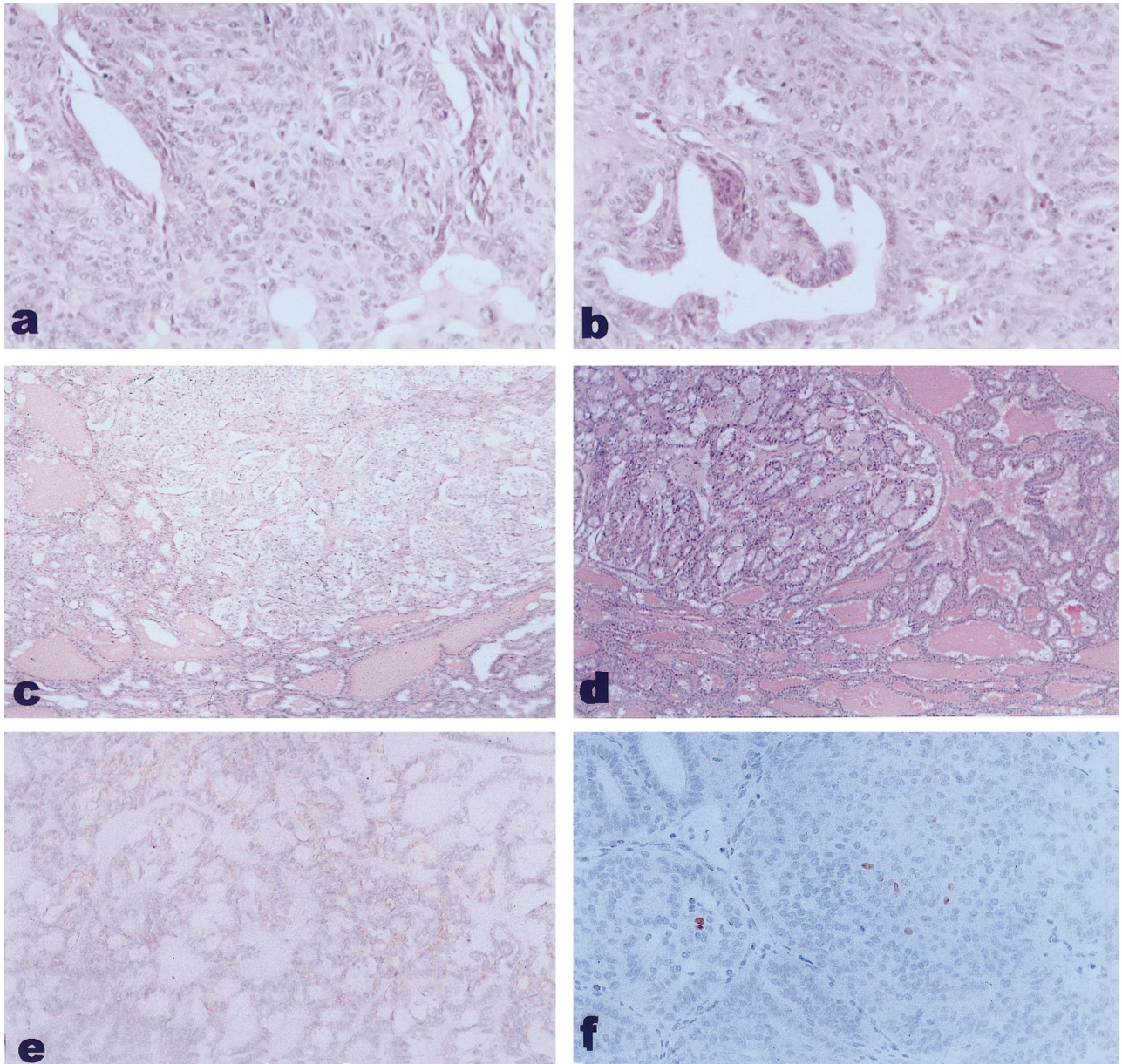


Figure 2. a, b: Histological features of the thyroid tumor of individual III-1 (kindred # 1). The tumor has a predominant solid spindle cell architectural pattern with whorls and squamoid nests (a) and focal papillary structures with more typical papillary morphology (b). H&E, Magnification, $\times 90$. c: Histology of the thyroid tumor of individual III-11 (kindred #1). This tumor is predominantly a follicular variant papillary carcinoma but contains scattered small papillary structures and focal solid areas. H&E, magnification, $\times 35$. d: Histology of the thyroid tumor of individual III-1 (kindred # 2). This tumor has papillary and follicular architecture and focally there is an unusual cribriform pattern (top left). H&E, magnification, $\times 35$. e: *ret* immunostaining localizes cytoplasmic immunoreactivity in tumor cells. Immunoperoxidase technique with hematoxylin counterstain, Magnification, $\times 90$. f: Immunostains for p53 show nuclear reactivity in scattered tumor cells within a solid area of the tumor of the patient III-11 (kindred #1). Immunoperoxidase technique with hematoxylin counterstain. Magnification, $\times 120$.

illary carcinoma. Capsular invasion was seen in both specimens. Immunoreactivity for thyroglobulin and keratins was strong. The p53 antibodies have a short half-life for normal protein, resulting in only focal faint or negative staining, whereas mutant p53 usually accumulates in the nucleus, producing intense positivity. In our tumor specimens, p53 was identified only in nuclei of cells in solid nests (Figure 2f).

In individual III-1 (kindred#2), the thyroid gland contained multiple nodules in both lobes varying from 0.6 to 2.3 cm in diameter. Three nodules, including the largest,

had cytologic features of papillary carcinoma; the remainder were considered hyperplastic nodules. The carcinomas had the architecture of follicular variant type papillary carcinomas with follicles storing hypereosinophilic colloid and occasional papillae. They also contained areas of cribriform architecture (Figure 2d) or formation of solid nests. The lesions were unencapsulated and infiltrated surrounding parenchyma but vascular involvement was not seen. The tumors contained high molecular weight cytokeratins and thyroglobulin but immunoreactive p53 was not identified.

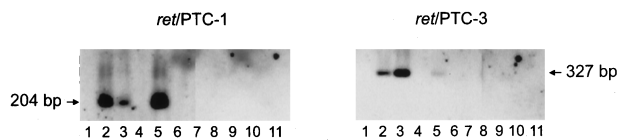


Figure 3. Representative *ret/PTC* analysis from individual III-11 (kindred #1), lanes 2–5. Lanes 1, 6 and 7: Negative controls for PCR. Lanes 8–11: Samples subjected to PCR but after omission of reverse transcriptase.

ret/PTC and Somatic APC Gene Analyses in Thyroid Cancers

Thyroid tumor blocks from 3 FAP patients were screened by RT-PCR for *ret/PTC* gene rearrangements. In patient III-1 (kindred #1) the dominant tumor and multifocal microcarcinomas were positive for *ret/PTC-1*. In patient III-11 (kindred #1) the dominant tumor was positive for *ret/PTC-1* and three of four blocks showed focal positivity for *ret/PTC-3* (Figure 3). In patient III-1 (kindred #2) multifocal papillary carcinoma was found. The three largest tumors were all positive for *ret/PTC-1*. No positivity for *ret/PTC-2* was observed in any tumor specimen.

We screened a total of nine tumor sections representative of different regions of four thyroid tumors for somatic *APC* mutations. The *APC*-MCR region (codons 1286–1513), which includes over 60% of all somatic *APC* mutations, was screened by SSCP analysis.³² An altered SSCP conformant was observed in one tumor specimen from individual III-11 (kindred #1). DNA sequence analysis showed a deletion of 205 bp and a concomitant insertion of 160 bp between *APC* nucleotides 4366 and 4571 (Figure 4), which was confirmed by two independent PCR and sequencing reactions. Further, in an independent assay by PCR amplification of the same tumor we observed two PCR products with sequences corresponding to the wild-type and the inserted sequence. GenBank search indicated that this sequence shared a significant homology (75%) with long interspersed nuclear element-1 (L1) sequences (AC 003012, human PAC clone DJ0169K13). Somatic *APC* mutations in the MCR were not identified in the remaining eight thyroid tumor sections.

ret Immunohistochemistry

ret immunoreactivity was positive but focal in all tumors (Figure 2e). The cytoplasmic staining was of variable intensity in different areas of any individual lesion. Immunoreactivity correlated with the results of RT-PCR; one of the four tissue blocks from a single large thyroid tumor (III-11, kindred #1) was negative for *ret* by immunohistochemistry and did not express *ret/PTC* rearrangements by RT-PCR analysis.

Discussion

In the present study we describe two FAP kindreds harboring two novel *APC* germline mutations with a variable polyposis phenotype and an association with thyroid cancer. The first kindred is a large multigenerational family whose phenotype corresponds to classical FAP: > 100 adenomas distributed throughout the colon and rectum, upper gastrointestinal polyposis, osteomas, epidermoid cysts, desmoid tumors, and dental abnormalities (Table 1). Average age at polyposis diagnosis was 25.4 years (range, 8–50 years). Two female patients developed thyroid cancer at ages 24 and 38, respectively. An additional male FAP patient was diagnosed with thyroid cancer at age 24. Both females had recurrent thyroid cancer, although it is important to note that the initial surgical procedure was not a total thyroidectomy. Interestingly, in individual III-1, endometrial cancer was diagnosed at 54 years of age. Another remarkable clinical feature of this kindred is the early onset of FAP in one branch of the family (individuals IV-28, 30, 44, and 46). Recently, this aggressive polyposis feature has been correlated with *APC* germline mutations occurring at the proximal end of exon 15.^{36,37} Our findings of an *APC* mutation at codon 698 of exon 15 further supports these observations. With respect to the second kindred (Table 2), the germline mutation is located within exon 9, one of the three regions of the *APC* gene corresponding to the attenuated variant,⁹ and accordingly the phenotypic expression consists of mild polyposis. AAPC kindreds are characterized by fewer colonic adenomas (<100), frequent right-sided colonic tumors, later onset of colorectal cancer, and rectal polyp sparing. In this kindred, two female patients developed thyroid cancer at 36 and 49 years of age; individual III-2 was also diagnosed with carcinoma *in situ* of the endometrium at age 35. In this AAPC family, mean age at polyposis diagnosis was 42.4 years (range, 25–68 years). Table 3 summarizes the reported *APC* germline mutations in FAP kindreds associated with thyroid cancer. Of these, mutations at codons 1061–1063 comprise one of the hot spots for germline *APC* mutations and may account for approximately 10% of all germline mutations in FAP patients.³⁸ Paraf et al²⁰ reported three additional FAP kindreds with thyroid cancer harboring mutations within exon 14, at the beginning of exon 15, and in the noncoding region of *APC*, but without further specific characterization of the *APC* mutations.²⁰ Results from our study and those published previously suggest that there are no specific genotype-phenotype correlation with respect to the location of the *APC* mutation and the occurrence of thyroid cancer in FAP.

Histological analysis of thyroid tumor specimens in three mutation carriers (III-1 and III-11 of kindred #1 and

APC nt # 4334, 5'
 CAAACAGCTCAAACCAAGCGAGAAGTACCTAAatgatgtatgttttacctctcatccttccaggctctccgctt
 ccgaacctccatatacattgtatcactctgtatattcttgcacatgccatagcttacctccaactaaattgacaacatgtggatttggttttctt
 ccogattacttacttaGAATAATGCCTCCAGTTCAGGAAAATGACAAT *APC* nt # 4602, 3'

Figure 4. 160-bp sequence insertion in MCR of the *APC* gene. Novel L1-like sequence insertion is shown in lower case and normal *APC* sequence in upper case letters. Forward and reverse primers are underlined. The 8-bp AT-rich consensus target sequence (nucleotides 4571–4578) is shown in italics.

Table 3. APC Germline Mutations in FAP-Associated Thyroid Carcinoma

Author (reference)	APC Mutation	Codon	Exon	Polyposis Phenotype	Extracolonic Manifestations
Kashiwagi (21)	AAA→TAA	848	15	classical FAP	thyroid carcinoma, desmoid tumor, duodenal polyposis
Civitelli (19)	del ACAA	1061	15	classical FAP	thyroid carcinoma, desmoid tumor, CHRPE, osteoma, duodenal polyposis, hepatoblastoma, dental abnormalities
Paraf (20)	del ACAA	1061	15	classical FAP	thyroid carcinoma, desmoid tumor, duodenal polyposis, brain tumor, hepatoblastoma
Present study	del GA TTA→TGA	312 698	9 15	AAPC classical FAP	thyroid carcinoma thyroid carcinoma, desmoid tumor, duodenal polyposis, osteoma, dental abnormalities, epidermoid cysts

AAPC, attenuated adenomatous polyposis coli; CHRPE, congenital hypertrophic retinal pigment epithelium; del, deletion.

III-1 of kindred #2) demonstrates the unusual microscopic appearance of these tumors. As with sporadic papillary carcinoma, clinical tumors were associated with multifocal microcarcinomas and these lesions exhibited papillary architecture. In almost all tumor specimens, immunoreactivity for thyroglobulin and high molecular weight keratins was strong. In addition, there were unusual areas of cribriform architecture, and sometimes spindle-cell components with whorled architecture. These features have been described previously.³⁹⁻⁴¹ It is important to recognize these unusual morphological aspects because thyroid cancer may be the first manifestation of FAP.¹⁸ Not all FAP-associated thyroid cancers have these features but when they are present, the pathologist should alert the clinician to undertake gastrointestinal investigations to consider the diagnosis of familial polyposis. Finally, it is worth emphasizing that because of the multifocal nature of FAP-associated thyroid carcinoma, total thyroidectomy is the preferred surgical procedure as in sporadic papillary thyroid cancer.

Somatic APC mutation analysis of thyroid tumors identified an insertion of L1-like sequence in individual III-11 (kindred #1). Other tumor specimens from this individual did not show this L1 insertion, possibly due to the heterogeneity of thyroid tumors. This frameshift insertion may have caused a truncated APC protein. A similar case of APC disruption by an L1 insertion has been reported in a colon cancer, where a duplication of an AT-rich 8-bp consensus sequence (GAATAATG) was observed at the target site.⁴² In our thyroid tumor specimen, the APC sequence flanking the insertion also harbors the same 8-bp consensus sequence (nucleotide 4571-4578, Figure 4) although it was not duplicated. This sequence insertion may be in the same mutant germline allele or the remaining wild-type allele. We were unable to verify this because the APC germline mutation in this patient is located upstream from L1 insertion (at nucleotide 2092) and both mutations could not be checked in a single PCR assay due to the limited availability of thyroid tumor specimen. L1 repeats are consensus elements of 6-7 kbp, consisting of three major regions: (1) at the 5' end, ~1 kb of sequence containing numerous stop codons in all reading frames, (2) a sequence consisting of several hundred base pairs at the 3' of element with no coding potential, and (3) a region of 5 kbp between the 5' and 3' end capable of coding for one or more proteins.⁴³ L1 repeats vary in length and insertions ranging from 60 bp

to several kilobase pairs have been reported. The biological function of L1 is not yet fully understood but, because of its mobility, its insertion is predicted to cause disruption of protein function. Insertions of truncated L1 repeats in *factor VIII* and *DMD* genes have been identified as disease-causing mutations.⁴³ We did not find somatic APC mutations other than this L1 insertion in the other thyroid tumor specimens but we cannot exclude that some mutations may exist outside the MCR in these tumors. In addition, because of insufficient tumor sample availability, a search for loss of heterozygosity and screening of the entire gene for somatic APC mutations were not possible.

We have previously demonstrated that *ret*/PTC rearrangements represent an early event in papillary thyroid carcinoma, are not present in tumors with aggressive morphological features, and are found in young patients (<45 years) with small thyroid carcinomas showing a predisposition for lymphatic involvement.²² Furthermore, *ret*/PTC rearrangements are early and multifocal events in thyroid tumorigenesis, in particular in microcarcinomas;²³ the diversity of *ret*/PTC profiles also suggest that individual tumors arise independently in a background of genetic and/or microenvironmental susceptibility.²³ The immunoreactivity for *ret* correlated with the RT-PCR expression profile of the gene rearrangements that allow expression of the tyrosine kinase domain of *ret* in thyroid follicular epithelial cells. Taken together, these findings suggest that the loss of function of the APC gene is associated with gain of function of *ret*/PTC-1 and -3 in FAP-associated thyroid cancer. However, it is not clear if tumor progression is similar to the multistep colorectal tumorigenesis in these patients.⁴⁴ Further investigations are needed to clarify the exact role of *ret*/PTC in FAP-associated thyroid carcinoma and its interactions with the APC gene. The role of other genes such as β -catenin, implicated in cell adhesion and signal transduction, may also be relevant^{45,46} and requires further studies to clarify possible interactions of APC and β -catenin in thyroid tumors. Moreover, the modifier gene(s) may contribute to the phenotypic variations of FAP; ie, in kindred #1, a branch of the family presents with a very early onset of adenomatous polyposis.

Finally, nuclear positivity for p53 was identified only in the recurrent tumor lesions. We have previously reported that p53 immunoreactivity is mainly detected in advanced and aggressive thyroid tumors, and is likely a

useful prognostic index of clinical behavior.²⁶ Taking these findings together, we suggest that p53 is a rather late event in thyroid tumorigenesis.

In summary, although specific *APC* mutation genotype may not correlate with manifestations of thyroid tumors in FAP kindreds, rearrangements in *ret/PTC-1* and *-3* have a more relevant effect in FAP-associated thyroid tumorigenesis.

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