PAPILLARY CARCINOMA OF THE THYROID ASSOCIATED TO FAMILIAL ADENOMATOSIS.

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XX CYCLE
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1. BACKGROUND

1.1. THYROID CARCINOMA.

Thyroid cancer is the most common malignancy of the endocrine system and accounts for approximately 1% of all newly diagnosed cancer cases (1). The incidence of this neoplasia is increasing, and is the fastest among common human cancers, becoming the seventh most common tumor in women. Age-adjusted global incidence rates vary from 0.5 to 10 cases per 100,000 population, occurring in most of the cases between 20 and 50 years of age (2). The thyroid gland, which is the largest endocrine organ in humans, regulates systemic metabolism through thyroid hormones. It is composed of two distinct hormone-producing cell types that have been designated follicular cells and parafollicular C cells. Follicular cells comprise most of the epithelium and are responsible for iodine uptake and thyroid hormone synthesis. C cells are scattered intrafollicular or parafollicular cells that are dedicated to the production of the calcium-regulating hormone calcitonin.

Thyroid carcinoma derived from follicular cells (papillary and follicular thyroid carcinoma (FTC), poorly differentiated thyroid carcinoma, and anaplastic thyroid carcinoma) is the most common endocrine malignancy.
Approximately 95% of thyroid cancers are non-medullary, which arise from follicular cells. Papillary carcinoma (FIG. 1) is the most common type of thyroid malignancy, comprising about 80–90% of all carcinomas (3-4).

**FIG. 1**

![Image of papillary thyroid carcinoma](image)

Fig. 1 Classic papillary thyroid carcinoma is formed of papillae with fibrovascular cores (H&E, original magnification _100).

Medullary thyroid carcinoma (MTC) refers to those neoplasms arising from the calcitonin-producing C thyroid cells derived from neural crest, and represents approximately 5% of all thyroid tumors (5-7).
1.2. MODEL OF CARCINOGENESIS OF PAPILLARY THYROID NEOPLASMS.

Recent years have been marked by dramatic expansion in the understanding of the molecular basis of thyroid carcinogenesis. A model of thyroid carcinogenesis has been proposed, based on general concepts and specific pathways. In this model a three-way interaction between environmental risk factors, genetic alterations and genomic instability is supported. It has become apparent that thyroid tumors, especially those of the papillary type, frequently have genetic alterations leading to activation of the mitogen-activated protein kinase (MAPK) signaling pathway. This crucial intracellular cascade regulates cell growth, differentiation, and survival in response to growth factors, hormones, and cytokines that interact with receptor tyrosine kinases present on the cell surface. Oncogenic activation of MAPK signalling increases genomic instability, leading to later genetic alterations that involve other signalling pathways, cell-cycle regulators and various adhesion molecules. Accelerating the interactions between genomic instability and genetic alterations promotes progression from well-differentiated to undifferentiated thyroid carcinoma.
1.2.1. ENVIRONMENTAL RISK FACTORS.

Radiation exposure, reduced iodine intake, lymphocytic thyroiditis, hormonal factors are putative risk factors for thyroid carcinoma. Radiation exposure as a consequence of nuclear fallout is associated with papillary carcinoma, as evidenced by the sequelae of the atomic bombs of Hiroshima and Nagasaki (1945), nuclear testing in the Marshall Islands (1954) and Nevada (1951–1962), and the more recent nuclear accident in Chernobyl (1986) (2,8) After the Chernobyl disaster, the effects of radiation exposure were most pronounced in children; it is not clear if this is because the thyroid is more susceptible to radiation damage in childhood, most of the thyroid cell mitoses happen before 10 years of age, whether it is a reflection of the fact that children drank more contaminated milk, increasing their exposure to radioactive iodine, or both (8-10). External beam-radiation exposure in childhood for the treatment of benign conditions of the head and neck also increases the risk of papillary carcinoma (11). The predilection to radiation induced injury seems to be closely linked to chromosomal rearrangement as opposed to intragenic point mutation as a mode of aberrant gene activation (12).

Iodine is required for thyroid hormone organification. Dietary iodine deficiency results in thyroid proliferation, known as goitre, as a compensatory mechanism. The incidence of follicular carcinoma in areas of iodine deficiency is higher than in areas of an iodine-rich diet (2,13) By contrast, papillary
carcinoma is the most frequent type of thyroid cancer in iodine-sufficient regions (2,14). Interestingly, in animal models, iodine supplementation causes experimental thyroid cancers to change from follicular to papillary morphology, indicating that one of the roles of iodine in thyroid carcinogenesis is modulating tumour morphology, rather than cancer initiation (13). Despite these well-documented relationships, the role of iodine in thyroid carcinogenesis is still unclear.

Lymphocytic infiltration is frequently observed in papillary carcinoma, indicating that immunological factors might be involved in tumour progression. Recent molecular analyses indicate that chronic lymphocytic thyroiditis harbours potential precursor lesions of malignancy (15). Most well-differentiated thyroid carcinomas manifest in patients who are 20–50 years of age, and the disease is 2–4 times more frequent in females than in males (1,2). These sex and age distributions of incidence indicate that female hormones might regulate thyroid carcinogenesis. Recently evidences have been collected that prove a close relationship between PTC and sex hormones.

1.2.2. GENETIC ALTERATIONS OF MAPK SIGNALLING PATHWAY.

Molecular alterations found in papillary carcinomas involve genes coding for the receptor tyrosine kinases, RET and NTRK1, and two intracellular effectors
of the MAPK pathway, a GTP-binding protein RAS and a serine-threonine kinase BRAF. Mutation of one of these genes can be found in more than 70% of papillary carcinomas and they rarely overlap in the same tumor, suggesting that activation of this signaling pathway is essential for tumor initiation and alteration of a single effector of the pathway is sufficient for cell transformation (17-19). BRAF and RET which are the two most frequently affected genes will be reviewed.

FIG. 2. Schematic representation of the MAPK pathway

RET-PTC
The RET protooncogene is localized on chromosome 10q11.2 and codes for a cell membrane receptor tyrosine kinase (20,21). It contains three functional domains: an extracellular ligand binding domain, a hydrophobic transmembrane domain, and an intracellular tyrosine kinase (TK) domain. The ligands of the RET receptor are growth factors belonging to the glial cell line-derived neutrophic factor family (GNDF) (22). Binding of the ligand causes receptor dimerization, autophosphorylation of tyrosine residues within the intracellular domain, and activation of the signaling cascade. In the thyroid gland, RET is expressed at high level in parafollicular C-cells but not in follicular cells, in which it can be activated by chromosomal rearrangement, resulting in the fusion of the 3’ portion of the RET gene to the 5’ portion of several unrelated genes, known as RET/PTC rearrangement. At least 11 types of RET/PTC have been reported to date, formed by the RET fusion to different partners (Fig. 3) (23,24). The partner genes share some common characteristics: they are expressed in thyroid follicular cells and therefore provide an active promoter for the expression of RET TK domain, that are essential for dimerization and ligand-independent activation of the truncated RET protein.

The RET/PTC development is directly linked to the amount of radiation exposure to which patients are exposed and its contribute is higher in papillary carcinomas from patients with the history of radiation exposure, including those subjected to either accidental or therapeutic irradiation.
Virtually all breakpoints in the RET gene leave intact the TK domain of the receptor and enabling the RET/PTC oncoprotein activate the RAS-RAF-MAPK cascade \((25)\). The two most common rearrangement types are RET/PTC1 and RET/PTC3, which account for the vast majority of all rearrangements found in papillary carcinomas. RET/PTC1 is formed by fusion with the H4 (D10S170) gene \((24)\) and RET/PTC3 by fusion with the NCOA4 (ELE1, RFG, or ARA70) gene \((26,27)\). RET/PTC1 and RET/PTC3 are intrachromosomal paracentric inversions because both genes participating in the fusion are located on chromosome 10q \((28,29)\). In contrast, RET/PTC2 and other rare types of RET/PTC are interchromosomal translocations. RET/PTC is tumorigenic in thyroid follicular cells; it transforms thyroid cells in culture \((30)\) and gives rise to thyroid carcinomas in transgenic mice \((31–33)\).

In most studies, RET/PTC is found in 20–40% of adult sporadic papillary carcinomas. Indeed, the distribution of RET/PTC rearrangement within each tumor can vary from involving almost all neoplastic cells (clonal RET/PTC) to being detected only in a small fraction of tumor cells (nonclonal RET/PTC) \((34,35)\). This has to be taken into account when selecting patients for the RET receptor-targeted therapy because tumors with nonclonal RET/PTC frequently have other genetic alterations and are unlikely to respond to such a treatment. Among papillary carcinomas from children affected by the Chernobyl nuclear accident, RET/PTC was found in up to 80% of tumors removed 5–8 yr after
the accident and 50–60% of those removed 7–11 yr after exposure (36–39). The formation of RET/PTC1 and RET/PTC3 rearrangements after radiation exposure is likely to be predisposed by close positioning of the RET chromosomal locus to its fusion partners within the nuclei of normal thyroid cells, which would facilitate the simultaneous breakage of both genes and their end joining (40,41). RET/PTC is also found more frequently (40–70%) in sporadic papillary carcinomas from children and young adults (42-44). Among several functional clusters of genes found to be activated after RET/PTC expression, many genes are involved in regulating the inflammatory and immune responses (45, 46).

This suggests a link between RET/PTC signaling and inflammatory infiltrates, which are frequently seen within thyroid tumor nodules and in surrounding thyroid tissue and may play a role in promoting tumor progression and invasion.
Figure 3 Schematic drawing of the RET protein with the four extracellular cadherin-like domains, the cysteine-rich box adjacent to the plasma membrane, the juxtamembrane domain and the split tyrosine kinase domain (TK). In PTCs, RET is rearranged with diverse genes, encoding protein dimerization motifs (highlighted) that mediate ligand-independent RET dimerization. The arrowheads indicate RET breakpoints.
BRAF

BRAF belongs to the family of RAF proteins (ARAF, BRAF, CRAF), which are intracellular effectors of the MAPK signalling cascade. Upon activation triggered by RAS binding and protein recruitment to the cell membrane, these serine-threonine kinases phosphorylate activate the MAPK cascade. Among the three functional human RAF proteins, BRAF has the highest basal kinase activity and is the most potent activator of MAPK (47-49). There have been more than 40 mutations identified in the BRAF gene, among which the T1799A point BRAF mutation is the most common and accounts for more than 90% of all the mutations found in the BRAF gene (50). This mutation has been found to occur frequently in thyroid cancer (51-53). The T1799A BRAF mutation causes a V600E amino acid change in the BRAF protein, resulting in the constitutive and oncogenic activation of the mutated BRAF kinase (48,54). BRAF mutation represents a somatic genetic alteration and is not a germline mutation in familial thyroid cancer (55). A striking finding on BRAF mutation in thyroid cancer is its exclusive occurrence in PTC, tall cell and PTC-derived anaplastic thyroid carcinoma, and it does not occur in follicular thyroid carcinomas of in other types of thyroid tumors (51). In those tumors, mutant BRAF is detectable in both well differentiated and poorly differentiated or anaplastic tumor areas, providing evidence that it occurs early in tumorigenesis and predisposes to tumor dedifferentiation. Many studies have investigated the
relationship of BRAF mutation with clinicopathological characteristics of PTC (56,57). Although the results are not entirely consistent, most of the studies from various ethnic and geographical backgrounds demonstrate a significant association of BRAF mutation with one or more conventional high-risk clinicopathological characteristics of PTC such as older age of patients, more frequent extrathyroidal extension, advanced tumor stage at presentation, and tumor recurrence (58,59). In a large comprehensive international multicenter study, Xing et al. (60) reported a close association of BRAF mutation with extrathyroidal invasion, lymph node metastasis, and advanced disease stages. Importantly, BRAF mutations have also been associated with the decreased ability of tumors to trap I-131 and treatment failure of the recurrent disease (61). Recently another mechanism of BRAF activation has been identified. It involves inversion of chromosome 7q that leads to an in-frame fusion between BRAF and the AKAP9 gene (62). This fusion is rarely found in sporadic papillary carcinomas and is more common in tumors associated with radiation exposure.

1.2.3 GENOMIC INSTABILITY.
Chromosome instability is broadly classified into microsatellite instability (MIN) associated with mutator phenotype, and chromosome instability (CIN)
recognized by gross chromosomal abnormalities. It has been proposed that genomic instability has a crucial role in the progression of thyroid neoplasms (63).

Transfection of mutant HRASV12 or mutant BRAFV600E induces genomic instability in the PCCL3 rat thyroid cell line, manifesting as loss of chromosomal material, mitotic bridge formation and misaligned chromosomes (64). These findings indicate that constitutive oncogenic activation of the mitogen-activated protein kinase (MAPK) signalling pathway might further promote genomic instability of thyroid carcinoma cells, possibly causing additional somatic mutations during cancer progression.

1.3. FAMILIAL FORMS OF PAPILLARY THYROID CARCINOMA.

Familial forms of thyroid papillary neoplasms have been only acknowledged in recent years. Presently, approximately 5% of papillary thyroid cancers are considered to be of familial origin. The papillary tumors encompass a heterogeneous group of diseases, including both syndromic associated tumors, and non-syndromic tumors. The first group has an increased prevalence of papillary thyroid carcinoma within a familial cancer syndrome with a
preponderance of non-thyroidal tumors. Thyroid carcinomas in multi-tumor genetic syndromes are heterogeneous diseases (Table 1) and tend to share some similar characteristics including early age onset, multicentricity, and bilaterality. The familial syndromes associated with papillary thyroid neoplasia include familial adenomatous polyposis (FAP) which will be discussed further, Carney complex, MEN1, MEN2A, and Werner syndrome.

- Carney Complex is an autosomal dominant disease, characterized by skin and mucosal pigmentation, diverse pigmented skin lesions, non-endocrine and a variety of endocrine neoplasias (pituitary adenoma, pigmented nodular adrenal disease, Sertoli and Leydig cell tumors, and thyroid tumors) (65). Patients with Carney Complex may share similar components with other familial multiple endocrine neoplasia. The thyroid is usually multinodular with multiple adenomatous nodules, follicular adenomas, and both PTC and FTC are present in about 15% of patients with Carney Complex.

- Werner syndrome is an autosomal recessive connective tissue disease, characterized by premature aging, bilateral cataracts, gray hair, and skin atrophy. Patients with this syndrome have increased risk of a variety of neoplasias, including benign thyroid lesions and an increased incidence of PTC (only tumor present in white patients), and the most common tumor in Japanese patients (84%), followed by FTC (14%) and
anaplastic thyroid carcinomas (2%). This latter neoplasm occurs in this syndrome at a higher frequency as compared to the general population. Thyroid carcinoma occurs at a younger age (mean age of 34) with a lower female to male ratio (2:1) (66,67).

- **Multiple Endocrine Neoplasia 2A (MEN2A):** The frequency of microscopic PTC is approximately twice as great in thyroid glands of MEN2A patients if compared with general population. These cases usually present with multiple microscopic PTCs. These microcarcinomas are likely to carry only modest clinical significance, as microscopic PTCs often remain clinically silent and affected subjects carrying germline RET mutations undergo thyroidectomy at a young age (68-69).

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Chromosomal location</th>
<th>Gene</th>
<th>Inheritance</th>
<th>Thyroid pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTEN hamartoma tumor syndrome (PHTS)</td>
<td>10q22-23</td>
<td>PTEN</td>
<td>AD</td>
<td>MAN, FA, FC, PTC thymiditis</td>
</tr>
<tr>
<td>Familial adenomatous polyposis (FAP)</td>
<td>5q21</td>
<td>APC</td>
<td>AD</td>
<td>CMv PTC sclerosis</td>
</tr>
<tr>
<td>Carney complex</td>
<td>2p15-16, 17q22-24</td>
<td>unknown PRKAR1a</td>
<td>AD</td>
<td>MAN, FA, FA, PTC</td>
</tr>
<tr>
<td>Werner syndrome</td>
<td>8p11-21</td>
<td>WRN</td>
<td>AR</td>
<td>PTC, FTC, anaplastic carcinoma</td>
</tr>
<tr>
<td>MEN1</td>
<td>11q13</td>
<td>MEN1</td>
<td>AD</td>
<td>Rare</td>
</tr>
<tr>
<td>MEN2A</td>
<td>10q11.2</td>
<td>RET</td>
<td>AD</td>
<td>Micro PTC</td>
</tr>
<tr>
<td>McCune Albright syndrome</td>
<td>20q13.1-13.2</td>
<td>GNAS1</td>
<td>Mosaic</td>
<td>Follicular adenoma and PTC</td>
</tr>
</tbody>
</table>

Tab. 1 Non-medullary thyroid carcinoma as a component of a familial tumor syndrome
In the second group the predominant neoplasm is papillary thyroid carcinoma (familial tumor syndromes characterized by a predominance of PTC; fPTC), although other neoplasms may occur with increased frequency. Non-syndromic or familial tumor syndromes characterized by a predominance of PTC, are subclassified in different groups as pure fPTC with or without oxyphilia, fPTC with renal papillary tumor, and fPTC with multinodular goiter (Table 2). Familial papillary thyroid carcinoma (fPTC) is characterized by three or more first degree relatives with papillary thyroid carcinoma and occurs regardless of the presence of another familial syndrome.

Familial papillary thyroid carcinoma has a high incidence of multifocality and association with multiple benign nodules. PTC patients have shorter disease-free survival than do sporadic disease patients because of frequent locoregional recurrence (70-73). The genetic inheritance of PTC remains unknown, but it is believed to be an autosomal dominant mode with incomplete penetrance and variable expressivity. (74,75)

1.3.1. Familial papillary thyroid carcinoma (fPTC) is characterized by multicentric tumors and multiple adenomatous nodules with or without oxyphilia. fPTC with oxyphilia has been mapped to chromosomal region 19p13 and fPTC without oxyphilia has also been mapped to 19p13.
1.3.2. The familial non-medullary thyroid carcinoma type 1 (fNMTC1) syndrome (chromosomal region 2q21) is characterized by PTC without any distinguishing pathologic features and without an obvious increase in frequency of non-thyroidal neoplasms in kindred members (74).

1.3.3. Familial PTC associated with renal papillary neoplasia, presents with the usual classical variant of PTC, and with no special features. The papillary renal neoplasia syndrome (fPTC/PRN), mapped to chromosomal region 1q21, includes not only PTC and the expected benign thyroid nodules but also papillary renal neoplasia and possibly other malignancies as well (75).

1.3.4. In familial multinodular goiter (FMNG) syndrome, which is mapped to 14q, some patients may develop an associated PTC (70). FNMTC has been shown to be associated with the presence of multiple benign nodules, to behave in a more aggressive clinical behavior, and to have a worse prognosis than sporadic non-medullary thyroid cancer. Individuals with FNMTC have an increased risk of multifocal disease, local invasion, and increased local or regional recurrence and lymph node metastases (Tables 2). These aggressive features appear to contribute to the higher recurrence rate and decreased disease-free survival seen in FNMTC patients compared to those with sporadic differentiated thyroid cancer. Compared to the patients with sporadic disease,
the FNMT patients were more likely to have intraglandular dissemination.

FNMTC is an independent predictor of shorter disease-free survival (76).

Tab. 2 Familial tumor syndromes characterized by a predominance of non-medullary thyroid carcinoma

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Chromosomal location</th>
<th>Gene</th>
<th>Inheritance</th>
<th>Thyroid pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Familial papillary thyroid carcinoma with oxyphilia</td>
<td>19p13.2</td>
<td>(TCO) unknown</td>
<td>AD</td>
<td>PTC with or without oxyphilia, multicentric</td>
</tr>
<tr>
<td>Familial papillary thyroid carcinoma without oxyphilia</td>
<td>19p13.2</td>
<td>Unknown</td>
<td>AD</td>
<td>PTC without oxyphilia, multicentric</td>
</tr>
<tr>
<td>Familial papillary thyroid carcinoma with papillary renal cell neoplasia (PTCPRN)</td>
<td>1q21</td>
<td>Unknown</td>
<td></td>
<td>Classical PTC</td>
</tr>
<tr>
<td>Familial papillary thyroid carcinoma (FPMTC1)</td>
<td>2q21</td>
<td>Unknown</td>
<td></td>
<td>Classical PTC</td>
</tr>
<tr>
<td>Familial multinodular goiter with papillary thyroid carcinoma</td>
<td>14q31</td>
<td>Unknown</td>
<td>AD</td>
<td>PTC in a background multinodular cyst formation</td>
</tr>
</tbody>
</table>

1.4. FAMILIAL ADENOMATOUS POLYPOSIS SYNDROME.

Familial adenomatous polyposis (FAP) is a colon cancer predisposition syndrome, characterized by the presence of hundreds to thousands of adenomatous colorectal polyps, which is inherited in an autosomal dominant
It is caused by germline mutations in the adenomatous polyposis coli (APC) gene mapped at chromosome 5q21. Since the first description of FAP in 1847 (77), the syndrome has been extensively investigated and described in the literature. In 1975, the clinical characteristics and natural history of FAP were described by Bussey.

The prevalence of FAP is estimated at 1 in 5000–10,000 (78). Patients develop hundreds to thousands of adenomatous polyps in their colorectum during their second and third decade of life (Fig.3). These polyps if not surgically treated inevitably develop colorectal carcinoma by the age of 40–50 years. A clinical diagnosis of FAP can be made when more than 100 adenomatous polyps are identified in the colorectum (77). About 10% of FAP patients have a milder course of disease with less than 100 colorectal adenomas and a later onset of disease. This variant is termed attenuated FAP (AFAP) (79).

In FAP patients not only colorectal adenomas but also various extracolonic manifestations are observed, including desmoid tumours, osteomas, dental abnormalities, congenital hypertrophy of the retinal pigment epithelium (CHRPE), lipomas, epidermoid cysts and upper gastrointestinal polyps. Moreover, cancers of the thyroid, brain and hepatobiliary tract are found to be associated with FAP.
Fig. 4 Example of familial adenomatous polyposis with a severe phenotype. Both the small adenomas carpeting the mucosa (too numerous to count) and the two larger adenomas were tubular adenomas with low-grade dysplasia.

Fig. 5 Colorectal microadenoma in a case of familial adenomatous polyposis (Hematoxylin and Eosin).

Periodical screening of the colorectum by sigmoidoscopy is recommended, starting between 10 and 12 years. In most patients, a preventive colectomy is performed by the age of 20 years (80).

1.4.1. MOLECULAR GENETICS.

In 1991, the APC gene (chromosome 5q21-22) was identified and found to be mutated in FAP patients (81-83). The coding region of the gene consists of 15
exons, encoding a protein consisting of 2843 amino acids. The APC gene is a tumour suppressor gene. It contains a variety of functional domains and is involved in several cellular processes, including transcription, cell cycle control, migration, differentiation and apoptosis (84,85). Mutations follow the classical two-hit model of tumour suppressor inactivation. FAP patients inherit one germline mutation and develop tumours from those cells in which a second hit, or loss of the other allele of APC, is somatically acquired (84-86). Most mutations will result in stop codons and lead to truncation of the APC gene product resulting in a non-functional protein. These mutations have a nearly complete penetrance of the colonic phenotype, but a variable penetrance of extra-colonic manifestations of the disease (Fig. 6). Modifier genes, variable interference of different mutant APC proteins on the wild-type APC function and environmental factors may play a role in extra- intestinal tumor formation (87). The APC protein is a large scaffolding protein with several functions (84). It is involved in the Wnt signalling cascade.
Fig. 6 Genotype–phenotype correlations of extra-intestinal familial adenomatous polyposis (FAP) manifestations according to the available literature. The APC gene consists of 15 exons. The highest cumulative frequencies of extra-colonic manifestations are found between codons 976–1,067 and 1,310–2,011. The margins of codon regions associated with extra-intestinal manifestations are not absolute and merely provide a guidelineCHRPE congenital hypertrophy of the retinal pigment epithelium.

As part of a multiprotein complex, the APC protein downregulates β-catenin activity (88). In the absence of a Wnt signal, APC forms a complex with the protein β-catenin, allowing it to be targeted for destruction. When APC function is lost, β-catenin accumulates in the cytoplasm and binds to several transcription factors, thereby altering the expression of various genes affecting
proliferation, differentiation, migration and apoptosis of cells (84). In addition, APC stabilizes microtubules, leading to chromosomal stability. Inactivation of APC can lead to defective chromosome segregation and aberrant mitosis (89-91). Since the identification of the APC gene, more than 825 germline mutations have been reported to the APC mutation database http://www.perso.curie.fr/Thierry.Soussi/APC.html (92). Mutational hotspots are located at codons 1309 and 1061, accounting for approximately 17% and 11% of all germline APC mutations, respectively (92). Because of the accumulation of mutations from codon 1250 to 1464, this region is termed the “mutation cluster region” (MCR) (93,94). Data were retrieved from the online APC mutation database at http://www.perso.curie.fr/Thierry.Soussi/APC.html.

In 30–50% of patients with the FAP or AFAP phenotype, no germline APC mutation is detected (94,95). In 10–15% of mutation-negative patients with the classical phenotype large genomic deletions were detected. These deletions were not found in AFAP patients. Recently, another polyposis causing gene was detected on chromosome 1p33-34, the MUTYH gene. Mutations in this gene have been found to be associated with a recessively inherited form of colonic polyposis. MUTYH mutations appear to cause an attenuated phenotype and have been reported in 10–30% of FAP and AFAP patients without an APC mutation (96-99).
1.4.2. PAPILLARY THYROID CARCINOMA IN FAP.

Papillary thyroid carcinoma is one of the extracolonic manifestations of FAP, and occurs in approximately 2% of patients. Thyroid cancer in a patient with FAP was first reported by Crail in 1964, but the importance of this association was fully appreciated when Camiel et al. reported two sisters with FAP who developed thyroid carcinoma and suggested thyroid carcinoma to be another manifestation of FAP (100,101). In 1987, Plail et al. reviewed 998 patients with FAP and found that thyroid carcinoma occurred at a higher frequency in FAP patients than in the general population (102). 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, with a female to male ratio of 17/1, while in sporadic papillary thyroid carcinomas the ratio is 2.5/1. PTC occurs in FAP patients with a frequency of about 10 times greater than that expected for sporadic PTC. The mean age of diagnosis of thyroid carcinoma has been reported at 28 years. Approximately in one third of the patients FAP is diagnosed before the onset of thyroid carcinoma, in one third FAP and thyroid carcinoma are diagnosed simultaneously, and in one third thyroid carcinoma is diagnosed before FAP is recognized (103).
Thyroid carcinomas associated with FAP are usually bilateral and multifocal (Fig. 7). The histological features of these tumours are represented by the classic, the follicular and by the cribriform-morular variants (Figs. 8, 9, 10). The cribriform-morular variant of papillary thyroid carcinoma (CMV-PTC) described originally as only FAP-associated thyroid carcinoma is a very rare subtype of papillary thyroid carcinoma representing approximately 0.1–0.2% of all papillary carcinoma cases. Among patients with FAP who

Fig. 7 Gross appearance of a multinodular thyroid in an 18-year-old female, associated with FAP syndrome and FAP mutation. The cut surface of the thyroid shows numerous multicentric white firm, well-circumscribed nodules measuring from 0.1 cm to 1.8 cm
have synchronous PTC, between 12 and 90% of these cases have been reported to exhibit histologic features of the cribriform-morular variant (103-105).

Fig. 8 Low power microscopy (×100) view of thyroid tumor associated with FAP syndrome. The cribriform appearance and encapsulation is characteristic of the tumor.

This variant is characterized by lobules of tumor separated by fibrous septa. The tumor lobules have cribriform architecture characterized by rigid spaces in the lobules formed by arches of cells with no fibrovascular cores (Figure 8).
Spindles cells and squamous morules also can be identified. There is a report of a somatic APC gene mutation rather than a germline mutation in one example of this tumor. Although it is rare, the pathologist should be aware of this variant and raise the possibility of underlying APC germline mutations in patients with these variants of PTC.

Fig. 9 Prominent cribriform pattern, with interspersed squamoid islands or morules, with minimal nuclear features of papillary carcinoma (×600)

Fig 10. Beta catenin immunohistochemistry demonstrating strong expression in cytoplasm and nuclei in the morular and cribriform areas in CMvPTC (×400)

The characteristic cellular and nuclear findings of sporadic PTC, such as nuclear grooving, overlapping, intranuclear inclusions, and clear nuclei are rare to absent in this subtype (104,105). The overall prognosis of the CMV-PTC is
similar to that of classical variant of PTC with less than 10% of cases demonstrating an aggressive clinical behavior. The distinct CMV-PTC seen in FAP-related thyroid carcinomas is very unusual in sporadic PTC and its identification should raise the possibility of this familial tumor syndrome. Any patient presenting with this rare subtype of papillary carcinoma should be evaluated for FAP. A recent study (106) showed a 12% prevalence of thyroid cancer in FAP, which is significantly higher than previously reported (2%), and recommended close follow-up ultrasound screening. Thyroid carcinomas associated with FAP show a very good prognosis, with rare metastases or poor outcome reported in most series with a long-term follow-up (103,107). Recent reports suggest that APC germline mutations in patients with FAP associated thyroid carcinoma are significantly more common in the 5’ portion of the APC gene, outside the mutation cluster region (MCR: codon 1250–1464) (108-113). Specifically, a mutation at codon 1061 is the most frequently reported mutation in patients with FAP associated thyroid cancer (103,108,113).

The adenomatous polyposis coli (APC) gene is ubiquitously expressed in normal tissue, and it is a negative regulator of Wnt pathway. Inactivation of the APC tumor suppressor gene initiates colorectal neoplasia and is also involved in FAP-related thyroid tumors. Mutations of the APC gene lead to a truncated protein that lacks the β-catenin binding site and therefore cannot degrade β-catenin. One of the biochemical activities associated with the APC protein is
down-regulation of transcriptional activation mediated by β-catenin. Colorectal tumors with intact APC genes were found to contain activating mutations of β-catenin that altered functionally significant phosphorylation sites. These results indicate that regulation of β-catenin is critical to APC tumor suppressive effect.

Uchino et al. (114) found no mutation of the β-catenin gene on the thyroid (12 CMV-PTC) specimen or in peripheral blood of a FAP patient. Somatic mutation in exon 3 of β-catenin was reported in two patients with FAP-associated thyroid carcinoma and in three cases considered “sporadic” CMV-PTC (Xu et al. (115). Cetta et al. (116) recommended intensive screening for thyroid nodules after the age of 15 years if a single patient or entire kindred have CHRPE and/or mutations in the 5’-portion of exon 15.
2. AIM OF THE STUDY.

The present research is a part of a long term study which have been conducted by our group concerning all the extracolonic manifestation of the FAP syndrome.

In particular the aims of the study will be:

a. A review of the literature about the FAP associated PTC and a report of the cases collected by our group in order to obtain
   i. a better definition of the details concerning the genotype-phenotype correlations.
   ii. a deeper insight into the pathogenesis of FAP associated PTC according to multifactor hypothesis based on gene function and environmental interaction.

b. Evaluate the feasibility of a screening method to detect FAP patients in a particular subset of subjects affected by PTC.
3. MATERIAL AND METHODS.

3.1. A review of the literature have been conducted to recruit the cases of FAP associated PTC from 1999, according to the data base of PubMed library. Then we have included in our data base the 18 patients with FAP-associated PTC selected during an international cooperative study among various European countries including different FAP registers. Only patients available for genetic analysis were included. Eight of these patients were observed in a single institution. In particular, 3 of them, all females, belonged to the same kindred. The extended pedigree of this kindred (23 siblings in 4 generations) has been reported previously together with a detailed list of all extracolonic manifestations (117). All living patients underwent colonoscopy, upper gastrointestinal endoscopy (supplemented by x-ray examination of the gastrointestinal tract in selected cases), and multiple biopsies. In addition, patients were screened for osteomas, dental abnormalities, desmoid tumors and other extracolonic manifestations of FAP. The fundus oculi was examined for congenital hypertrophy of the retinal pigmented epithelium (CHRPE) in 12 patients. All patients underwent ultrasound examination of the thyroid gland. Fine needle aspiration (FNA) of nodules larger than 5 mm was performed. Some patients underwent multiple ultrasound and FNA procedures. Cytological examination of the FNA specimens was performed according to
standard methods. In 8 patients, search for LOH for the APC gene and for activation of the Ret/PTC oncogene in the thyroid tumoral tissue was also performed. In 6 patients, β-catenin activation by immunohistochemical staining was also performed.

3.2. 260 patients, below the age of 30 years, were included in a multi-institutional study developed by a collaboration between the Department of Surgery of Pisa University and the Institute of Surgical Pathology of Siena University. Between 2000 and 2005 all of these patients were submitted to a total thyroidectomy at the same Institution (the Department of Surgery of Pisa University) after a diagnosis of papillary carcinoma was achieved. There were 63 men (24.2%) and 197 women, the mean age was 23 years. Each one of these patients was collected for this study. An envelope was sent to everyone which contained a letter explaining the aim of the study and a questionnaire (Fig 11). It consisted of several questions regarding the presence in the patient and in the kindred of diseases associated to the papillary carcinoma which could be found in the FAP syndrome.
NAME…………………………
LAST NAME…………………………………….
DATE OF BIRTH………………………………………………………….
ADDRESS ……………………………………………
E-MAIL ...................... ZIP CODE....................
PHONE NUMBER………………………………

QUESTIONNAIRE

1) After you underwent your surgical procedure (thyroidectomy) did you suffer of any other disease, in particular:

- Benign tumours of the colon (polyps of the colon)? □ yes □ no
- Malignant neoplasia of the colon? □ yes □ no

2) Did your relatives present or die because of:

- Benign tumours of the colon (polyps of the colon)? □ yes □ no
• Malignant neoplasia of the colon

3) Did you or your relatives present a retina disease called congenital hypertrophy of the pigmented retinal epithelium (CHRPE). (Please specify who was affected).

4) Did any of your relatives have a diagnosis or a treatment for tumours in one of these sites.

<table>
<thead>
<tr>
<th>Site</th>
<th>yes</th>
<th>no</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Breast</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Pancreas</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Liver</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Gallbladder, biliary tract</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Bones</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Brain</td>
<td>yes</td>
<td>no</td>
</tr>
<tr>
<td>Desmoid tumours</td>
<td>yes</td>
<td>no</td>
</tr>
</tbody>
</table>
3.3. Histological techniques

All grossly identifiable nodules as well as normal thyroid areas were extensively sampled. Sections were routinely stained with hematoxylin and eosin. Immunohistochemistry was carried out using the following monoclonal antibodies: thyroglobulin (BioGenex Laboratories, Inc., San Ramon, CA; diluted 1:500), chromogranin A (Dakopatts, Glostrup, Denmark; diluted 1:200), carcinoembryonic antigen (Immunotech, Marseilles, France; diluted 1:10), and cytokeratin AE1/AE3 (Roche Molecular Biochemicals, Mannheim, Germany; diluted 1:1000). Color was developed using the APAAP method. A polyclonal antibody against calcitonin (BioGenex Laboratories, Inc. diluted 1:200) was also used, and the color was developed with 3,3’-diaminobenzidine tetrahydrochloride (118).

3.3.1. DNA extraction.

Extraction of normal and tumor DNA from fresh samples was performed using standard methods. Formalin-fixed, paraffin-embedded sections, 5–10 µm in thickness, were collected on glass slides and stained with hematoxylin. After pathological review, areas of normal tissue and tumor were marked and microdissected; if the areas of interest could not be clearly separated from the surrounding tissue, selective ultraviolet radiation fractionation was performed.
To extract genomic DNA, microdissected samples were incubated in xylene, spun in a microcentrifuge, and washed twice with absolute ethanol. Pellets were resuspended in 100 µl digestion buffer containing 100 µg/ml proteinase K. After overnight digestion at 55 C, the samples were heated at 80 C for 10 min to inactivate proteinase K, rapidly cooled, and stored at 4 C. One microliter of DNA was used to set up 10-µL PCR reactions.

3.3.2. Single strand conformation polymorphism (SSCP) and sequencing. The entire coding region (8532 bp) of the APC gene was analyzed by the PCR-SSCP method in all patients. All the amplified segments were 250–400 nucleotides long. PCR-SSCP analysis was performed as previously described (119-121). To increase PCR specificity, a two-step protocol was used, consisting of a nonradioactive external PCR followed by a radioactive internal PCR that used a 1:10,000 final dilution of the primary PCR as a template. The external PCR was performed in 10 µl of a mixture containing 10 mmol/L Tris (pH 8.3), 1.5 mmol/L MgCl2, 50 mmol/L KCl, 200 µmol/L of each deoxynucleotide triphosphate, 10 pmol/L of each primer, 0.1 µg complementary DNA, and 0.3 U Taq polymerase (Perkin-Elmer Corp./Cetus, Norwalk, CT). Samples were denatured at 94 C for 5 min and processed through 30 temperature cycles, consisting of 90 s at 58 C, 90 s at 72 C, and 1
min at 94 C, followed by 1 cycle at 72 C for 10 min. One microliter of the resulting PCR product was used as DNA template in a 10-µL reaction containing the internal pair of primers. PCR products were denatured, cooled on ice, and electrophoresed overnight through a 6% polyacrylamide gel under 2 conditions: 4 C (25 watts) in a buffer containing 45 mmol/L Tris-borate, 1 mmol/L ethylenediamine tetraacetate, and 24 C (7 watts) in the same buffer plus 5% glycerol. Gels were autoradiographed for 1–2 days without intensifying screens. PCR products corresponding to samples showing unique SSCP conformers were directly sequenced as previously described (119-120). Sequence variants were also confirmed using DNA from independent blood samples.

3.3.3. Search for germline mutations of the APC gene.

To extract genomic DNA (gDNA), 1 mL whole fresh blood from each sample was spun in a microcentrifuge, and peripheral blood lymphocytes were washed twice with phosphate-buffered saline (1x). Then, gDNA was isolated using the QIAmp Blood Kit 50 (QIAGEN, Chatsworth, CA). One microliter of DNA was used to set up 10-µL PCR reactions.
3.3.4. Search for somatic mutations of the APC gene.

Paraffin-embedded sections from thyroid tumor tissue were collected on microscope slides. Areas representative of tumor and normal tissue were identified within single deparaffinized sections lightly counterstained with hematoxylin and microdissected into 1.5-mL polypropylene vials using a hematoxylin/eosin-stained step section from the same block as a guide. The samples were incubated in xylene for 15 min and pelleted at full speed in a microcentrifuge. The xylene was then removed, and the pellet was washed in ethanol. One hundred microliters of digestion buffer containing 1 mol/L Tris-HCl, 0.5 mol/L ethylenediamine tetraacetic acid (EDTA), 0.02% Tween-20, and 100 mg/mL proteinase K were added to each tube. After an incubation of 3 h at 55 C, the samples were pelleted, and the supernatant was stored at -20 C until use (120).

3.3.5. Analysis of LOH.

We used PCR amplification of polymorphism at the following loci to assess LOH on chromosome arm 5q: four dinucleotide repeats (CA) at D5S644 (5q14–15), at D5S82 and D5S299 (5q15–23) proximal to the APC gene, and at D5S346 (30–70 kb downstream from the APC gene) (110). A two-step protocol was used, consisting of a non radioactive external PCR, followed by a
radioactive internal PCR (nested PCR), and using a 1:104 dilution of primary PCR products as template. The radioactively amplified PCR products were then run on a formamide denaturing acrylamide gel electrophoretic system and analyzed by autoradiography. Loss of a chromosomal marker was considered to be present when the PCR assay showed the absence or more than 50% loss of intensity of a heterozygous band from a tumor sample compared with the corresponding nontumor sample.

3.3.6. Search for Ret/PTC activation.

The ret gene, which is also involved in multiple endocrine neoplasm 2A and B, encodes a receptor-type tyrosine kinase for neurotropic molecules belonging to the glial cell-line derived neurotropic factor family. After ribonucleic acid extraction from paraffin-embedded samples, RT-PCR, as described previously (117), was used for subsequent identification of Ret/PTC expression as Ret/PTC1, -2, or -3 (which are the most frequent isoforms of the Ret/PTC). In fact, the ret/PTC oncogene derives from the fusion of the tyrosine kinase domain of the ret protooncogene with the 5’-terminal region of other genes. Ret/PTC1 is fused with another gene, named H4, also located on the long arm of chromosome 10, whereas the 5’-portions of the Ret/PTC2 and Ret/PTC3 are represented, respectively, by the regulatory subunit R1a of the cAMP-
dependent protein kinase A and the RFG/ELE1 gene. The sequences of the forward primers used were: Ret/PTC1, 5’-ATTGTCATCTCGCCGTTTC-3’ (nucleotides 196–214), Ret/PTC2, 5’-TATCGCAGGAGAGACTGT-3’ (nucleotides 483–503), and Ret/PTC3, 5’-AAGCAACCTGCCAGTGG-3’ (nucleotides 697–714). The sequence of the reverse primer, synthesized accordingly to the ret tyrosine kinase sequence, was 5’-TGCTTCAGGACGTGAAC 3’ (16). Further details of this method were reported previously (110,111).

3.3.7. Immunohistochemical analysis for β-catenin accumulation.

The surgical specimen was fixed in neutral, phosphate-buffered, 10% formalin, and paraffin-embedded sections were stained with H&E. Immunostaining for β-catenin was performed using β-catenin-1 antibody (DAKO, Glostrup, Denmark), following the manufacturer’s instructions, dilution 1:300. Results were considered positive when, in addition to membranous pattern staining, strong cytoplasmatic and nuclear staining were also observed (122).
4. RESULTS.

Table 3 shows data from the personal series collected thanks to an European cooperation. Data concerning cases 1 to 15 have previously been published in part (14), whereas cases 16-18 are new cases. All patients were females, (18 of 18, mean age 25.6; range 18-39). CHRPE was present in 12 of 15, in whom CHRPE was available (86.7%); 2 patients (3 and 9) also had Brain tumors (BTs). In one kindred there was also one patient with Hepatoblastoma (HB). None had LOH for the APC gene (n = 9); 6 of 8 had Ret/PTC activation; 4 of 6 had β-catenin nuclear accumulation in the tumoral tissue. Concerning extracolonic manifestations in our personal series (n = 18, belonging to 15 kindred) 12 of 15 (80%) had CHRPE, whereas there was 1 member of the kindred with patients 7, 8 and 9, who had hepatoblastoma and later hepatocarcinoma, at age 2 and 12, respectively, and 2 members who had brain tumors.

There were 9 patients in whom the definitive diagnosis was CMV, (mean age 23.8 yr; range 18-31 yr), 7 patients with conventional PTC (mean age 29.7 yr; range 20-39 yr) and 2 aged 19 and 20, respectively, in whom pathologists recognized in one case the prevalently solid variant, and in the other the so-called encapsulated follicular variant.
Actually, a wide range of different histological variants was observed, in particular in patients with multiple nodules. Tumors were considered conventional PTC when, on a background of follicular and trabecular architecture, papillary architecture was prevalent and nuclear chromatin pattern showed a typical ground glass appearance.

On the contrary, they were considered CMV when, even in the presence of follicular trabecular architecture, with focal papillary formation, they also contained a great proportion of areas with cribriform architecture. In these cases, tumors consisted of empty cribriform spaces adjacent to focal squamous morules, and cribriform spaces were formed by bridges 1-2 cells wide. Nuclei of malignant cells were oval, pale and grooved.

However, even in patients belonging to the same kindred (patients 7-9, with a germline mutation at codon 1061) a wide range of architectures and also of genetic alterations were found. In particular, patient 7 had a conspicuous proportion of papillary foci, with few cribriform morular areas, patient 8 had a prevalence of CMV with a few papillary foci, whereas patient 9 had conventional PTC. Interestingly, whereas the former 2 patients had Ret/PTC activation (Ret/PTC1 isoform) and β-catenin nuclear staining, the latter had neither detectable Ret/PTC activation, nor β-catenin nuclear staining.

In addition to the striking female prevalence (18:0), in the present series the prevalence of CMV was also associated with younger age. In particular, if we
set an arbitrary cut-off point at age 30, there were 8 patients with CMV aged 18 to 30 and 1 aged 31 or more, whereas there were 2 patients with conventional PTC aged 30 or less than 30, and 5 aged 31-39 ($\chi^2 = 6.11 \ p<0.05$) (Table 3).

Table 5 shows PTC in FAP patients with known germline APC mutations reported in the literature after year 2000. There were 45 cases, all females but one. They were pooled up to FAP associated PTCs previously published by our team in year 2000.

In this series, even if data concerning the specific PTC variant were not always reported in detail in every patient, among those with sufficient indications, there were 31 additional cases. Concerning age, there were 17 patients with CMV aged 30 or less and 4 with older age, whereas patients with conventional PTC were 7 younger and 7 older than age 30. Pooling all data together, there were 25 and 5 patients with CMV and 9 and 12 patients with conventional PTC, aged less or more than 30 respectively.

Table 4 reports PTC associated with typical FAP in patients without detection of the APC germline mutation. These patients were added to the 97 previously reported without APC mutations for a total number of 182 (171 F:11M; F:M ratio = 16:1). They include either patients with detected APC mutation or patients with clinical diagnosis of FAP.

However, it must be outlined that a great proportion of these patients are historical case reports (often single case reported), in the absence of APC
mutation detection. In particular, there were 24 out of 112 patients (23%) (101F:11M) with APC germline mutations in the former series, (up to 1999) and 45 of 65 with APC gene mutations (68%) in the present series.

Concerning the sites of germline APC mutations, they are listed in Table 3 (personal series n = 18-2=16), Table 5 (series from the literature after year 1999, n = 45) and in Cetta et al. Table 5 (n = 7).

Pooling together all of them, a total of 68 patients with FAP associated PTC and APC mutations have been accrued. Interestingly, in most of cases they tend to cluster outside the MCR (mutation cluster region = codons 1286-1513), where most of sporadic APC mutations are found.

In particular, setting an arbitrary cut-off point at codon 1220, there were 57 patients with germ line mutations 5’ to codon 1220 and 10 3’ to this codon, and comparing with a reference population of FAP patients without PTC, there was a statistically significant difference ( $\chi^2 = 11.77$ p< 0.001). However, since there were 21 patients who belonged kindreds with 2 siblings (n = 6) or 3 siblings (n = 3) with FAP associated PTC, comparison was made not only among individuals (57 to 10), but also among kindreds (48 to 9). Difference was also statistically significant ($\chi^2 = 9.39$, p<0.002).
B)
Of 260, 95 envelopes came back. In our series we found that none of the patients was affected by any of the diseases reported in the questions. Only in three cases (3%) relatives were affected by intestinal polyps, in six cases (6.3%) relatives were diagnosed of a colon cancer, and in one case a relative died because of that tumour. We had 4 diagnosis of CHPRE (4.2%) in relatives of our patients. We also had 2 (2.1%) gastric cancers, 5 breast cancers (5.2%), one pancreatic cancer (1%) and 2 (2.1%) liver cancers in the kindred. We carefully considered the cases of CHPRE positive patients’ relatives. The specimens of the histological analyses were reviewed by a pathologist of the Pathology Department of Pisa University looking for the cribriform and morular hystotype. Patients with relatives affected by CHPRE were proposed in continuing the study by a genealogical tree and a genetic analyses.
Table 3. PTC in FAP patients with APC germline mutations (n = 18, personal series).

<table>
<thead>
<tr>
<th>No.</th>
<th>Sex</th>
<th>Age</th>
<th>Histologic variant</th>
<th>No. codon</th>
<th>No. exon</th>
<th>CHRP E</th>
<th>BT</th>
<th>LOH for APC gene</th>
<th>ret/PTC activation</th>
<th>β-catenin accumulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>30</td>
<td>PTC</td>
<td>140</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>n.p.</td>
<td>n.p.</td>
<td>n.r.</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>19</td>
<td>Prevalently solid</td>
<td>593</td>
<td>14</td>
<td>+</td>
<td>-</td>
<td>n.p.</td>
<td>n.p.</td>
<td>n.r.</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>22</td>
<td>PTC</td>
<td>778</td>
<td>15</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>n.r.</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>18</td>
<td>CMV</td>
<td>976</td>
<td>15</td>
<td>+</td>
<td>-</td>
<td>n.p.</td>
<td>n.p.</td>
<td>n.r.</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>27</td>
<td>CMV</td>
<td>993</td>
<td>15</td>
<td>+</td>
<td>-</td>
<td>n.p.</td>
<td>n.p.</td>
<td>n.r.</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>26</td>
<td>CMV</td>
<td>1061</td>
<td>15</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7*</td>
<td>F</td>
<td>22</td>
<td>CMV ¹</td>
<td>1061</td>
<td>15</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8*</td>
<td>F</td>
<td>36</td>
<td>CMV ²</td>
<td>1061</td>
<td>15</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9*</td>
<td>F</td>
<td>24</td>
<td>PTC</td>
<td>1061</td>
<td>15</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>n.r.</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>20</td>
<td>Encapsulated follicular variant</td>
<td>1309</td>
<td>15</td>
<td>+</td>
<td>-</td>
<td>n.p.</td>
<td>n.p.</td>
<td>n.r.</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>27</td>
<td>PTC</td>
<td>1309</td>
<td>15</td>
<td>n.a.</td>
<td>-</td>
<td>n.p.</td>
<td>n.p.</td>
<td>n.r.</td>
</tr>
<tr>
<td>13</td>
<td>F</td>
<td>22</td>
<td>CMV-follicular</td>
<td>n.a.</td>
<td>15</td>
<td>n.a.</td>
<td>-</td>
<td>n.p.</td>
<td>n.p.</td>
<td>n.r.</td>
</tr>
<tr>
<td>14</td>
<td>F</td>
<td>20</td>
<td>PTC</td>
<td>n.a.</td>
<td>n.a.</td>
<td>n.a.</td>
<td>-</td>
<td>n.p.</td>
<td>n.p.</td>
<td>n.r.</td>
</tr>
<tr>
<td>15</td>
<td>F</td>
<td>31</td>
<td>CMV</td>
<td>937</td>
<td>15</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>n.r.</td>
</tr>
<tr>
<td>16°</td>
<td>F</td>
<td>25</td>
<td>CMV</td>
<td>1068</td>
<td>15</td>
<td>+</td>
<td>-</td>
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<td>+</td>
<td>+</td>
</tr>
<tr>
<td>17°</td>
<td>F</td>
<td>34</td>
<td>PTC</td>
<td>1105</td>
<td>15</td>
<td>-</td>
<td>-</td>
<td>n.p.</td>
<td>n.p.</td>
<td>-</td>
</tr>
</tbody>
</table>

FAP = Familial Adenomatous Polyposis; APC = Adenomatous Polyposis Coli; PTC = Papillary Thyroid Carcinoma; CMV = Cribriform Morular Variant; CHRPE = Congenital Hypertrophy of the Retinal Pigment Epithelium; BT = Brain tumor.

n.p. = not performed
n.a. = not available

* - Hepatoblastoma and hepatocellular carcinoma in a member of this kindred.
No. 1-15 = patients already reported in Cetta et al. (2000)
° - No. 16-18 = new cases
¹ - PTC with few CMV areas.
² - CMV with some solid areas.
Table 4. Papillary Thyroid Carcinoma (PTC) in FAP patients with unknown APC germline mutations from the literature after year 2000 (n = 22).

<table>
<thead>
<tr>
<th>No.</th>
<th>Authors, Year</th>
<th>Pts. no.</th>
<th>Sex</th>
<th>Age (PTC)</th>
<th>TC Histology</th>
<th>Age (FAP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Xu, 2003</td>
<td>1. F</td>
<td>34</td>
<td>CMV-PTC</td>
<td></td>
<td>n.r.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. F</td>
<td>23</td>
<td>CMV-PTC</td>
<td></td>
<td>n.r.</td>
</tr>
<tr>
<td>2.</td>
<td>Truta, 2003</td>
<td>3. F</td>
<td>27</td>
<td>PTC</td>
<td></td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4. F</td>
<td>34</td>
<td>PTC</td>
<td></td>
<td>34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5. F</td>
<td>35</td>
<td>PTC</td>
<td></td>
<td>35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7. F</td>
<td>16</td>
<td>CMV-PTC</td>
<td></td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8. F(s)</td>
<td>19</td>
<td>CMV-PTC</td>
<td></td>
<td>19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9. F(s)</td>
<td>17</td>
<td>CMV-PTC</td>
<td></td>
<td>17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11. F</td>
<td>32</td>
<td>PTC</td>
<td></td>
<td>32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14. F</td>
<td>34</td>
<td>CMV-PTC</td>
<td></td>
<td>34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16. F</td>
<td>30</td>
<td>CMV-PTC</td>
<td></td>
<td>n.r.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18. F</td>
<td>34</td>
<td>CMV-PTC</td>
<td></td>
<td>20</td>
</tr>
</tbody>
</table>

FAP = Familial Adenomatous Polyposis; APC = Adenomatous Polyposis Coli; PTC = Papillary Thyroid Carcinoma (conventional); CMV = Cribriform Morular Variant of PTC; TC = Thyroid Carcinoma.

Pts. = patients.

(s) = siblings

n.r. = not reported
Table 5. PTC in FAP patients with known APC germline mutations from the literature after year 2000 (n = 45). CHRPE = Congenital Hypertrophy of the Retinal Pigment Epithelium;

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Pts. no.</th>
<th>Sex</th>
<th>Age (FAP)</th>
<th>Histotype</th>
<th>Age (PTC)</th>
<th>APC mutations</th>
<th>ECM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miyaki, 2000</td>
<td>1</td>
<td>F</td>
<td>n.r.</td>
<td>PTC</td>
<td>26</td>
<td>175</td>
<td>n.r.</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>F</td>
<td>n.r.</td>
<td>PTC</td>
<td>21</td>
<td>1110</td>
<td>n.r.</td>
</tr>
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<td>Chong, 2000</td>
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BT = Brain tumor.; ECM = Extracolonic Manifestation; MB = Medulloblastoma; PB = Pinealoblastoma; UGIP = Uper Gastrointestinal polyps; MEN 1 = Multiple Endocrine Neoplasia 1; S&EC = Sebaceous and Epidermoid Cysts; DT = Desmoid Tumor.
5. DISCUSSION

Papillary thyroid carcinoma is one of the extracolonic manifestations of FAP, and occurs in approximately 2-12% of patients (123,124). PTC occurs in FAP patients with a incidence much higher than that expected for sporadic PTC, which is between 0.5 to 10 cases per 100000 in the general population (2). The mean age of diagnosis of FAP associated thyroid carcinoma has been reported at 28 years. 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, with a female to male ratio of 17/1, while in sporadic papillary thyroid carcinomas the ratio is 2.5/1 (125-128). All these epidemiological data show that PTC development in FAP population is not casual but that it is the result of the cooperation of coexisting determining agents. A total of 182 patients was collected as it is represented by Table 3, 4 and 5, they resulted: 1) from a personal series of 18 patients (Table 3: n = 15 plus 3 new cases); 2) from the literature review until year 1999 (116); 3) from the literature review after year 2000 (Table 4-5) (107,114,114,122,124,128-147).
5.1. GENOTYPE PHENOTYPE CORRELATION.

The review of the literature and the analysis of our patients have confirmed our preliminary findings that there is an evident genotype-phenotype correlation for FAP associated PTCs. In 87% of our patients (14/16, Table 3) and in 66% of the patients reviewed by the literature, in whom the genetic analysis was performed (28/45, Table 4), PTC were associated with APC germ-line mutations 5' to codon 1220, i.e. outside the mutation cluster region (MCR). Moreover our data show a frequent co-segregation of the PTC with CHRPE in FAP patients. In our personal series 80% of the patients, in whom this phenotype was evaluated (12/15 Table 3), showed an association with PTC. This data have been confirmed by the genetic analysis, that point out a very similar clustering between the germline’s APC mutations in the CHRPE and in the PTC affected patients. In fact CHRPE patients have been proved to be related with APC germline mutations localized between codons 311-1444, outside the MCR (148,149).
5.2. GENE FUNCTION AND ENVIRONMENTAL INTERACTION.

The germline mutation of the APC gene is crucial for the development of the colon cancer in the FAP syndrome. In FAP affected patients the colonic polyps, if not surgically treated, invariably transform into colorectal cancer before the age of 40 years. As previously reported the APC gene is a tumour suppressor gene which is involved in several cellular processes, including transcription, cell cycle control, migration, differentiation and apoptosis (84,85). Mutations, leading to colorectal cancer, follow the classical two-hit model of tumour suppressor inactivation. FAP patients inherit one germline mutation and develop tumours from those cells in which a second hit, or loss of the other allele of APC, is somatically acquired. This is a pathogenetic model that explains the onset of colonic cancer but it is not exhaustive for the description of the development of FAP associated extracolonic neoplasia in particular the PTC. In FAP associated PTC, as in all tumors belonging to a multitumoral syndrome, the inherited germline mutation of the tumor suppressor gene (the APC germline mutation) confers an increased susceptibility to tumor development, but the germline mutation is insufficient by itself for the occurrence and clinical manifestation of the PTC. In our data we have proved the absence of LOH for APC in the thyroid tumoral tissue (8 out of 8 cases), (Table 3). This means that other factors affect dramatically the occurrence of
FAP associated PTC, suggesting that, in individuals with the same germline mutations, different mechanisms determine the occurrence of extra-colonic tumors, with infrequent or absent LOH of the APC gene (110).

In particular, analysis of sporadic APC mutations in the thyroid tumoral tissue has been performed in 3 different series (150-152). There was only one patient with somatic mutation of the APC gene. These data suggest that the APC gene is usually not involved in the development of most of sporadic thyroid tumors (150-152). On the other hand, the frequent occurrence of PTC in siblings with APC germline mutations suggests a causative or facilitating role of this genetic alteration. In fact, we have collected, only among subjects with APC germline mutations, 3 kindreds, with 3 siblings showing FAP associated PTC including our own, and 6 kindreds with at least a couple of siblings. But, adding historical cases, (100-101, 125) the number of PTC in siblings belonged to the same FAP kindreds is even greater.

The investigation of the model of carcinogenesis for the sporadic PTC may contribute to better define which are the genetic pathways that cooperate with the germline APC mutation, in the occurrence of FAP associated PTC. As previously reported RET/PTC oncogenes are among the most frequent genetic alteration in the sporadic PTC. The products of these rearranged genes are constitutively active oncoproteins, that have lost the transmembrane domain (153-155). Experimental evidence supports RET/PTC rearrangements as
causative factors in the pathogenesis of sporadic PTC. Expression of RET/PTC in human thyrocytes has been shown to stimulate their proliferation (45) and to induce typical changes in nuclear envelope and chromatin, which are diagnostic for PTC (156). The ability of RET/PTC to initiate carcinogenesis has been confirmed in transgenic mice (157). Recent studies have investigated the contribution of RET/PTC1 and β-catenin mislocation, in the pathogenesis of sporadic PTC. This study has been accomplished using in vitro model of human thyroid carcinogenesis and the human PTC cell line, TPC-1, endogenously expressing the RET/PTC1 oncogene. It has been demonstrated that RET/PTC1 effects include β-catenin nuclear translocation, associated with an increased expression of its transcriptional target gene cyclin D1. This effect have suggested a β-catenin functional switch from adhesive to transcriptional activity (158). Other AA have identified a novel RET- β-catenin signalling pathway that, even if demonstrated for medullary thyroid carcinoma, may be applied also for our research. This pathway is a critical contributor to enhanced cell proliferation and tumor progression in thyroid cancer. This study have shown that RET induces β-catenin-mediated transcription, cell proliferation and transformation in vitro and that β-catenin nuclear localization and the resultant RET-mediated β-catenin signalling is a key secondary event in tumor growth and spreading in vivo (159).
These data seems to be confirmed by our study. In fact, in addition to APC germline mutation, in the same thyroid tumors, a very high rate of activation of the Ret/PTC gene was found (Table 3). Furthermore, in 4 of 6 patients, who had sufficient tumoral tissue, there was a conspicuous nuclear staining of the cell nucleus for β-catenin, with a moderate staining in the remaining 2 cases (Table 3).

It has been suggested that β-catenin nuclear staining and CD+ in morules in tumors with the CMV of PTC is a quite specific marker of tumors showing disruption of the Wnt-pathway (122). The hypothesis that can be drawn by these data is that, in the absence of LOH for the APC gene, the second hit in the multi-step tumorigenesis process, can be represented by the RET/PTC activation. β-catenins could represent the common substrate of two different signalling pathway: the Wnt and the MAPK with the result of β-catenins nuclear translocation and promotion of thyroid tumorigenesis.

Analyzing our data it is possible to observe the distribution of FAP associated PTCs according to the sex of the patients. There were 170 females and 12 males; with a F:M ratio of 16:1 in the overall population. Considering that the F:M ratio in sporadic PTC is usually 3:1, this female preponderance is noteworthy. The temporal series also seems to be of importance. However, a great proportion of these patients were historical case reports (often single case reports) with thyroid carcinoma in kindreds with clinical diagnosis of FAP, but
with no detected APC germline mutation. In particular, due to the increased diffusion of genetic analysis during the last decade, there were only 24 of 112 patients (23%, 101 F and 11 M) with APC germline mutation in the former series (up to 1999) versus 43 of 65, (68%) in the latter (after year 2000). Interestingly, whereas the F:M ratio was 9:1, i.e. 101 F to 11 M, in the former series (and we reported a F:M ratio of 17:1, because we restricted the analysis only to those kindreds with at least a couple of siblings in the same family, i.e kindreds in which FAP associated PTC could not be considered a casual finding), the F:M ratio in the latter series was 60:1. In particular, the only male was an atypical FAP patient with aggressive CMV, responsible for lung metastases at age 53, in the absence of colonic polyps (Germ-line mutation intron 1-3). The causes of this striking prevalence of PTC in FAP females deserves further evaluation.

Recent reports (159-161) have demonstrated that the gender difference in the development of sporadic thyroid cancer may be influenced by sex hormones, particularly by the estrogen 17β-estradiol (E2), which has been shown to interact and promotes the growth and progression of thyroid tumors. The molecular mechanisms by which estrogens affects the cell cycle regulatory apparatus to induce cellular proliferation have been elucidated (161). 17β-estradiol stimulates cell cycle progression early in G1 phase by induction of cyclin D1 gene expression (which is the same target gene of the β catenins)
The possible mechanism by which E2 promotes thyroid cells carcinogenesis is mainly due to the interaction with the estrogen receptor α (ERα), that results in the activation of the MAPK pathway, at the level of ERK1/2. This activation promotes expression of a family of antiapoptotic proteins Bcl-2 providing a survival signal for the thyroid tumoral cells (161). The mechanism by which E2 contributes to proliferation and population growth of thyroid cancer cells might explain, at least in part, the higher incidence of FAP associated PTCs in females.

It might be proposed a model of cooperation in female patients among the role of E2 and ERα in the activation of the ERK1/2, MAPK cascade, the same activation of this cascade by RET/PTC chimeric gene ligand independent activity and the interruption of the Wnt pathway promoted by the germline mutation of the APC gene.

An important environmental interaction in the pathogenesis of FAP associated PTC have been proposed during the last years. A yet undetermined role could be played by nuclear disasters, which could facilitate PTC occurrence in predisposed patients with APC germline mutation (112).

In particular, after a boom of reports during the period between 1995 and 2001, it seems that reports of this manifestation are decreasing in the last few years. One could “hypothesize” a “Chernobyl effect”, at least in reports from Europe, i.e., an increasing occurrence 8–15 years after the Chernobyl disaster,
suggesting that, in a subset of frail predisposed subjects, a long-term, long-distance effect of nuclear disasters should be envisaged. In particular, radiation could determine an increased incidence of thyroid carcinoma, namely in children, not only within a 250–300 km distance, but also at greater distance, in subjects with inherited predisposition (113,163).

5.2 FEASIBILITY OF A SCREENING METHOD TO DETECT FAP PATIENTS IN A PARTICULAR SUBSET OF SUBJECTS AFFECTED BY PTC.

The real incidence of FAP associated PTC has not been definitively evaluated, but it has been estimated approximately between 2-12% (123-124). According to these data a screening method for an early detection of the papillary thyroid carcinoma in all FAP patients has not been considered worthwhile. The studies of the genotype-phenotype correlation which have proved a frequent occurrence of the APC germline mutations 5’ to the codon 1220 and the association of the PTC with the CHRPE has offered some criteria that has permitted to restrict the range of patients at risk. In this subset of patients, a
screening method by intensive ultrasonography studies could be considered cost-effective.

The attempt we have done in this study, has been to consider this issue from the opposite point of view, namely to evaluate the feasibility of a screening method in the population of patients affected by thyroid carcinoma in order to early detect the FAP syndrome. The mean age at diagnosis of PTC and/or thyroidectomy was 25.7 year in our series, 28.1 year in patients from the literature, and 25.9 years in the entire series of 182 patients. In our personal series the histological type was always PTC and its variants were: CMV (cribriform morular variant) in 9 patients (mean age 23.8 yr; range 18-31 yr), conventional PTC in 7 patients (mean age 29.7 yr; range 20-39 yr) and 2, aged 19 and 20, respectively, with the so called encapsulated follicular variant in 1 case and the trabecular variant in another. Actually, a wide range of different histological variants was observed: classic encapsulated, trabecular, even in patients belonging to the same kindred with the same germline APC mutations (patients 7, 8, 9) (Table 3). The prevalence of CMV in subjects studied after 2000 was of 38/55 (69%). According to these data we considered as restriction criteria the patients’ age below 30 years and the papillary hystotype of thyroid cancer. We selected 260 patients, of whom 95 answered to our tests. None of these patients was affected by any of the diseases reported in the questions. In 3 cases patients’ relatives were affected by sporadic colonic polyps and in 6
cases we found, again in patients’ relatives, sporadic colon cancers. In 4 cases there was a diagnosis of CHRPE in the clinical history of 2 grandfathers and 2 parents of 4 different subjects of our study. We carefully considered the cases of CHPRE positive patients’ relatives. The specimens of the histological analyses were reviewed by a pathologist of the Pathology Department of Pisa University looking for the cribriform and morular hystotype, which has been shown to be strictly related to thyroid carcinomas associated to FAP syndrome, but the result was negative. Patients with relatives affected by CHPRE were however proposed in continuing the study by a genealogical tree and a genetic analyses and in all the cases we were not able to obtain a consent. These patients were followed-up for 3 years and there was no evidence of the occurrence of colonic polyps.

The number of patients obtained with our restriction criteria was really high if considered that we did not include the papillary thyroid carcinomas of patients aged more than 30 years. We have calculated that the ratio between our group and the one of all papillary thyroid carcinoma is 1/3. To obtain these numbers it has been necessary the collaboration with a big Italian referral centre for the thyroid surgery. Despite this we have not been able to find any FAP associated PTC, so even under these conditions it appears not cost effective to propose a screening method for the detection of FAP in a restricted cohort of PTC patients. On the contrary the diagnosis of the CMV variant of papillary thyroid
carcinoma in the specimen of patients with multifocal and multinodular thyroid disease should suggest to the pathologist a strict collaboration with the clinician in order to initiate in these subjects and in the family members a screening for detection of the colonic and extracolonic manifestations of the FAP.
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In a recent paper by Uchino et al.,1 reported on a patient with the cribriform-monolobular variant of papillary thyroid carcinoma (CMVPTC), associated with familiar adenomatous polyposis (FAP). They analyzed both exon 1–13 of the APC gene and exon 3 of the β-catenin gene in the peripheral blood and in 12 CMVPTC nodules from a 25-year-old woman with FAP and previous total colectomy. In particular, they found a germ-line mutation of the APC gene at codon 544 and six somatic mutations between codons 508 and 595. There were no mutations of the β-catenin gene either in peripheral blood leukocytes or in the 12 CMVPTC specimens. In particular, loss of heterozygosity was not observed in the tumor tissues without somatic APC mutations.1

We would like to comment on this paper, supporting some conclusions, while criticizing others.

The interesting data which are further documented by this study are following:

1. APC somatic mutations were detected in 6 out of 12 tumor nodules from the same patient. The germ-line APC mutation was at codon 544, whereas all 6 somatic APC mutations were 5’ to codon 545, i.e., out of the mutation cluster region (codons 1286–1513), where most somatic APC mutations occur in sporadic colorectal carcinoma and in colonic polype.

2. No β-catenin mutation, either germ-line or somatic, was observed in this patient, whereas the same Authors reported a somatic mutation in exon 3 of β-catenin in 7 CMVPTC. Five of these 7 had no APC mutations.2,3

The former data give further evidence to the fact that germ-line and somatic APC mutations in patients with FAP and associated PTCs occur in the 5’ position of the gene, usually associated with CHPRE (Congenital hypertrophy of the retinal pigment epithelium). This was first documented in our series of 15 patients with FAP associated PTC, and subsequently confirmed by others.4,6

The latter suggest that APC mutations and β-catenin mutations are mutually exclusive of each other in disrupting the Wnt signal transduction in thyroid carcinoma as well as in hepatoblastoma. β-catenin mutations usually occur in the absence of APC mutations and viceversa.6

In addition, we would make some critical remarks: 1) somatic mutations of the APC gene are very rare in FAP associated thyroid carcinoma, and in sporadic thyroid carcinoma. They were almost never found in multiple consecutive series of patients with sporadic PTC; 2) LOH of the APC gene is extremely rare even in FAP associated thyroid carcinoma. We found no LOH for APC in 6 of 6 patients with FAP associated PTC.7

Other criticisms are more specific. They include: 1) an incorrect quotation of our papers. In fact, in a series of 9 patients, only 4 (not 12) PTCs had at least some cribriform-monolobular aspects.6,7 The remaining were typical papillary carcinomas occurring in patients with typical FAP. Twenty-two of 24 germ-line mutations occurred in the genomic area between codons 140 and 5219 (not codons 463 and 387 as reported in the paper by Uchino

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APPENDIX
The attempt at drawing general conclusions on the basis of data from a single patient. In our series the most frequent germ line mutation (in 5 out of 16) was a 5p deletion at codon 1061 whereas Ubino et al. in their conclusion restrict their ‘critical region’ between codons 338 and 935.

In conclusion, cumulative data, associated with 1) the low incidence of PTC in FAP patients (< 2%), 2) the almost exclusive occurrence in females (P:S ratio > 17:1), (3) a very high rate of RET/PTC activation (50%), similar to that observed in post Chernobyl children, suggest that mechanisms other than blastic loss of function of the APC gene, which is found only in a minority of patients, are responsible for the occurrence of most FAP associated PTCs. Interestingly, in 6 of the 12 tumoral nodules reported by Ubino, CMV-PTC developed in the absence of LOH of APC or of β-catenin mutations. Therefore, we suggest that in most of cases APC germ line mutations only give a genetic susceptibility to thyroid cancer development, whereas other concomitant factors (sex-related factors, modifier genes, environmental factors, namely exposure to radiation or even long distance nuclear disasters) all play a role in the actual occurrence of PTC in FAP patients. However, there are 2 recent biological data which could be relevant from a clinical point of view: 1) most of APC germ line mutations (90%) are located 5’ to codon 1287, in the same genomic area associated with an easily detectable extracellular feature of FAP, namely CHRIPE; 2) at least one third of FAP associated PTCs (the remaining are typical papillary tumours associated with typical FAP) show CMV-PTC, an otherwise extremely rare histologic variant (0.16 % of sporadic tumours).

Therefore, even if intensive screening for PTC has not been suggested in all FAP patients or relatives, due to the very low incidence of PTC, it could be recommended in those kindreds that show CHRIPE in one affected member or have a germ line APC mutation 5’ to 1287. Conversely, screening for undiagnosed FAP is recommended in thyroidectomized patients with CMV/PTC.

Anyway, in a complex and certainly multifactorial matter such as FAP associated PTC which is far from being completely elucidated, only suggestions deriving from cumulative data (instead of single cases) could be of importance for both clinical and pathogenetic purposes.

REFERENCES


