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RAPID COMMUNICATION

Gene variants associated to malignant thyroid disease in familial adenomatous polyposis: A novel APC germline mutation

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ABSTRACT. ***Background and aim:*** **Familial adenomatous polyposis (FAP) is an autosomal inherited syndrome characterized by hundreds to thousands colorectal adenomatous polyps with oncological transformation lifetime risk of 100%. FAP is mainly associated with mutations in APC (autosomal dominant inheritance) or MUTYH (autosomal recessive inheritance) genes. Affected individuals are at increased risk of developing extra-intestinal tumors. Lifetime risk of developing thyroid carcinoma has been described in previous reports of about 2-12%, mainly in females, and the mean age is below 30 yr. About 95% of cancers are papillary thyroid carcinomas (PTC), mostly multifocal. The aim of this study was to evaluate the frequency of PTC among our series of FAP patients and to assess the type of gene mutation associated with the disease.** ***Methods:*** **Fifty-four subjects from 36 FAP families were selected (29 females/25 males) and the mean age (±SD) at diagnosis was 28.8±10.8 yr. All patients underwent blood examination for thyroid hormones and antibodies, germline mutational analysis of APC and/or MUTYH genes, thyroid ultrasound, and endocrinological evaluation.** ***Results:*** **In 13/54 (24.1%) subjects, an eumetabolic thyroid disease was found: plurinodular disease in 7/54 (13.0%); single nodule in 4/54 (7.4%); in 2/54 patients (3.7%), we found a malignant nodule characterized after total thyroidectomy as a classical PTC. Both patients were female and showed a classic FAP phenotype. Mutational analysis revealed in the first patient the APC germline mutation 3183_87del ACAAA and in the second patient the del9-10 (del9080dup11) novel APC variant; the first mutation has been already reported in association with PTC; to our knowledge the second mutation has never been previously reported in association with FAP.** ***Conclusions:*** **In the population examined, the estimated prevalence of thyroid malignant diseases was 3.7%. In both patients, the identified APC gene pathogenetic variants mapped within the 5' region of the gene, previously reported as a PTC-associated mutational hot spot. Both patients had classic FAP phenotype and genetic analysis revealed two pathogenetic APC mutations: c.3183_87delACAAA, a recurrent pathogenetic variant and del9-10 (del9080dup11), a novel, not previously described genomic rearrangement. In agreement with previous studies, the morpho-functional surveillance of thyroid in FAP series should be recommended. A better insight into the overall genotype-phenotype correlation of APC gene mutations would be helpful for the identification of at-risk individuals.**
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INTRODUCTION

Familial adenomatous polyposis (FAP) is an autosomal dominant inherited syndrome associated to APC (adenomatous polyposis coli) gene germline mutations. It is characterized by hundreds to thousands colorectal adenomatous polyps with cancerization lifetime risk of 100%. FAP can be associated to a wide variety of extracolonic manifestations including gastrointestinal adenomas, cutaneous cystis, osteomas, CHRPE (congenital hypertrophy of retinal pigmented epithelium), desmoid tumors and thyroid carcinomas (1, 2). A milder form displaying an attenuated phenotype (AFAP) is characterized by <100 polyps, with delayed onset and usually diagnosis occurs when there are already one or more colorectal adenocarcinomas (3, 4). Extracolonic tumors are not frequently observed in patients with AFAP and their incidence is not yet well established.

APC (MIM#175100) is a tumor suppressor gene located at chromosome 5q21-22. More than 1060 germline mutations have been described to date in the APC mutation database [according to HGMD (Human Gene Mutation Database)]. More than 60% of APC mutations are found in the central region (between codons 1284 and 1580) of the protein, which is called the mutation cluster region (MCR) (5). In addition, there are several recurrently described mutations, of which two, at codon positions 1309 (c.3927_3931delAAAGA) and 1061 (c.3183_87delACAAA), account for as much as 30% of the germline APC mutations. The vast majority of pathogenetic variants found in the APC gene represents truncating mutations (46% small deletions; 10% small insertions; 28% nonsense mutations). However recent reports have indicated that intragenic deletions and genomic rearrangements also account for a further up to 20% of pathogenetic mutations. In the last decade, biallelic mutations in the MUTYH gene (human homolog of the E. coli mutY) (MIM#604933), one of the key components of the base excision repair (BER) pathway, have been associated to an autosomal recessive form of polyposis called MAP

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(MUTYH-associated polyposis), usually characterized by an attenuated feature (6). MUTYH biallelic (homozygous and compound heterozygous) germline mutations have been associated to about 7-29% of APC negative adenomatous polyposis (4).
The association of FAP with PTC was first reported by Crail in 1949 (7); thereafter, multiple other studies on this association were reported (8-10). FAP-associated PTC exhibit a marked female preponderance (female to male ratio of 10:1) and are more common under the age of 30 yr. The cribriform-morular variant of PTC is reported more often in patients with FAP than in the general population, therefore its finding should lead to screening for FAP (11, 12). APC germline mutations associated with PTC have been reported overall the gene, even though some authors report more often a mutation in exon 15 and in the 5' portion of the APC gene outside the Mutational Cluster Region (MCR) (codons 1286-1513) (2, 13-15). In MAP patients PTC has been reported in very few cases (1) and to date no genotype-phenotype correlation data are available.
We report of a novel pathogenetic variant in a patient affected with classical FAP associated to papillary thyroid carcinoma.

PATIENTS, MATERIALS, AND METHODS

We performed a retrospective review of 54 patients with a proven diagnosis of FAP, with classic or attenuated phenotype in follow-up at Hereditary Colorectal Cancer Outpatients of Regina Elena National Cancer Institute since 1985. FAP diagnosis, treatment, and endoscopic follow-up were defined by International Guidelines Criteria based on endoscopic feature and/or genetic analysis (APC and/or MUTYH) results (16).

Morpho-functional thyroid evaluation

All patients underwent yearly, or earlier if necessary, blood examination for thyroid hormones (TSH, free T_4, free T_3), calcitonin, and anti-thyroid antibodies [thyroglobulin (TgAb), thyroperoxidase (TPOAb)] using the common commercial kits, thyroid ultrasound and endocrinologic specialistic evaluation. Patients with suspicious nodules found at ultrasonographic evaluation underwent fine needle aspiration biopsy (FNAB); in particular FNAB is recommended for nodules ≥10 mm and is suggested for nodules <10 mm only if clinical information or ultrasound features are suspicious for malignancy. Thyroid FNAB is reliable and safe, and smears should be interpreted by an experienced pathologist (17).

DNA and RNA extraction

Genomic DNA was prepared from the patients' fresh or frozen lymphocytes obtained from 10 ml EDTA blood using the protocol described by the QiaAmp DNA blood mini kit (Qiagen; Valencia, CA, USA). Total RNA was extracted using the Blood RNA kit (AB Analitica; Padova, Italy). RT-PCR was performed using SuperScript One-Step RT-PCR with Platinum Taq kit (Invitrogen, Cat. No.: 10928-042) with 1 µg of RNA as template. Long range PCR was performed using GeneAmp®XL PCR kit (Applied Biosystems).

Mutation analysis

Mutation analysis was performed through all the coding regions and the exon/intron boundaries of the APC and MUTYH genes using PCR - Single Strand Conformation Polymorphism (SSCP) and direct sequencing. Samples were run and analyzed on an ABI PRISM 3130 genetic analyzer. The primers used for PCR-SSCP and for direct sequencing were as described previously by Groden et al. (18) and by Miyoshi et al. (19) for APC; supplementary information by Al-Tassan et al., for MUTYH (20). Analysis for large deletions was performed using the multiplex ligation-dependent probe amplification (MLPA) assay (21) in cases where the above-written screening methods did not identify any mutation. To describe MUTYH mutations we used the most up-to-date annotation, see the LOVD (Leiden Open Variation Database) (22). Nucleotide numbering is based on GenBank sequence NM_000038 (APC gene) and NM_012222.2 (MUTYH gene) with +1 as the A of the initial Met codon.

Statistical analysis

Descriptive statistics were computed for all variables of interest. Continuous data were reported as the mean±SD or median and categorical data were represented with frequencies and percentage values.

RESULTS

Fifty-four subjects from 36 families with clinical and/or genetic criteria for FAP were enrolled in the study, 29 females and 25 males with a median age of 26.5 (14-53) yr. A classic phenotype was observed in 49 individuals and an attenuated phenotype in 5. All patients have been previously treated with a total colectomy or proctocolectomy. In 34 out of 49 patients with the classic phenotype a pathogenetic mutation in the APC gene was detected and in 2 patients biallelic pathogenetic variants in the MUTYH gene were detected. In 3 out of 5 patients with the attenuated phenotype biallelic pathogenetic variants of the MUTYH gene were found and in the remaining 2 individuals monoallelic pathogenetic variants of this gene were detected. APC gene mutations included 7 small deletions, 4 nonsense mutations, 3 small insertions, 2 gross deletions, and 1 splice site mutation (data not shown). MUTYH gene mutations included 4 missense mutations, 2 splice site mutations, and 1 small in-frame deletion (data not shown).
Regarding thyroid function in 13/54 (24.1%) subjects, an eumetabolic thyroid disease was found. A nodular disease was found in 11/54 patients; in particular, plurinodular disease in 7/54 (13.0%) and single nodule in 4/54 (7.4%). Two of 54 patients (3.7%) with FAP had a diagnosis of a PTC after FNAB evaluation. Both patients were females and showed a classic FAP phenotype. The first patient was diagnosed with FAP at the age of 17 yr and with PTC 3 yr later; mutational analysis revealed a common variant of APC: the c.3183_87delACAAA mutation in exon 15. The second patient was diagnosed with FAP at the age of 18 yr and with PTC 7 yr later. APC mutational analysis in this patient showed a never previously described large genomic deletion including exons 9 and 10 (del9080dup11) (Fig. 1A); long-range PCR (Fig. 1B)

revealed a small DNA fragment of 2572bp compatible with a 9080bp deletion. Furthermore, direct sequencing provided evidence for a 11bp (del9080dup11) APC duplication next to the deletion (Fig. 1C). Exactly the same complex deletion/duplication pattern was found in 3 individuals belonging to two, to our knowledge unrelated, Italian families (data are shown for 2 of them in Figure 1B). The 2 patients with cytological diagnosis of PTC underwent total thyroidectomy that confirmed the diagnosis of a classical PTC (non-cribiform variant).

DISCUSSION

Slightly in excess to FAP registry estimates (2), assessing a lifetime risk of 1-2% to develop PTC, the FAP population examined herein (54 patients) displayed an estimated prevalence of PTC of 3.7%. Recently, a retrospective study by Herraiz and collegues (12) detected a much higher overall prevalence (12%) in 51 FAP patients. The small size of the populations examined by individual investigators, as well as the contribution of environmental factors, genetic modifiers, and/or uncovered complex genetic alterations affecting the APC gene (23) may account for the variable prevalence of PTC within FAP case collections. However, inclusion of FAP cases characterized poorly or not at all by molecular genetic analysis possibly introduces an even greater element of bias. For instance, it is unclear whether or not PTC occurs in MUTYH-mutated AFAP patients at a prevalence higher than that seen in the general population (3). Although 5 such patients were observed in our series, none had clinically evident PTC.

APC germline mutations associated with papillary carcinoma have been reported overall the gene, even though some authors report more often a mutation in exon 15 and in the 5′ portion of the APC gene outside the MCR (codons 1286-1513). In the population examined, the 2 patients affected with PTC, carried respectively the APC mutation 3183_3187delACAAA and del9-10; the first mutation maps within the 5′ portion of the APC gene and is considered a hot spot for PTC (14). The second mutation, a gross deletion including exons 9 and 10 of

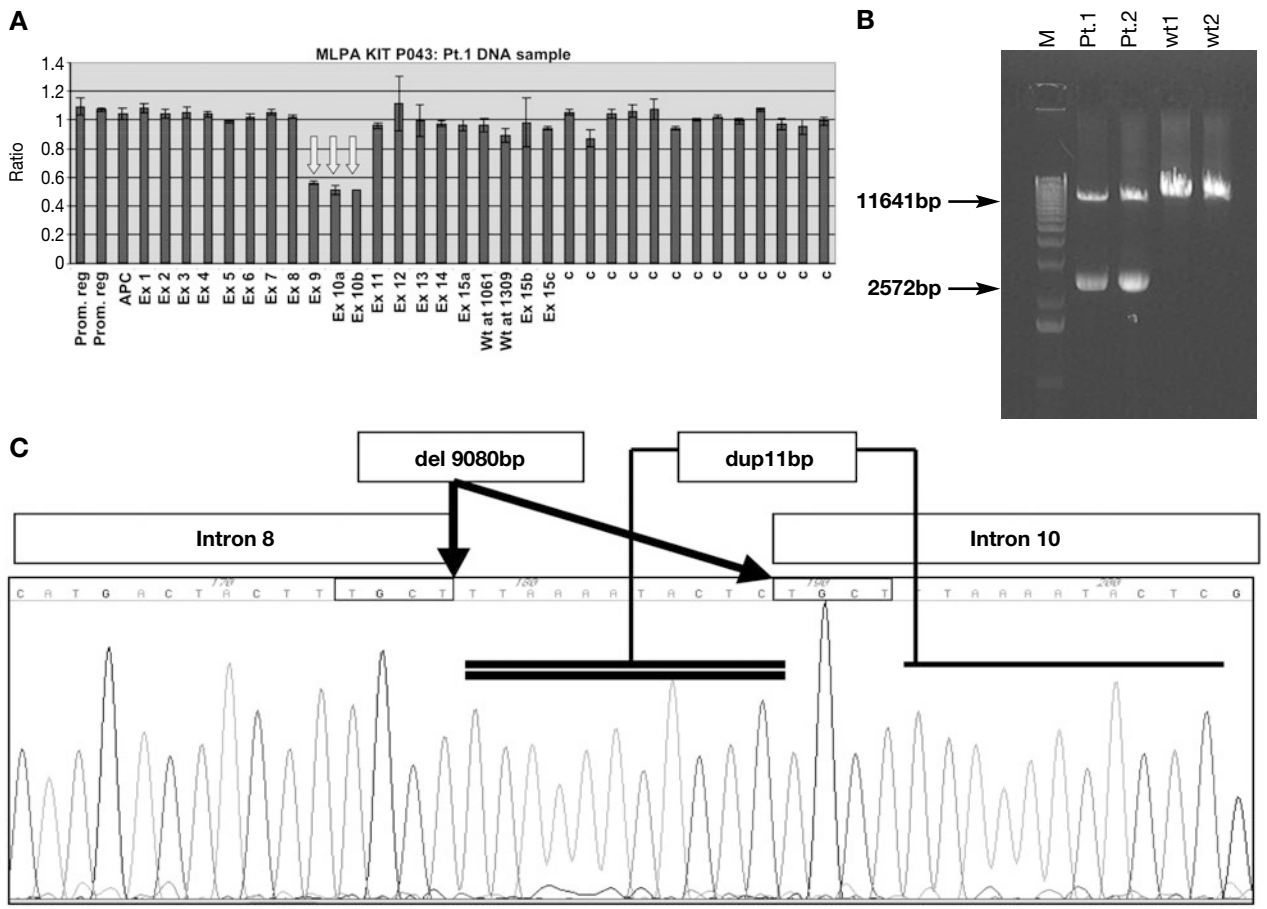

Fig. 1 - *A) Multiplex ligation-dependent probe amplification analysis: copy number decrease in exons 9 and 10 (arrows). Bars represent deleted exons of APC as a function of copy number. B) Long range PCR: Pt.1 is the papillary thyroid carcinoma affected familial adenomatous polyposis (FAP) patient; Pt.2 is a FAP patient with the same genomic rearrangement as Pt.1; M: 1Kb DNA ladder (Invitrogen); wt1/2: samples from normal individuals; arrows indicate the wild type (wt) (11641bp) and the Mutant (2572bp) alleles. C) Partial sequence of the mutant allele showing the breakpoints in intron 8 and intron 10 (arrows) and the 11-bp duplication (double horizontal bar).*

APC, was found in 3 individuals belonging to two families in our series and to our knowledge has never been reported in previous studies. Interestingly, the rearrangement pattern (del9080dup11) was exactly the same in both families suggesting a "founder" effect. This genomic alteration maps within the 5' region of the gene in accordance with previously described APC mutations associated with thyroid cancer. This novel mutation indeed represents the first large genomic rearrangement of the APC gene detected in a PTC-affected FAP patient: the causative role of this rearrangement with the patient's adenomatous polyposis is confirmed by the huge amount of data on similar genomic alterations leading to the formation of a truncated aberrant protein; on the other hand, however, the genotype-phenotype correlation of this APC gene mutation on the clinical manifestation of PTC requires further investigation. Furthermore, in agreement with previous studies the morpho-functional surveillance of thyroid in FAP series should be recommended. Our results suggest that a better insight into genotype-phenotype correlations of APC gene is likely to improve the accuracy in the identification of patients at risk of PTC.

Thyroid surveillance in FAP is still controversial and screening guidelines are neither consistent nor well-established. In our series a classical PTC was found in 3.7% of patients, whereas the estimated prevalence in general population is reported to be 0.8%. Furthermore, we found 2 cancers among the 11 patients with thyroid nodular disease (18.1%) compared to 5-10% reported in the general population (12). These results are in agreement with other studies supporting the need of thyroid surveillance in FAP. As to clinical surveillance, the starting age is not well established, thus to clarify this critical area, we should focus on the observation that in our series patients with malignant nodules were diagnosed at age of 20 and 25 yr and that to our knowledge, the youngest case of DTC associated with FAP reported in scientific literature was at 15 yr of age. In view of this early onset (respect to general population) surveillance should be recommended starting puberty.

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