Clinical and genetic characterization of classical forms of familial adenomatous polyposis: a Spanish population study

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Background: Classical familial adenomatous polyposis (FAP) is characterized by the appearance of >100 colorectal adenomas.

Patients and Methods: We screened the APC and MUTYH genes for mutations and evaluated the genotype–phenotype correlation in 136 Spanish classical FAP families.

Results: APC/MUTYH mutations were detected in 107 families. Sixty-four distinct APC point mutations were detected in 95 families of which all were truncating mutations. A significant proportion (39.6%) had not been previously reported. Mutations were spread over the entire coding region and great rearrangements were identified in six families. Another six families exhibited biallelic MUTYH mutations. No APC or MUTYH mutations were detected in 29 families. These APC/MUTYH-negative families showed clinical differences with the APC-positive families. A poor correlation between phenotype and mutation site was observed.

Conclusions: Our results highlight that a broad approach in the genetic study must be considered for classical FAP due to involvement of both APC and MUTYH and the heterogeneous spectrum of APC mutations observed in this Spanish population. The scarcely consistent genotype–phenotype correlation does not allow making specific recommendations regarding screening and management. Differences observed in APC/MUTYH-negative families may reflect a genetic basis other than mutations in APC and MUTYH genes for FAP predisposition.

Key words: APC gene, colorectal cancer, familial adenomatous polyposis, genotype–phenotype correlation, MUTYH gene, Spanish population

Introduction

Familial adenomatous polyposis (FAP) is an autosomal dominant syndrome characterized by the development of multiple adenomas throughout the colorectum. Some of the polyps will inevitably evolve into carcinomas, if no surgical measures are taken. Approximately 70% of patients also suffer from a variety of extracolonic alterations. In addition to a high risk for colorectal cancer, patients also have increased risk for other cancers like papillary thyroid cancer, hepatobiliary tract tumors, and brain tumors [1]. There are two FAP phenotypes: the classical form of familial adenomatous polyposis with manifestations of >100 colorectal adenomas and with a de novo rate of 30%–40% of cases, while the attenuated form of familial adenomatous polyposis (AFAP) is characterized by a milder course of the disease [2–4]. Adenomatous polyposis coli (APC) is a tumor suppressor gene that controls β-catenin turnover in the Wnt pathway. Mutations in APC gene are responsible for the majority of FAP cases [5].

The germline mutation detection rate in APC depends on the clinical features of the FAP patients and the technique used. On average, mutations can be detected in 85% of classical FAP families, while only 20%–30% of AFAP cases will exhibit a germline APC mutation [2, 6].

Molecular screening of APC is a time-consuming process. More than 1000 different APC germline mutations have been reported and are stored in public databases (www.hgmd.cf.ac.uk/ac, www.lovd.nl/2.0/index_list.php and http://www.ncbi.nlm.nih.gov/pmc/articles/PMC147178/pdf/260269.pdf). These mutations are distributed along the gene and frequently produce a truncated protein [7]. In spite of the large number of APC mutations already described, it is still common to find novel APC mutations in patients affected by polyposis, which underscores the
heterogeneity of APC mutations causing FAP [8, 9]. However, no germline mutations are detected in 30%–50% of clinically diagnosed FAP or AFAP patients [1], suggesting the existence of other loci responsible for the disease. MUTYH is the second FAP-related gene [10]. The MUTYH gene product is involved with base-excision repair of DNA damaged by oxidative stress [9]. MUTYH mutations account for 10%–20% of classical FAP cases without a mutation in APC and for 30% of AFAP cases [11]. MUTYH-associated polyposis (MAP) may be considered a phenocopy of FAP [12].

Genotype–phenotype correlation in FAP syndrome (reviewed by Nieuwenhuis and Vasen) has clinical interest since it contributes to better genetic counseling and simplifies mutation screening. In some studies, an association between location of the mutation and clinical manifestations could be established. However, it is not known if these correlations are population specific, and therefore, it is important to incorporate genetic and clinical data of FAP families from other populations. The present study shows the analysis of the APC/MUTYH genes in a large series of 136 Spanish families classified as having classical FAP as well as the correlation of APC/MUTYH mutations with clinical and familial characteristics of the patients. We studied only classical FAP families with the aim of increasing homogeneity in our series, permitting us to carry a comprehensive and in-depth analysis to accurately describe mutation frequencies and further define the FAP-associated phenotype.

patients and methods

One hundred and thirty-six probands suspected of having FAP received genetic counseling and were molecularly studied at two different Spanish centers (Spanish National Cancer Research Centre (CNIO), Madrid, Spain, and FIGTP-Catalan Institute of Oncology, Barcelona, Spain). Both centers are considered reference centers that receive samples from different Spanish Autonomous Communities. A detailed family pedigree and clinical history of all affected members were collected. All patients provided informed consent.

DNA extraction and genetic testing

DNA was obtained from peripheral blood lymphocytes using the MagNA Pure LC Instrument (Roche) following the manufacturer’s recommendations. Denaturing high-performance liquid chromatography and direct sequencing were used to detect point mutations in the APC gene. Primers, probes, and PCR conditions are available upon request. Samples negative for point mutations were studied for gross rearrangements (GR) using the MLPA test kit (SALSA P043 APC exon deletion test kit, MRC-Holland) following the manufacturer’s protocol or by Quantitative Multiplex PCR of Short Fluorescence fragments as described by Castellsague et al. [13], depending on the center where they were studied. When GR APC tests were negative, the two recurrent variants (Tyr165Cys and Gly382Asp) of the MUTYH gene were screened.

Mutations in APC or MUTYH were considered deleterious if they were classified as pathological in the HGMD (Human Gene Mutation Database) database. In cases where we identified a previously undescribed mutation, we studied 1000 healthy volunteers and carried out segregation studies among the affected members of the family. Once a mutation was identified in a family, genetic counseling and molecular testing were offered to other relatives.

statistical tests

Statistical tests were carried out using SPSS 17.0, applying chi-square or Fisher’s exact tests when required to analyze categorical data. Analysis of variance and Student’s t-test were employed to analyze continuous data comparing the four groups of families: APC positive, MUTYH positive, APC-GR positive, and APC/MUTYH negative.

results

A total of 136 families from two Spanish centers with a clinical diagnosis of classical FAP were included in this study. Of these patients, 81 (59.6%) had a family history of adenomatous polyposis. The remaining 55 (40.4%) patients were apparently the first case in the family. In 12 cases, it was possible to confirm the de novo nature of FAP, while difficulties in obtaining DNA samples (death of parents or lack of collaboration of other family members) from other relatives prevented us from unequivocally confirming the de novo origin in other 43 remaining cases.

We identified mutations in 107 families (78.7%) with similar mutation detection rates and types for both participant Spanish centers. Table 1 summarized types and frequency of mutations identified. APC and MUTYH mutations are described in supplemental Tables S1 and S2 (available at Annals of Oncology online) and in Figure 1. All the APC mutations were truncating. No missense APC mutations were detected in this series of classical FAP patients. All MUTYH-positive cases exhibited a recessive inheritance pattern.

| Table 1. Types of mutations and rates of novel and recurrent mutations in the APC gene |
|---|---|---|---|
| | Familial FAP, n (%) | Nonfamilial FAP, n (%) | Total, n (%) |
| Cases | 83 (61) | 53 (39) | 136 (100) |
| APC mutation positive | 65 (64.4) | 36 (35.6) | 101 (74.3) |
| Type of mutations | | | |
| Small deletion | 34 (52.3) | 14 (38.9) | 48 (47.5) |
| Nonsense | 20 (30.7) | 10 (27.8) | 30 (29.7) |
| Small insertion | 4 (6.2) | 8 (22.2) | 12 (11.9) |
| Splicing | 3 (4.6) | 2 (5.6) | 5 (4.9) |
| Great rearrangements | 4 (6.2) | 2 (5.6) | 6 (5.9) |
| Different mutations | 41 | 28 | 64 |
| Previously described mutations | 26 | 18 | 38 (59.4) |
| Novel mutations | 15 | 10 | 25 (39.6) |
| Recurrent* mutations | | | |
| c.423-2A>G | 2 | 0 | 2 |
| c.646C>T | 1 | 2 | 3 |
| c.694C>T | 1 | 1 | 2 |
| c.847C>T | 1 | 1 | 1 |
| c.2327_2328delTA | 2 | 0 | 2 |
| c.2805C>A | 2 | 0 | 2 |
| c.3183_3187delAAGAA | 4 | 1 | 5 |
| c.3202_3205delTCACA | 3 | 0 | 3 |
| c.3562_3565delCCTT | 3 | 0 | 3 |
| c.3880C>T | 2 | 0 | 2 |
| c.3927_3931delAAAGA | 6 | 6 | 12 |
| c.4394_4395delAG | 3 | 0 | 3 |

*Recurrent mutations are considered those that appear in more than one family in the present series.
Mutations were identified between codons 49 and 1569 of the APC gene (Figure 1). Exon 15 harbored 58.4% of all mutations, and no mutations were detected in exons 1, 4, 7, and 10. Twenty-four point mutations (25.3%) were located in the mutation cluster region (MCR; codons 1286–1513 [14, 15]). The hot spots at codons 1061 and 1309 appeared mutated in 4.9% (5/101) and 11.9% (12/101) of our families, respectively.

Of the sixty-four different mutations identified, 25 (39.1%) had not been previously described (Table 1 and Figure 1). The frequency and type of extracolonic manifestations (EM) as well as the age of onset associated with these novel mutations were indistinguishable from previously described APC mutations.

We analyzed haplotypes associated with two mutations that appear in five or more unrelated families (data not shown) but failed to identify a common haplotype for families with either mutation, indicating that a common origin for these mutations is improbable.

**genotype-phenotype correlation**

**age of onset**

The average age of onset was 33 years (range 1–81) across the entire series with 32 and 35 years for familial cases (1–81) and cases without family history (range 8–63), respectively. The mean age of onset of APC-positive cases was 31 (range 1–81) years and 45 (range 22–63) in MAP cases. In general, mutations in the proximity of codon 1309 in the MCR were associated with the earliest age of onset. In patients with MCR mutations, the age of onset was 20 years (range 1–43), while the average age was 34 years (range 12–54) for mutations in the remainder of exon 15, 34 years (range 13–81) for mutations in other parts of APC, and 33 years for GR of APC (range 18–51) (P = 0.001). In this regard, an average onset of 21 years (range 8–33) was observed in our patients with mutations in codon 1309.

**number of polyps**

We identified a total of seven families with profuse polyposis (>1000 polyps), one of them without detectable mutations in APC or MUTYH (Figure 2). Only one patient (ID 54, supplemental Table S3, available at *Annals of Oncology* online) carried a mutation in codon 1309 (Figure 2), and the remaining patients carried mutations in codons 49, 216, 805, 1075, and 1113. Interestingly, we did not observe profuse phenotypes associated to gross APC rearrangements. Two novel mutations in this series were associated with the presence of >1000 polyps (c.145delAAAC and c.3337_3338delAA, supplemental Table S3, available at *Annals of Oncology* online).

**extracolonic manifestations**

EM were reported in 62 cases (45.6%): 45 (55.5%) familial cases and 17 (30.9%) nonfamilial cases. The frequencies of the various EM were probably product of underreporting, and therefore, the figures given represent minimum estimations. Figure 2 shows the distribution of EM according to location of the genetic alteration in the APC gene. The highest proportion of EM (14/18; 77.8%) was observed in patients harboring mutations between codons 500 and 1000. More than half of all point mutations (49/95) clustered in the region between codons 1000 and 1569, and almost half of these (24/49, 49%) were associated with some type of EM. We noticed an important variability in the type of EM linked to codons 1061–1068 (six different EM) and 1465 (five different EM).

Upper gastrointestinal (UGI) polyps were the most common EM. They were reported in 29 families (21.3%): 24 APC-positive families, 2 MAP families, and 3 families without detectable APC/MUTYH mutations (UGI polyps were identified in carriers where mutations were distributed all over the gene).

Desmoids were observed in 19 families (14%): 18 were APC positive and 1 had no detectable APC/MUTYH mutation. Desmoids appear to be associated with different types of mutations, including GR, mainly between codons 232 and 499 and 805 and 1465. Interestingly, only three of these families harbored mutations beyond codon 1400. We observed only one family (ID 28, supplemental Table S3, available at *Annals of Oncology* online) with more than one relative affected with desmoid tumors.

Osteomas were detected in 15 families (11%) and were associated with all mutation types concentrated between codons 500 and 1000 (Figure 2): 13 APC-positive, 1 MAP family, and 1 APC/MUTYH-negative family.

Congenital hypertrophy of the retinal pigment epithelium (CHPRE) was concentrated between codons 1000 and 1569 (Figure 2) and was reported in only 10 families (7.4%); all of them were APC-positive, except APC/MUTYH-negative family.

Hepatoblastoma was reported in three families: two of which were APC positive and one was APC/MUTYH negative. Papillary thyroid carcinoma appeared in two families, one APC positive and one APC/MUTYH negative. Finally, a duodenal carcinoma was detected in one APC-positive family and one

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**Figure 1.** Schematic representation of the location of mutations and great rearrangements (GR) along the APC gene. Gray diamonds indicate previously described mutations, whereas black circles indicate novel mutations. Exons are numbered. MCR, mutation cluster region.
MUTYH-positive family, and gastric carcinoma was observed in two cases without detectable APC/MUTYH mutations.

FAP families with a negative genetic testing

We compared main clinical characteristics between families with APC aberrations (n = 101) and those without detectable APC/MUTYH mutations (Table 2).

Considering only nonfamilial cases, in order to avoid bias introduced by earlier age of detection in cases with a family history of FAP, mean age of onset was 30 years in APC-positive and 42 in APC/MUTYH-negative cases (P = 0.004).

Differences were also apparent regarding the presence of EM: 50.3% of the APC-positive cases had some type of EM, while this percentage dropped to 27.6% of APC/MUTYH-negative cases (P = 0.024). The proportion of cases without family history was modestly higher, while presence of profuse polyposis appeared to be lower in the APC/MUTYH-negative group, but these differences were not statistically significant. The frequency of CHPRE, desmoid tumors, UGI polyps, and osteomas was nearly half for APC/MUTYH-negative patients relative to APC-positive patients. By contrast, the presence of tumors other than colorectal was higher in APC/MUTYH-negative families.

Discussion

APC mutations

There is limited and scattered information on the APC mutation spectrum in the Spanish population. Here, we provide results of the mutation screening of the APC and MUTYH genes in a series of 136 Spanish families with classical FAP from many different geographical areas of the country, which has allowed us to expand the knowledge about the APC mutational spectrum in the Spanish population. Previous studies were carried out on Spanish populations from Northwestern and Southern regions and from the Balearic Islands. These data suggest various differences with other populations [16–19]. Thus, we carried out a comprehensive analysis in a large Spanish series to assess the validity of these
differences. We detected deleterious germline mutations in 78.7% of the patients, and, this number increased to 82.7% when only familial cases were considered. This detection rate is at the upper end of the range (50%–85%) previously described for classical FAP [20, 21]. The proportions of the different mutation types in our series overlap with those appearing in the HGMD database, with small deletions representing ~40% of all mutations. In our series, GR represent 5.9% of all APC mutations, similar to the 6.7% registered in HGMD, although in a recent paper, a GR frequency of 12% was reported in FAP cases [22]. The number of novel mutations (25 in our series) was also similar to that described for other European populations [23–26]. Besides, we identified 13 mutations, 3 of which were previously undescribed, that appear in more than one family. A founder effect of the c.3183_3187delACAAA APC mutation was previously reported in the Spanish Balearic Islands [18]. We failed to demonstrate a common haplotype in cases with this mutation as well as those 12 cases with the 1309 mutation.

APC mutations, all of them truncating, were distributed between codons 49 and 1569. Importantly, more than half of all mutations were located in the first half of exon 15. The MCR contained 25.3% of all point mutations, which coincides with the range reported in the Italian, German, and Norwegian populations (3.6%–34%) [8, 27–31]. Mutations in codons 1061 and 1309 represented 5.3% and 12.6%, respectively, of the APC point mutations in our series, clearly below the 8% and 20% previously reported [32, 33] but similar to that described in other Spanish and European populations [19, 23, 34–37]. We detected eight classical FAP cases with mutations in areas reportedly associated with attenuated forms of FAP (four mutations upstream of codon 157 and four mutations downstream of codon 1516). This underscores the need to include these areas in mutational screening of the APC gene [38, 39].

Mutations in MUTYH have been suggested to be the major cause of the attenuated version of the syndrome, although they have also been reported in classical FAP patients [40]. However, we found six cases of classical FAP associated with MUTYH mutations. The distribution and frequency of APC mutations and MUTYH mutations in our population suggest that a broader approach must be considered for classical FAP studies, including the 3’ and 5’ extremes of the APC gene in order to increase mutation detection yield and MUTYH screening in APC-negative cases.

**genotype–phenotype correlation**

Genotype–phenotype correlation studies may help defining the most likely phenotype associated with a given mutation. The identification of APC mutation carriers with a well-documented phenotype will allow for the establishment of more accurate surveillance programs and the adjustment of prophylactic surgical treatment.

Mutations affecting the MCR, especially codon 1309, relate with the youngest ages of diagnosis [41, 42], reinforcing the notion that this location associates with a more severe phenotype [1, 30, 43]. We found that the mean age of onset in nonfamilial APC-positive patients was 30 years, similar to that reported in recent publications [41, 42, 44, 45]. It should be noted that the age of onset was only considered in nonfamilial cases to avoid introducing bias related to an earlier detection of cases with a positive family history.

Seven families presented profuse forms of polyposis; one case resulted APC/MUTYH negative, while the remaining six patients had mutations along the entire APC gene. These include a mutation in codon 1309, classically associated with profuse forms, but also one in codon 49, previously associated with AFAP. The latter finding does not support the hypothesis that a 5’-terminal mutation effect can be partially compensated by the activation of a second translation start at position 184, yielding a partially active APC protein [46]. Furthermore, the latter again highlights the great expression variability exhibited by different mutations along the APC sequence. Thus, we suggest that these two novel mutations associated to profuse forms (c.145delAAAC and c.3337_3338delAA, supplemental Table 3, available at Annals of Oncology online) be taken into account for genetic counseling.

The distribution pattern of mutations in relation with EM showed some differences with previously described series (Figure 2). Half of our APC-positive or MUTYH-positive families developed EM. This proportion likely reflects an underdiagnosis of benign lesions, but not severe complications. Eighteen of the 19 families presenting desmoid tumors had a detectable APC mutation mainly located between codons 232 and 499 and 805 and 1465 (Figure 2). In contrast, only three desmoids were associated with mutations beyond codon 1400, an area classically associated with the presence of desmoid tumors [1]. Although desmoids have been linked to a strong family history [47], only one family with several desmoid-affected members was reported.

We also observed an important proportion of UGI polyps and CHPRE associated to mutations located outside the classically described sites. Finally, we detected mutations in AFAP-related areas of the APC gene associated with profuse forms of FAP. The lack of an evident genotype–phenotype correlation in our population, together with the intrafamilial and interfamilial variability observed in our series (data not shown), complicates phenotype predictions based on knowledge of the mutation site. These results preclude making recommendations regarding surveillance and prophylactic measures for Spanish FAP patients.

**APC/MUTYH-negative cases**

Patients and families with classical FAP without detectable APC/MUTYH mutations exhibited some distinct features, such as a lower percentage of EM (desmoids tumors, CHPRE, osteomas and UGI polyps) and a later age of onset (42 versus 30 years in nonfamilial cases). Furthermore, a higher incidence of extracolonic tumors was observed among APC/MUTYH-negative families. We observed two gastric carcinomas: one papillary thyroid carcinoma and one hepatoblastoma among these negative cases. It remains elusive whether or not these differences reflect APC or MUTYH inactivation via mechanisms yet to be identified or by a distinct etiology all together. Although the number of cases were limited, these APC/MUTYH-negative families probably constitute a heterogeneous group [48]. Notwithstanding, until underlying mechanisms have been elucidated, members of these negative
families should be managed according to guidelines for surveillance and treatment measures established for classical FAP patients.

In conclusion, the data from the present series expand our knowledge of the genetic basis and the clinical manifestations of classical FAP. The detection rate and the type of APC mutations in the Spanish population are similar to those described for other European populations. No missense mutations were identified; in fact, all of them led to protein truncation. According to our results, even if broad genetic screening for mutations in APC and MUTYH was carried out in all cases, some 20% of classical FAP patients will remain without a detectable APC/MUTYH mutation. These negative cases present clinical differences that could be related to a different genetic basis. Finally, the lack of a clear genotype-phenotype correlation in our population should be kept in mind for genetic counseling and the clinical management of these patients.

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**disclosure**

The authors declare no conflicts of interest.

**references**


