Clinical and molecular aspects of MUTYH- and APC-associated polyposis

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Germline mutations in *APC* and *MUTYH* are responsible for the majority of families with attenuated familial adenomatous polyposis

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ABSTRACT

A small fraction of families with familial adenomatous polyposis (FAP) display an attenuated form of FAP (AFAP). We aimed to assess the presence of germline mutations in the *MUTYH* and adenomatous polyposis coli (*APC*) genes in AFAP families and to compare the clinical features between the two causative genes. Families with clinical AFAP were selected from the Dutch Polyposis Registry according to the following criteria: (a) at least two patients with 10–99 adenomas diagnosed at age >30 years or (b) one patient with 10–99 adenomas at age >30 years and a first-degree relative with colorectal cancer (CRC) with a few adenomas, and, applying for both criteria, no family members with more than 100 polyps before the age of 30 years. All probands were screened for germline mutations in the *APC* and *MUTYH* genes. Twenty-five of 315 Dutch families with FAP (8%) met our criteria for AFAP. These families included 146 patients with adenomas and/or CRC. Germline *APC* mutations were identified in nine families and biallelic *MUTYH* mutations in another nine families. CRC was identified at a mean age of 54 years (range 24–83 years) in families with *APC* and at 50 years (range 39–70 years) in families with *MUTYH* ($p = 0.29$). *APC* and biallelic *MUTYH* mutations are responsible for the majority of AFAP families. Based on our results and those reported in the literature, we recommend colonoscopy once every 2 years in AFAP families, starting surveillance from the late teens in *APC* mutation carriers and from age 20–25 years in biallelic *MUTYH* mutation carriers.

INTRODUCTION

Approximately 1% of all cases of colorectal which can be identified in 60–80% of families cancer (CRC) are attributed to familial adeno-with FAP.[1, 2]In 1975, Bussey defined FAP as matous polyposis (FAP). FAP is caused by the an autosomal dominant disorder characterized dominant inheritance of a constitutional adeno-by the development of more than 100 adenomas matous polyposis coli (*APC*) gene mutation, in the colorectum during adolescence and young adulthood.[3] For practical purposes, this definition of the phenotype has been used ever since. However, it has become clear that there is a variance in expression; some families have been described in which patients have fewer adenomas (<100).[4–8] For this phenotypic variant, the term attenuated FAP or AFAP was coined, but clear clinical definitions have not been described so far. Clinically, families with AFAP are characterized by the development of adenomas and CRC at a more advanced age than classical FAP and a predilection of the adenomas to the proximal colon.[9] Recognition of the variants of polyposis has important implications for their

management. Several studies have indicated that mutations located in three specific regions in the *APC* gene are associated with AFAP: (a) the 5# part of the *APC* gene, (b) the alternative spliced region in exon 9 and (c) the extreme 3# part of the gene. [7, 8, 10–13] It has been suggested that, in these cases, in addition to a somatic mutation on the wild type allele, another (second) somatic mutation is necessary on the mutant germline allele for tumor progression to start ('the three-hit model'), thereby explaining the relatively mild phenotype.[4–16] However, modification by environmental or other genetic factors has also been suggested, as individuals with identical mutations, even within one family, show variation in their clinical phenotype. [17, 18]

Notably, in some AFAP families, patients have been described (with or without a germline *APC* mutation) with colon cancer and just a few adenomas, [7, 8, 19]making the distinction between Lynch syndrome and AFAP sometimes difficult. [20]

In 2002, Al Tassan *et al*. showed an unsuspected role for base excision repair (BER) in hereditary CRC.[21] They identified biallelic mutations in the BER gene *MUTYH* (previously known as *MUTYH*) ina British family with three affected members and recessive inheritance of multiple colorectal adenomas and carcinoma. Further studies found homozygous and compound heterozygous (biallelic) *MUTYH* mutations in approximately 26–29% of patients with 10–100 polyps and in 7–29% of patients with 100–1000 polyps.[22–24] In these studies, no biallelic germline *MUTYH* mutations were found in patients with less than 10–15 adenomas, but biallelic mutations have been reported in some patients with CRC only.[25, 26]The aims of our present study were to (a) evaluate the role of *APC* and *MUTYH* in well-defined families with AFAP and (b) compare the clinical features between those families associated with *APC* mutations, those with biallelic *MUTYH* mutations, and those without known mutations.

PATIENTS AND METHODS

Dutch Polyposis Registry

In 1985, the Netherlands Foundation for the Detection of Hereditary Tumors set up a registry for families with FAP. The organization and methods used by this foundation have been described elsewhere.[27] All patients with multiple polyps (>10–15 polyps), regardless of their family history, were invited for registration. The genealogical studies were performed by genetic field workers connected with the registry before 1999 and by clinical geneticists after 1999. Personal data, results of investigations, pathology reports and results of treatment are recorded in this registry. In January 2006, the Dutch Polyposis Registry covered 315 families with polyposis. Families with AFAP were defined as follows:

(a) at least two first-degree relatives with 10–99 colorectal adenomas diagnosed after the age of 30 years, (b) one patient with 10–99 adenomas diagnosed after age of 30 years plus a first-degree relative with CRC and a few <10 adenomas, and, applying for both criteria, (c) no family members with 'classic FAP' (i.e. more than a 100 polyps) before the age of 30 years. We calculated the mean age at diagnosis of CRC and

Table 1. Clinical features of all AFAP families and outcome of genetic testing

Number	Family id	Number of patients	One to nine polyps	10–100 polyps	>100 polyps	Colon cancer (confirmed)		Number of affected generations	Germline mutation
						Total	>100 polyps		
1	19235	12	4	6	2	2	1	≥2	*APC* exon 15, p.Asp1636fs (c.4906delG)
2	19025	3	0	2	1	0	0	≥2	*APC* intron 9, c.1312+3G>A, splice donor
3	19061	22	4	13	4	12	2	≥2	*APC* exon 15, p.Ser1861X (c.5582_5585delCTTT)
4	19033	17	1	9	6	8	0	≥2	*APC* exon 9, p.Ile357fs (c.1069dupA)
5	51161	17	2	10	2	9	1	≥2	*APC* exon 11, p.Lys516Asn (c.1548G>C, splice donor)
6	54822	3	0	3	0	2	0	≥2	*APC* exon 9, p.Val368fs (c1102_1103delGT)
7	50846	11	1	6	4	3	1	≥2	*APC* deletion exon 1–5 (including promoter region)
8	53344	5	3	2	0	3	0	≥2	*APC* deletion exon 7–13 (in frame)
9	50773	3	0	3	0	1	0	1	*APC* exon 3, p.Leu134X (c.401T>A)
10	19047	8	5	2	1	2	0	≥2	*MUTYH* G382D/G382D [c.1145G>A, p.Gly382Asp]+ [c.1145G>A, p.Gly382Asp]
11	20090	6	2	1	0	5	0	≥2	*MUTYH* Y165C/P391L [c.494A>G p.Tyr165Cys]+ [p.Pro391Leu c.1172C>T]
12	55356	3	1	1	0	2	0	≥2	*MUTYH* P391L/1105delC [c.1172C>T, p.Pro391Leu]+ [c.1105delC p.Ala371fs]
13	19049	5	1	2	2	2	2	1	*MUTYH* Y165C/Y165C [c.494A>G p.Tyr165Cys]+ [c.494A>G p.Tyr165Cys]
14	247	2	0	2	0	1	0	1	*MUTYH* Y165C/Y165C Y165C [c.494A>G p.Tyr165Cys]+ [c.494A>G p.Tyr165Cys] (in tissue)
15	54962	3	2	1	0	1	0	1	*MUTYH* G382D/P391L [c.1145G>A, p.Gly382Asp]+ [p.Pro391Leu c.1172C>T]
16	54186	3	1	2	0	2	0	1	*MUTYH* Y165C/Y165C Y165C [c.494A>G p.Tyr165Cys]+ [c.494A>G p.Tyr165Cys]
17	53029	2	0	2	0	1	0	1	*MUTYH* Y165C/P391L [c.494A>G p.Tyr165Cys]+ [p.Pro391Leu c.1172C>T]
18	19045	2	0	2	0	0	0	1	*MUTYH* Y165C [c.494A>G p.Tyr165Cys]+[c.649-1G>A, splice acceptor]
19	51628	2	1	1	0	1	0	≥2	*APC* and *MUTYH* negative
20	53154	5	2	1	0	3	0	≥2	*APC* and *MUTYH* negative
21	54391	3	2	1	0	2	0	≥2	*APC* and *MUTYH* negative
22	54226	2	0	2	0	1	0	≥2	*APC* and *MUTYH* negative
23	54797	2	0	1	0	2	0	≥2	*APC* and *MUTYH* negative
24	19077	9	6	2	0	1	0	1	*APC* and *MUTYH* negative
25	52	4	2	1	0	3	0	1	*APC* and *MUTYH* negative

AFAP, attenuated familial adenomatous polyposis; *APC*, adenomatous polyposis coli.

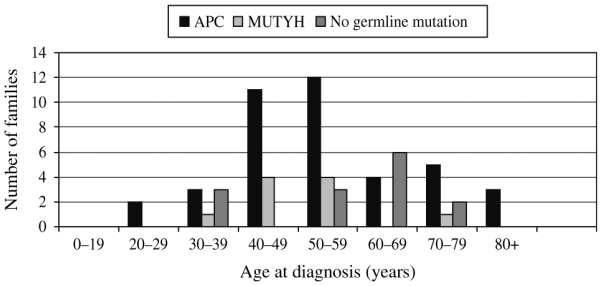

Fig. 1. Distribution of ages at diagnosis of colorectal cancer in 25 AFAP families.

second-degree relatives with a diagnosis of adenomas and/or CRC (Table 1). To compare clinical features in families associated with *APC* and *MUTYH*, we included all proven or obligate *APC* carriers and biallelic *MUTYH* carriers (Fig. 1, Table 2).

Molecular genetic analysis

Mutation analysis was performed at the Centre for Clinical and Human Genetics, Leiden University Medical Center, and in one case, it was performed at the Netherlands Cancer Institute, Amsterdam. All patients gave written consent for the testing of DNA sample according to protocols approved by the institutional review board. When an *APC* or biallelic *MUTYH* mutation was found in a proband, mutation analysis was performed in other relatives if their DNA was available. We performed *APC* germline mutation analysis using the protein truncation test (PTT, exon 15), denaturing gradient gel electrophoresis (DGGE, exons 2, 6, 8–13 and first 618 nucleotide of exon 15) and sequence analysis (exons 3, 4, 5 germline *APC* mutation. The coding regions of all 16 exons of *MUTYH* were sequenced as described by Nielsen *et al*.[24] In family 14, there were no living affected family members, so no DNA from peripheral blood was available for genetic testing. In this family, genomic DNA of the index patient was extracted from formalinfixed paraffin-embedded material as described by de Jong *et al*.[28] Sequence analysis of two fragments containing the three most common *MUTYH* mutations in the Netherlands [Tyr165Cys (Y165C), Gly382Aps (G382D) and Pro391Leu (P391L)] was performed in tissue DNA. The Y165C and G382D are established *MUTYH* hotspot mutations,[21–24] while the P391L mutation is a possible Dutch (North-West European) founder mutation,[24] which was not detected in 668 Dutch controls (manuscript in preparation). The primers and protocols used are available on request.

Statistics The Student's *t*-test was used for comparing means. Differences in percentages were assessed by the Fisher's exact test. A p value of <0.05 was considered as statistically significant. All tests were performed with SPSS 11.01 (SPSS, Chicago, IL).

RESULTS

Among the 315 registered families with polyposis, we identified 25 families (8%) that met our criteria for AFAP. The clinical features and results of mutation analysis are summarized in Table 1. A total of 140 patients (73 males, 67 females) had developed adenomas, with a mean age at diagnosis of 48 years (range: 19–83 years). The number of adenomas detected in these patients varied greatly: 40 (29%) had one to nine adenomas, 78 (56%) had 10–100 adenomas, and 22 (16%) had more than 100 adenomas (after the age of 30 years).

The 25 families included 69 CRC cases (33 males and 36 females) confirmed by medical reports or pathology reports. The mean age at diagnosis was 55 years (range: 24–83 years). The distribution of the ages at diagnosis is shown in Fig. 1. Twenty-seven (40%) tumors were diagnosed in the proximal part of the colon (proximal to the splenic flexure) and 41 tumors (59%) in the distal part. In one case, the precise location of the tumor was not known.

APC mutations

In nine of the 25 families, a pathogenic *APC* mutation was identified. Ninety-three family members were proven *APC* carriers or obligate carriers. In families 1, 3, 4, 6 and 9, the type of mutation and location agree with previous reports on AFAP-related *APC* mutations.[8, 9, 11] The *APC* exon 9 splice donor mutation c.131213A>C (as found in family

Table 2. Comparison of clinical features between families with *APC* mutations and biallelic *MUTYH* mutations

	APC mutation (proven or obligate carriers)	Biallelic *MUTYH* mutations	No *APC* or *MUTYH* mutations
Number of families	9	9	7
Number of patients	93	26	27
Age at diagnosis (years) of adenoma, mean (range)	46 (19–83)	47 (30–70)	52 (33–78)
Age at diagnosis (years) of CRC, mean (range)	54 (24–83)	50 (39–70)	57 (33–78)
Location (first) CRC (L/R/unknown)	26/14/0	4/7/0	9/3/1
Number of polyps 1–10/10–100/>100	11/41/15	6/10/3	13/9/0 (tested persons: 2/5/0)

APC, adenomatous polyposis coli; CRC, colorectal carcinoma; L, left; R, right.
Statistical analysis of differences between patients with *MUTYH* (biallelic) and *APC* mutations; age at diagnosis of adenomas: $p = 0.71$; age at diagnosis of CRC: $p = 0.29$; location of CRC: $p = 0.78$.

2) has been *APC*, adenomatous polyposis coli; CRC, colorectal carcinoma; L, left; R, right. Statistical analysis of differences between patients with *MUTYH* (biallelic) and *APC* mutations; age at diagnosis of adenomas: p = 0.71; age at diagnosis of CRC: p = 0.29; location of CRC: p = 0.78. reported to cause partial exon 9 skipping.[29] In family 5, a previously reported mutation in exon 11, p.Lys516Asn, was identified, probably also resulting in a partial splice donor defect.[2] The splice donor recognition score as calculated in http://www.fruitfly.org/seq_tools/splice.html dropped from 1.00 to 0.86, predicting a less efficient splicing. By using MLPA, a previously reported exon 1–5 deletion (including the promoter region) was found in family 7, and an unreported exon 7–11 frame deletion was found in family 8.[30]

MUTYH mutations

Nine of the 16 *APC*-negative families were found to harbor biallelic germline mutations in *MUTYH*. In family 14, biallelic *MUTYH* mutations were found in DNA isolated from archival tumor material. In total, 26 biallelic *MUTYH* mutation carriers were found. Six *MUTYH* mutations were identified in families with one affected generation and three mutations in families with two generations involved. In one of these latter families, family 10, the spouse of a patient with biallelic *MUTYH* (G382D/G382D) also carried a heterozygote *MUTYH* mutation, G382D. All three children of this couple had inherited two *MUTYH* mutations (G382D/ G382D), explaining the (pseudo)dominant inheritance in this family. In the two other families with apparently dominant *MUTYH* (families 11 and 12), both the parents of the patients with biallelic *MUTYH* had been diagnosed with CRC at a later age but without the development of polyps. Although it has been reported that patients with only a single *MUTYH* mutation have a slightly elevated risk of 1.5 of developing colon cancer,[26] in these cases, there may be additional genetic and/or environmental factors contributing to colon cancer pathogenesis.

APC vs MUTYH

A mutation was found in 50% of families with two affected members (3/6, all *MUTYH* mutations) and in 63% of families with three or more affected members (12/19, nine *APC* and three *MUTYH*). The mean ages at diagnosis of the adenomas and CRC were not statistically significantly different between families with a biallelic *MUTYH* mutation and families with an *APC* mutation, although we saw a few cases of CRC diagnosed between ages 20 and 30 years in families with an *APC* mutation (Table 2, Fig. 1). Of 25 patients with the mildest phenotype (one to nine adenomas and no carcinoma), 10 patients were tested for mutations: five patients had an *APC* mutation (age: 19,

23, 34, 44 and 46 years) and two patients had biallelic *MUTYH* mutations (age: 30 and 38 years). In the group of patients with 10–100 polyps, 59 patients were tested and 54 patients were diagnosed with a mutation (41 *APC* and 13 biallelic *MUTYH*). All 18 patients with the most severe phenotype (more than 100 polyps, after age of 30 years) were tested, and all were diagnosed with a mutation (15 *APC* and three biallelic *MUTYH*).

DISCUSSION

Knowledge of the natural history in AFAP families is important for making screening protocols and clinical management. All the previous clinical studies on AFAP reported in the literature were based on a single or only a few families. Therefore, the information derived from our present study, the largest group of AFAP families clinically described so far, is relevant for clinical practice. We have shown that 8% of families with FAP registered in the Dutch Polyposis Registry have an AFAP. We have identified constitutional *MUTYH* and *APC* defects in 18 of 25 (72%) families. The mutation detection rate was somewhat higher in families with three or more affected members than that in families with only two affected members (63% vs 50%) (p = 0.3, Fisher's exact test). In patients with more than 100 polyps, the mutation detection rate was also higher than that in patients with 1–10 polyps and no CRC [100% (18/18) vs 50%,[7/10] p = 0.04, Fisher exact].

In all our AFAP families, we saw a significantly higher age at diagnosis of adenomas and CRC than that reported for classic FAP. Moreover, a large intrafamilial variation in the number of adenomas and age at diagnosis was observed in the AFAP families, as has been reported earlier in families with attenuated *APC* or *MUTYH* mutations.[17, 18, 24] The clinical features of *MUTYH* families did not differ significantly from those in families with an *APC* mutation (Table 2).

For a family with an established AFAP diagnosis and a pedigree suggesting autosomal dominant inheritance, we would advise starting with an *APC* mutation screening. Mutation analysis should include the whole *APC* gene because, besides well-documented genotype–phenotype associations, large variations in the clinical expression for all mutations in the *APC* have also been reported.[31] However, the relatively high frequency of heterozygous *MUTYH* mutation carriers may also induce the occurrence of two generations with polyposis, as illustrated in one-third of our families with *MUTYH*-associated polyposis (MAP). If a family with AFAP shows evidence for recessive inherited disease, i.e. one or more cases in one generation, the

first step should be to screen the index patient for *MUTYH* mutations, although de novo or mosaic *APC* mutations should also be anticipated in these families.

Especially for recessive inherited disorders such as *MUTYH*, mutation screening may be hampered by the fact that no living patients are available for testing. In such families, mutation analysis of archival tumor material for *MUTYH* mutations can be helpful. This would re-open the possibility of detecting patients with an inherited pre-disposition for multiple colorectal adenomas and carcinomas in AFAP families. In 28% (7/25) of our AFAP families, no germline mutations in *APC* or *MUTYH* could be detected. The underlying gene defects for these AFAP families may include mutations in regions other than the open reading frame in the *APC* or *MUTYH* gene, defects in other (base excision) repair genes, genes involved in the Wnt pathway, or as yet unidentified genes.

The decision regarding the age at which screening should start in FAP is generally based on the age distribution of CRC. As CRC cases have been (albeit rarely) reported in classic FAP in the late teens, most authors recommend beginning screening between age 10 and 15 years.[32–34] In the present series of families with AFAP associated with *APC*, we found that the mean age at diagnosis of CRC (54 years) was about 15 years later than in classical FAP (average age 40 years).[35] In addition, no cases of CRC were observed in persons younger than 20 years. The youngest case of CRC was diagnosed at age of 24 years. In support of our results, Burt *et al*. reported comparable ages of diagnosis in a large pedigree of a family with AFAP with an *APC* mutation, a mean age of 41 years for adenomas and 58 years (range: 29–81 years) for CRC.[19] These observations may justify starting surveillance of AFAP families with an *APC* mutation in the late teens.

For patients with AFAP with biallelic *MUTYH* germline mutations, we believe surveillance can be started somewhat later (20–25 years) because CRC associated with *MUTYH* before age of 30 years has been reported only twice.[24, 36] Patients with AFAP without identified germline *APC* and *MUTYH* mutations were diagnosed with CRC at a mean age of 57 years, and no CRC cases occurred before the age of 30 years (Table 2). In such families with an unidentified cause, we recommend the same screening strategy as in *MUTYH*-related patients. We did not include sporadic patients with AFAP in our study because they did not meet our diagnostic criteria. To be prudent, we advise screening sporadic patients with AFAP according to FAP guidelines until more phenotypical family data are available and, if biallelic *MUTYH* mutations are found, screening should of course comply with MAP guidelines.

What are the implications of our findings for managing the disease? The management depends on the number and size of the colorectal adenomas; whenever possible, the adenomas should be removed endoscopically at the time of the diagnosis. When

endoscopic intervention is not possible, colectomy should be the next step. In view of the mild form (i.e. the low number of adenomas and the late onset of CRC) of polyposis in *MUTYH*, colectomy with an ileorectal anastomosis seems to be the preferred surgical procedure. In the present study, 25% of *APC*-associated CRC and 63% of *MUTYH*-associated CRC were located in the proximal part of the colon. Burt *et al*. found an even higher percentage of right-sided carcinomas (75%) in two large AFAP pedigrees with an *APC* mutation [19]. On the basis of these findings, we recommend performing colonoscopy instead of sigmoidoscopy (which is advised in classic FAP) every 2 years. In previous studies on AFAP, there were no clear definitions or diagnostic criteria used systematically; however, the criteria that we have used in this study appear to be appropriate for identifying families with AFAP. We feel that it is essential to have clear clinical criteria in general practice and would like to suggest our criteria be adopted for the clinical diagnosis of AFAP families.

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