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

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Genotype-phenotype correlations in 19 Dutch cases with *APC* gene deletions and a literature review

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Partial and whole gene deletions represent a large proportion (4–33%) of the *APC* mutations found in polyposis patients, who previously had negative test results. The genotype–phenotype correlations for these *APC* deletions have not been studied in detail. We aimed to assess the number of germ line *APC* deletions in Dutch polyposis patients, to describe the clinical phenotype(s), and to review the current literature. We screened 296 index patients with polyposis, who previously had negative test results for *APC* or *MUTYH* mutations, for germ line *APC* gene deletions using Multiplex Ligation-dependent Probe Amplification. *APC* deletions were identified in 19 polyposis patients; seven had a whole gene deletion, nine had a deletion involving two or more exons, and three had single exon deletions. Most of the deletion families (83%) displayed a classic familial adenomatous polyposis (FAP) phenotype (100–2000 adenomas). We saw no patients with *APC* deletions and a severe phenotype (ie >2000 polyps); on the contrary, two families carrying a deletion of exons 7–13 and one family with a deletion of exons 1–5 showed a distinctly attenuated FAP phenotype. *APC* deletions were found in a considerable proportion of polyposis patients previously tested negative for *APC* or *MUTYH* (6%, 19/296) and represent 8% of all *APC* mutations found at our clinics (19/242). Methods to identify such deletions should therefore be included in routine germ line *APC* mutation analysis. While most total and partial *APC* deletions lead to a classic FAP phenotype, specific (in-frame) deletions may lead to an attenuated polyposis phenotype.

INTRODUCTION

Familial adenomatous polyposis, FAP (MIM175100), is an autosomal dominant inherited disease caused by germ line mutations in the *APC* gene; it affects approximately one in 13 500 people.[1] Mutation carriers are predisposed in the majority of cases to develop hundreds or even thousands of polyps and subsequently go on to develop colorectal cancer (CRC).[1,2] In addition, extracolonic features such as fundic gland polyps (FGP), duodenal polyps, congenital hypertrophy of the retinal pigment epithelium (CHRPE) and desmoid tumors are often present, while a more attenuated form of FAP has also been described in which patients develop less than a hundred polyps at a relatively late age.3 Another inheritable form of polyposis is caused by recessive mutations of the *MUTYH* gene. *MUTYH*-associated polyposis (MAP) is found in 10–20% of polyposis patients.[5–7]

The *APC* gene is a relatively large gene, containing 15 exons and encoding a protein of 2843 amino acids. Exon 15 is by far the largest exon containing over three-quarters of the coding sequence.[8] Depending on clinical features and mutation detection techniques applied, germ line mutations in the *APC* gene are found in 30–80% of patients with polyposis[2,9,10] and most *APC* mutations occur in the 50 half of its coding region. Genotype– phenotype relations have been reported extensively for small nucleotide alterations (point mutations). A classic phenotype (ie 100–2000 polyps) is associated with mutations in codon 168–1250 (exons 4/5–exon 15) or codon 1400–1580 (exon 15). Mutations occurring between codon 1250 and 1464 (exon 15), but particularly at codon 1309;[2,11] lead in most cases to a more severe phenotype (>2000 polyps at a relatively young age). A more attenuated form of polyposis is associated with mutations in three regions: (i) in the 5' part of the *APC* gene; (ii) alternative spliced region in exon 9 and (iii) mutations in the extreme 30 site of the gene.[12,13]

Partial and whole gene deletions have been shown to represent a large proportion (4–33%) of *APC* mutations in polyposis patients who previously had negative test results.[14–17] Large deletions have also been found in other oncogenes such as BRCA1, *BRCA2*, *MLH1*, *MSH2* and – 20 *MSH6*.[17] Recently, *APC* duplications of exon 4 and 15 have also been described in FAP patients.[21,22] Using the new MLPA (Multiplex Ligation-dependent Probe Amplification) technique,[23] we searched for deletions of the whole gene of one or more exons in 296 *APC*-and *MUTYH*-negative index patients with polyposis. Here we report on genotype–phenotype correlations in 19 index patients with identified deletions and review the current literature on *APC* deletions.

Patients and methods

Between 1995 and 2005, a total of 599 index polyposis patients were referred for FAP testing to the Molecular Genetics Laboratory, Center for Human and Clinical Genetics (Leiden, The Netherlands). Informed consent was obtained for DNA testing according to protocols approved by LUMC Ethics Review Board.

The 599 index patients were all screened for germ line mutations in the *APC* gene by DGGE, PTT and sequence analysis. In 223/599 patients, we were able to identify a pathogenic *APC* mutation (37%). Mutations in the *APC* gene involve frameshift mutations (n = 124, 55%), nonsense mutations (n = 70, 31%) or splice mutations (n = 26, 12%), and three large cytogenetically detected deletions (cytogenetic analysis is only done when developmental disorders are present).

In 296 polyposis index patients with available DNA, in whom no *APC* mutations were detected and *MUTYH* was ruled out in most of the cases (225/296), we went on to perform MLPA to look for *APC* deletions.

For MLPA, the P043 kit was used (http://www. mrc-holland.com). This kit contains 20 probes covering the *APC* region, three probes for the promoter regions, 14 for exons 1–14 and three for exon 15. The MLPA analysis was performed according to the MRC Holland protocol23 with some adaptation: all reagents in the kit were used at 1/ 4 of the advised volume and hybridization time was cut from 16 to 2.5 h. To exclude false-positive results due to variants underlying the probe, single exon deletions containing only one MLPA probe (exons 11 and 14) were confirmed by a long-range PCR (see Figure 1) with the following primers:

The valued size of the deleted exons 11 and 14 was approximately 0.7 and 2.6 kb, respectively. A false-positive deletion was unlikely in the case of the single exon 15 deletion, since three MLPA probes were used for this exon. Clinical and pathological data were obtained from patients' records to confirm the FAP diagnosis. The polyposis patients and their families were grouped according to the following phenotype definitions:

Figure 1

Deletion	*Primer location*	*Primer sequence 5′-3 (nt_006899.11)*
Exon 14 deletion	Intron 13 (24)	TTCTTTGGAGCACTTTGTTTCA
	Exon 15	TGTCGATTGGTGTCAAAAACA
Exon 11 deletion	Intron 9	TTGATCCACTAAAATTCCGTGA
	Intron 12	ACTGAGCAACAATCTAAATCAGG

1. Severe: >2000 polyps.
2. Classic: 100–2000 polyps before the age of 30–40.
3. Attenuated: 10–99 adenomas diagnosed at a more advanced age (over 30–40 years). If other family members had a classic polyposis phenotype, in most cases the family was considered as classic FAP.

We describe the clinical details of Dutch index cases and their family members. Furthermore, we combined our results for index cases with whole and partial *APC* gene deletions with those of previous studies. For this, we searched the PubMed and EMBASE databases up to December 2006 using the terms 'Adenomatous Polyposis Coli', 'Genes, *APC*', 'Gene Deletion', 'Gene Dosage', 'Gene Disruption, 'Gene Loss' and 'Gene Dosage', with relevant subheadings. We also searched reference lists in articles identified by this strategy and selected additional articles that we considered relevant, including only those describing large germ line *APC* deletions in patients that could not be detected by cytogenetic techniques. We calculated Pearson's R to analyze trends and considered Po0.05 statistically significant. All our results were analyzed with SPSS 11.01 (SPSS, Chicago, IL, USA).

Table 1 Clinical characteristics of 19 index patients with *APC* deletions found by our MPLA study

Patient no.	*Family ID*	*Sex*	*Exons deleted*	*Age at diagnosis*	*Polyp count*	*CRC (age)*	*Extracolonic features*	*Family history*
1	54482	F	1–15	27	Polyposis			Sporadic: (seven sibs no abnormalities)
2	54878	M	1–15	34	>100	R (34)		Grandfather: CRC (age unknown)
3	53265	F	1–15	33	100–1000			Father: >100 polyps, age 62 years
4	55981	F	1–15	39	>100	CA (39) and R (39)		Daughter[a]: colon resection, age 14 years (spread small polyps) Son[a]: hepatoblastoma, age 2 years Mother: BrC, age 57 years
5	19076	M	1–15	45	Polyposis	S (45)		Sporadic: (10 sibs no abnormalities)
6	53045	F	1–15	ND				ND
7	50847	M	1–15	36	100–1000		DA and FGP (46)	Daughter[a]: >100 polyps at age 15 years and FGP at age 20 years, and DA at age 25 years
8	50846	M	1–5	38	>50	C (38)		Mother: numerous polyps, age 44 years Maternal uncle: 30 polyps, age 42 years Maternal aunt: >100 polyps, age 52 years Maternal aunt: >30 polyps, age 41 years Maternal aunt[a]: >100 polyps, age 48 years Maternal aunt[a]: 4–10 polyps, age 46 years Maternal grandmother: >100 polyps and CRC 2×, age 71 years Maternal great grand mother: <100 polyps and CRC, age 80 years Niece[a]: 10–100 polyps, age 22 years Niece: 10–100 polyps, age 32 years
9	57206	M	4–5	41	>100	R (41)	FGP and DA (41)	Parents both tested negative for the *APC* deletion
10	19038	M	6–15	27	70–100	—	FGP and DA (35)	Mother: numerous polyps, age 36 years Sister[a]: ±50 polyps, DA and FGP, and PC, age 41 years Son[a]: 10–50 polyps, age 15 years Brother[a]: 10s polyps age 27 years Niece[a]: polyposis coli at age 21 years and duodenal carcinoma at age 45 years Daughter[a]: 30 polyps, age 16 years Son[a]: >100 polyps, age 13 Nephew[a]: polyposis coli, age 19 years and DA and FGP
11	54262	M	7–13	45	Multiple/ 'AFAP'	CA (45)		Mother: multiple polyps (>10), age 45 years Aunt: CRC, age 50 years

Table 1 (*Continued*)

Patient no.	*Family ID*	*Sex*	*Exons deleted*	*Age at diagnosis*	*Polyp count*	*CRC (age)*	*Extracolonic features*	*Family history*
12	53344	M	7–13	39	±40	—		Mother: CRC at age 48 years and few polyps at age 50 years Aunt: multiple polyps and CRC, age 44 years Daughter: 1–10 polyps, age 36–48 years Uncle: 'polyps' and CRC, age 37
13	50557	F	9–15	25	Polyposis	—		Father and grandfather: FAP and CRC, ages unknown Brother[a]: FAP, epilepsy and learning disability Daughter[a]: no screening yet, learning disability
14	56071	F	9–15	27	100–1000	—	FGP and 1 DA (29)	Sporadic
15	53335	F	9–15	26	Numerous	—		Mother: CRC age 28 years
16	19026	M	11	36	50–100 polyps	—	DA and EC	Son[a]: age of diagnose 9 years (some polyps); age 16 years (numerous polyps and FGP) Brother: polyposis coli, age 25 years and CRC (R 56) Daughter[a]: operation age 28 years, unknown number of polyps Sister: >100 polyps, age 29 years and learning disability Daughter[a]: 50–100 polyps, age 15 years, stomach polyps and EC Sister: died at age 37, cause unknown Two daughters: numerous and multiple polyps, age 16 and 22 years, respectively Two grand daughters[a]: multiple and 10–100 polyps, age 16 and 14 years, respectively Sister: died at age 29 years, cause unknown Two sons: many and numerous polyps, age 16 and 17 years, respectively Two daughters[a]: many and 50–100 polyps, age 27 and between 13 and 20 years, respectively Grand daughter[a]: at age 15 years, no polyps Brother: died at age 42 years, cause unknown Son[a]: many/10s of polyps, age 28 years Grandson: multiple polyps, age 13 years Sister: died age at 40 years, cause unknown Two daughters[a]: polyposis coli and 'FAP', age 20 and 18 years, respectively Son: 'FAP', age 17 years Grand daughter: 'FAP', age 7 years Grand daughter[a]: 'polyposis coli', age 18 years More distant relatives: niece: numerous polyps, age 31 years and her son[a]: >50 polyps, age 20 years
17	56483	F	14	44	Multiple of 10s	R (44)		Sister[a]: polyposis coli and CRC (R), age 51 years Two sons: multiple polyps, age 21 and 26 years, respectively Mother and aunt: leukemia, age 41 years
18	52326	M	14 and 15	22	Polyposis	—		Father: CRC age 36 years
19	19082	F	15	30	Numerous	R (30)	BrC, age 40 and 47 years (adenocarcinoma and ductal), LuC (small cell) age 51 years	Father: stomach cancer, age 77 years Daughter[a]: age 22 years, numerous polyps Daughter[a]: five polyps at age 20 years and numerous polyps at age 22 years

BrC, breast cancer; C, caecum; CA, colon ascendens; CHRPE congenital hypertrophy of the retinal pigment epithelium; CRC, colorectal cancer; DA duenal adenomas/polyps; EC, epidermoid cysts; F, female; FAP, familiar adenomatous polyposis; FGP, fundic gland polyps; LuC, lung cancer; M, male; ND, no data; PrC, prostate carcinoma; R, rectum; S, sigmoid.
In all cases in which exon 1 was deleted, the promoter region was also deleted. Families of cases 8 and 12 have been reported previously.[38]
[a]*APC* deletion found.

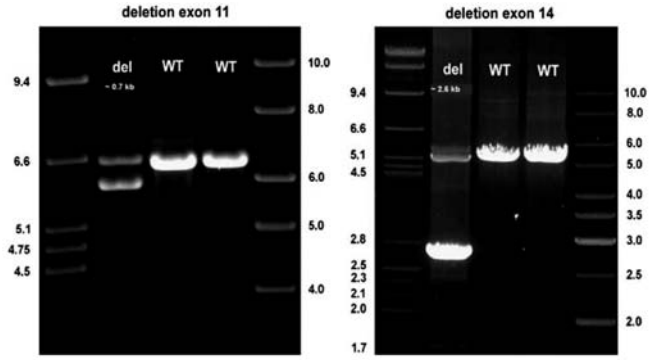

Figure 1 Two single-exon deletions (exons 11 and 14) confirmed by long-range PCR. An agarose gel shows the PCR products of two families with an exons 11 and 14 deletion, respectively (left), compared with controls (centre and right). In the patients, a smaller second fragment is also visible besides the normal product. WT, wild type; del, deletion.

RESULTS

APC deletions were detected in 19/296 index polyposis patients. Their clinical details and their family members are shown in Table 1. Deletions spanned the whole *APC* [1–7] gene in seven index cases, and clinical information was available for six of these; the remaining cases were classified as classic, but not severe. Cases with deletions of exons 9–15 (cases 13–15), exons 14–15 (case 18) and exon 15 (case 19) were also classified as classic. In four cases (9, 10, 16 and 17) with deletions of exons 4–5, 6–15, 11 and 14, no clear-cut phenotypic classification was possible. The index patients 9 and 10 developed less than 100 polyps, but they were young (27 and 20 years old, respectively) and were therefore classified as classic FAP. In cases 16 and 17, the index patients developed between 50 and 100 polyps at the ages of 36 and 44 years, respectively, but since a majority of family members showed a classic phenotype, both families were classified as classic. Two cases (11 and 12) with exons 7–13 deletion, and one case[8] with an exons 1–5 deletion had an apparently attenuated phenotype.

Five (1, 2, 5, 9 and 14) of 18 index patients (28%) were apparently *de novo* cases, because parents and siblings had no polyps or CRC; in one case, no family history was available. In five index cases (7, 9, 10, 14 and 16) and seven family members, extra colonic features such as duodenal adenomas, FGP and/or epidermoid cysts were diagnosed. Other FAP-related manifestations in family members included a deletion carrier with hepatoblastoma (case 4) and a deletion carrier with duodenal carcinoma (case 10). One index patient (case 19) developed breast cancer twice at age 40 and 47 years, histologically defined as an adenocarcinoma (no further details could be retrieved) and a ductal adenocarcinoma, respectively, and a small-cell lung cancer at age 51.

None of the families showed developmental disorders, which might be an indication of a deletion extending beyond the *APC* gene, although in case 13, two family members with an exons 9–15 deletion had a learning disability and one also had epilepsy.
Deletion of exons 7–13 is an in-frame deletion, because this region contains an exact threefold of nucleotides (corresponding with the codons), deletions of exons 4–5, 11 and 14 are out of frame. The in-or out-of-frame status in deletions including the promoter region and/or exon 15 cannot be predicted because it is not known to what extend these deletions reach.

DISCUSSION

We identified 19 large *APC* deletions in a MPLA screening of 296 polyposis patients in whom no *APC* and *MUTYH* mutations were detected: seven whole gene deletions, nine deletions of two or more exons and three single exon deletions. Most cases had classic polyposis, none could be classified as severe, and three families had AFAP. Remarkably, the 7–13 deletion found in two AFAP families is an in-frame deletion, and we therefore studied the literature to determine possible genotype–phenotype correlations in *APC* deletion carriers.
Up to August 2006, a total of 89 submicroscopic *APC* gene deletions, including this series of patients, were reported using MLPA, PCR and real-time PCR, quantitative PCR, polymorphic markers, and cDNA analysis.[14– 17,22,24 – 33] The reported deletions are summarized in Figure 2 and clinical details are given in table 2. Not in all index patients' clinical data were available, in 39 whole and 33 partial APC gene deletion cases, data were available for the presence or absence of CRC and extracolonic features. The age of diagnosis was reported in 27 cases with whole gene deletions.

Whole gene deletions

Including our cases, a total of 47 whole gene deletions have been reported in the literature. In most cases, the deletion included the promoter region. The mean age at diagnosis (as reported in 27 cases) was 32 years, ranging from 14 to 65 years, which is comparable to that reported by Friedl *et al*2 in classic FAP patients with *APC* mutations other than deletions (mean age: 30 years). Most cases with whole gene deletions can be classified as classic rather than severe, since the number of polyps in such carriers varies between 'multiple' and 2000 or profuse/numerous. Only one case with a whole gene deletion displayed a clearly attenuated phenotype,[32] ie, a proband with less then 70 polyps and CRC at an age of 65 years old. In six further whole gene deletion

cases, the number of polyps was described as 'multiple', which is generally reserved for a polyp count of less than 100, although patients were classified by the authors as 'typical' and 'FAP', probably because of their young age at presentation.[14,17]

Partial deletions

Including our cases, a total of 42 partial *APC* gene deletions have been reported in the literature. The most frequent deletions were exons 14 (n = 6), 14–15 (n = 4), 15 (n = 4), 8–15 (n = 4) and 9–15 (n = 4). Most cases with partial *APC* deletions were classified as classic FAP.

We found three index cases with an attenuated phenotype (8, 11 and 12), two of which had a previously unreported exons 7–13 deletion and one a 1–5 deletion. One obviously attenuated FAP case was reported by Su *et al* whose patient had an exon 15 deletion, 70–90 polyps at age of 42 years, and no other family members diagnosed with polyps before the age of 30.

Another deletion of exons 1–5 was reported by Aretz *et al*[14] in a patient with multiple colorectal polyps after the age of 31. Family members of this patient had a classic FAP phenotype, and the family was therefore classified as classic according to our definition. However, there were other index patients reported with deletions in exons 1–5 who did not show an attenuated phenotype: case (9) in our study had an exons 4–5 deletion but showed a classic FAP phenotype. A family reported by Aretz *et al*[14] with a single exon 1 deletion, including the promoter region, was also classified as classic. Renkonen *et al*[33] described a classic FAP family with an exon 4 deletion with an index patient who had 300 polyps at the of age 69 years.

The in-or out–of-frame status of the *APC* deletion could be relevant for the phenotype of *APC* deletion patients, as this is a well-known phenomenon in Duchenne and Becker dystrophies.[34] If the deletion is in-frame, a shorter but possibly functional *APC* product can be produced. The exons 7–13 deletion is an in-frame deletion, because this region contains an exact threefold of nucleotides (corresponding with the codons), possibly thereby explaining the milder phenotype. The in-or out-of-frame status in deletions including the promoter region and/or exon 15 could not be predicted. Without these regions, whether or not in-or out-of-frame, it is very unlikely that a functional *APC* product will be made. The promoter region is needed for starting transcription and exon 15 contains seven 20 amino acid repeats and three 15 amino acid repeats that are essential for regulating the β-catenin turnover and localization.[35,36] In our two AFAP families with a 7–13 deletion, the deletion was in frame. All the other partial deletions (without the promoter region or exon 15) reported here and in the literature were out frame.

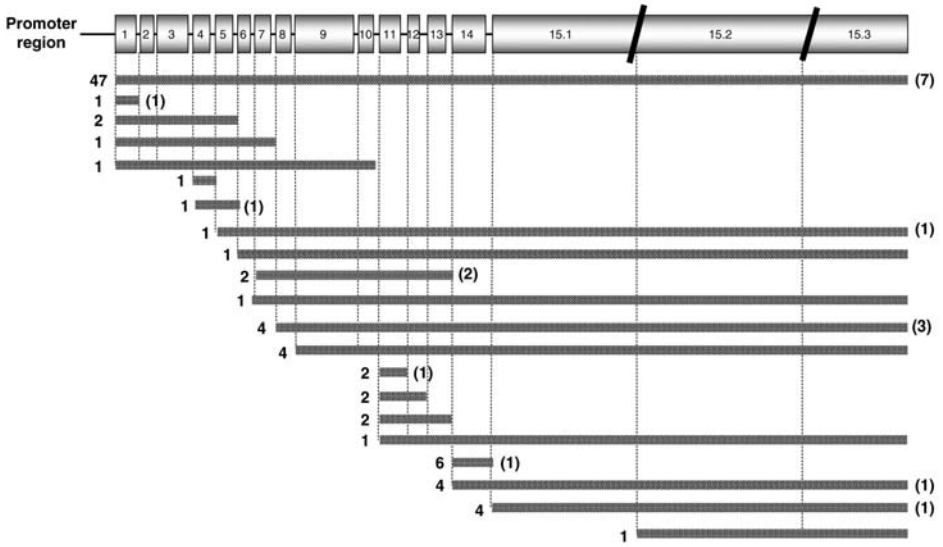

Figure 2 Exon deletions reported in our series and by previous studies.[14–17,22,24–33] The deletions found in this study are given between brackets. Deletions due to proven splice site mutations were not included. The exact breakpoint (in introns) of most deletions was not described exactly; the exonic deletions are shown here.

The diversity of the reported deletions does not indicate the presence of 'hot spot' breakpoints. We also found that the size of the exon 14 deletion in our study was larger than that reported by Aretz et (2.6 versus 2.0 kb), *et al*[14] indicating at least one different breakpoint. More breakpoints should be expected in large introns, but we did not find any relation between the length of the intron (ranging from 681 to 14 111 nucleotides (http://www.ensembl.org/, Gene ID ENSG00000134982) and the number of breakpoints (P = 0.4 for trend, Pearson's R). For this analysis, we included only studies that had looked for deletions in all the exons separately using MLPA.[14,17,24,28,29,33]

Colorectal Cancer

Almost half of our patients developed colon cancer (44%, 8/18), one patient with a whole gene deletion had two synchronous carcinomas, and in one patient no clinical data were available. Five of the nine carcinomas were located in the distal part of the colon (55%).

Combining our results with those from previous studies (see Table 2), we found no significant difference between partial and whole gene *APC* deletions for the occurrence of CRC. In 39 whole gene deletion cases with detailed clinical information, nine CRCs were found (23%) with a mean age of 40 years (range: 31–65). In 33 cases with one or more exon deletions – for whom detailed clinical information was reported – 8 (24%)

Table 2 Characteristics of all the index patients (one per family) reported with an *APC* deletion

Deleted exons (reference)[a]	*No. of patients*	*Age at diagnosis [range] [mean]*	*No. of polyps*							*CRC age [mean]*	*Extracolonic manifestations*
			50–100	*Multiple*	*100–1000 or 'polyposis coli*	*1000–2000*	*Profuse, numerous*	*'FAP'*	*Other*		
1–15 (A, 14–17,22, 24,26–33,40)	47	[14–65] [32] 8 ND	2 (age 24 and 65 years)	6 (age 14–46 years)	14 (14–45)	7 (18–39)	7 (19–55)	6 (ND)		31, 34, 34, 36, 38, 39, 42, 45, 65 [40]	CHRPE (3), desmoids (4), DA (6), FGP (5), osteomas (5), EC (2) and duodenal cancer (1)
1 (14)	1	25							'Several'		
1–5 (A, 14)	2	35, 38	1 (age 38 years)	1						36, 38	
1–7 (14)	1	27		1							
1–10 (14)	1	26									
4 (33)	1	69			1						DA and FGP (1)
4–5 (A)	1	41			1					41	DA and FGP (1)
5–15 (29)	1	25			1						Stomach polyps and DA, CHRPE
6–15 (A)	1	27		1							DA and FGP (1)
7–13 (A)	2	39, 45	2 (age 39 and 45 years)							45	
7–15 (17)	1	28			1						
8–15 (14,26)	4	21, ND, ND, ND			1			3			
9–15 (A)	4	25, 26, 27, 29			3		1			29	
11 (A, 40)	2	16, 36	1 (age 36 years)			1 (age 16)					FGP (1), DA and EC (1)
11–12 (14,30)	2	31, ND		1							
11–13 (28)	2	26, 37	1 (age 26 years)		1						
11–15 (14)	1	25					1				
14 (A, 14,24,31,40)	6	26, 43, 44, ND, ND, ND	1 (age 44 years)	1				1		44 and 1 case, age unknown	DA (1), desmoids (1) and duodenal cancer (1)
14–15 (A, 14,16)	4	15, 22, 23, 28			3						
15 (A, 26,30)	4	21, 30, 42, ND	1 (age 42 years)		1		1	1		30	DA, FGP and EC in one patient, BrC, LuC in one patient
15.2–15.3 (14)	1	40					1				

BrC, breast cancer; CHRPE, congenital hypertrophy of the retinal pigment epithelium; DA, duodenal adenomas/polyps; FGP, fundic gland polyps; LuC, lung cancer; ND, no data; ThC, thyroid cancer.
If no index patient was available, the family member with the most extensive clinical information is shown.
Methods: MLPA: (A) [14,17,24,28,29]; Linkage: [24,40]; (Semi) quantitative/double competitive PCR: [15,26,27]; Real-time PCR: [16,22,32]; cDNA analysis:[30,31,40].
[a]Refers to this study.

developed colon cancers with a mean age of 29 years (range: 29–45). The actual risk for developing CRC is most probably higher than this 23– 24%, since a large number of cases will probably have undergone colectomy at a younger age (ie < 30 years) to prevent the development of CRCs.

Extracolonic manifestations

We saw no essential difference between partial and whole gene *APC* deletions for the occurrence of extracolonic manifestations when we combined our results with those of previous studies (a total of 39 patients with whole *APC* gene deletions and 33 patients with partial exon deletions had available clinical data). Fourteen out of these 39 index cases (36%) had one or more extracolonic features, that is duodenal adenomas (n = 6), duodenal cancer (n = 1), FGP (n = 5), CHRPE (n = 3), desmoids (n = 4), osteomas (n = 5) and epidermoid cysts (n = 2). In 33 index cases with one or more exon deletions, nine (27%) developed one or more extracolonic features: duodenal adenomas (n = 7), duodenal cancer (n = 1), FGP (n = 5), stomach polyps (n = 1), CHRPE (n = 1), desmoids (n = 1) and epidermoid cysts (n = 2).

Consequences for surveillance

APC deletions show large intrafamilial as well as inter-familial variation in clinical phenotype, suggesting environmental and/or other genetic factors have a modifying influence. Consequently, advice for screening should not be based only on the extent and/or location of the mutation. As the mean age and range of CRC in patients with whole gene deletions is the same as for classic FAP patients, we advise screening according to FAP guidelines,[37] ie endoscopic colorectal screening every 2 years starting from age 10 to 12 years, and endoscopic screening of the upper gastrointestinal tract every 1–5 years from age 25 to 30 years, depending on the findings. In those families with two or more members with an apparently attenuated phenotype, screening should start in the late teens.[38,39] If there are more polyps than can be removed endoscopically, we advise performing a colectomy with ileorectal anastomosis, or a proctocolectomy with an ileoanal pouch anastomosis and follow-up screening of the rectum.

Conclusion

Using MLPA, we identified partial and total *APC* gene deletions in 6% (19/296) of patients who had previously undetected *MUTYH* and *APC* mutations; this represents 8% (19/242) of all the *APC* mutations found in our cohort of 599 polyposis patients. The majority of deletion patients displayed a classic polyposis phenotype, although we also identified three families with *APC* deletions (16%, 3/19) with a clearly attenuated FAP phenotype. We therefore recommend that MLPA should be used as a standard procedure for identifying *APC* mutations in polyposis patients with classic FAP as well as those with attenuated FAP.

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