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

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Chapter 2

Clinical phenotype

2.1

Multiplicity in polyp count and extracolonic manifestations in 40 Dutch patients with *MUTYH*-associated polyposis coli (MAP)

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Objective:

To investigate the contribution of *MUTYH*-associated polyposis coli (MAP) among polyposis families in the Netherlands, and the prevalence of colonic and extracolonic manifestations in MAP patients.

Methods:

170 patients with polyposis coli, who previously tested negative for *APC* mutations, were screened by denaturing gradient gel electrophoresis and direct sequencing to identify *MUTYH* germline mutations.

Results:

Homozygous and compound heterozygous *MUTYH* mutations were identified in 40 patients (24%). No difference was found in the percentage of biallelic mutation carriers between patients with 10–99 polyps or 100–1000 polyps (29% in both groups). Colorectal cancer was found in 26 of the 40 patients with MAP (65%) within the age range 21 to 67 years (median 45). Complete endoscopic reports were available for 16 MAP patients and revealed five cases with gastro-duodenal polyps (31%), one of whom also presented with a duodenal carcinoma. Breast cancer occurred in 18% of female MAP patients, significantly more than expected from national statistics (standardised morbidity ratio = 3.75).

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Conclusions:

Polyp numbers in MAP patients were equally associated with the attenuated and classical polyposis coli phenotypes. Two thirds of the MAP patients had colorectal cancer, 95% of whom were older than 35 years, and one third of a subset of patients had upper gastrointestinal lesions. Endoscopic screening of the whole intestine should be carried out every two years for all MAP patients, starting from age 25–30 years. The frequent occurrence of additional extraintestinal manifestations, such as breast cancer among female MAP patients, should be thoroughly investigated.

MAP (*MUTYH* associated polyposis) is an autosomal recessive disease characterised by the development of multiple adenomatous polyps and, eventually, colorectal cancer (CRC).[1] Carriers are homozygous or compound heterozygous for germline mutations in the mutY human homologue (*MUTYH*) gene, located on chromosome 1 between p32.1 and p34.3.[2] The MUTYH protein is a base excision repair (BER) glycosylase that is involved in the repair of DNA damage caused by the oxidation of a guanine leading to 8-oxo-7,8-dihydroguanine (8-oxoG).[3] In the process of replication it excises the adenine mispaired opposite the oxo-G. When the MUTYH protein is dysfunctional, G:C→T:A mutations can occur in genes such as *APC* and KRAS.[1 4]

Since the report by Al Tassan *et al* of germline biallelic *MUTYH* mutations in polyposis patients,[1] several clinical studies have provided additional insight into the clinical features of MAP.[5–8] The highest percentage (29%) of homozygous and compound heterozygous *MUTYH* mutations was found in patients with 15 to 100 adenomas.[5] In two series—with, respectively, 107 and 38 patients with classical FAP but without the *APC* mutation—biallelic *MUTYH* mutations were identified in 7% and 13% of the cases.[5 8] Accordingly, the majority of MAP cases were characterised by polyp counts in the range from 10 to 100. So far, six ''CRC only'' patients and one with more than 1000 polyps who carry biallelic *MUTYH* mutations have been described.[9–11]

Approximately 50% of the MAP patients develop CRC.[5 8 10] A few extracolonic manifestations—including duodenal adenomas (n = 3), osteoma (n = 2), congenital hypertrophy of the retinal pigment epithelium (CHRPE) (n = 4), and dental cysts (n = 1) [5] [7 8]—have been observed in MAP patients. In addition, one case of stomach cancer was reported in a 17 year old patient.[7]

It is likely that other reports focused mainly on the occurrence of colonic lesions. In this study we investigated the contribution of MAP among polyposis families in the Netherlands, and the prevalence of both colonic and extracolonic manifestations in MAP patients.

METHODS

We selected 170 index patients from all the patients with polyps referred for *APC* germline mutation analysis to the DNA Diagnostic Laboratory of Human and Clinical Genetics in Leiden from 1986 to 2002. Patients were recruited from throughout the Netherlands, the vast majority being of white northern European origin. We excluded those with a pathogenic *APC* or mismatch repair gene germline mutations, inadequate clinical data, or insufficient DNA quality for *MUTYH* mutation analysis. We did not exclude two

patients with the low penetrant I1307K mutation in the *APC* gene.[12] Informed consent was obtained for DNA testing according to protocols approved by local ethics review boards. Clinical and pathological data were obtained from patient records to confirm the diagnosis. The endoscopic reports were studied to assay the total number of polyps in the colon that the patient had developed over time (metachronous count). For practical reasons, the index patients were divided into four categories: less than 10 adenomas; between 10 and 100 adenomas; between 100 and 1000 adenomas; and more than 1000 adenomas. Unfortunately, the endoscopy and pathology reports for 29 index patients gave descriptions like ''polyposis'' or ''multiple'' rather than the exact number of adenomas.

Table 1 Primer sets for *MYH* gene mutation analysis

Exon	Purpose	Oligonucleotide sequence (5'-3')
1	Sequence	cggtgtacaacggaacttgt
		gacgtctgaacggaagttcg
2	Sequence	ccttggaaggcctcaaaat
		cctgaatctgcctttcatgg
2	DGGE	gccgcctgcagcccgcgcccccccgtgcccccgccccgcccccggcccgggcgccggtggatgagcggggagatagc
		gggcggggcgaatgagccttgggccacaac
3-4-5	Sequence	atactgccacaggctgct
		ctctcatctggggtctga
6-7-8	Sequence	ttggggtgggtgtagagaag
		agagggggccaaagagttagc
7	DGGE	gccgcctgcagcccgcgcccccccgtgcccccgccccgcccccggcccgggcgccgagggtgggggaaatagggaac
		gggcggggcgtataagacacccaagactcc
8	DGGE	gccgcctgcagcccgcgcccccccgtgcccccgccccgcccccggcccgggcgccgggtaaggggatggcaggogg
		ggctgggaggaaggaggctg
9-10-11	Sequence	gtgcccagcctccttcct
		agaactggaatggggcttct
10	DGGE	gccgcctgcagcccgcgcccccccgtgcccccgccccgcccccggcccgggcgccgggtgcttcaggggtgtctgc
		gggcggggcgcttctcactgcccccttcccc
12-13	Sequence	tctoggttggcccctaaagc
		aagggtcaagggggttcaaat
14	Sequence	cggaatagccaaggatgttg
		ttcatgtagaacatgtaggaaaacac
15	Sequence	tgaagttaagggcagaacacc
		actgatggctccacaattca
16	Sequence	gggaaaggggagagaggacaa
		caaaaataagcactttactaacaacag

Table 4 Results of MYH mutation analysis in relation to family history of polyposis patients

Number of polyps	Sporadic or autosomal recessive*		Autosomal dominant	
	Total	Biallelic	Total	Biallelic
1–9	5	None	27	None
10–99	37	17 (46%)	45	7 (16%)
100–1000	10	3 (30%)	14	4 (29%)
>1000	1	None	None	None
Polyposis	3	2 (67%)	13	4 (31%)
Multiple	7	2 (29%)	3	None
Unknown	1	None	1	None
Total	64	24 (38%)	103	15 (15%)

For assessing the mode of inheritance, first and second degree relatives were considered as patients if they had ⩾3 adenomas (or 2 at young age, ie, <40 years) and/or CRC. The family history was considered dominant if patients occurred in at least two generations, and recessive if patients were siblings. The family history for three index patients was not available and these three were not included in this table.
*26 patients with a recessive pattern of inheritance and 38 apparently sporadic cases with 9 and 15 biallelic MYH patients, respectively.

MYH mutation analysis

Polymerase chain reaction and denaturing gradient gel electrophoresis All DNA amplifications were carried out in a 25 µl volume containing 45 mM Tris HCl, pH 8.3, 11 mM $(NH_4)_2SO_4$, 45 mM $MgCl_2$, 0.2 mM of each dNTP, 110 µg/ml bovine serum albumin (BSA), 4 µM EDTA, 10% glycerol, 10 pmol of each primer, and 1 U polymerase and were subjected to 35 polymerase chain reaction (PCR) cycles (30 seconds at 94°C, 30 seconds at 55°C or 58°C, and 60 seconds at 72°C). Oligonucleotide sequences (table 1) were designed from intron–exon boundaries of the *MUTYH* gene using the DNA20 computer program (designed by HT Huisman, University of Groningen). For exons 2, 7, 8, and 10, DGGE analysis was undertaken and a 55 base pair (bp) GC clamp was present at the 59 of the forward oligonucleotide. Denaturing gradient gel electrophoresis (DGGE) analysis was carried out as described by Fodde *et al.*[13]

Direct sequence analysis

The PCR for the sequence analysis was carried out with oligonucleotide primers specific for the 16 coding exons of the *MUTYH* gene, extended with an M13 forward or reverse sequence. Some exons were combined into one fragment (table 1), and in all nine fragments covered the 16 coding exons of the *MUTYH* gene. The primer sequences are given in table 1. PCR products were sequenced on an ABI 3730 DNA analyser (Applied Biosystems, Foster City, California, USA) using their BigDye Terminator cycle sequencing ready reaction kit. The raw sequence data were analysed using SeqScape software for comparative sequence analysis (Applied Biosystems). Detected mutations were confirmed on a second, independent DNA sample when available.

Nucleotide numbering of the *MUTYH* gene was based on the sequence of the NCBI website (http://www.ncbi.nlm.nih.gov/, accession number NT_004852.14), changed according to published reports,1 with base 1 corresponding to the first base of the initiation codon, and the first 11 codons of exon 3 considered as non-coding). Splice prediction was carried out with Fruitfly and SpliceSiteFinder software, available from the internet: http://www.fruitfly.org/seq_tools/splice.html, http://www.genet.sickkids.on.ca/~ali/splicesitefinder.html.

Southern blot analysis of *MUTYH* was carried out for 95 of the 170 index patients and was done mainly as previously described.[14] We used HindIII and *Bg/II* digests, followed by hybridisation with two cDNA probes encompassing exons 1– 10 (*MUTYH* 59) and exons 10–16 (*MUTYH* 39), respectively.

Table 3 Mutations and clinical features of patients with MYH associated polyposis coli (MAP)

	Sex	Mutation 1	Mutation 2	Age at diagnosis (years)	Number of polyps	Location CRC	CRC stage	MSI	Age at CRC diagnosis (years)	Upper GI tract manifestations	Family history	Other cancer/other manifestations
1	M	Y165C	Y165C	65	10-100	C	B2		65	Duodenum carcinoma, 2 adenomatous polyps	Sporadic	
2	M	Y165C	Y165C	45	35	C	D	MSS	45		AD	Basal cell carcinoma skin (nose)
3	F	Y165C	Y165C	45	>25		D	MSS	45		Sporadic	
4	F	Y165C	Y165C	39	50-100	HF	B	MSS	39	NA	Sporadic	
5	F	Y165C	Y165C	33	50-100	SF	A		44	FGP and antrum; 20 polyps	Sporadic	
6	M	Y165C	Y165C	34	>100	HF; R/S	B1; B1	MSS	45; 68	Duodenal polyps	AD	
7	M	Y165C	Y165C	45	>100					Ulcus ventriculi	Sporadic	
8	M	Y165C	Y165C	36	>100	R; S	B; A		40; 40	Duodenal polyps	AD	
9	F	Y165C	Y165C	40	10-49						Sporadic	
10	M	Y165C	Y165C	46	Multiple						AR	Papillary urothelial carcinoma bladder age 60
11	M	Y165C	Y165C	41	Multiple	2C; 2AC; I	1 D, 4 NR		41; 41; 41; 41		Sporadic	Breast cancer (intracystic papillary carcinoma in situ) age 76 & Brc infiltrating ductal carcinoma, minor in situ component age 78 (unilateral)
12	F	Y165C	Y165C	54	10-100	R; AC; I; left	A or B; NR; carcinoid; NR	MSS	54; 57; 77; 77		AR	
13	M	Y165C	Y165C	49	Polyposis	S	B2		49	Barrett oesophagus with carcinoma	AD	
14	F	Y165C	Y165C	45	Polyposis	AC	D	MSS	45		AR, parents consanguineous	Basal cell carcinoma (chin) age 41
15	F	Y165C	G382D	54	10-100						AR	Breast cancer age 49, both sides (T1N0M0)
16	F	Y165C	G382D	59	>20	HF	B2	MSS	59		AD	
17	F	Y165C	G382D	58	30	C	Tis	MSS	58		AD	
18	F	Y165C	G382D	43	10-50	C; TC	B1; B2		43; 46	NA	Sporadic	
19	M	Y165C	G382D	52	>50	AC	C2	MSS		NA	AR	
20	M	Y165C	G382D	67	>100	AC	B		67	NA	AD	
21	F	Y165C	R233X	46	50-100				46		Sporadic	
22	F	Y165C	P391L	47	12						Sporadic	
23	M	Y165C	P391L	44	10-100			MSS		NA	AR	
24	M	Y165C	A371fs	37	10-100			MSS		NA	Sporadic	
25	M	Y165C	A371fs	42	Polyposis						AD	
26	F	Y165C	P391L	21	36	R	A	MSS	21	NA	AD	
27	F	G382D	P391L	44	50-100	S	B2	MSS	44		AD	
28	F	G382D	P391L	41	50-100			MSS			AR	
29	M	G382D	P391L	48	100-1000	C	A		48	Duodenal polyps	AD	
30	M	G382D	A371fs	42	10-50	DC	A or B		42	NA	Sporadic	I1307K APC mutation
31	M	G382D	G382D	45	>15	AC/C	A				Sporadic	
32	F	G382D	G382D	59	Polyposis				59		AD	
33	F	G382D	G382D	40	Polyposis						AD	
34	F	P391L	P391L	57	Polyposis	R	D		57		NR	Breast cancer age 55 (ductal carcinoma in situ with a micro-invasive component)
35	M	P391L	P391L	37	100-1000	C; AC	D; ?	MSS	37; 37		AR	
36	F	P391L	A371fs	40	Polyposis	R	B		42		AD	
37	M	P391L	891-3A→C	40	50-100	C	D		40		AD	
38	F	1185_1186dupGG	1185_1186dupGG	42	10-50	R	B	MSS	42		AD, parents consanguineous Moroccan	Cervix ca (squamous, HPV status unknown) age 27, Breast cancer (T1N0M0) age 49

Table 3 Continued

	Sex	Mutation 1	Mutation 2	Age at diagnosis (years)	Number of polyps	Location CRC	CRC stage	MSI	Age at CRC diagnosis (years)	Upper GI tract manifestations	Family history	Other cancer/other manifestations
39	M	Q377X	Q377X	37	110–120						Sporadic Turkish	
40	F	Y165C	649-1G→A	42	10–50						AR	
41	M	Y165C	not detected	46	Multiple	S			46		AD	
42	M	Y165C	not detected	52	10–50						AR	
43	M	G382D	not detected	47	100–1000	AC	A	MSS	44	NA	AD	
44	M	G382D	not detected	50	10–50			MSS		NA	Sporadic	Age 27 mesenteric desmoid tumour
45	F	P366T (UV)	not detected	35	2					NA	Sporadic	
46	M	D91N (UV)	not detected	42	30	R	A		42			

Mutations: Asp91Asn (c.271G→A), Tyr165Cys (c.494A→G), Arg233X (c.697C→T), Pro366Thr (c.1096C→A), Ala371fs (c.1105delC), Gln377X (c.1129C→T), Gly382Asp (c.1145G→A), c.1172C→T (Pro391Leu), Glu396fs (c.1185_1186dupGG).

AC, ascending colon; AD, autosomal dominant; AR, autosomal recessive; C, caecum; CRC, colorectal cancer; DC, descending colon; FGP, fundic gland polyposis; HF, hepatic flexure; I, ileum; MSS, microsatellite stable; NA, no abnormalities; NR, not reported; R, rectum; S, sigmoid colon; SF, splenic flexure; TC, transverse colon; Tis, carcinoma in situ; UV, unclassified variant.

Statistics

Differences in percentages were assessed by the χ^2 test. The F test was used for comparing age of diagnosis and percentages in more than two groups. A probability (p) value of <0.05 was considered as statistically significant. All tests were carried out with SPSS 11.01 (SPSS, Chicago, Illinois, USA). We calculated the expected number of breast cancer cases using rates for each five year calendar period and age group from the Dutch Cancer Registry.[15] SMR-exact was used to calculate the significance and (Poisson) confidence interval for the standardised morbidity ratio (SMR)—that is, the ratio between observed and predicted morbidity of breast cancer among the female *MUTYH* patients compared with breast cancer in the Dutch population (http://home. clara.net/sisa/ smrhlp.htm).

RESULTS

MYH mutation analysis

Of the 170 index patients analysed, 40 (24%) were found to carry (presumed) homozygous or compound heterozygous *MUTYH* germline mutations (table 2). In addition, we identified six carriers of a heterozygous mutation (3.5%)—four pathogenic mutations and two unclassified variants. Two index patients with biallelic *MUTYH* mutations had consanguineous parents (table 3). Southern blot analysis in 95 patients did not reveal any gross genomic rearrangements of the *MUTYH* gene. One novel missense change, P391L, was found in 14% of the patients with biallelic mutations. Two other novel nonsense mutations, Q377X and R233X, were found: the first in a homozygous patient of Turkish origin, and the second in a patient who was compound heterozygote for R233X and Y165C. We also identified one novel, c649-1G→A (IVS8) and one previously reported splice site mutation, c891+3A→C (IVS10), affecting the wild type splice acceptor and splice donor recognition sites of exons 8 and 10, respectively. Two further unclassified variants were found in patients where no additional *MUTYH* variant could be identified: D91N in exon 3, and P366T in exon 13, within a stretch of evolutionarily conserved and non-conserved residues, respectively. The pathogenicity of both substitutions is unclear from the evidence available.

Colorectal polyp multiplicity and cancer risk in MAP

Equal frequencies of biallelic *MUTYH* germline mutations were observed in patients with 10–99 (29%) and 100–1000 adenomas (29%) (table 2). No biallelic mutations were found in patients with 1–9 polyps or in a patient with more than 1000 polyps.

The mean age at diagnosis of MAP patients was 45 years, ranging between 21 and
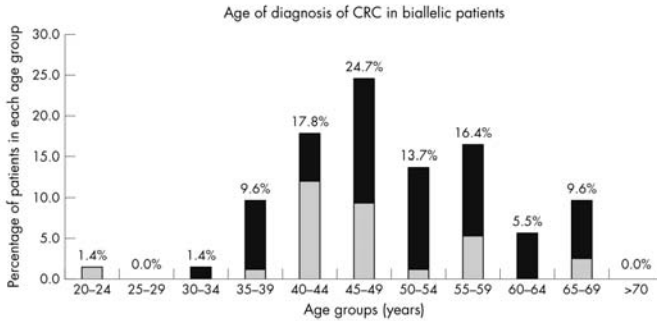
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Figure 1 Age at diagnosis of homozygous and compound heterozygous MAP patients with colorectal carcinoma (CRC). In all, 73 patients are included, 26 from our series (light bars) and 47 (dark bars) from other studies.[5, 7-9, 24, 25]

67 (median, 45). In most MAP patients, the adenomatous polyps were distributed throughout the colon.

Of the 40 patients with homozygous or compound heterozygous *MUTYH* mutations, 26 (65%) were diagnosed with CRC, in 130 patients without biallelic *MUTYH* mutations the percentage of CRC patients was 45% (59/130). In MAP patientsx CRC was found at first endoscopy in a majority of cases (24 of 26). Sixteen of the 26 CRC cases (62%) were right sided (that is, proximal to the splenic flexure). Two patients presented with two and one patient with five synchronous carcinomas (patients 9, 35, and 11, respectively). A second(metachronous) CRC was identified during follow up in two other patients, while a third patient developed three adenocarcinomas in the colon and a carcinoid tumour in the ileum metachronously (patients 7, 18, and 12, respectively, table 3). In all, 37 CRCs were found in 26 patients. The Dukes classification was variable: seven of the 26 cases were stage D (27%), five of which were right sided. Microsatellite instability (MSI) was analysed in CRCs from 16 index patients and all the 16 tumours tested showed stable microsatellites. The median age of onset of CRC in our series is 45 years, ranging from 21 to 67. Figure 1 shows the distribution of age of onset of CRC in MAP patients both in our series and those in published reports.

Thirty six patients with biallelic *MUTYH* gene mutations underwent bowel surgery at a mean age of 46 years (ranging from 21 to 67 years): 12 because of their high polyp counts, and 24 because of the presence of colorectal carcinoma. Thirty two patients underwent a total colectomy, carried out in one or more surgical procedures.

Extracolonic manifestations in MAP

Of 16 MAP patients who underwent upper gastrointestinal endoscopy, five (31%) showed an FAP associated extra-colonic manifestation, namely duodenal adenomas in four cases and gastric fundic gland polyposis in one. Duodenal carcinoma was diagnosed in one of the four patients with duodenal adenomas. The duodenal adenomas were diagnosed at a mean age of 52 years (range 36 to 65). One patient was reported to have

oesophageal cancer in the presence of a Barrett's oesophagus with dysplasia.

Of the 22 female MAP patients, four (18%) have been diagnosed with breast cancer. The first patient had a unilateral breast cancer twice, though with different histologies, diagnosed at age 76 and 78 years old (intracystic papillary carcinoma in situ and an infiltrating ductal carcinoma with a minor in situ component; patient 12). The second patient was diagnosed with breast cancer at age 55 years (ductal carcinoma in situ with a microinvasive component; patient 34). The third patient had bilateral breast cancer at age 49 years (pathology unknown; patient 15), and the fourth patient had breast cancer at age 49 years (pathology unknown; patient 38) but had also had cervical carcinoma at age 27 years (squamous cell carcinoma, human papillomavirus (HPV) status unknown).

Family history

Family history data were available for 167 of the index patients. In all, 103 index patients had a family history compatible with an autosomal dominant pattern of inheritance (that is, more than one generation with either polyposis or CRC), whereas 64 cases suggest recessive inheritance. The frequency of biallelic *MUTYH* mutations in these two groups is shown in table 4. We were able to obtain family history data for 39 of the 40 patients with biallelic *MUTYH* mutations, and we identified a total of 164 siblings and 78 parents. Of 92 siblings examined, 29 (32%) were found to have polyps or colorectal carcinomas. Of 78 parents, five were diagnosed with CRC, four with polyps, while endoscopies revealed no abnormalities in six parents.

Table 4 Results of MYH mutation analysis in relation to family history of polyposis patients

Number of polyps	Sporadic or autosomal recessive*		Autosomal dominant	
	Total	Biallelic	Total	Biallelic
1–9	5	None	27	None
10–99	37	17 (46%)	45	7 (16%)
100–1000	10	3 (30%)	14	4 (29%)
>1000	1	None	None	None
Polyposis	3	2 (67%)	13	4 (31%)
Multiple	7	2 (29%)	3	None
Unknown	1	None	1	None
Total	64	24 (38%)	103	15 (15%)

For assessing the mode of inheritance, first and second degree relatives were considered as patients if they had ≥3 adenomas (or 2 at young age, ie, <40 years) and/or CRC. The family history was considered dominant if patients occurred in at least two generations, and recessive if patients were siblings. The family history for three index patients was not available and these three were not included in this table.
*26 patients with a recessive pattern of inheritance and 38 apparently sporadic cases with 9 and 15 biallelic MYH patients, respectively.
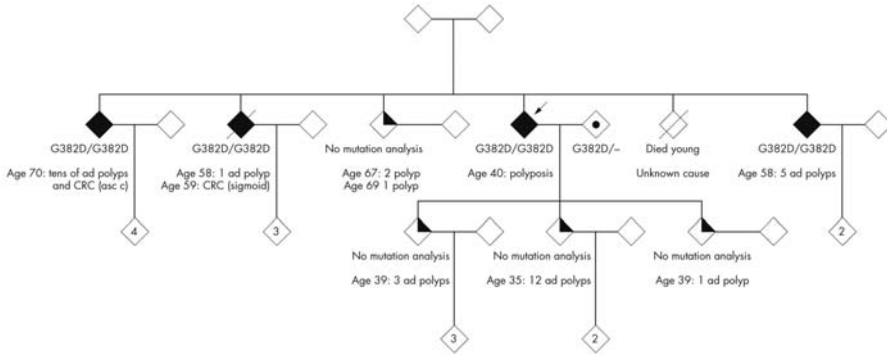
Figure 2 Pseudodominant inheritance pattern in an MAP (*MYH* associated polyposis coli) pedigree (patient 33). ad, adenomatous; asc, ascending colon; CRC, colorectal carcinoma.

DISCUSSION

In this study we describe the largest series of patients with homozygous and compound heterozygous mutations in the *MUTYH* gene reported so far. We found that in the Dutch population, MAP polyp counts are equally associated with the attenuated and florid forms of polyposis coli. This is in sharp contrast with previous studies in which the yield of the biallelic *MUTYH* mutation carriers was higher in patients with 10–100 polyps than in those with 100–1000 polyps.[5 8] As in these and other studies, we investigated patients who previously tested negative for the presence of germline *APC* mutations. Hence we assume that the actual prevalence of *MUTYH* biallelic mutations among polyposis patients is lower. Considering that *APC* mutations are detected in approximately 60–80% of classical FAP patients and in 10–30% of attenuated FAP patients, *MUTYH* mutations are likely to account for about 10% and 20–25% of patients in these groups, respectively.[16 17]

MYH mutation spectrum
DGGE and direct sequencing identified 40 biallelic germline *MUTYH* gene point mutations in 170 index patients. Apparently, larger genomic rearrangements do not play an important role in MAP as Southern blot analysis in a subset of patients did not reveal any deletions or duplications.

In our series we found a relatively high frequency (14%) of a previously unreported mutation, P391L in exon 13, which may suggest a founder mutation. Although haplotype analysis was not carried out, P391L carriers were found in different regions of the central and southern parts of the Netherlands and are apparently unrelated. Population specific *MUTYH* mutations have already been reported in India (E466X)[7] and Italy (c1395_1396delGGA).[8]

MAP colonic manifestations

CRC was found in 65% of biallelic *MUTYH* mutation carriers. The mean age of onset was 47 years. One case (patient 26) presented with CRC at age 21 and is the youngest MAP patient with CRC reported so far. Figure 2 shows that the vast majority of MAP patients (95%) develop CRC after the age of 35 years. In contrast with the findings of Lipton *et al*, who reported 71% (17/24) of the carcinomas being confined to the left side of the colon,[4] we found CRC in our MAP patients was predominantly right sided (62%). The percentage of Dukes stage D colorectal cancers found in MAP patients (27%) is higher than the percentage found in general Dutch CRC patients (19%).[18] The latter observation can partly be explained by the fact that five of these CRC patients had not been recognised with polyposis before, and their CRCs were right sided, which may explain their late symptoms.

Extracolonic manifestations

We found a relatively high percentage (31%) of upper gastrointestinal tract manifestations in MAP patients who underwent endoscopy (15). One patient (No 1) was found to have a duodenal carcinoma. To date, clinical data of approximately 100 MAP patients have been described and only three cases of duodenal adenomas without duodenal carcinoma have been reported.[5 7] Unfortunately, the number of MAP patients who underwent upper gastrointestinal screening was not reported. As endoscopy of the upper tract is not routinely carried out in patients with 10–100 polyps, 3% is most likely to represent an underestimation. Among FAP patients, the cumulative incidence of duodenal adenomas is 90% and 4.5% developed duodenal carcinomas.[19] In attenuated FAP, duodenal adenomas are seen often and duodenal cancer has been described in a few cases.[20]

The percentage of cases of breast cancer found in female MAP patients was 18% (4/22), of whom two had double breast cancer (bilateral and unilateral). The SMR for developing breast cancer for female MAP patients compared with the Dutch population is significantly higher than 1 (3.75, 95% confidence interval, 1.02 to 9.57; $p = 0.019$). Unfortunately, the prevalence of double tumours in the Dutch population is unknown and this factor could not therefore be included in the calculation. To exclude the possibility that polyposis patients develop breast cancer for other reasons, we also calculated the SMR in female polyposis patients without *MUTYH* mutations (n = 48). A nonsignificant SMR of 0.88 was found (95% confidence interval, 0.11 to 3.16; $p = 0.4$). It is noteworthy that the BRCA1 and BRCA2 tumour suppressor proteins, loss of function of which predisposes to breast or ovarian cancer, participate in the base excision repair of 8-oxo-G lesions.[21] Accordingly, loss of BER function owing to biallelic *MUTYH* mutations may underlie the increased breast cancer risk observed among

Dutch MAP patients. Moreover, *MUTYH* knock-out mice that also carry heterozygous *APC* mutations are more prone to develop mammary tumours than *APC*-heterozygotes only.[22]

MAP family history

As expected, more MAP patients were identified in cases with a family history compatible with an autosomal recessive mode of inheritance or apparently sporadic presentation (i.e. 24/64, 38%) than in dominant cases (15/103, 15%) (p = 0.001) (table 4). Given an estimated frequency of heterozygotes in the population of 1–2%,[19 10] pseudodominant inheritance of MAP is not uncommon, as illustrated in patient 33's pedigree (fig 2). Polyps or CRC were found in 32% of the siblings screened, which is again in agreement with an autosomal recessive mode of transmission.

Genotype–phenotype correlations

In E coli it has been found that the corresponding mutation of Y165C (Y82D) has a more deleterious effect on the rate of adenine removal than the corresponding mutation of G382D (G253D).[1] In addition, heterozygous Y165C mutation carriers are associated with a higher risk for CRC than G382D heterozygotes.[9] In this study, we detected a non-significantly earlier age of onset of colorectal polyps in patients homozygous for *MUTYH* Y165C compared with G382D and all other biallelic carriers (data not shown). Combining our data with 93 MAP patients reported worldwide, age at diagnosis of homozygous Y165C mutation carriers was on average five years earlier than all other biallelic mutation carriers (p = 0.011; 95% confidence interval, 1.1 to 8.8). [5 7 8 10 11 23] However, there was no significant difference in age of onset between homozygotes for Y165C and G382D. We found no other significant differences among the three groups relative to polyp multiplicities or CRC risk.

Conclusions

We show that patients with a polyp count compatible with either the classical or attenuated form of polyposis coli should be screened for the presence of *MUTYH* mutations. As the vast majority of MAP patients presents with CRC from the age of 35 years and there is a relatively high prevalence of lesions of the upper gastrointestinal tract, we recommend endoscopic screening of the lower and upper gastrointestinal tracts every two years for MAP patients from 25 to 30 years of age. Limited numbers of colonic adenomas may initially be treated by polypectomy, but in patients with large numbers of polyps a subtotal colectomy is indicated. The relatively high prevalence of breast cancer warrants further exploration of the involvement of *MUTYH* in breast cancer.

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