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MUTYH-associated polyposis: 70 of 71 patients with biallelic mutations present with an attenuated or atypical phenotype

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To determine the frequency, mutation spectrum and phenotype of the recently described autosomal recessive *MUTYH*-associated polyposis (MAP), we performed a systematic search for *MUTYH (MYH)* mutations by sequencing the complete coding region of the gene in 329 unselected *APC* mutation-negative index patients with the clinical diagnosis of familial adenomatous polyposis (FAP) or attenuated FAP (AFAP). Biallelic germline mutations in *MUTYH* were identified in 55 of the 329 unselected patients (17%) and in another 9 selected index cases. About one-fifth (20%) of the 64 unrelated MAP patients harboured none of the 2 hot-spot missense mutations Y165C and/or G382D. Including 7 affected relatives, almost all MAP patients presented with either an attenuated (80%) or with an atypical phenotype (18%). Fifty percentage of the MAP patients had colorectal cancer at diagnosis. Duodenal polyposis was found in 18%, thyroid and stomach cancer in 1 case, other extraintestinal manifestations associated with FAP were not observed. In 8 families, vertical segregation was suspected; in 2 of these families, biallelic mutations were identified in 2 generations. Monoallelic changes with predicted functional relevance were found in 0.9% of the 329 patients, which is in accordance with the carrier frequency in the general population. In conclusion, biallelic *MUTYH* mutations are the underlying genetic basis in a substantial fraction of patients with adenomatous polyposis. The phenotype of MAP is best characterised as attenuated or atypical, respectively. Colorectal surveillance starting at about 18 years of age is recommended for biallelic mutation carriers and siblings of MAP patients, who refuse predictive testing.

Key words: *MUTYH*-associated polyposis; MAP; *MYH*; *MUTYH*; FAP; APC; multiple colorectal adenomas

Highly penetrant tumour predisposition syndromes associated with adenomatous polyps contribute to approximately 5% of colorectal cancers (CRC) and are mainly subdivided into 2 entities: *familial adenomatous polyposis* (FAP) (OMIM 175100), caused by germline mutations in the tumour suppressor gene *APC*, and *hereditary nonpolyposis colorectal cancer* (HNPCC, Lynch syndrome) (OMIM 114500), caused by germline mutations in DNA mismatch repair (MMR) genes such as *MLH1*, *MSH2* and *MSH6*. Both the conditions are autosomal dominant diseases and accompanied by a family history of early onset CRC; in typical cases, the germline mutation detection rate is high (70–90%).[1–4] In HNPCC, usually only few adenomas occur, and tumours exhibit high microsatellite instability.[5] Typical FAP is characterised by the appearance of hundreds to thousands of colorectal adenomas, which usually arise within the second decade of life and become symptomatic during the third decade, often associated with duodenal polyposis and extraintestinal manifestations.[6,7] Patients with fewer (10–100) colorectal adenomas obviously represent a heterogeneous and yet poorly characterised group between FAP and HNPCC. The phenotype is often referred to as attenuated FAP (AFAP) or multiple colorectal adenomas (MCA); however, *APC* germline mutations were detected in only 20–30% of AFAP cases.[8–10] Both adenomas and CRC occur later than in typical FAP, extraintestinal lesions are uncommon, and most cases are sporadic.

MUTYH-associated polyposis (MAP) (OMIM 608456) is a recently discovered autosomal recessive precancerous condition of the colorectum, which is caused by germline mutations in the base excision repair (BER) gene *MUTYH* (*MYH*), the human homolog of the *E. coli mutY* gene (Human Gene Mutation Database (HGMD): http://uwcmml1s.uwcm.ac.uk/uwcm/mg/search/9315115.html).[11] *MUTYH* is located on chromosome 1p35 and consists of 16 exons encompassing 1608 bp (GenBank: U63329.1) or 1641 bp (GenBank: NM_012222.1). It encodes a protein that is responsible for the excision of adenosine mismatched with 8-oxo-7,8-dihydroxy-2′-deoxyguanosine (8-oxoG), the most stable product of DNA damage caused by reactive oxygen species. Biallelic mutations in this highly conserved enzyme increase 8-oxoG–induced somatic G:C>T:A transversions in other genes, including *APC*. No germline mutations have been detected in the 2 other members of the BER pathway, *OGG1* or *MTH*.[11–13]

Biallelic germline mutations in the *MUTYH* gene were found to correlate with a predisposition to MCA and carcinomas. The missense mutations Y165C and G382D are the most frequently observed disease-causing mutations. To date, the impact of *MUTYH* mutations has been examined in patients with different phenotypes including FAP, MCA, HNPCC and sporadic CRC.[12,14–21] However, several reports are restricted to the mutational hot spots and to selected patient samples; thus, the results vary considerably depending on inclusion criteria, methods used for mutation detection and ethnic background of the patients. Accordingly, there is still limited information about the general frequency of *MUTYH* mutations among patients with MCA. The spectrum of clinical manifestations, the risk of developing CRC and the consequences with respect to surveillance and recommendations for mutation screening are yet unclear. Here, we present results of a comprehensive mutation screening for the *MUTYH* gene in 329 German patients with clinically suspected FAP or AFAP, in whom no APC mutation was detected, and discuss the clinical implications.

Material and methods

Patients

Since 1991, blood samples from around 1,170 apparently unrelated patients with the clinical diagnosis of typical *familial adenomatous polyposis* (FAP) or attenuated FAP (AFAP) have been referred to the Institute of Human Genetics, University of Bonn for mutation analysis in the *APC* gene.[22] Of these, 660 unrelated patients (see Fig. 1 for phenotype distribution) were screened extensively for germline mutations in the *APC* gene by the protein truncation test (PTT) for mutations in exon 15, denaturing high performance liquid chromatography (DHPLC) for mutations in exons 1–14 and the first 400 base pairs of exon 15, and multiplex

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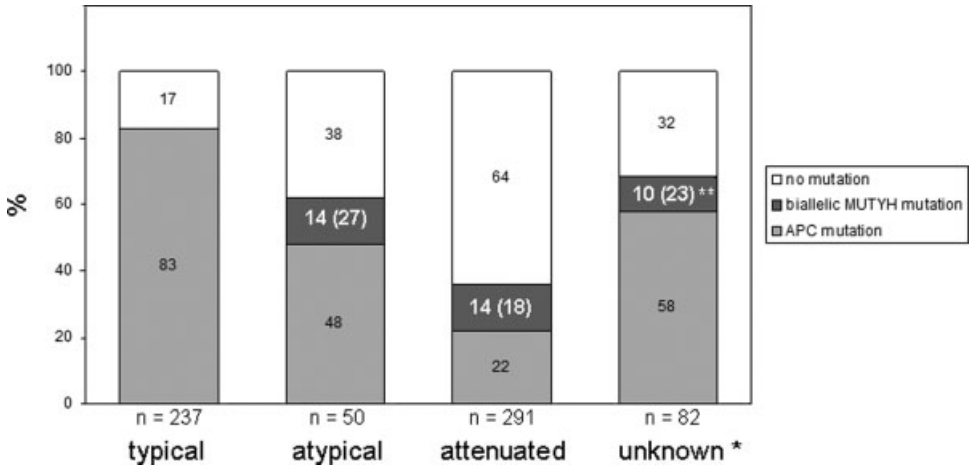

FIGURE 1 – *APC* and biallelic *MUTYH* mutation detection rates in 660 unrelated polyposis patients grouped according to the polyposis phenotype. The numbers in brackets indicate the incidence of biallelic *MUTYH* mutations in the APC mutation-negative patients of the corresponding polyposis phenotype. *Because of lack of clinical information, a classification of the polyposis subtype (typical, attenuated, atypical) was impossible, when *MUTYH* examination was started. **For the 8 patients with biallelic *MUTYH* mutations of this group subsequently clinical information was obtained, 7 were classified as attenuated and 1 as atypical.

ligation-dependent probe amplification (MLPA) for the presence of large genomic deletions, as described.[10,23] A pathogenic *APC* mutation was detected in 331 patients. The overall mutation detection rate was 50%; the highest incidence was found in patients with typical FAP (83%) and the lowest in the attenuated phenotype (22%) (Fig. 1).

To determine the incidence of *MUTYH* germline mutations in APC mutation-negative polyposis, screening of the *MUTYH* gene was performed in the remaining 329 unrelated *APC* mutation-negative index patients, who were not selected with regard to polyposis subtype (typical, atypical, attenuated) and assumed mode of inheritance, and in 116 normal controls (healthy anonymous German blood donors).

To describe the mutation spectrum and the phenotype pattern, additional biallelic *MUTYH* carriers identified in unrelated *APC* mutation-negative polyposis patients selected by phenotype (attenuated or atypical polyposis) or family history (consistent with autosomal recessive inheritance) and affected relatives with biallelic *MUTYH* mutations were included. The study was approved by the ethics committee of the University of Bonn.

Phenotype classification

All patients were referred for mutation analysis in the *APC* gene because of an adenomatous polyposis. Clinical information was obtained during genetic counselling sessions, from a questionnaire, or through telephone interviews and/or medical records. The classification of different colorectal polyposis phenotypes was based on the number of colorectal adenomas, age at diagnosis of polyposis and occurrence of colorectal cancers (CRC). The polyposis phenotype was classified as typical when the patient presented with more than 100 colorectal adenomas before 35 years of age; in the case of unavailable or unclear colonoscopic data, the classification was based on the occurrence of clinical symptoms before 35 years of age. The diagnostic criterion for the attenuated phenotype (AFAP) was the occurrence of a smaller number of adenomas (10–100) after 25 years of age or more than 100 polyps diagnosed for the first time after 45 years of age, respectively. When the polyp number was unknown, AFAP was assigned if first symptoms or diagnosis of CRC occurred after 45 years of age. The phenotype was classified as *atypical* in patients who meet the criteria neither for typical nor AFAP, so that an unambiguous attribution was impossible. Patients with atypical course usually present with more than 100 polyps, diagnosed between 35 and 45 years of age; in addition, cases with an obvious discrepancy between age at diagnosis (symptoms) or the occurrence of CRC and number of colorectal adenomas were considered as atypical (*e.g.* patient no. 26 with few adenomas but CRC at 29 years of age, or patient no. 1,114 with around 1,000 polyps, diagnosed not until 68 years of age). If clinical information on the colorectal disease was not available, the phenotype was denoted as unknown.

Screening for germline mutations in the MUTYH *gene*

Genomic DNA was extracted from peripheral EDTA-anticoagulated blood samples, according to the standard salting-out procedure. Screening for *MUTYH* mutations was performed by amplifying and sequencing the whole coding region (exons 1–16) and the flanking exon–intron boundaries of the *MUTYH* gene on an ABI 3100 automated sequencer (Applied Biosystems, Darmstadt), using the cycle sequencing procedure and the BigDye terminator kit version 1.1 (primers and PCR conditions are available upon request). To allow comparison of results, we applied the description of the coding sequence used by Al-Tassan *et al.*[11] (GenBank accession: U63329.1) for mutation description and not the actual reference sequence (GenBank: NM_012222.1), which has 11 additional codons in exon 3. All mutations were confirmed by a second independent PCR reaction. Informed consent was obtained from all the patients examined.

Statistical analysis

The statistical comparison of features (frequency of biallelic *MUTYH* mutations, age at diagnosis) was performed using the Fisher's exact test for categorical variables or the Student's *t* test for continuous variables. A p value of <0.05 was considered to be statistically significant.

Results

Frequency and spectrum of MUTYH *mutations*

We screened the whole coding region of the *MUTYH* gene for point mutations in 329 unrelated patients with clinically diagnosed adenomatous polyposis, in whom no germline mutation in the *APC* gene had been identified. These patients were not selected according to family history or polyposis subtype (typical, atypical, attenuated). The mean age at diagnosis was 44 years (range, 5–76 years). The majority of patients (227; 69%) was classified as AFAP.

Overall, biallelic germline mutations in the *MUTYH* gene were detected in 55 of the 329 patients (17%). The incidence clearly depends on the polyposis subtype: in the attenuated phenotype, biallelic mutations were identified in 18% (40/227) and in the atypical phenotype in 27% (7/26); no biallelic mutation was found in 41 cases with typical course (Fig. 1).

Another 9 patients with biallelic *MUTYH* mutations were identified in 26 families selected by phenotype or family history. These patients were included in the evaluation of the mutation spectrum and phenotype (Table I). The mutation spectrum encompassed 21 different mutations, 7 of which are first described to our knowledge (Table II). All but one (V479F) of the missense variants were located at highly conserved sites, occurred together with

TABLE I – BIALLELIC *MUTYH* MUTATIONS AND THE CORRESPONDING CLINICAL PHENOTYPES IN 71 PATIENTS WITH BIALLELIC MUTATIONS (64 INDEX PATIENTS AND 7 AFFECTED RELATIVES)

FAP No.	Patient	*MUTYH* mutations	Polyposis[1] phenotype	Age at diagnosis (years)	Number of colorectal adenomas at diagnosis	Colorectal adenoma distribution	CRC (site and age at diagnosis in years)[2]	Assumed mode of inheritance[3]	Family history
26	Index[4]	Y165C; p.466delE	Atypical	29	Few	Irregular	29 (S, D)	R	
26	Sister	Y165C; p.466delE	Attenuated	31	1 at 31 y and 25 at 48 y	Mainly proximal	No	R	
26	Sister	Y165C; p.466delE	Typical	33	200	Mainly distal	33 (R)	R	
94	Index[4]	Y165C; G382D	Attenuated	68	>100	Mainly proximal	No	S	
189	Index[4]	Y165C; p.466delE	Attenuated	47	>100	Diffuse	47 (R)	S	
213	Index[4]	G382D; G382D	Atypical	72	Hundreds		72	R	
213	Brother	G382D; G382D	Attenuated	71	20	Distal	71 (D)	R	
370	Index[4]	Y165C; c.1105delC	Atypical	25	>100 at 37 y	Mainly proximal	No	S	
395	Index[4]	G382D; P391L	Attenuated	37	20–50 at 49 y	Diffuse	No	S	
489	Index[4]	Y165C; G382D	(Attenuated)	36	Multiple at 49 y	Diffuse	No	R	
489	Sister	Y165C; G382D	Attenuated	45	<100		No	R	
521	Index[4]	Y165C; V479F	Atypical	44	Multiple (in part dense)	Diffuse	44 (C, T)	R	Affected brother (30–40 adenomas) mother with CRC
548	Index[4]	R231H; c.1105delC	Atypical	36	100–200			D/S	
620	Index	G382D; G382D	Attenuated	60	>100	Diffuse		R	
641	Index	Y165C; c.1105delC	Atypical	31	<100	Proximal and distal	No	S	
659	Index	Y165C; c.891+3A>C	Attenuated	52				S	
660	Index	Y165C; G382D	Attenuated	46	Multiple	Proximal and distal	46 (C)	R	3 affected siblings
676	Index	G382D; G382D	Attenuated	38	30–40		No	S	
693	Index	c.891+3A>C; p.466delE	Attenuated	45	30	Proximal and distal	No	R	2 affected brothers (CRC 34 and 54 years)
698	Index	Y165C; Y165C	Attenuated	53	Multiple	Mainly proximal	49 (R, A)	S	
719	Index	Y165C; Y165C	Attenuated	47	Multiple		47 (T)	U	
757	Index	Y165C; c.891+3A>C	Attenuated	40	50–100	Mainly proximal	No	S	
760	Index	G382D; G382D	Attenuated	55	Numerous	Mainly distal	57 (S, D)	S	
774	Index	G382D; G382D	Attenuated	48	20–50		No	R	2 sisters CRC (44 and 63 years)
786	Index	G382D; G382D	Attenuated	39	30	Mainly proximal	No	S	
787	Index	G382D; G382D	Attenuated	51	30–50	Proximal and distal	No	S	
810	Index	Y165C; Y165C	Attenuated	54				D	Father and cousin affected?
818	Index	Y165C; G382D	Atypical	37	>100	Diffuse	No	R	Affected brother
826	Index	Y165C; c.891+3A>C	(Attenuated)	28	Multiple at 37 y		No	R	Affected sister, uncle CRC (50 years)
848	Index	c.1105delC; p.466delE	Attenuated	38	<40	Mainly proximal	38 (C)	R	Affected brother
852	Index	R168H; G382D	Attenuated	49	50	Mainly proximal	No	R	Affected sister (5 adenomas, CRC 49 years)
858	Index	Y165C; p.263insAG	Attenuated	49	>100	Irregular	49 (R, C)	S	
872	Index	Y165C; Y165C	Attenuated	36	50–100	Diffuse	No	S	
885	Index	P143L; G382D	Attenuated	46	>100	Diffuse	46 (R)	D/S	Mother and grandfather CRC
914	Index	R83X; P391L	Attenuated	51	80	Proximal and distal	51 (R)	S	
925	Index	Y165C; Y165C	Attenuated	48	36		48 (C, S)	U	
957	Index	p.466delE; p.466delE	Attenuated	49				R	Affected brother
966	Index	Q377X; Q377X	Attenuated	51	40	Diffuse	51 (R)	D/R	Sister and father CRC
973	Index	Y165C; G382D	Attenuated	50	20–30	Proximal	No	R	Affected brother (CRC with 47 years)
973	Brother	Y165C; G382D	Attenuated	43	20		43 (R)	R	
979	Index	G382D; G382D	Attenuated	30	Some tiny at 30 y and 50–100 at 52 y	Mainly proximal	No	S	

TABLE 1 – BIALLELIC *MUTYH* MUTATIONS AND THE CORRESPONDING CLINICAL PHENOTYPES IN 71 PATIENTS WITH BIALLELIC MUTATIONS (64 INDEX PATIENTS AND 7 AFFECTED RELATIVES) (CONTINUED)

FAP No.	Patient	*MUTYH* mutations	Polyposis[1] phenotype	Age at diagnosis (years)	Number of colorectal adenomas at diagnosis	Colorectal adenoma distribution	CRC (site and age at diagnosis in years)[2]	Assumed mode of inheritance[3]	Family history
982	Index	Y165C; Y165C	(Attenuated)	39			54 (R)	R	
994	Index	P281L; p.466delE	(Atypical)	39	Multiple		39	U	
1003	Index	c.1105delC; G382D	Attenuated	57	>100	Diffuse	57 (R)	S	
1035	Index	R168H; p.466delE	(Attenuated)	44				U	
1062	Index	Y165C; G382D	Attenuated	48	Multiple	Mainly proximal	48 (C)	S	
1065	Index	Y165C; Y165C	Attenuated	42	Multiple	Mainly proximal	42 (R)	D	Affected son
1065	Son	Y165C; Y165C	Attenuated	28	<50	Diffuse	No	D	
1068	Index	R260W; c.1476+2T>C	Attenuated	60	50–100	Mainly proximal	No	U	
1077	Index	Y165C; G382D	Attenuated	44	Multiple	Mainly proximal	No	D	Affected father
1077	Father	Y165C; G382D	Attenuated	64	<100		64 (R)	D	
1083	Index	Y165C; Q324X	Atypical	24	<100 (35 removed)		No	R	Affected brother
1086	Index	p.137insIW; p.137insIW	(Attenuated)	40	Numerous	Proximal and distal	No	S	
1087	Index	Y165C; Y165C	Attenuated	55				U	
1111	Index	Y165C; Q377X	Attenuated	49	30–50	Diffuse	No	U	
1114	Index	R231H; c.1105delC	Atypical	68	1000		68 (A)	R	2 siblings with CRC
1125	Index	Y165C; G382D	Attenuated	57			57	U	
1126	Index	Y165C; G382D	Attenuated	38	25–30	Diffuse	38 (C)	D/S	Father CRC with 58 years
1175	Index	Y165C; R231H	Atypical	36	100–150	Diffuse	No	S	
1180	Index	Y165C; G382D	Attenuated	35	1 at 35 y and >100 at 47 y	Mainly proximal	47 (A)	R	Affected brother
1211	Index	c.1105delC; G382D	Atypical	38	Many small at 51 y	Diffuse	38 (R)	U	
1222	Index	R231H; G382D	Attenuated	52	40–60	Proximal	No	U	Father CRC with 60 years
1229	Index	G382D; G382D	Attenuated	63	Multiple	Proximal	63 (C, A)	S	
1241	Index	Y165C; G382D	Attenuated	52	20–30	Diffuse	No	D	Affected brother and father
1257	Index	Y165C; Y165C	Attenuated	48			48 (A, R)	U	
1258	Index	Y165C; Y165C	Attenuated	49	50–100	Proximal	49 (A)	S	
1260	Index	Q196X; c.1105delC	Attenuated	45	>50		No	S	
1286	Index	Y165C; P391L	Attenuated	39	5 at 39 y and 20 at 42 y	Proximal	No	R	
1293	Index	Y165C; c.891+3A>C	Attenuated	44	50		44 (R)	U	
1309	Index	c.421-1G>C; c.1105delC	Attenuated	32	50	Diffuse	No	R	
1315	Index	Y165C; G382D	Atypical	69	>200			R	Affected sister

[1]Words in brackets indicate cases where the phenotype was strongly suggested on the basis of the available data or the assessment of the clinician, who had examined the patient.–[2]CRC (site): R: rectum; D: colon descendens; T: colon transversum; A: colon ascendens; C: caecum.–[3]Assumed mode of inheritance: D: apparently dominant, with affected persons in 2 consecutive generations; R: autosomal recessive (at least 2 affected siblings, no polyposis reported in the parents); S: single case; U: family history unknown.–[4]These 9 cases were identified among 26 patients selected according to family history or polyposis subtype.

TABLE II – DESCRIPTION OF THE MUTATIONS AND RARE VARIANTS IDENTIFIED IN THE *MUTYH* GENE IN 329 POLYPOSIS PATIENTS

Exon	Mutation	Consequence	Allele frequency in 329 patients
Assumed pathogenic mutations			
3	c.247C>T	R83X	1/658 (0.15)[5]
5	c.411_416dupATGGAT	p137insIW	2/658 (0.3)
6	c.421–1G>C	splice	1/658 (0.15)
6	c.428C>T[1,2]	P143L	1/658 (0.15)
7	c.494A>G[2]	Y165C	40/658 (6.1)
7	c.503G>A[2]	R168H	2/658 (0.3)
8	c.586C>T	Q196X	1/658 (0.15)
9	c.692G>A[2]	R231H	3/658 (0.46)
10	c.778C>T	R260W	1/658 (0.15)
10	c.782–787dupCAGGAG[1]	p263insAG	1/658 (0.15)
10	c.817delG[1,3]	p.A273PfsX32	1/658 (0.15)
10	c.842C>T[1,2]	P281L	1/658 (0.15)
10	c.891+3A>C	splice	5/658 (0.76)
12	c.1129C>T[1]	Q377X	3/658 (0.46)
12	c.970C>T	Q324X	1/658 (0.15)
12	c.1105delC	p.L369LfsX25	7/658 (1.1)
13	c.1145G>A[2]	G382D	31/658 (4.7)
13	c.1172C>T[2]	P391L	1/658 (0.15)
14	c.1395_1397delGGA	p.466delE	8/658 (1.22)
15	c.1435G>T[1,4]	V479F	
15	c.1476+2T>C[1]	splice	1/658 (0.15)
Rare variants (identified in monoallelic state only)			
2	c.42C>T[1]	p.I14	1/658 (0.15)
3	c.270C>T	p.Y90	1/658 (0.15)
5	c.347–46G>A[1]		1/658 (0.15)
5	c.408C>T[1]	p.T136	1/658 (0.15)
10	c.883C>T	R295C	1/658 (0.15)
12	c.956–27G>A[1]		2/658 (0.3)
12	c.956–9C>T		1/658 (0.15)
13	c.1145–27C>T		3/658 (0.46)
13	c.1234C>T	R412C	1/658 (0.15)
14	c.1407C>T	p.T469	1/658 (0.15)

[1]Novel mutations.–[1]Substitutions located at highly conserved sites (bacteria: *Bacillus stearothermophilus, Escherichia coli*; yeast: *Schizosaccharomyces pombe; Homo sapiens*).–[3]All but one of the 21 different mutations with assumed pathogenic relevance were identified in patients with a biallelic mutation state.–[4]identified in one of the selected patients.–[5]Values in parentheses indicate percentages.

another mutation of assumed pathogenic relevance, and none was detected in 232 chromosomes of normal controls. The variant c.891+3A>C reduces the splicing efficiency of the splice donor site from 0.70 to 0.02 as calculated by the splice prediction programme BDGP (Berkeley Drosophila Genome Project: (http://www.fruitfly.org/seq_tools/splice-instrucs.html)). In family no. 26, ten relatives (siblings, parents) were screened for the *in frame* mutation p.466delE identified in the index patient; in all cases, the results were consistent with autosomal recessive inheritance.

In 31 of the 64 unrelated index patients (48%), the missense mutations Y165C and/or G382D were identified in a biallelic state, 20 patients (31%) were compound-heterozygous for either Y165C or G382D and another mutation. Thirteen patients (20%) harboured none of the 2 hot spot mutations, but were either homozygous or compound-heterozygous for other variants (Table I).

Monoallelic *MUTYH* variants in the coding region were found in 9 of the 329 patients (2.7%), 2 of which are novel. However, only 3 of the patients (0.9%) harboured a mutation of predicted functional relevance (c.817delG, G382D, c.891+3A>C); the other substitutions include 4 silent changes (I14, Y90, T136, T469) and 2 missense variants at unconserved sites (R295C, R412C). Rare variants in intron 4 (c.347-46G>A) and intron 11 (c.956-27G>A and c.956-9C>T) were detected in 4 patients (Table II). The frequencies of the previously reported polymorphisms were similar in patients and controls and consistent with the published data. The polymorphisms in intron 6 (c.462+35G>A) and intron 14 (c.1435-40G>C) were in strong linkage disequilibrium.

Phenotype of MAP patients

For phenotype description, all 64 index patients and 7 affected relatives with biallelic *MUTYH* mutations were considered (Table I). The mean age at diagnosis was 45 years (range, 24–72). Most MAP index patients were diagnosed because of symptoms; only 7 underwent presymptomatic surveillance because of an affected relative; another 2 were diagnosed by chance.

Fifty-seven patients (80%) presented with an attenuated polyposis phenotype, 13 (18%) with an atypical course, and only 1 case (a relative of index patient no. 26, Table II) had an assumed typical polyposis (200 adenomas and CRC at 33 years).

The polyp number ranged between 20 and a few hundred. Only 1 proband (no. 1114, Table I) had around 1,000 polyps; the phenotype in this patient was classified as atypical, since age at diagnosis was 68 years. The colorectal distribution of adenomas was reported in 47 patients: in 19 of these (40%), a mainly proximal distribution was noticed, in only 3 cases (6%), the adenomas were located mainly in the distal colorectum. Probands compound-heterozygous for the frameshift mutation c.1105delC and another mutation tended to possess a higher polyp number; no other genotype–phenotype correlation was observed.

With respect to the colorectal polyp number alone, the highest incidence of biallelic *MUTYH* mutations was found in patients with 15–100 adenomas (20%), followed by those with more than 100 polyps (15%) (Table III). However, the difference in the incidence of biallelic *MUTYH* mutations between both groups was not significant ($p > 0.1$). Despite the high polyp number, the latter group is referred to as having an attenuated or atypical phenotype,

TABLE III – INCIDENCE OF BIALLELIC *MUTYH* MUTATIONS RELATED TO THE NUMBER OF COLORECTAL ADENOMAS IN 329 UNRELATED PATIENTS

Polyp number	Examined patients		Patients with biallelic *MUTYH* mutations	
	No.	Mean age at diagnosis (years)	No.	Mean age at diagnosis (years)
1–15	30	46 (17–66)	0	
>15–100	85	45 (5–76)	17 (20%)	43 (30–60)
>100	55	45 (11–71)	8 (15%)	50 (35–68)
Multiple	75	44 (12–65)	13 (17%)	45 (24–63)
Unknown	84	43 (14–55)	17 (20%)	48 (39–55)

since the mean age at diagnosis (50 years; range, 35–68) was significantly delayed ($p < 0.001$) when compared with our 156 *APC* mutation-*positive* patients with typical FAP and known age at diagnosis (25 years; range, 8–44). No biallelic mutations were found in patients with 1–15 colorectal adenomas.

Of the 56 index patients about whom clinical information was available, 28 (50%) had CRC at the time of diagnosis (mean age 48 years; range, 29–72); in 13 out of 16 cases, the tumour stage was advanced (T3, T4). Thirty-three patients underwent duodenoscopy. In 6 of them (18%) a duodenal polyposis was diagnosed, including 1 case (no. 925) with severe and 5 (no. 26, 370, 719, 872 and 848) with mild course. In 1 patient (no. 1293), a follicular thyroid carcinoma (age at diagnosis 37 years) and a stomach cancer (mucosa type; age at diagnosis 48 years) were diagnosed. No other FAP-associated extraintestinal manifestations were reported; however, most of the patients have not been examined systematically for desmoids and benign lesions such as osteomas, epidermoid cysts, or CHRPE. Two sisters (no. 26) each had a lipoma.

Family history was known in 52 MAP index patients. In 85% (44/52) it was compatible with autosomal recessive inheritance. Notably, in 8 families, there was evidence for vertical segregation: In 5 families, a clustering of CRC and in 3 families the clinical diagnosis of adenomatous polyposis in 2 consecutive generations was reported. In 2 of the latter families (no. 1065, 1077), biallelic *MUTYH* mutations were identified in both affected parent and offspring.

Discussion

Spectrum of biallelic MUTYH *mutations*

The reported frequencies of biallelic *MUTYH* mutations in probands with MCA vary between 7% and 42%, depending on the inclusion criteria, the relative number of different phenotypes in the examined patient groups and the methods used for mutation detection.[12,16,18,20,21,24] We identified biallelic *MUTYH* mutations in 55 (17%) of 329 unrelated and unselected *APC* mutation-negative polyposis patients.

The predominant mutation type in the *MUTYH* gene are missense changes. The mutations span the whole gene except for the first 2 exons. We detected 21 different mutations in all of our MAP patients, 7 of which are novel (Table II). In Caucasian populations, a biallelic status for the hot spot mutations Y165C and/or G382D is reported in up to 70% in MAP patients; in up to 93% of biallelic mutation carriers, at least one of the 2 hot spot changes was identified.[12] We found that only 48% of the unrelated MAP index patients carried biallelic mutations at the 2 hot spots. Twenty percentage had neither Y165C nor G382D; thus, up to one-fifth of the probands would not have been identified by a screening protocol restricted to exons 7 and 13. The mutations c.891+3A>C, c.1105delC and p.466delE were identified in 4 (9%), 7 (15%), and 7 (15%) patients, respectively. All other substitutions occurred only once or a few times. However, ethnic and geographic differences in the mutation spectrum have been observed.[14,17,20,25]

We cannot rule out that we missed some mutations not detectable by routine procedures, in particular large genomic deletions. However, because of the low incidence of monoallelic *MUTYH* mutations in our polyposis patients, it is not very likely that these variants contribute substantially to the mutation spectrum and incidence of *MUTYH*.

Frequency and phenotype of MAP

The highest incidence of biallelic *MUTYH* mutations has been reported in patients with 15–100 adenomas (16–42%),[12,16,17] followed by those with more than 100 polyps (7–19%).[13,14,18] Our findings (20% and 15%, respectively) are in line with these data (Table III). However, the difference between both groups is not significant and another study has found the reverse (16% and 19%, respectively),[18] suggesting that there is no general preference concerning the polyp number (15–100 or >100, respectively) in MAP.[24] Consistent with our data, no biallelic *MUTYH* mutations were found in 470 probands with 0–10 polyps,[14,16,19] and in about 3,700 controls.[15,21,26–28]

In light of our findings, data on genotype–phenotype relationship from the literature must be reinterpreted. Often patients with more than 100 polyps are referred to as typical FAP, regardless of age at disease onset. Therefore, a substantial number of cases with pretended typical polyposis was linked to biallelic *MUTYH* mutations. In fact, the mean age at diagnosis in MAP index patients with more than 100 adenomas is significantly increased both in our patients (50 years, Table III) and in those of most other studies[12,16,18,24] when compared with typical FAP (25 years, own data). Moreover, as in AFAP, the colorectal adenoma distribution is accentuated in the proximal colorectum in around 40%. Essentially, the vast majority of biallelic *MUTYH* mutation carriers is best characterised by an attenuated or atypical colorectal polyposis (98% in our study). Comparable to MMR deficiency in HNPCC, this is in accordance with the assumption that 2 somatic mutations in the *APC* gene are needed to develop the phenotype, which will take a longer time when compared with *APC*-related polyposis where only 1 somatic mutation is necessary beside of the inherited germline mutation.

In our sample of 660 polyposis patients, the mutation detection rate in the group of attenuated cases was considerably increased when biallelic *MUTYH* mutations were included (Fig. 1). The highest incidence of biallelic *MUTYH* mutations was identified in *APC* mutation-negative patients with atypical course (27%). In contrast, the incidence among patients with typical polyposis seems to be very low, indicating that the most characteristic feature in MAP patients is the advanced age at onset rather than the polyp number.

The risk of CRC in MAP patients is high (50–60% at the time of diagnosis, penetrance of CRC approximately 100% by age 65 years[24,29,30]), and advanced tumour stages are frequently observed.[12,14] The frequency of duodenal polyposis varies between 4% and 25%; our data and those of others indicate that severe manifestations seem to be rare. However, 1 case of duodenal cancer[24] and 1 patient with stomach cancer (own study) were reported. FAP-associated extraintestinal lesions such as desmoids, osteomas or CHRPE are not typical for MAP neither in our patients nor in most of the earlier reports. Few patients had an osteoma[16]; CHRPEs were reported in 4 patients, but whether diagnosis was certain is unclear.[12,16] Thyroid cancer occurred in 2 patients[31] (own study), the histology was different (papillary and follicular carcinoma, respectively). Recently, 2 affected siblings with multiple pilomatricomas[32] and a patient with multiple seba-

ceous adenomas on the forehead and neck[31] were described, indicating some kind of phenotype variability. In another study, breast cancer has been diagnosed in 4 (18%) of 22 female MAP patients (age at diagnosis of breast cancer 49–76 years).[24] In our 32 female patients (mean age at diagnosis of polyposis 43 years) no case of breast cancer was reported.

Consistent with other studies,[12,33] a significant number of our MAP patients had a family history of CRC in antecedents, suggesting vertical transmission. In 2 of them, biallelic *MUTYH* mutations were identified in 2 generations, in both families the patients carry the hot spot mutations. Autosomal dominant transmission can be mimicked by CRC in parents or by pseudodominant inheritance either as a result of consanguinity or the carrier frequency in the general population. As a consequence, *MUTYH* mutation screening should also be performed in pedigrees with a polyposis in 2 generations.

Data on phenotype consequences of monoallelic *MUTYH* mutation carriers are controversal[12,13,17,27,28]; however, the CRC risk is quite likely to be low.[14,27,30] Only 0.9% of our 329 unselected polyposis patients harboured monoallelic mutations of suspected pathogenic relevance, which is in accordance with the carrier frequency in the general population (1–2%).[19,27] To assess a true heterozygosity effect, the number of adenomas identified in heterozygous siblings of MAP patients must be compared with an age-matched control group.

Surveillance recommendations

To prevent CRC, endoscopic surveillance is recommended in persons at risk and in proven carriers of *APC* mutations in families with FAP.[34–36] To date, no specific screening guidelines have been established for MAP.[29]

In MAP patients, the risk of CRC is comparable to that in FAP. However, as in AFAP, the age at onset is delayed: the youngest patient with CRC in our sample was 29 years of age, but in accordance with literature data the vast majority occurred between the fifth and seventh decade of life[17,18,27,37]; only 1 MAP patient was reported to present with CRC at 21 years of age.[24] Thus, beginning and frequency of colonoscopic surveillance as advised in AFAP seems sufficient. Assuming a carrier frequency of about 1–2% in the general population, the recurrence risk of biallelic mutations in children of MAP patients is assumed to be low (about 0.5–1%). Consequently, regular screening of the entire colon should be restricted to proven biallelic mutation carriers and to siblings of MAP patients, who refuse predictive testing, starting at about 18 years of age and continuing throughout life. Accordingly, predictive molecular testing can also be offered at that age. Severe duodenal affection occurred in a few patients, thus, upper gastrointestinal surveillance seems to be worthwhile, but detailed recommendations with respect to onset and frequency of screening cannot be given so far.

In conclusion, *MUTYH* screening substantially increases the mutation detection rate in *APC* mutation-negative patients with attenuated or atypical adenomatous polyposis and should be performed regardless of the presence of 15–100 or >100 adenomas, respectively. Since the hot spot mutations Y165C or G382D were not found in approximately 20% of MAP patients, mutation screening may start with exons 7 and 13, but should finally encompass the whole gene. The most striking feature of MAP is the later age of onset of both adenomas and CRC; duodenal polyposis seems to be quite frequent and sometimes severe, extraintestinal manifestations are rarely observed. The risk of CRC is high, thus, regular colonoscopic screening and prophylactic colectomy are important for cancer prevention. As in FAP, surgical therapy should depend on clinical and endoscopic findings rather than on mutation analysis. MAP should be considered as an important differential diagnosis to FAP since phenotype and mode of inheritance have consequences for surveillance and genetic counselling of patients and their relatives.

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