MUTATION IN BRIEF

Low Frequency of *AXIN2* Mutations and High Frequency of *MUTYH* Mutations in Patients With Multiple Polyposis

Sophie Lejeune[1], François Guillemot[5], Jean-Pierre Triboulet[3,7], Stéphane Cattan[4], Christine Mouton[2], PAFNORD Group[†], Nicole Porchet[2,6,7], Sylvie Manouvrier[1,7], and Marie-Pierre Buisine[2,6,7*]

[1]Service de Génétique Clinique, Hôpital J. de Flandre, [2]Laboratoire de Biochimie et Biologie Moléculaire, Pôle de Biologie-Pathologie, [3]Service de Chirurgie Générale et Endocrinienne, [4]Service des Maladies de l'Appareil Digestif et de la Nutrition, Hôpital C. Huriez, CHRU de Lille; [5]Service de Gastro-Entérologie, Hôpital V. Provo, CH de Roubaix; [6]Unité 560 INSERM, Lille; [7]Faculté de Médecine H. Warembourg, Université de Lille II, France

[†]PAFNORD Group is a multidisciplinary group for clinical management of patients from North of France with colorectal cancer susceptibility

*Correspondence to: Marie-Pierre Buisine, Laboratoire de Biochimie et Biologie Moléculaire, Pôle de Biologie-Pathologie - Eurasanté, CHRU de Lille, 59045 Lille Cedex, France ; E-mail: mp-buisine@chru-lille.fr

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Familial adenomatous polyposis has been linked to germline mutations in the *APC* tumor suppressor gene. However, a number of patients with familial adenomatous polyposis (with either classical or attenuated phenotype) have no *APC* mutation. Recently, germline mutations in the *Wnt* pathway component gene *AXIN2* have been associated with tooth agenesis-colorectal cancer syndrome. Moreover, biallelic mutations in the base excision repair gene *MUTYH* have been associated with polyposis and early-onset colorectal cancer. The aim of this study was to further assess the contribution of *AXIN2* and *MUTYH* to hereditary colorectal cancer susceptibility. *AXIN2* and *MUTYH* genes were screened for germline mutations by PCR and direct sequencing in 39 unrelated patients with multiple adenomas or colorectal cancer without evidence of *APC* mutation nor mismatch repair defect. Two novel *AXIN2* variants were detected in one patient with multiple adenomas, but no clearly pathogenic mutation. In contrast, nine different *MUTYH* mutations were detected in eight patients, including four novel mutations. Biallelic *MUTYH* mutations were only found in patients with multiple adenomatous polyposis (7 out of 22 (32%)). Interestingly, five *MUTYH* mutation carriers had a family history consistent with dominant inheritance. Moreover, one patient with biallelic *MUTYH* mutations presented with multiple adenomas and severe tooth agenesis. Therefore, germline mutations are rare in *AXIN2* but frequent in *MUTYH* in patients with multiple adenomas. Our data suggest that genetic testing of *MUTYH* may be of interest in patients with pedigrees apparently compatible with autosomal recessive as well as dominant inheritance. © 2006 Wiley-Liss, Inc.

KEY WORDS: multiple polyposis; tooth agenesis; familial adenomatous polyposis; mismatch repair; *AXIN2*; *MUTYH*

INTRODUCTION

Familial adenomatous polyposis (FAP; MIM# 175100) is a well-described inherited syndrome characterized by

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an increased susceptibility to multiple adenomatous polyps in the colon and rectum, and thence to colorectal cancer, as well as to various upper gastrointestinal and extraintestinal manifestations. A variant of FAP, referred as attenuated familial adenomatous polyposis (AFAP), is characterized by 100 or less colorectal adenomas with a tendency to rectal sparing, later age of onset of polyps and cancer, and a more limited expression of the extracolonic features, although gastric and duodenal adenomas are frequently encountered (for review, Fearnhead et al., 2001). A subset of AFAP, with a few colorectal adenomas (< 10), states the problem of differential diagnosis with another form of inherited colorectal cancer, *i.e.* the HNPCC (hereditary non-polyposis colorectal cancer) syndrome.

Most of FAP patients (80% when classical phenotype) have a germline mutation in the adenomatous polyposis coli gene (*APC*; MIM# 175100). Nevertheless, a subset of patients with classical FAP phenotype and most patients with multiple adenomas have no germline *APC* mutation, suggesting the implication of additional genes in colorectal adenoma susceptibility. Recently, a germline mutation in the *AXIN2* gene (MIM# 604025) has been described in a large Finnish family in which severe tooth agenesis co-segregated with the existence of colorectal adenomatous polyps (Lammi et al., 2004). AXIN2 (also named conductin or axil) is with APC another important component of the canonical *Wnt* signal transduction pathway (Behrens et al., 1998). Moreover, germline recessive mutations in the human Mut Y homolog gene *MUTYH* (MIM# 604933) (also named *MYH*), that encodes a member of the base excision repair (BER) pathway, have been identified in patients presenting a phenotype of FAP or AFAP with neither dominant transmission nor evidence of *APC* mutation (Sampson et al., 2003; Sieber et al., 2003; Venesio et al., 2004). Furthermore, biallelic *MUTYH* mutations can be associated with early-onset colorectal cancer (Fleischmann et al., 2004; Wang et al., 2004).

To further assess the contribution of *AXIN2* and *MUTYH* to hereditary colorectal cancer susceptibility, we screened these genes for germline mutations in 39 unrelated patients with multiple adenomas (including one with severe tooth agenesis) without *APC* mutation or with colorectal cancer without MisMatch Repair (MMR) defect that characterizes the HNPCC syndrome.

MATERIALS AND METHODS

Patients

Thirty nine unrelated patients recorded in the registry of the regional multidisciplinary center PAFNORD were selected for this study. Twenty three patients (median age at diagnosis: < 43 years, range 18-66) with a phenotype resembling FAP (≥ 100 adenomatous polyps) or AFAP (≥ 5 and < 100 adenomatous polyps) without germline mutation in the *APC* gene were screened for *AXIN2* and *MUTYH* mutations. Absence of mutation in *APC* gene was ascertained by sequencing of all exons, exon-intron boundaries and the promoter region, as well as by Multiplex Ligation-dependant Probe Amplification (MLPA) of exons and promoter to detect large deletions or duplications. Five out of these 23 patients were only screened for mutations in *MUTYH* because of either identification of biallelic mutations in this gene or insufficient DNA. To complete the study, 16 additional patients (median age at diagnosis: 47 years, range 24-66) with a suspected HNPCC syndrome and no evidence of MMR defect were screened for *AXIN2* and/or *MUTYH* mutations, depending on available DNA. These patients had no microsatellite instability in their tumor, as determined using the Bethesda panel (Boland et al., 1998), and/or no germline mutation in mismatch repair genes *MLH1*, *MSH2*, *MSH6*, as ascertained by sequencing all exons, exon-intron boundaries and the promoter region, as well as by MLPA or quantitative multiplex PCR of short fragments analysis of exons. Clinical features are summarized in Table 1. All patients gave full informed consent for the study.

Table 1. Clinical features of patients with the results of previous analyses

Patient	Sex	Age at diagnosis (years)	Family history of polyps[a] or CRC	Colorectal findings	Additional features	Results of *APC* and *MMR* genes analyses	Status of the tumor	Genes analyzed in the present study
Li-1	**H**	**39**	**No**	**> 100 polyps[a], CRC**	**Severe tooth agenesis**	***APC* -**		***AXIN2, MUTYH***
Li-2	H	39	Yes (4)[b], AD[c]	> 100 polyps	-	*APC* -		*AXIN2, MUTYH*
Li-3	M	66	No	> 100 polyps	2 duodenal polyps	*APC* -		*AXIN2, MUTYH*
Li-4	H	< 52	No	15 polyps, 2 CRC	Duodenal and jejunal polyps	*APC* -		*AXIN2, MUTYH*
Li-5	**H**	**37**	**Yes (2), AD**	**~ 40 polyps**		***APC* -**		***AXIN2, MUTYH***
Li-6	H	46	No	> 50 polyps		*APC* -		*AXIN2, MUTYH*
Li-7	**F**	**43**	**Yes (3), AD**	**> 50 polyps**		***APC* -**		***AXIN2, MUTYH***
Li-8	F	< 30	No	~ 60 polyps		*APC* -		*AXIN2, MUTYH*
Li-9	F	18	No	30 polyps		*APC* -		*AXIN2, MUTYH*
Li-10	H	43	No	~ 20 polyps		*APC* -		*AXIN2, MUTYH*
Li-11	F	53	Yes (2), AD	29 polyps		*APC* -, *MMR* -[f]		*AXIN2, MUTYH*
Li-12	F	55	No	> 30 polyps, CRC	4 duodenal polyps	*APC* -	MSS[e]	*MUTYH*
Li-13	**M**	**54**	**Yes (1), AR**	**55 polyps, CRC**		***APC* -**		***AXIN2, MUTYH***
Li-14	M	60	Yes (1)[d], ?	30 polyps, 3 CRC		*APC* -	MSS	*AXIN2, MUTYH*
Li-15	**M**	**35**	**Yes (2)[d], AD**	**18 polyps, CRC**		***APC* -**		***AXIN2, MUTYH***
Li-16	M	47	No	10 polyps, CRC		*APC* -	MSS	*AXIN2, MUTYH*
Li-17	F	51	Yes (1)[d], ?	10 polyps, CRC		*APC* -	MSS	*MUTYH*
Li-18	F	52	No	45 polyps, CRC		*APC*-		*MUTYH*
Li-19	**M**	**40**	**Yes (1), AD**	**A few polyps; multiple polyps (at 51)**		***APC* -**		***MUTYH***
Li-20	M	13	No	Numerous polyps		*APC* -		*MUTYH*
Li-21	M	42	Yes (4), AD	< 10 polyps, CRC		*APC* -, *MMR* -		*AXIN2, MUTYH*
Li-22	**F**	**32**	**Yes (2), AR**	**> 10 polyps, CRC**		***APC* -, *MMR* -**	**MSS**	***AXIN2, MUTYH***
Li-23	F	52	Yes (3), AD	20 polyps		*APC* -, *MMR* -	MSS	*AXIN2*
Li-24	M	26	Yes (1)[d], ?	A few polyps; CRC (at 31)		*APC* -, *MMR* -		*AXIN2, MUTYH*
Li-25	F	62	Yes (2), AR	1 polyp, CRC		*APC* -, *MMR* -	MSS	*AXIN2, MUTYH*
Li-26	F	65	Yes (8), AD	Several polyps		*MMR* -	MSS	*AXIN2*
Li-27	F	63	Yes (4), AR	4 polyps, CRC		*MMR* -		*AXIN2, MUTYH*
Li-28	F	50	Yes (2), AD	CRC			MSS	*AXIN2, MUTYH*
Li-29	M	66	Yes (3), AD	2 polyps, CRC		*MMR* -	MSS	*AXIN2, MUTYH*
Li-30	M	63	Yes (2), AD	2 polyps, CRC			MSS	*AXIN2, MUTYH*
Li-31	**M**	**44**	**Yes (4), AD**	**1 polyp; CRC (at 53)**	**Colonic arterioveinous malformations**		**MSS**	***MUTYH***
Li-32	M	46	Yes (2), AD	CRC		*MMR* -	MSS	*AXIN2*
Li-33	M	44	Yes (1), AD	CRC		*MMR* -	MSS	*AXIN2*
Li-34	M	43	Yes (1), AR	1 polyp			MSS	*AXIN2, MUTYH*
Li-35	F	24	Yes (2), AD	CRC		*MMR* -	MSS	*AXIN2, MUTYH*
Li-36	M	54	Yes (2), AD	CRC		*MMR* -		*AXIN2*
Li-37	F	44	Yes (4), AD	CRC		*MMR* -	MSS	*AXIN2*
Li-38	F	49	Yes (5), AD	CRC		*MMR* -	MSS	*MUTYH*
Li-39	F	29	No	CRC		*MMR* -		*MUTYH*

[a]adenomatous polyps; [b]number of cases; [c]apparent transmission: AD, autosomal dominant; AR, autosomal recessive; [d]second or third degree; [e]MSS, microsatellite stable; [f]MMR-, no mutation detected in *MLH1*, *MSH2*, *MSH6.*
Patients with mutations in *MUTYH* are in bold.

AXIN2 and *MUTYH* Genes Analysis

DNA isolation. DNA was extracted from peripheral blood using the QIAamp® DNA Blood Maxi kit or the EZ1 DNA Blood kit with the BioRobot® EZ1 (Qiagen, Courtaboeuf, France).

***AXIN2* and *MUTYH* genes analysis.** Coding regions and exon-intron boundaries of *AXIN2* (GenBank accession number NM_004655.2) (exons 1-10) and *MUTYH* (Genbank accession number U63329.1) (exons 1-16) were screened using PCR and direct sequencing. Primers sequences are available on request. PCR reactions were performed in 50 µl reaction volumes consisting of 1X AmpliGold Buffer II (100 mM Tris/HCl, 15 mM $MgCl_2$, 500 mM KCl, pH 8.3) (Applied Biosystems, Courtaboeuf, France), 200 µM desoxynucleoside triphosphates, 0.3 mM of each primer, 2.5 units of AmpliTaq-Gold DNA polymerase (Applied Biosystems) and 200 ng of template DNA. PCR were performed in a GeneAmp® PCR System 9700 (Applied Biosystems) with the following conditions: initial denaturation at 95°C for 5 min, followed by 35 cycles at 94°C for 30 s, 58-60°C for 30 s, and 72°C for 1-2 min, followed by a final extension at 72°C for 7 min. PCR products were then purified and sequenced using the BigDye™ Terminator v3.0 DNA sequencing kit (Applied Biosystems) with the ABI 3100-Avent Genetic

Analyser (Applied Biosystems). Sequences were analyzed using Seqscape v2.5 software. All sequence variations were confirmed on a second independent PCR product. Mutation nomenclature follows the Human Genome Variation Society (HGVS) guidelines (www.hgvs.org/mutnomen/) and is referred to the cDNA sequences of *AXIN2* (NM_004655.2) and *MUTYH* (U63329.1) respectively.

Missense variants analysis. Missense variants were analyzed by comparison of amino-acid sequences for Axin family proteins and for HhH-GPD superfamily base excision DNA repair proteins and using Polyphen software. Their prevalence was evaluated in 50 healthy control subjects. Moreover, RT-PCR was performed in order to study the effect of sequence variations onto splicing. Total RNA was extracted from blood using the RNA Blood Mini kit (Qiagen). RNA was reverse transcribed using the Advantage™ RT-for-PCR kit (ClonTech, Heidelberg, Germany) and oligodT according to the manufacturer's instructions. First strand cDNA was then amplified as described above using primers selected in exons flanking that contained the sequence variation.

Confirmation of biallelic mutations. In absence of parents DNA samples, allele-specific PCR (AS-PCR) was performed in cases where two mutations were found in order to assess if the variations were present on the same allele or on different alleles. PCR amplifications were conducted on genomic DNA as described above using allele-specific primers (wild type and mutated).

RESULTS

Mutations in genes *AXIN2* and *MUTYH*, as well as clinical features of patients carrying mutations and their relatives, are shown in Table 2 and Figure 1.

AXIN2 Gene Analysis

The entire *AXIN2* gene was screened for mutations in 31 patients with multiple adenomatous polyps or colorectal cancer. No clearly pathogenic mutations (frameshift or nonsense mutations) were found in any of our patients, including the one patient with multiple polyps and severe tooth agenesis (Li-1). We found however two novel sequence variations in exon 5 of *AXIN2* in one patient aged 18 presenting with 30 adenomatous polyps (Li-10): one apparently missense mutation c.1235A>G (p.Asn412Ser) and one apparently silent mutation c.1530G>A (p.Thr510Thr). As some sequence variants have been reported to have an effect onto splicing (Sharp et al., 2004), we analyzed transcripts by RT-PCR using primers within exons 3 and 7. RT-PCR and sequencing of the product showed the presence of the two variants at a similar proportion as observed in the genomic DNA, indicating no splicing alteration. Moreover, Polyphen software predicted the missense mutation p.Asn412Ser as benign based on the limited conservation of Asn residue across species. Although these variations were not found neither in 30 other patients nor in 50 healthy control subjects, they are likely to be polymorphisms. Several common polymorphism were also found in exon 1 (c.148T>C (p.Ser50Pro)) and exon 5 (c.1365G>A (p.Pro456Pro); c.1386T>C (p.Pro463Pro)), as well as in non-coding sequences (c.1060-17C>T; c.1712+19G>T).

MUTYH Gene Analysis

The entire *MUTYH* gene was screened for mutations in 33 patients with multiple polyposis or colorectal cancer. One patient was found to carry one *MUTYH* mutation and seven carried two mutations. Of these, one was homozygous and six were compound heterozygous. In these cases, mutations were proved to be present onto different alleles, as ascertained by AS-PCR. Mutations consisted in two frameshift mutations and one nonsense mutation that are predicted to generate a truncated protein due to a premature stop codon and in six missense mutations. Frameshift and nonsense mutations were detected in exon 12 (c.1059dupC (p.Arg354GlnfsX164)) and exon 13 (c.1204C>T (p.Gln402X); c.1185_1186dupGG (p.Glu396GlyfsX43)). Missense mutations were detected in exon 7 (c.494A>G (p.Tyr165Cys); c.497A>G (p.Tyr166Ser)), exon 10 (c.805C>A (p.Met269Val); c.842C>T (p.Pro281Leu)), exon 12 (c.1121T>C (p.Leu374Pro)), and exon 13 (c.1145G>A (p.Gly382Asp)). If the deleterious character of both p.Tyr165Cys and p.Gly382Asp amino acid substitutions has been clearly demonstrated by functional assays showing that mutated proteins have a remarkably reduced activity (Chmiel et al., 2003), the significance of the four other missense mutations described here remains to be proved. Nevertheless, these variations were not found in 50 healthy control subjects tested. All missense mutations were present in highly conserved regions across phylogeny as assessed by multiple sequence alignments. Moreover, all missense mutations were located within important functional domains of the protein: p.Tyr166Ser within the pseudo helix-hairpin-helix (HhH) motif, p.Met269Val within the adenine-recognition motif, p.Pro281Leu within the [4Fe-4S] cluster loop, and p.Leu374Pro within the catalytic core of the glycosylase (Guan et al., 1998; Yang et al., 2001). Furthermore, all but one (p.Met269Val) were predicted to be damaging by the Polyphen software. Several

common polymorphism were also found in exons 2 (c.64G>A (p.Val22Met)), 12 (c.972G>C (p.Gln324His)), 16 (c.1502C>T (p.Ser501Phe)), as well as in non-coding sequences.

Biallelic *MUTYH* mutations were detected in seven patients out of 22 (32%) with FAP or AFAP without *APC* mutation. The median age at diagnosis was 39 years (range, 32-54). Only one *MUTYH* mutation was detected in a 44-year old patient diagnosed with only one adenomatous polyp. One patient appeared to be sporadic and seven patients had a family history of adenomatous polyps or colorectal cancer. In two cases, only siblings in a single generation were affected, which was consistent with a recessive transmission, whereas in five cases, at least two successive generations were affected hardly suggesting a dominant transmission (Fig. 1).

No *MUTYH* mutation was detected in patients who developed a colorectal cancer without associated adenomatous polyps. However, only one polyp was detected in the patient (Li-31) heterozygous for *MUTYH* mutation. No *MUTYH* mutation was detected in three patients with duodenal and jejunal polyps. Intriguingly, the patient presenting with severe oligodontia and multiple adenomatous polyps (> 100) (Li-1) was found to be compound heterozygous for *MUTYH* mutation, carrying c.1185_1186dupGG (p.Glu396GlyfsX43) and c.842C>T (p.Pro281Leu).

Table 2. Results of *AXIN2* and *MUTYH* analyses

AXIN2

Patient	Nucleotide change[a]	Consequence
Li-9	c.[1235A>G (+) 1530G>A]	p.[Asn412Ser (+) Thr510Thr]

[a]Numbering is based on the cDNA sequence of *AXIN2* (GenBank accession number NM_004655.2)

MUTYH

Patient	Nucleotide change[a]	Consequence
Li-1	c.[842C>T]+[1185_1186dupGG]	p.[Pro281Leu]+[Glu396GlyfsX43]
Li-5	c.[805A>G]+[1059dupC]	p.[Met269Val]+[Arg354GlnfsX164]
Li-7	c.[494A>G]+[494A>G]	p.[Tyr165Cys]+[Tyr165Cys]
Li-13	c.[494A>G]+[1145G>A]	p.[Tyr165Cys]+[Gly382Asp]
Li-15	c.[494A>G]+[497A>C]	p.[Tyr165Cys]+[Tyr166Ser]
Li-19	c. [1121T>C]+[1204C>T]	p. [Leu374Pro]+[Gln402X]
Li-22	c.[494A>G]+[1185_1186dupGG]	p.[Tyr165Cys]+[Glu396GlyfsX43]
Li-31	c.1145G>A	p.Gly382Asp

[a]Numbering is based on the cDNA sequence of *MUTYH* (GenBank accession number U63329.1)

DISCUSSION

Germline mutations of the *APC* tumor-suppressor gene result in FAP. A subset of AFAP has also been associated with mutations in *APC*. The mutations associated with AFAP have been mainly detected in the 5' and 3' regions of the gene, as well as in exons 9 and 14 that are alternatively spliced (Heinimann et al., 1998; Fearnhead et al., 2001). Nevertheless, a significant proportion of patients with AFAP and some patients with classic FAP do not harbor germline mutation in *APC*.

APC is an important component of the canonical *Wnt* signal transduction pathway. It functions with AXIN2 and the serine/threonine kinase GSK3β as a negative regulator of Wnt signaling. APC induces degradation of cytosolic β-catenin, preventing its interaction with transcription factors such as lymphoid enhancer-binding factor 1/T cell-specific transcription factor (LEF/TCF) and subsequent expression of a whole battery of genes regulating cell proliferation, including c-*MYC* and *CCND1* (Behrens et al., 1998). It is thus tempting to speculate that germline mutations in *AXIN2*, as well as in other members of the pathway, could substitute for *APC* mutations in FAP or AFAP without evidence of *APC* mutations. Several studies failed to show germline mutations in *CTNNB1* (the gene encoding β-catenin) and *GSK3β* in patients with FAP or AFAP without mutation in *APC* (Cao et al., 1999; Venesio et al., 2003). In contrast, a germline *AXIN2* mutation (c.1966C>T (p.Arg656X)) has been recently described in a large Finnish family in which severe tooth agenesis-colorectal adenomatous polyps phenotype co-segregated with dominant inheritance (number of polyps varied among relatives from one to 68) (Lammi et al., 2004). Another *de novo AXIN2* mutation (c.1994insG (p.Asn666GlyfsX41)) was found in a patient with a similar tooth phenotype, but cancer predisposition could not be confirmed in this case due to the very young age of the patient (13 years) (Lammi et al., 2004).

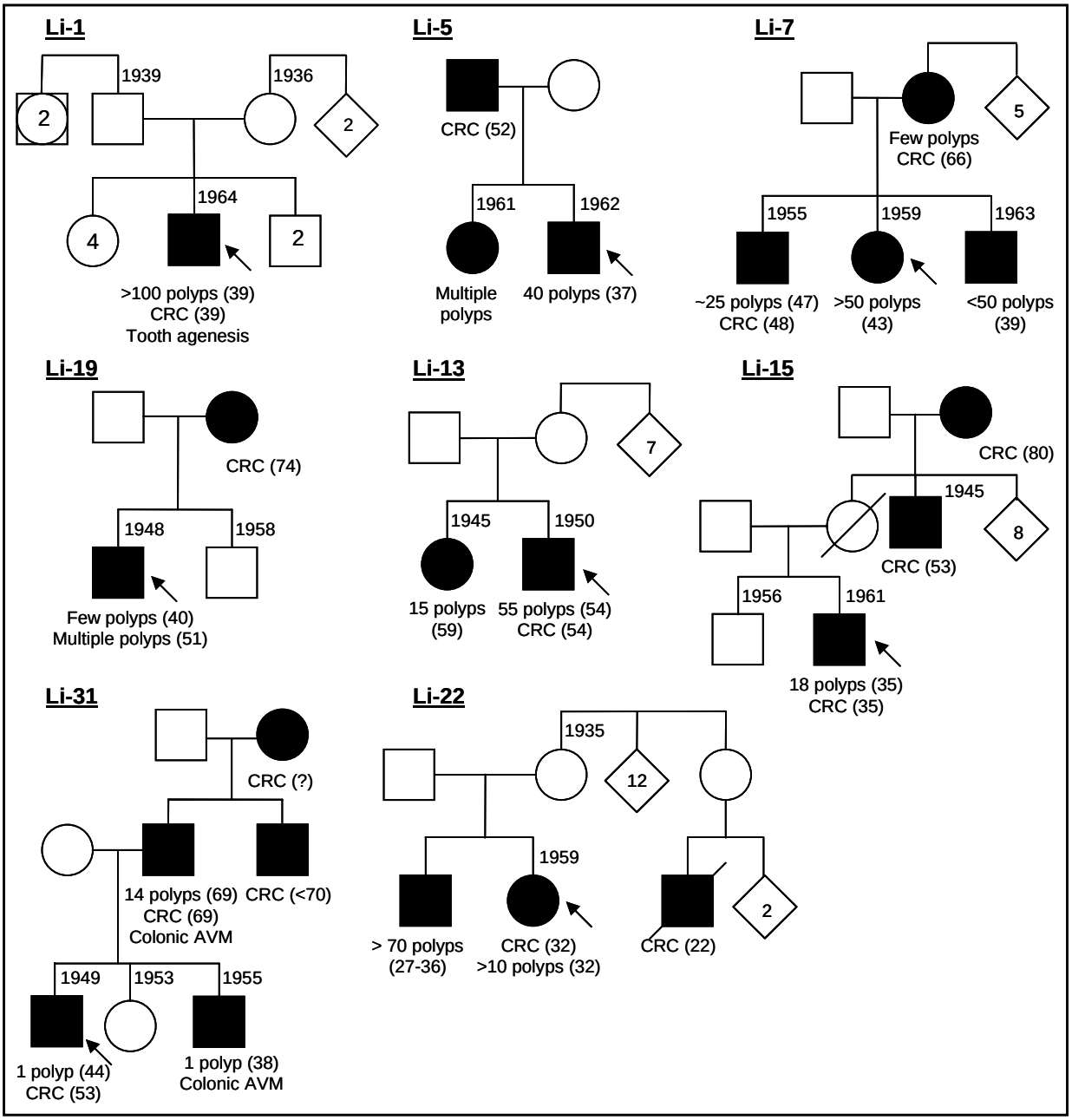

Figure 1. Abbreviated pedigrees of the eight families with mutations in *MUTYH*. *Squares*, males; *circles*, females; *diamonds*, individuals whose sex is not specified. *Solid symbol*, colorectal cancer or polyp. Numbers *above* the symbols indicate date of birth; numbers *below* the symbols in ( ) indicate age at diagnosis. Numbers *within* the symbols indicate the number of offspring. *Arrows* indicate the proband cases. Abbreviations: *CRC*, colorectal cancer; *AVM*, arterioveinous malformation.

To further evaluate the role of *AXIN2* in colorectal cancer susceptibility, we screened 18 patients with FAP/AFAP, as well as 13 patients with colorectal cancer without evidence of MMR defect. Among patients with AFAP, one presented with severe oligodontia. Unfortunately, we did not find clearly pathogenic mutations in any of the patients analyzed. More particularly, we did not find *AXIN2* mutation in the patient with multiple adenomatous polyps associated with severe oligodontia. Nevertheless, we found two novel sequence variations in the coding sequence of *AXIN2* in an 18-year old patient with 30 polyps and no family history of polyposis or colorectal cancer. These changes were absent in the 30 other patients and the 50 healthy control subjects analyzed,

and have not been previously reported. However, these two variations did not alter splicing, and the missense mutation p.Asn412Ser did not concern a highly conserved amino-acid, suggesting no functional effect. Our data strongly suggest that *AXIN2* germline mutations are rare in AFAP patients without *APC* mutations, and do not account for all AFAP associated with severe tooth agenesis. Moreover, *AXIN2* does not appear to be involved in colorectal cancer susceptibility without polyposis.

MUTYH is a DNA glycosylase that plays a key part in the base excision repair (BER) pathway. It is involved in the removal of adenines misincorporated into DNA opposite guanine or 7,8-dihydro-8-oxodeoxyguanine during DNA replication or recombination. Recently, germline mutations in *MUTYH* have been associated with autosomal recessive forms of FAP/AFAP and early-onset colorectal cancers (Al-Tassan et al., 2002; Jones et al., 2002; Sampson et al., 2003; Sieber et al., 2003; Venesio et al., 2004; Wang et al., 2004).

Here, we screened 33 patients with FAP/AFAP or colorectal cancer without evidence of MMR defect for germline mutations in *MUTYH*. We detected five mutations already described, including the two most frequent missense mutations, namely p.Tyr165Cys and p.Gly382Asp, the frameshift mutation p.Glu396GlyfsX43 and the missense mutations p.Leu374Pro and p.Pro281Leu (Al-Tassan et al., 2002; Jones et al., 2002; Venesio et al., 2004; Aceto et al., 2005; Aretz et al., 2006), as well as four novel mutations. The two novel mutations p.Arg354GlnfsX164 and p.Gln402X are expected to result in loss of the carboxy-terminal domain of the MUTYH protein required for substrate recognition (Chmiel et al., 2001) and proliferating cell nuclear antigen necessary for long patch BER (Parker et al., 2001). The four missense mutations different from the two most frequent are also predicted to be pathogenic since they are located in important functional domains of the protein. This is particularly true for the p.Tyr166Ser mutation that is located within the pseudo-HhH motif involved in substrate recognition. p.Tyr166Ser is therefore expected to reduce the adenine glycosylase activity of the enzyme, as observed for the p.Tyr165Cys by Chmiel et al. (2003). Similarly, the p.Pro281Leu substitution is assumed to be pathogenic since it is located within the Cys-X_6-Cys-X_2-Cys-X_5-Cys loop implicated in coordinating the $[4Fe\text{-}4S]^{2+}$ cluster that has been shown to be critical for substrate binding and catalysis (Porello et al., 1998) and believed to function in DNA-mediated charge transport for DNA repair (Boon et al., 2003). These hypotheses remain to be demonstrated using functional tests.

Biallelic mutations were only found in patients with multiple adenomatous polyposis. Thirty two percent of patients with FAP/AFAP without *APC* mutation were found to be homozygous or compound heterozygous for *MUTYH* mutations. Our results are in accordance with previous studies reporting *MUTYH* germline mutations in 20 to 45% patients presenting with 10 or more adenomas and no evidence of *APC* mutation (Sampson et al., 2003; Sieber et al., 2003; Venesio et al., 2004; Wang et al., 2004). However, patients described here developed polyps at a median age of 39 years, which was much younger than reported previously (range, 48-58 years) (Sampson et al., 2003; Sieber et al., 2003; Venesio et al., 2004; Wang et al., 2004). Moreover, whereas in previous studies patients with *MUTYH* mutations were either apparently sporadic cases or had family history consistent with recessive inheritance, we show evidence in some cases of apparent vertical transmission suggesting dominant inheritance. This finding prompts investigation of patients with apparently recessive but also dominant inherited FAP/AFAP without *APC* mutation for the presence of *MUTYH* mutation. If the excess risk of colorectal cancer in biallelic *MUTYH* mutation carriers is now largely accepted, the question of whether monoallelic *MUTYH* mutation carriers are at increased risk of colorectal cancer remains debated (Croitoru et al., 2004; Fleischmann et al., 2004; Wang et al., 2004; Peterlongo et al., 2005). Recently, in a very large systematically collected population-based association study, Farrington et al. (2005) reported a 93-fold excess risk of colorectal cancer in individuals with biallelic *MUTYH* mutations and an only 1.35-fold excess risk of colorectal cancer in heterozygous carriers. Apparently dominant inheritance in some families could be explained by a higher risk for *MUTYH* mutation carriers with family history of multiple polyps or colorectal cancer, in relation with modifier genes. Moreover, given the frequency of heterozygotes of about 1% in the general population, we cannot exclude the fact that parents of some index patients may be also biallelic mutation carriers. Since conclusions of such a study may have important consequences in genetic counseling and clinical management, further investigations in families with identified *MUTYH* mutations are required.

Little is known regarding *MUTYH* mutations and extra-colonic features. Duodenal adenomas have been reported in some patients with biallelic *MUTYH* mutations (Sampson et al., 2003; Sieber et al., 2003). However, we did not found *MUTYH* mutations in three patients presenting with duodenal and jejunal adenomas. In contrast, the patient with multiple adenomas and severe tooth agenesis we expected to carry a germline *AXIN2* mutation was in fact compound heterozygous for *MUTYH* mutations. The presence of severe tooth agenesis in this patient could just be a coincidence. However, should this association be confirmed, tooth agenesis-colorectal cancer syndrome (MIM# 608615) should not be exclusively limited to mutations in *AXIN2*.

We were unable to detect a mutation in *AXIN2* or *MUTYH* in 14 out of 23 patients with classic FAP or AFAP and no *APC* mutation, nor in any patients with colorectal cancer and no MMR defect, although most of them had family history of multiple adenomatous polyps or colorectal cancers and early onset clinical features. Several other candidate genes have been unsuccessfully screened for germline mutations in patients with FAP/AFAP or colorectal cancer, including other main components of the *Wnt* pathway (*CTNNB1*, *GSK3β*) (Cao et al., 1999; Venesio et al., 2003) and of the BER system (*OGG1*, *MTH1*) (Sieber et al., 2003; Croitoru et al., 2004). Of note, two variants in *MTH1* (p.Arg31Gln) and *OGG1* (p.Arg197Trp) were detected in rare patients (3 out of 84 (3.6%)) with monoallelic *MUTYH* mutations (Farrington et al., 2005), but the significance of these variants is unclear. These observations hardly suggest the implication of additional genes in hereditary adenomatous polyp and colorectal cancer susceptibility.

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