

ORIGINAL ARTICLE

## Multiple Colorectal Adenomas, Classic Adenomatous Polyposis, and Germ-Line Mutations in MYH

Oliver M. Sieber, B.Sc., Lara Lipton, M.B., B.S., Michael Crabtree, M.B., B.S., Karl Heinimann, Ph.D., Paulo Fidalgo, M.D., Robin K.S. Phillips, M.D., Marie-Luise Bisgaard, M.D., Torben F. Orntoft, M.D., Lauri A. Aaltonen, Ph.D., Shirley V. Hodgson, D.M., Huw J.W. Thomas, Ph.D., and Ian P.M. Tomlinson, Ph.D.

### ABSTRACT

#### BACKGROUND

Germ-line mutations in the base-excision–repair gene MYH have been associated with recessive inheritance of multiple colorectal adenomas. Tumors from affected persons displayed excess somatic transversions of a guanine–cytosine pair to a thymine–adenine pair (G:C→T:A) in the APC gene.

#### METHODS

We screened for germ-line MYH mutations in 152 patients with multiple (3 to 100) colorectal adenomas and 107 APC-mutation–negative probands with classic familial adenomatous polyposis (>100 adenomas). Subgroups were analyzed for changes in the related genes MTH1 and OGG1. Adenomas were tested for somatic APC mutations.

#### RESULTS

Six patients with multiple adenomas and eight patients with polyposis had biallelic germline MYH variants. Missense and protein-truncating mutations were found, and the spectrums of mutations were very similar in the two groups of patients. In the tumors of carriers of biallelic mutations, all somatic APC mutations were G:C→T:A transversions. In the group with multiple adenomas, about one third of patients with more than 15 adenomas had biallelic MYH mutations. In the polyposis group, no patient with biallelic MYH mutations had severe disease (>1000 adenomas), but three had extracolonic disease. No clearly pathogenic MTH1 or OGG1 mutations were identified.

#### CONCLUSIONS

Germ-line MYH mutations predispose persons to a recessive phenotype, multiple adenomas, or polyposis coli. For patients with about 15 or more colorectal adenomas — especially if no germ-line APC mutation has been identified and the family history is compatible with recessive inheritance — genetic testing of MYH is indicated for diagnosis and calculation of the level of risk in relatives. Clinical care of patients with biallelic MYH mutations should be similar to that of patients with classic or attenuated familial adenomatous polyposis.

From the Molecular and Population Genetics Laboratory, London Research Institute, Cancer Research UK, London (O.M.S., L.L., M.C., I.P.M.T.); the Cancer Research UK Colorectal Unit and Polyposis Registry, St. Mark's Hospital, Harrow, United Kingdom (L.L., M.C., R.K.S.P., H.J.W.T.); the Department of Clinical Genetics, Guy's Hospital, London (L.L., S.V.H.); the Research Group on Human Genetics, Division of Medical Genetics, University Clinics, Basel, Switzerland (K.H.); the Instituto Portugues de Oncologia, Lisbon, Portugal (P.F.); the Danish Polyposis Register, Department of Gastroenterology, Hvidovre University Hospital, Hvidovre, Denmark (M.-L.B.); the Department of Clinical Biochemistry, Aarhus University Hospital, Aarhus, Denmark (T.F.O.); and the Department of Medical Genetics, Biomedicum Helsinki, University of Helsinki, Helsinki, Finland (L.A.A.). Address reprint requests to Dr. Tomlinson at the Molecular and Population Genetics Laboratory, Cancer Research UK, 44 Lincoln's Inn Fields, London WC2A 3PX, United Kingdom, or at [ian.tomlinson@cancer.org.uk](mailto:ian.tomlinson@cancer.org.uk).

Mr. Sieber and Dr. Lipton contributed equally to this article.

N Engl J Med 2003;348:791-9.

Copyright © 2003 Massachusetts Medical Society.

**M**OST MENDELIAN PREDISPOSITIONS to colorectal tumors are dominant and involve tumor-suppressor genes. Recently, Al-Tassan et al.<sup>1</sup> reported on a single Welsh family with three affected members and recessive inheritance of multiple colorectal adenomas and carcinoma. The patients' tumors had an excess of somatic mutations consisting of the substitution of a thymine–adenine pair for a guanine–cytosine pair (G:C→T:A) in the adenomatous polyposis coli (APC) gene, which is typical of changes caused by oxidative damage to DNA.<sup>2-7</sup> This damage produces the stable guanine adduct 8-oxo-7,8-dihydroxy-2'-deoxyguanosine, which tends to mispair with adenine, leading to the observed mutation. Levels of 8-oxo-7,8-dihydroxy-2'-deoxyguanosine are increased in carcinomas of the breast, lung, and kidney.<sup>8-11</sup> Al-Tassan et al.<sup>1</sup> therefore tested oxidative-repair genes for germ-line changes in the family they studied. They found that the affected persons carried two missense variants, Y165C and G382D, in the base-excision–repair gene MYH. Jones et al.<sup>12</sup> subsequently tested 21 patients in the United Kingdom who had multiple adenomas and found biallelic MYH mutations in 7 of these patients, all of whom were of Welsh, Indian, or Pakistani origin.

The products of three human base-excision–repair genes, MTH1, OGG1, and MYH, act synergistically to prevent mutagenesis induced by 8-oxo-7,8-dihydroxy-2'-deoxyguanosine. In the nucleotide pool, MTH1 hydrolyzes 8-oxo-7,8-dihydroxy-2'-deoxyguanosine triphosphate to 8-oxo-7,8-dihydroxy-2'-deoxyguanosine monophosphate<sup>13-17</sup>; OGG1 detects and removes 8-oxo-7,8-dihydroxy-2'-deoxyguanosine incorporated into the DNA<sup>18-22</sup>; and MYH, an adenine-specific DNA glycosylase, removes adenines mispaired with 8-oxo-7,8-dihydroxy-2'-deoxyguanosine or guanine.<sup>23-26</sup>

The cause of the phenotype of multiple (3 to 100) colorectal adenomas is probably heterogeneous and would be elucidated by molecular classification; such classification would permit differentiation between sporadic and hereditary disease and would indicate appropriate approaches to the care of patients and their families. Classic adenomatous polyposis (involving >100 to >8000 colorectal adenomas) may also be genetically heterogeneous, given that no germ-line APC mutation can be found in some patients despite extensive testing. We therefore studied both a group of patients with multiple colorectal adenomas and a group of patients with classic adenomatous polyposis who tested negative for APC mutations to determine whether they had

germ-line mutations in MYH. We screened for somatic APC mutations in the tumors of selected patients and tested subgroups of patients for mutations in MTH1 and OGG1.

---

## METHODS

---

### PATIENTS WITH MULTIPLE COLORECTAL ADENOMAS AND CONTROLS

We identified 152 patients seen in genetics departments in the United Kingdom (St. Mark's Hospital, Harrow; Churchill Hospital, Oxford; and Guy's Hospital, London) with multiple (3 to 100) synchronous or metachronous colorectal adenomas. Patients had been referred to these centers either because of a family history of colorectal tumors or because they had presented with symptoms and multiple polyps, suggesting the presence of a genetic disease. All patients were receiving colonoscopic screening and had given written informed consent for the testing of a blood or DNA sample according to protocols approved by ethics review boards. Clinicopathological data were obtained from patients' records to confirm diagnoses. In all cases, either a precise count of adenomas had been reported or, more rarely, a rounded or approximate count had been given. Family histories (of tumors or other major disease) were recorded as reported by the patients and were confirmed, when possible, on the basis of hospital records, although precise counts of adenomas in patients' relatives were rarely available.

We used 107 anonymous controls from the United Kingdom: the unaffected spouses of patients recruited for a study of multiple leiomyomas.<sup>27</sup> We also studied 26 patients with multiple adenomas from Finland and Denmark, all of whom had been reported to have between 5 and 100 adenomas.

### PATIENTS WITH CLASSIC ADENOMATOUS POLYPOSIS

We contacted polyposis registries in the United Kingdom, Switzerland, Finland, Portugal, and Denmark with a request to study all APC-mutation–negative patients with more than 100 adenomas (whether synchronous or metachronous). We identified 107 probands and confirmed that local laboratories had rigorously ruled out germ-line changes in APC. All probands gave written informed consent. Full clinicopathological details and family histories were obtained. For some patients, exact counts of polyps at the time of colectomy had been recorded; for others, counts were given as a range (for example, “100 to 1000” or “several thousand” to classify disease as

mild or severe classic polyposis, respectively); and for others, counts were provided essentially for diagnostic purposes (with >100 being diagnostic of classic adenomatous polyposis).

**ANALYSIS OF MUTATIONS**

Coding regions and exon–intron boundaries of MYH (GenBank accession number NM\_012222), MTH1 (GenBank accession number AB025241), and OGG1 (GenBank accession numbers NM\_002542 and NM\_016821) were screened by fluorescence single-strand conformation polymorphism analysis. Polymerase-chain-reaction (PCR) products were analyzed at 18°C and 24°C on an automated DNA sequencer (ABI 3100, Perkin Elmer Applied Biosystems) and studied with the use of Genotyper 2.5 software (Perkin Elmer Applied Biosystems). Samples with band shifts were sequenced in forward and reverse orientations from new PCR product with the use of ABI BigDye Terminator Mix (Applied Biosystems) and a semi-automated sequencer (ABI 377, Applied Biosystems).

Sections were cut from paraffin-embedded adenomas and stained with hematoxylin and eosin. Dysplastic regions were identified and dissected manually. DNA was extracted by digestion for 48 to 72 hours in 1× PCR buffer (Promega) containing 0.02 percent proteinase K (BDH Laboratory Supplies). APC was screened for somatic mutations in regions G and H of exon 15 (the part of the gene in which somatic mutations most commonly occur) by fluorescence single-strand conformation polymorphism analysis.

**LOSS OF HETEROZYGOSITY**

Loss of heterozygosity (allelic loss) and genotyping analyses at the microsatellite locus D1S2677 (2.5 kb from MYH) were performed according to standard protocols with the use of dye-labeled oligonucleotides and the ABI 377 sequencer. Samples were scored as having allelic loss if the dose of one allele in the tumor was at least 50 percent lower than that of the other allele, after correction for the relative peak areas of the alleles found in germ-line DNA of the same patient.

**RESULTS**

**GERM-LINE MYH MUTATIONS IN PATIENTS WITH MULTIPLE ADENOMAS**

The 152 patients in the United Kingdom who had multiple adenomas (Table 1) presented between

1970 and 2001 at a median age of 56 years (range, 18 to 77). As of the date of follow-up (December 31, 2001), the mean number of synchronous or metachronous adenomas per patient was 16 (median, 7; range, 3 to 100). Twenty-six patients presented with a synchronous colorectal carcinoma, but none were known to have cancer that developed subsequently, all patients having undergone regular colonoscopic surveillance. A family history of colorectal cancer was reported by 75 patients; no patient had a family history of adenomas without also having a family history of colorectal cancer. No patients reported a consanguineous marriage in their family history.

Six patients carried biallelic MYH mutations (Table 2 and Fig. 1). Of these patients, three were compound heterozygotes, as shown by sequencing of cloned PCR products; the remaining three were presumed to be homozygotes. The previously reported missense changes, Y165C (A→G at position 494) and G382D (G→A at position 1145), were the most common alterations. Both Y165C and G382D target highly conserved residues, the former mapping to the pseudo-helix–hairpin–helix protein domain, which probably confers specificity of recognition of mismatches, and the latter mapping to the predicted NUDIX hydrosylase domain and thus possibly affecting the catalytic core of the glycosylase (residues 366 through 497). We also found novel

**Table 1. Characteristics of Patients from the United Kingdom with Multiple Adenomas, in Relation to Germ-Line MYH-Mutation Status.**

Characteristic	Germ-Line MYH-Mutation Status		
	Negative (N=140)	Single Mutation (N=6)	Biallelic Mutation (N=6)
Age at presentation			
Age known — no. of patients (%)	139 (99)	6 (100)	6 (100)
Median — yr	56	64	56
Range — yr	18–77	25–72	45–59
Polyps			
Precise count given — no. of patients (%)	114 (81)	6 (100)	6 (100)
Median — no.	7	4	55
Range — no.	3–100	3–12	18–100
Colorectal cancer — no. of patients (%)			
Yes	19 (14)	2 (33)	3 (50)
None reported	121 (86)	4 (67)	3 (50)
Family history of colorectal cancer — no. of patients (%)			
Yes	66 (47)	4 (67)	5 (83)
None reported	74 (53)	2 (33)	1 (17)

**Table 2.** Patients from the United Kingdom with Multiple Adenomas and Germ-Line *MYH* Mutations.\*

Patient No.	First <i>MYH</i> Mutation	Second <i>MYH</i> Mutation	Sex	Age at Diagnosis yr	No. of Polyps	Colorectal Cancer	Family History of Adenomas, Colorectal Cancer, or Both
1	Y165C	Y165C	Male	52	40	None reported	Yes
2	Y165C	G382D	Female	45	100	Yes	None reported
3	Y165C	1419delC	Female	57	18	Yes	Yes
4	1103delC	G382D	Male	55	70	None reported	Yes
5	G382D	G382D	Male	56	40	Yes	Yes
6	G382D	G382D	Female	59	100	None reported	Yes
7	R83X	None detected	Female	69	6	Yes	Yes
8	Y165C	None detected	Male	72	3	None reported	Yes
9	Y165C	None detected	Female	58	5	None reported	Yes
10	R295C	None detected	Female	25	3	None reported	None reported
11	G382D	None detected	Male	52	3	Yes	Yes
12	G382D	None detected	Female	70	12	None reported	None reported

\* Two of the patients with a single mutation had novel *MYH* variants, R83X (C→T at position 247) and R295C (C→T at position 883). It is not known whether these variants would be pathogenic in compound heterozygotes or homozygotes, although R83X is likely to be so.

frame-shift changes, 1103delC (at codon 368) and 1419delC (at codon 473), which are suspected to abolish glycosylase function.

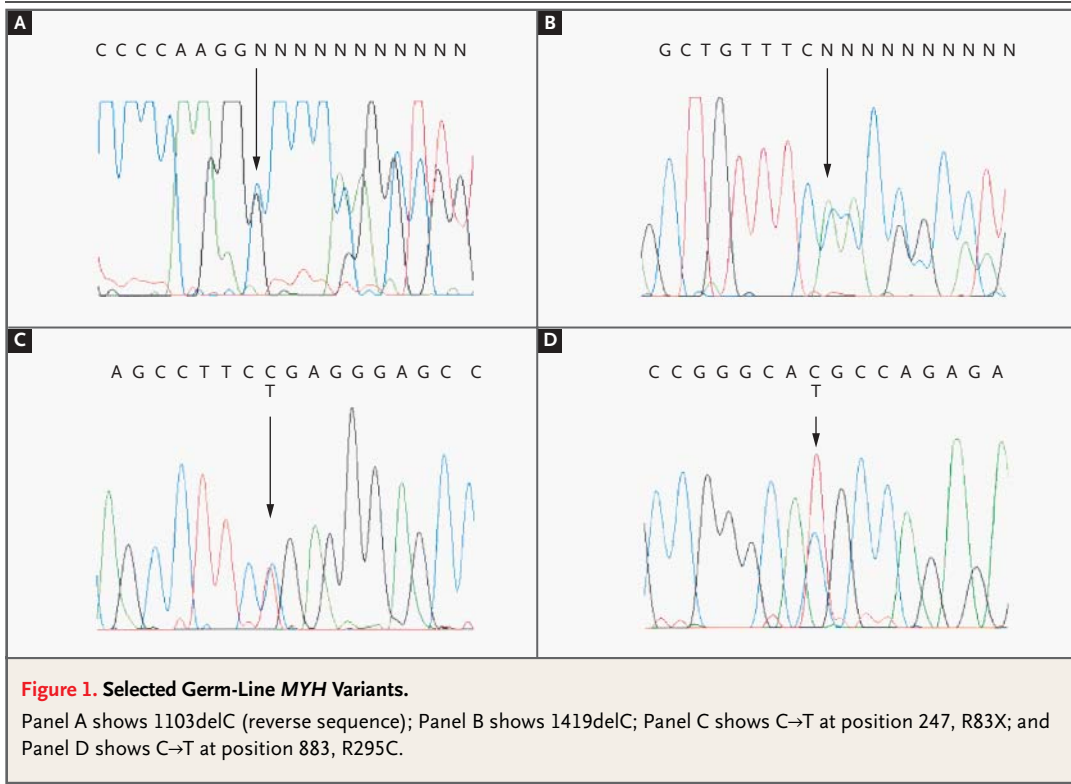
None of the 107 controls in the United Kingdom carried two *MYH* mutations. Y165C was found in just two controls, and none of the other mutations associated with disease were present in the control group. The previously described *MYH* polymorphisms in exon 2 (G→A at position 64, V22M), exon 12 (G→C at position 972, Q324H), and exon 16 (C→T at position 1502, S501F) were detected in our patients, with allele frequencies of 10 percent, 21 percent, and 2 percent, respectively—similar to the frequencies previously reported in a control population.<sup>1</sup>

In order to provide further evidence of the pathogenicity of the *MYH* mutations, we screened available relatives for the changes carried by the proband (Table 2). In all cases, the results were consistent with recessive inheritance. An affected sister of Patient 1 was also homozygous for the Y165C mutation. An unaffected daughter of Patient 2 was a heterozygous carrier of the Y165C mutation. The sister of Patient 4 carried both mutations seen in

that patient and had had multiple adenomas and two colorectal cancers. Only unaffected siblings of Patient 6 were still alive, and they were both heterozygotes.

In order to determine the frequency of Y165C and G382D in other northern European populations, we also studied 26 Finnish and Danish patients with multiple adenomas. Two of the patients (8 percent) were compound heterozygotes for Y165C and G382D. In the entire group of patients, Y165C and G382D were not consistently associated with specific alleles at D1S2667 (data not shown), thus providing no evidence that these are ancestral rather than recurrent changes.

Twenty-five adenomas from three patients who were compound heterozygotes for *MYH* mutations (one with Y165C and 1419delC, one with 1103delC and G382D, and one with Y165C and G382D) were screened for somatic *APC* mutations (in regions G and H of exon 15). Three mutations were detected, one in each patient, and all of them were G:C→T:A transversions (C→A at position 4230, C1410X; G→T at position 4381, E1461X; and G→T at position 4480, E1494X). Loss of heterozygosity at *MYH* was



found in 3 of the 25 adenomas. Given that G382D reportedly retains some enzyme activity,<sup>1</sup> the low frequency of allelic loss may indicate that the absence of MYH function is not necessary for tumorigenesis.

Six patients were heterozygous for an MYH mutation and the wild-type allele (Table 2). In these patients, we sequenced the entire MYH gene but found no further changes. We screened seven adenomas from one of the carriers of the G382D mutation for somatic APC mutations and found two changes, one C→G transversion (S1346X) and one 1-bp deletion (4244delG). Neither change was a G:C→T:A transversion, thus providing no evidence of defective MYH activity.

**SCREENING FOR MTH1 AND OGG1 MUTATIONS IN PATIENTS WITH MULTIPLE ADENOMAS**

A total of 127 patients with multiple adenomas who were negative for MYH mutations were screened for MTH1 mutations, and 42 of these patients were also screened for mutations in OGG1. No obviously pathogenic or biallelic MTH1 or OGG1 mutations were detected. The allele frequencies of previously described polymorphisms of MTH1<sup>1</sup> were not sig-

nificantly different from those found among the controls (data not shown). A novel missense variant of MTH1 (G→A at position 92, R31Q) was identified in one patient (and not in any of the controls), but this mutation did not cosegregate with the multiple adenoma phenotype. With regard to OGG1, aside from the well-described polymorphism in exon 7 (C→G at position 977, S326C),<sup>28-30</sup> no further DNA sequence variants were detected.

**ASSOCIATIONS BETWEEN MYH GENOTYPE AND PHENOTYPE IN PATIENTS WITH MULTIPLE ADENOMAS**

The ages at presentation in the six patients with biallelic MYH mutations (median, 56 years; range, 45 to 59) were similar to those of the other patients with multiple adenomas in our study (Tables 1 and 2). Five of these patients with biallelic mutations had symptoms at presentation, and one was found to have polyps at the time of a colonoscopy performed because of a family history of colorectal tumors. Polyps were predominantly small, mildly dysplastic tubular adenomas, with a minority of tubulovillous adenomas and very few hyperplastic polyps. Three of the six patients had colorectal cancer at presen-

tation. Five of the six had a family history of colorectal cancer, in all cases involving more than one generation. Carriers of biallelic MYH mutations were no more likely to have a family history of colorectal cancer than other patients in the study ( $P=0.10$  by Fisher's exact test). Two patients with biallelic MYH mutations had a confirmed family history of multiple adenomas, but the disease occurred only in siblings, which is consistent with recessive inheritance of MYH-associated disease. No notable extracolonic tumors or other clinical features were reported.

The one phenotypic difference that clearly distinguished patients with biallelic MYH mutations from carriers of single mutations and MYH-mutation-negative patients with multiple adenomas was the number of tumors (median, 55, 4, and 7, respectively;  $P=0.02$  for the three-way comparison by the Kruskal-Wallis test) (Table 2). Of 21 patients with 15 to 100 adenomas each, 6 (29 percent) had biallelic MYH mutations.

The frequency of colorectal cancer among carriers of biallelic MYH mutations (3 of 6) was greater than that among the other patients in our study (21 of 146;  $P=0.05$  by Fisher's exact test) as well as that in the general population (3.86 percent among people 0 to 80 years of age).<sup>31</sup> These data suggest that persons with biallelic MYH mutations have an increased risk of colorectal cancer, although these results should be interpreted with caution because, although our patients were recruited on the basis of their adenoma phenotype alone, they were more likely to have come to clinical attention if they also had carcinoma.

#### GERM-LINE MYH MUTATIONS IN PATIENTS WITH CLASSIC ADENOMATOUS POLYPOSIS

Eight of 107 probands with classic polyposis (7.5 percent) carried biallelic pathogenic MYH mutations (Tables 3 and 4). Y165C and G382D were again the most common changes. Three other mutations were found: a frame shift (252delG at codon 84); an unusual in-frame duplication (411dupATGGAT at codon 137, 137insIW); and a nonconservative missense change (G→T at position 694, V232F). Four patients carried single MYH mutations (Y165C in two patients, I209V in one, and G382D in one).

All probands with polyposis who had biallelic MYH mutations had a family history compatible with recessive inheritance, in that only the proband or siblings in a single generation were affected by polyposis. Although it is necessary to exercise some caution, given the variation among centers in clin-

ical practice and in the precision of the counting of polyps, it is probable that patients with MYH mutations had mild classic adenomatous polyposis: none had more than 1000 polyps; two had exact counts of 115 and 210 adenomas; and none had early-onset cancer. All patients with two MYH mutations had been treated by total colectomy with ileorectal anastomosis or ileal pouch, at a mean age of 47.6 years (median, 47; range, 30 to 70), as compared with a mean of 28 years (median, 23; range, 13 to 65) among patients with APC mutations who were included in the polyposis registry of St. Mark's Hospital (data not shown).<sup>32</sup>

In several respects, the clinicopathological features of patients with biallelic MYH mutations were the same as those of patients with polyposis resulting from APC mutations: macroadenoma morphologic features were the same (largely small tubular lesions with mild dysplasia); microadenomas were present, despite the fact that such lesions were previously thought to be pathognomonic of classic adenomatous polyposis; and some patients had extracolonic disease. Severe (Spigelman stage IV) duodenal polyposis developed in Patient 15, and Patient 16 had duodenal polyps at diagnosis. Congenital hypertrophy of the retinal pigment epithelium was diagnosed in Patient 13 (although it was not specifically noted to be of a type associated with polyposis). No desmoid tumors were reported.

---

#### DISCUSSION

---

We have characterized a new genetically defined class of disease that applies to some patients with the multiple adenoma phenotype and some patients with classic adenomatous polyposis. Germ-line MYH mutations predispose persons in a variety of European populations to recessive inheritance of multiple colorectal adenomas and classic adenomatous polyposis. All patients with biallelic MYH mutations probably have an increased risk of colorectal cancer. Of patients with 3 to 100 adenomas, about 5 percent had disease attributable to MYH, and of those with more than 15 adenomas, nearly one third had biallelic MYH mutations. Of patients with a phenotype of classic polyposis and no APC mutation, 7.5 percent had two germ-line MYH mutations. Extracolonic disease was present in some patients with MYH-associated polyposis, indicating that these features are not restricted to those with germ-line APC mutations. The presence of extracolonic disease is consistent with the model of tumor-

igenesis resulting from defective MYH activity in the colon — namely, hypermutability of APC and perhaps  $\beta$ -catenin (with which APC interacts).

Patients with biallelic MYH mutations thus tend to have milder disease than most patients with classic adenomatous polyposis but more severe disease than most patients with multiple adenomas. It is difficult to distinguish between patients with APC mutations and those with biallelic MYH mutations on the basis of clinicopathological features, although family history can be useful. MYH mutations appear to be a more common cause of the multiple adenoma (or attenuated classic polyposis) phenotype than are APC mutations,<sup>28-30,33</sup> but they are evidently a less common cause of classic adenomatous polyposis. More tumors develop in carriers of biallelic MYH mutations than in patients with hereditary nonpolyposis colorectal cancer, but progression of adenoma to carcinoma appears to be slower in the MYH-mutation carriers.<sup>34</sup>

We identified 10 patients who had only one mutated MYH allele. Might patients who are heterozygous for an MYH mutation have somewhat increased susceptibility to colorectal tumors, given that allelic loss on chromosome arm 1p<sup>35-37</sup> is apparently an early event in colorectal tumorigenesis that could inactivate the wild-type MYH allele? Persons who carried a single MYH mutation were not overrepresented among our patients as compared with our control group or with the control group studied by Al-Tassan et al.<sup>1</sup>; and the two somatic APC mutations in polyps from patients who were heterozygous for an MYH mutation were not G:C→T:A transversions. Nevertheless, several of our patients with a single MYH mutation had a family history that suggested dominant inheritance of colorectal cancer (although not of multiple adenomas). Formal exclusion of MYH as an allele conferring somewhat increased susceptibility will require analysis of a large group of patients with colorectal cancer and controls. We cannot yet answer the question of why MYH, rather than MTH1 or OGG1, is important in creating a predisposition to tumors. Specifically, we cannot rule out the possibility that carriers of biallelic MTH1 or OGG1 mutations are predisposed to tumors, although we found no such persons in our group of patients.

We suggest that genetic testing for changes in MYH should be performed in patients who have tens or hundreds of colorectal adenomas, with the proviso that almost all patients with MYH-associated polyposis will have a family history consistent only

**Table 3. Characteristics of Patients with Classic Adenomatous Polyposis in Relation to Germ-Line MYH-Mutation Status.**

Characteristic	Germ-Line MYH-Mutation Status		
	Negative (N=95)	Single Mutation (N=4)	Biallelic Mutation (N=8)
Age at presentation			
Age known — no. of patients (%)	55 (58)	4 (100)	8 (100)
Median — yr	30	31	48
Range — yr	7–72	30–54	30–70
No. of polyps — no. of patients (%)			
100–1000	68 (72)	4 (100)	8 (100)
>1000	27 (28)	0	0
Family history of colorectal cancer — no. of patients (%)			
Yes	29 (31)	0	4 (50)
None reported	66 (69)	4 (100)	4 (50)

with recessive inheritance of multiple adenomas. Screening of APC and MYH may be performed in parallel in some patients, such as those with isolated cases of multiple adenomas. Evidently, if biallelic MYH mutations are identified in a proband, testing of siblings is worthwhile, even if they are asymptomatic in their sixth or seventh decade. It should, however, be borne in mind that in 2 to 3 percent of cases, a carrier of two MYH mutations will produce children with a partner who carries a single mutation; in this case, the disease will appear to be dominantly inherited. Whether it is worthwhile to undertake genetic testing in the partners of patients with biallelic MYH mutations is a question that remains open.

All but three of our patients with biallelic MYH mutations required colectomy, since their disease could not be controlled by colonoscopic polypectomy. For patients with relatively mild disease, regular screening by colonoscopy may be used initially — although the optimal intervals between screenings must be determined empirically — and may prevent the need for colectomy, as it has in the three patients in our study who had fewer than 50 adenomas. Unfortunately, the recessive nature of the disease means that it will prove difficult to identify carriers of MYH mutations early enough to prevent the need for colectomy in all cases. In the absence of any evidence of an increased risk of colorectal tumors in persons who are heterozygous for an MYH mutation, there is currently little justification for aggressive colonoscopic screening of any such fam-

**Table 4. Patients with Classic Adenomatous Polyposis and Germ-Line MYH Mutations.**

Patient No.	First MYH Mutation	Second MYH Mutation	Sex	Age at Diagnosis <i>yr</i>	No. of Polyps	Colorectal Cancer	Family History of Classic Adenomatous Polyposis	Extracolonic Features
13	Y165C	Y165C	Male	41	100–1000	None reported	Yes	Congenital hypertrophy of the retinal pigment epithelium
14	Y165C	G382D	Female	50	100–1000	None reported	Yes	None reported
15	G382D	G382D	Male	30	100–1000	None reported	None reported	Duodenal adenomas
16	252delG	137insIW	Male	38	100–1000	Yes	None reported	Duodenal adenomas
17	Y165C	Y165C	Female	45	100–1000	Yes	Yes	None reported
18	Y165C	V232F	Male	70	100–1000	None reported	None reported	None reported
19	Y165C	G382D	Male	51	210	Yes	None reported	None reported
20	Y165C	G382D	Female	69	115	Yes	Yes	None reported
21	G382D	None detected	Male	32	100–1000	None reported	Yes	None reported
22	I209V	None detected	Female	54	100–1000	None reported	None reported	Osteoma
23	Y165C	None detected	Male	30	100–1000	None reported	None reported	None reported
24	Y165C	None detected	Male	30	750	None reported	Yes	None reported

ily member. We do suggest that all persons with two identified MYH mutations have regular endoscopy of the upper gastrointestinal tract, primarily for the detection and management of duodenal polyposis.

We conclude that molecular methods should be used to classify disease in patients with multiple adenomas or adenomatous polyposis. Patients with identified germ-line mutations should be classified as having APC-associated or MYH-associated polyposis. The level of risk in relatives and the likely severity of disease can then be accurately assessed. Patients with polyposis but no identified germ-line mutation may then be further classified as having presumed classic adenomatous polyposis if they have a dominant family history of classic disease, severe polyposis (>1000 colorectal adenomas), or both. Patients with no detected germ-line mutation

in APC or MYH with either mild polyposis (100 to 1000 adenomas) or fewer than 100 adenomas and a family history consistent with recessive inheritance should be classified as having polyposis or multiple adenomas of unknown origin.

Supported by Cancer Research UK and by grants from the Boehringer Ingelheim Fonds (to Mr. Sieber), the Bobby Moore Fund (to Dr. Lipton), the Center of Excellence Program of the Academy of Finland (project number 44870, to Dr. Aaltonen), the Swiss National Foundation (3200-067571, to Dr. Heinimann), and the Fifth European Community Framework Program (QLG 2-CT-2001-01861, to Drs. Orntoft, Aaltonen, and Tomlinson).

We are indebted to the patients for their participation in this study; to their doctors and pathologists for contributing clinical information; and to Andrew Rowan, Ella Barclay, Kay Neale, the Polyposis Registry of St. Mark's Hospital, Ian Frayling, the staff of the Cancer Research UK Equipment Park, Carol Cummings, and the Cancer Research UK Family Cancer Clinic at St. Mark's Hospital for their help and support.

## REFERENCES

1. Al-Tassan N, Chmiel NH, Maynard J, et al. Inherited variants of MYH associated with somatic G:C→T:A mutations in colorectal tumors. *Nat Genet* 2002;30:227-32.
2. Halliwell B. Mechanisms involved in the generation of free radicals. *Pathol Biol (Paris)* 1996;44:6-13.
3. Wang D, Kreutzer DA, Essigmann JM. Mutagenicity and repair of oxidative DNA damage: insights from studies using defined lesions. *Mutat Res* 1998;400:99-115.
4. Shibutani S, Takeshita M, Grollman AP. Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxodG. *Nature* 1991;349:431-4.
5. Nghiem Y, Cabrera M, Cupples CG, Miller JH. The mutY gene: a mutator locus in *Escherichia coli* that generates G.C→T.A transversions. *Proc Natl Acad Sci U S A* 1988;85:2709-13.
6. Michaels ML, Miller JH. The GO system protects organisms from the mutagenic effect of the spontaneous lesion 8-hydroxyguanine (7,8-dihydro-8-oxoguanine). *J Bacteriol* 1992;174:6321-5.
7. Thomas D, Scot AD, Barbey R, Padula M, Boiteux S. Inactivation of OGG1 increases the incidence of G.C→T.A transversions in *Saccharomyces cerevisiae*: evidence for endogenous oxidative damage to DNA in eukaryotic cells. *Mol Gen Genet* 1997;254:171-8.
8. Malins DC, Haimanot R. Major alterations in the nucleotide structure of DNA in cancer of the female breast. *Cancer Res* 1991;51:5430-2.
9. Olinski R, Zastawny T, Budzbon J, Skokowski J, Zegarski W, Dizdaroglu M. DNA base modifications in chromatin of human cancerous tissues. *FEBS Lett* 1992;309:193-8.
10. Jaruga P, Zastawny TH, Skokowski J, Dizdaroglu M, Olinski R. Oxidative DNA base damage and antioxidant enzyme activities in human lung cancer. *FEBS Lett* 1994;341:59-64.
11. Okamoto K, Toyokuni S, Uchida K, et al. Formation of 8-hydroxy-2'-deoxyguanosine and 4-hydroxy-2-nonenal-modified proteins in human renal-cell carcinoma. *Int J Cancer* 1994;58:825-9.
12. Jones S, Emmerson P, Maynard J, et al. Biallelic germline mutations in MYH predispose to multiple colorectal adenoma and somatic G:C→T:A mutations. *Hum Mol Genet* 2002;11:2961-7.
13. Sakumi K, Furuichi M, Tsuzuki T, et al. Cloning and expression of cDNA for a human enzyme that hydrolyzes 8-oxo-dGTP, a mutagenic substrate for DNA synthesis. *J Biol Chem* 1993;268:23524-30.
14. Mo JY, Maki H, Sekiguchi M. Hydrolytic elimination of a mutagenic nucleotide, 8-oxodGTP, by human 18-kilodalton protein: sanitization of nucleotide pool. *Proc Natl Acad Sci U S A* 1992;89:11021-5.
15. Kang D, Nishida J, Iyama A, et al. Intracellular localization of 8-oxo-dGTPase in human cells, with special reference to the role of the enzyme in mitochondria. *J Biol Chem* 1995;270:14659-65.
16. Oda H, Nakabeppu Y, Furuichi M, Sekiguchi M. Regulation of expression of the human MTH1 gene encoding 8-oxo-dGTPase: alternative splicing of transcription products. *J Biol Chem* 1997;272:17843-50.
17. McLennan AG. The MutT motif family of nucleotide phosphohydrolases in man and human pathogens. *Int J Mol Med* 1999;4:79-89.
18. Lu R, Nash HM, Verdine GL. A mammalian DNA repair enzyme that excises oxidatively damaged guanines maps to a locus frequently lost in lung cancer. *Curr Biol* 1997;7:397-407.
19. Radicella JP, Dherin C, Desmaze C, Fox MS, Boiteux S. Cloning and characterization of hOGG1, a human homolog of the OGG1 gene of *Saccharomyces cerevisiae*. *Proc Natl Acad Sci U S A* 1997;94:8010-5.
20. Roldan-Arjona T, Wei YF, Carter KC, et al. Molecular cloning and functional expression of a human cDNA encoding the antimutator enzyme 8-hydroxyguanine-DNA glycosylase. *Proc Natl Acad Sci U S A* 1997;94:8016-20.
21. Shinmura K, Kasai H, Sasaki A, Sugimura H, Yokota J. 8-Hydroxyguanine (7,8-dihydro-8-oxoguanine) DNA glycosylase and AP lyase activities of hOGG1 protein and their substrate specificity. *Mutat Res* 1997;385:75-82.
22. Nishioka K, Ohtsubo T, Oda H, et al. Expression and differential intracellular localization of two major forms of human 8-oxoguanine DNA glycosylase encoded by alternatively spliced OGG1 mRNAs. *Mol Biol Cell* 1999;10:1637-52.
23. Slupska MM, Baikalov C, Luther WM, Chiang JH, Wei YF, Miller JH. Cloning and sequencing a human homolog (hMYH) of the *Escherichia coli* mutY gene whose function is required for the repair of oxidative DNA damage. *J Bacteriol* 1996;178:3885-92.
24. Slupska MM, Luther WM, Chiang JH, Yang H, Miller JH. Functional expression of hMYH, a human homolog of the *Escherichia coli* MutY protein. *J Bacteriol* 1999;181:6210-3.
25. Takao M, Zhang QM, Yonei S, Yasui A. Differential subcellular localization of human MutY homolog (hMYH) and the functional activity of adenine:8-oxoguanine DNA glycosylase. *Nucleic Acids Res* 1999;27:3638-44.
26. Ohtsubo T, Nishioka K, Imaiso Y, et al. Identification of human MutY homolog (hMYH) as a repair enzyme for 2-hydroxyadenine in DNA and detection of multiple forms of hMYH located in nuclei and mitochondria. *Nucleic Acids Res* 2000;28:1355-64.
27. Tomlinson IP, Alam NA, Rowan AJ, et al. Germline mutations in FH predispose to dominantly inherited uterine fibroids, skin leiomyomata and papillary renal cell cancer. *Nat Genet* 2002;30:406-10.
28. Kohno T, Shinmura K, Tosaka M, et al. Genetic polymorphisms and alternative splicing of the hOGG1 gene, that is involved in the repair of 8-hydroxyguanine in damaged DNA. *Oncogene* 1998;16:3219-25.
29. Wikman H, Risch A, Klimek F, et al. hOGG1 polymorphism and loss of heterozygosity (LOH): significance for lung cancer susceptibility in a Caucasian population. *Int J Cancer* 2000;88:932-7.
30. Hanaoka T, Sugimura H, Nagura K, et al. hOGG1 exon7 polymorphism and gastric cancer in case-control studies of Japanese Brazilians and non-Japanese Brazilians. *Cancer Lett* 2001;170:53-61.
31. Surveillance, epidemiology, and end results. Bethesda, Md.: National Cancer Institute, 2003. (Accessed January 18, 2003, at <http://seer.cancer.gov>.)
32. The Polyposis Registry 1996-1998. (Accessed January 18, 2003, at <http://www.polyposisregistry.org.uk>.)
33. Lamlum H, Al Tassan N, Jaeger E, et al. Germline APC variants in patients with multiple colorectal adenomas, with evidence for the particular importance of E1317Q. *Hum Mol Genet* 2000;9:2215-21.
34. Lynch HT, Smyrk T, Jass JR. Hereditary nonpolyposis colorectal cancer and colonic adenomas: aggressive adenomas? *Semin Surg Oncol* 1995;11:406-10.
35. Tanaka Y, Yanoshita R, Konishi M, et al. Suppression of tumorigenicity in human colon carcinoma cells by introduction of normal chromosome 1p36 region. *Oncogene* 1993;8:2253-8.
36. Lothe RA, Andersen SN, Hofstad B, et al. Deletion of 1p loci and microsatellite instability in colorectal polyps. *Genes Chromosomes Cancer* 1995;14:182-8.
37. Praml C, Finke LH, Herfarth C, Schlag P, Schwab M, Amler L. Deletion mapping defines different regions in 1p34.2-pter that may harbor genetic information related to human colorectal cancer. *Oncogene* 1995;11:1357-62.

Copyright © 2003 Massachusetts Medical Society.