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Report

Penetrance and Expressivity of *MSH6* Germline Mutations in Seven Kindreds Not Ascertained by Family History

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Hereditary nonpolyposis colorectal cancer (HNPCC) is caused by inherited mutations in DNA mismatch-repair genes, most commonly *MLH1* or *MSH2*. The role *MSH6* plays in inherited cancer susceptibility is less well defined. The aim of this study was to investigate the penetrance and expressivity of *MSH6* mutations in kindreds ascertained through endometrial cancer probands unselected for family history. Detailed pedigrees were constructed for six *MSH6* mutation carriers. All reported cancers and precancers were confirmed, and tissues were obtained when available. Tumors were analyzed for microsatellite instability (MSI) and for expression of *MSH2*, *MLH1*, and *MSH6*. *MSH6* mutation status was determined for 59 family members. Of these 59 individuals, 19 (32%) had confirmed cancers and precancers. There was an excess of mutation carriers among the 19 affected family members (11 [58%] of 19) compared with those among the 40 unaffecteds (8 [20%] of 40, $P = .0065$, odds ratio = 5.5, 95% CI = 1.66–18.19). In four of the seven tumors analyzed from mutation carriers other than the probands, MSI and/or MMR protein expression was consistent with the involvement of *MSH6*. Overall estimated penetrance of the *MHS6* mutations was 57.7%. Of the tumors in mutation carriers, 78% were part of the extended HNPCC spectrum. This study demonstrates that *MSH6* germline mutations are, indeed, associated with increased cancer risk and that the penetrance of mutations may be higher than appreciated elsewhere. A combination of MSI and immunohistochemistry analyses may be helpful in screening for *MSH6* mutation carriers.

Hereditary nonpolyposis colorectal cancer (HNPCC) is an autosomal dominant cancer-susceptibility syndrome characterized by early onset cancers of the colorectum, endometrium, small bowel, ureter, and renal pelvis (Amsterdam criteria II; see Vasen et al. [1999]). Additional extracolonic lesions associated with the syndrome include cancers of the stomach, ovary, brain, and hepatobiliary tract and benign sebaceous adenomas (Vasen et al. 1999). HNPCC is caused by inherited mutations in DNA mismatch-repair genes, most commonly involving *MLH1* (MIM 120436) or *MSH2* (MIM 120435) (Fishel et al. 1993; Bronner et al. 1994). Defective DNA mismatch repair in HNPCC-associated tumors results in the microsatellite instability (MSI) tumor phenotype (Aaltonen et al. 1993). MSI is found in >90% of colorectal tumors and ~75% of endometrial tumors associated with HNPCC (Risinger et al. 1993; Fujiwara et al. 1998).

Germline mutations in *MSH6* (MIM 600678) have been reported elsewhere as an uncommon cause of HNPCC (Miyaki et al. 1997; Wijnen et al. 1999; Wu et al. 1999). In fact, most *MSH6* mutations have been found in kindreds with suspected HNPCC that do not fulfill the classic diagnostic criteria (Kolodner et al. 1999; Shin et al. 1999; Wijnen et al. 1999; Wagner et al. 2001). *MSH6* kindreds are often characterized by multiple endometrial cancers, low penetrance of colorectal cancer, and older age at cancer diagnosis. An additional distinguishing feature of *MSH6*-associated HNPCC is the observation that many cancers arising in mutation carriers

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Table 1

Details of Family Size, Number of Individuals with Tumors, and *MSH6* Mutation Status for Seven Kindreds of *MSH6* Germline Mutation Carriers

Kindreds	No. of Reported Relatives	No. of Relatives with Cancers and Precancers Reported/Confirmed[a]	No. of Relatives with Known Mutation Status	
			Affected Carriers/ Noncarriers[a,b]	Unaffected Carriers/ Noncarriers[a]
ID:				
1524	71	12/12	3/2	5/4
1064	57	7/4	1/2	0/7
1497	2	1/0	0/0	0/0
1319	27	8/0	0/0	0/2
1335	27	9/4	2/1	2/3
1401	58	9/8	5/2	1/11
1389	38	7/1	0/1	0/5
All	280	53/29	11/8	8/32[c]

[a] Mean age of relatives with cancers and precancers reported/confirmed is 58.4/63.8 years, of affected carriers/noncarriers is 65.6/64.9 years, and of unaffected carriers/noncarriers is 42.8/47.0 years.

[b] Affected includes only confirmed cancers and precancers.

[c] Of the affected relatives, 11 of 19 were mutation carriers, whereas 8 of 40 unaffected relatives had an *MSH6* mutation ($P = .0065$, Fisher's exact test).

exhibit an MSI-low (MSI-L) or MS-stable (MSS) tumor phenotype (Wijnen et al. 1999; Wu et al. 1999; Berends et al. 2002). Functional redundancy in the DNA mismatch-repair system could explain the less extensive MSI observed in tumors in *MSH6* germline mutation carriers compared with the MSI of *MSH2*- or *MLH1*-associated cancers (Acharya et al. 1996; Marsischky et al. 1996).

The exact role of *MSH6* germline mutations in inherited cancer susceptibility is uncertain at this time. Whether inherited defects in *MSH6* cause HNPCC, when some *MSH6*-associated tumors do not appear to be deficient in DNA mismatch repair, is a subject of debate. One possible explanation is that *MSH6* defects may affect pathways preceding mismatch-repair function (Kariola et al. 2002). Alternatively, some of the *MSH6* mutations found in kindreds with atypical HNPCC may not be the cause of their cancer susceptibility but, rather, an incidental finding. Lastly, it is also possible that *MSH6* mutations seemingly associated with MSS tumors cause low-level MSI that is not detected by the NCI consensus panel (Boland et al. 1998) used by most investigators.

We investigated the penetrance and expressivity of presumed pathogenic *MSH6* germline mutations in seven kindreds identified through endometrial cancer probands that were not ascertained on the basis of family or medical history (Goodfellow et al. 2003). The loss-of-function mutations in these kindreds are as follows (kindred identification numbers are in boldface): **1401** L634Z; **1335** R911Z; **1497** R911Z; **1389** frameshift, insert TA at codon 1066; **1064** alanine insertion, insert CTG at codons 1163/1164; **1319** frameshift, del CAAG at 1320/1321, and **1524** frameshift, insert TCAAAAGGGACATAGAAAA at 1320. All of the probands' cancers had high-level microsatellite instability (MSI-H). Detailed family histories were obtained for six of the seven probands. Case 1497 declined to participate in the study, and we had only the reported cancer status for her parents. Information regarding cancers or precancers was obtained for 278 relatives of the 6 other endometrial cancer probands (mean 46 per family, a range of 27–71) over four generations. Reported cancers, possible precancers (including colonic polyps and endometrial hyperplasia), and any tumors of uncertain malignant potential, as well as ages at diagnosis, were verified by a review of medical records, pathology reports, and/or death certificates when available. Among 53 relatives (including 1 parent of case 1497) reported as having cancers or precancers, 29 cases (55%) were confirmed via medical record review, yielding detailed information on 32 (54%) of 59 reported lesions.

MSH6 mutation status was determined for 59 relatives (21% of those reported). The PCR primers and methods used to determine mutation status are included in table A1 (online only). The mean age of these 59 relatives was 51.3 years. Of the 59 family members, 19 (32%) had confirmed cancers or precancers (affected). The remaining 40 relatives we analyzed were unaffected by any confirmed cancers or precancers. There was a statistically significant excess of *MSH6* mutation carriers among the affected family members. As shown in table 1, 11 (58%) of 19 affected relatives were mutation carriers, whereas 8 (20%) of 40 unaffected relatives had an

MSH6 mutation ($P = .0065$, odds ratio = 5.5, 95% CI = 1.66–18.19, two-tailed Fisher's exact test).

Overall, the affected family members were older than the unaffected ones. The mean age at diagnosis of the 19 affected family members was 64.7 years, whereas the unaffected family members had a mean age of 46.1 years ($P = .0018$, Student's *t*-test). A similar age difference was found between the mean ages of the 11 affected mutation-carriers (65.6 years) and the 8 unaffected mutation-carriers (42.8 years). Table 1 summarizes the information on confirmed cancers and mutation status for each of the seven kindreds. The pedigrees for the seven kindreds, including mutation status and age at onset of tumors, are presented in figure 1.

In an effort to further determine whether inherited *MSH6* mutations confer an increased risk for cancers in relatives, we compared the rates of confirmed cancers in the first-degree relatives of our 6 *MSH6* probands and 40 MSS endometrial cancer probands from the same patient series (described in Whelan et al. [2002]). We reasoned that this MSS cohort is representative of sporadic endometrial cancer families. As the risk of developing cancer increases with age, only first-degree relatives >60 years old and free from cancer or individuals affected with cancer at any age were considered informative and were included in the analysis. A significantly higher number of cancers were found in first-degree relatives of the 6 *MSH6* probands than in those of the 40 MSS probands. Among 16 informative first-degree relatives in the *MSH6* cohort, 7 cancers were confirmed, versus 17 confirmed cancers in 150 informative first-degree relatives in the MSS cohort (44% vs. 11.3%, $P = .0027$, two-tailed Fisher's exact test).

We assessed tumors for MSI and mismatch-repair protein expression by immunohistochemistry. Archival tissue specimens were available for seven cancers/precancers from seven *MSH6* mutation carriers. Results of the combined MSI analysis and immunohistochemistry for *MSH6* are presented in table 2. For three of the seven mutation carriers (1064:III-1 with a squamous cell carcinoma [SCC] of the tongue, 1524:II-2 with prostate cancer, and 1335:II-3 with a colonic adenoma), the molecular analyses did not suggest an association between *MSH6* germline mutation and the development of their tumors. The specimens showed no evidence of MSI at any marker and stained positively for all three mismatch-repair proteins. Because the MSI phenotype is variable in *MSH6* mutation carriers, it may be unreliable to use solely MSI to make a connection between mutation and disease, but, if the expression data is considered as well, it seems unlikely that these three tumors have a defect in mismatch repair.

For two of the seven mutation carriers (1524:III-2 with a pituitary adenoma and 1335:II-2 with colon cancer) the molecular analysis of the tumors suggested a causative role for *MSH6*. The pituitary adenoma had MSI at BAT25 (fig. 2*A*) as well as loss of the wild-type copy of *MSH6* (fig. 2*B*), whereas the colon cancer had MSI at all markers (data not shown). *MSH6* staining was absent for both the pituitary adenoma and colon carcinoma (fig. 2*C* and additional data, not shown). For the remaining two mutation carriers (1524:II-3 with a colonic adenoma and 1401:III-4 with prostate cancer), evidence for the involvement of *MSH6* was inconclusive. The adenoma from 1524:II-3 showed focally positive staining for *MSH6* but had MSI at D17S250 (data not shown). The prostate carcinoma from 1401:III-4 had absent *MSH6* staining (data not shown); however, there was insufficient tissue to perform MSI analysis on this tumor.

The overall penetrance of *MSH6* germline mutations was 69.2% (18 affected individuals among 26 mutation carriers, including the probands), on the assumption that all cancers/precancers were associated with *MSH6* mutation. Figure 3 illustrates the fraction of penetrant family members plotted against age in years. The estimated penetrance decreases to 57.7% if we exclude the three affected mutation-carriers for whom no evidence for *MSH6* involvement in their tumors was found.

The different types of confirmed cancers and precancers found in *MSH6* mutation carriers and noncarriers, excluding the probands, are presented in table 3. Overall, 9 (69%) of 13 cancers/precancers in mutation carriers were HNPCC-associated lesions in accordance with the ICG Research Criteria (Vasen et al. 1999), compared with 4 (50%) of 8 cancers/precancers in the nonmutation carrier group. As described elsewhere (Buttin et al. 2004), two of the seven probands, 1064 and 1497, had second HNPCC-associated cancers: rectal and ovarian carcinomas, respectively.

Although the link between *MSH2, MLH1,* and HNPCC has been firmly established, the exact role of *MSH6* in inherited cancer susceptibility continues to evolve. *MSH6* germline mutations are a cause of HNPCC, although most families with mutations do not conform to the Amsterdam II criteria. Screening for *MSH6* mutation is recommended for kindreds with suspected inherited cancer susceptibility suggestive of HNPCC who test negative for *MSH2* and *MLH1* germline mutations (Lynch and de la Chapelle 2003). It should be emphasized that mutations in *MSH6* are more frequent in kindreds that fail to meet HNPCC diagnostic criteria. The true incidence of *MSH6* germline mutations in HNPCC is yet to be determined.

Efforts to define the role of *MSH6* in HNPCC, and in cancer susceptibility in general, have been hampered by the fact that *MSH6* mutations may be associated with tumors that lack features usually associated with defective DNA mismatch repair and the fact that kindreds segregating *MSH6* mutations may be lacking the clinical

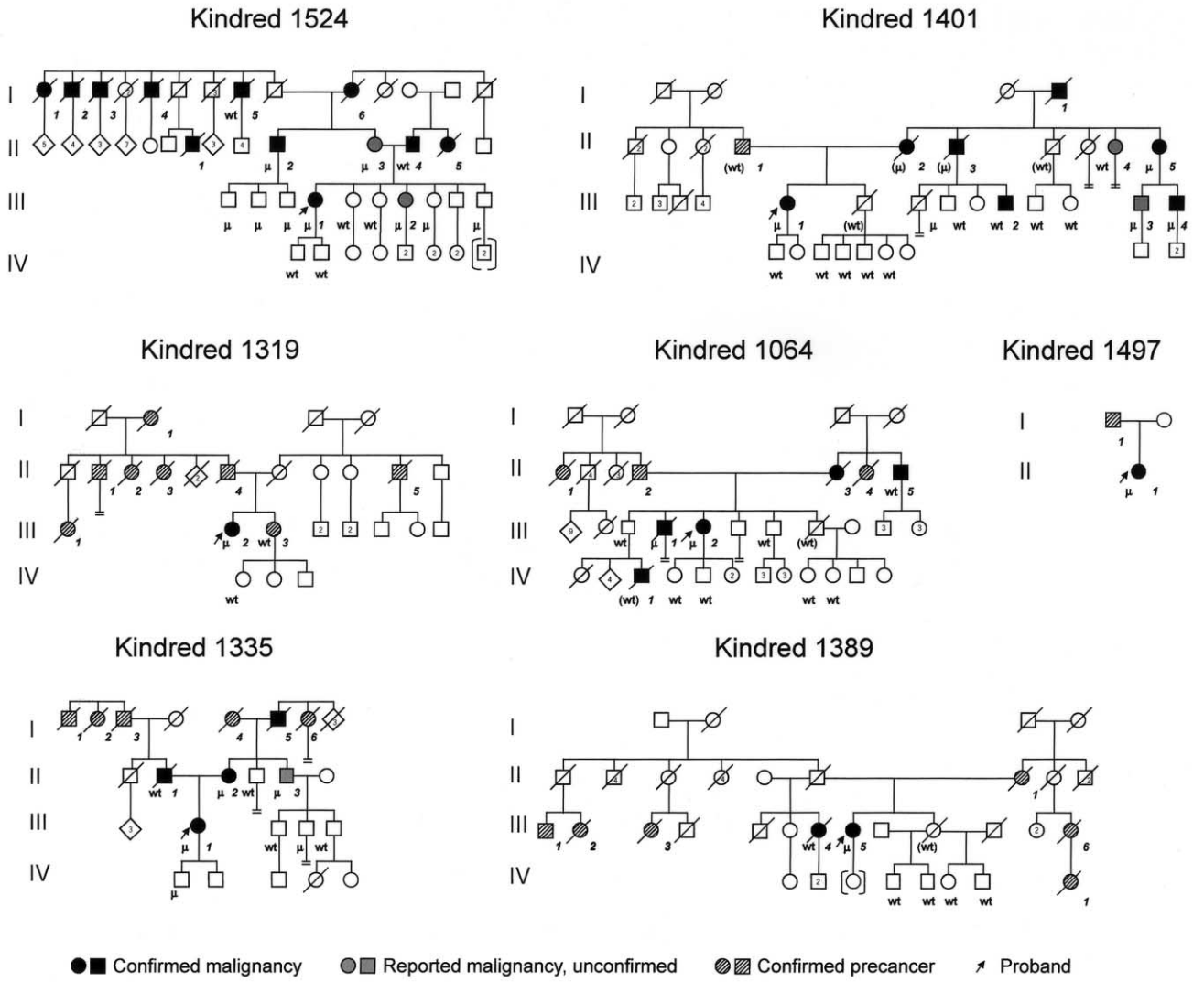

Figure 1 *MSH6* kindreds. *Kindred 1524,* I−1 Hodgkin lymphoma, age 58; I−2 Hodgkin lymphoma, age 44; I−3 lung cancer, age 36; I−4 laryngeal cancer, age 61; I−5 colon cancer, age 92; I−6 endometrial cancer, age 65; II−1 lung cancer, age 69; II−2 prostate cancer, age 73; II−3 colonic tubular adenoma, age 69; II−4 lung cancer, age 74; II−5 lung cancer (small cell), age 71, and lung cancer (oat cell), age 78; III−1 endometrial cancer, age 46; III−2 atypical pituitary adenoma, age 38. *Kindred 1401,* I−1 colon cancer, age 80; II−1 pancreatic cancer, age 73; II−2 cholangiocarcinoma, age 86; II−3 glioblastoma, age 80s; II−4 uterine cancer, age 40s (unconfirmed), and adenomatous colon polyps, age 86; II−5 renal cell cancer, age 50s (unconfirmed), and breast cancer, age 97; III−1 endometrial cancer, age 57; III−2 basal cell cancer, age 56; III−3 adenomatous colon polyps, age 53; III−4 prostate cancer, age 62, and adenomatous colon polyps, age 66. *Kindred 1319,* I−1 skin cancer, age 60s; II−1 cancer, unknown primary, age 40s; II−2 cancer, unknown primary, age 61; II−3 cancer, unknown primary, age 40; II−4 cancer, unknown primary, age 60; II−5 multiple myeloma, age 70s; III−1 endometrial cancer, age 40s; III−2 endometrial cancer, age 74; III−3 skin cancer, age 50s. *Kindred 1064,* II−1 breast cancer, age late 20s; II−2 brain cancer, age 60s; II−3 oat cell lung cancer, age 66; II−4 ovarian cancer, late 30s, and colon cancer, age 40s; II−5 rectal cancer, age 65; III−1 tongue squamous cell carcinoma, age 63; III−2 endometrial cancer, age 53, and rectal cancer, age 61; IV−1 medulloblastoma, age 3. *Kindred 1497,* I−1 esophageal cancer, age unknown; II−1 endometrial cancer, age 52, and ovarian cancer, age 52. *Kindred 1335,* I−1 lung cancer, age unknown; I−2 liver cancer, age unknown; I−3 prostate cancer, age 40s; I−4 breast cancer, age 60; I−5 prostate cancer, age 60; I−6 endometrial cancer, age 50s; II−1 pancreatic cancer, age 71; II−2 endometrial cancer, age 35, and colon cancer, age 67; II−3 adenomatous colon polyps, age 65; III−1 endometrial cancer, age 44. *Kindred 1389,* II−1 endometrial cancer, age 30; III−1 cancer, unknown primary, age unknown; III−2 lung cancer, age 60s; III−3 breast cancer, age 70s; III−4 bladder cancer, age 72; III−5 endometrial cancer, age 61; III−6 cancer, unknown primary, age 76; IV−1 cancer, unknown primary, age unknown.

Table 2

Results of MSI Analysis and IHC of MMR Proteins on Available Tumors of *MSH6* Mutation Carriers

				MSI Markers							IHC		
Kindred ID	Relationship to Proband	Tumor Type[a]	Age at Diagnosis	D17S250	D2S123	D5S346	BAT25	BAT26	BAT40	MSI Status	MSH6	MSH2	MLH1
1064:III-*1*	Brother	SCC tongue	63	NI	NL	NL	NI	NI	NI	MSS	+	+	+
1524:II-*3*	Mother	Colonic adenoma	69	MSI	NL	NL	NI	NI	NI	MSI-L	Focal weak	+	+
1524:II-*2*	Maternal uncle	Prostate ca	73	NL	NL	NL	NI	NI	NL	MSS	+	+	+
1524:III-*2*	Sister	Pituitary adenoma	38	LOH	LOH	NL	MSI	NI	NI	MSI-L	−	+	+
1335:II-2	Mother	Colon ca	67	MSI	MSI	MSI	MSI	MSI	MSI	MSI-H	−	+	+
1335:II-*3*	Maternal uncle	Colonic adenoma	65	NL	NL	NI	ND	NI	NI	MSS	+	+	+
1401:III-4	Maternal cousin	Prostate ca	62	ND	ND	ND	ND	ND	ND	ND	−	ND	+

Note.—MSI analysis was performed using five consensus markers (Boland et al. 1998). Since *MSH6* mutations tend to have lower levels of MSI and MSI is more frequently found in mononucleotide repeats, an additional mononucleotide marker (BAT40) was typed. Samples were classified as MSI-H if instability was detected at two or more consensus markers, as MSI-L if instability was confined to one consensus marker, and as MSS if none of the consensus markers revealed instability. IHC for *MLH1*, *MSH2*, and *MSH6* was performed with the use of 5-μm thick paraffin sections mounted on charged slides, as described elsewhere (Buttin et al. 2004). Sections of normal appendix served as external positive controls. Nuclear staining was read as positive (+), and absence of nuclear staining of any of these was read as negative (−). Abbreviations in the table are as follows: ca = carcinoma, LOH = loss of heterozygosity, NL = no loss, NI = not informative, and ND = no data.

[a] Includes only confirmed cancers and precancers (see "Subjects and Methods").

hallmarks of HNPCC. Hence, screening on the basis of either tumor MSI status or cancer family history may fail to identify a subset of patients with *MSH6* germline defects. There is a clear need to further characterize the natural history of *MSH6* germline mutations, to better define *MSH6*-associated HNPCC and, ultimately, to devise appropriate identification, clinical screening, and treatment strategies.

We evaluated seven kindreds in an effort to clarify the role of *MSH6* germline mutations in inherited cancer susceptibility. Unlike prior studies, we investigated families of *MSH6* germline mutation carriers that were identified independent of family history or age at diagnosis. The *MSH6* germline mutations were detected in women with MSI-H endometrial cancers. Given the reported association between *MSH6* germline mutations and MSI-L or MSS tumors (Wu et al. 1999; Wagner et al. 2001; Berends et al. 2002), our results may suggest that the mutations are different from those described elsewhere. It is possible that kindreds ascertained through probands with MSI-L or MSS tumors have different penetrance and expressivity from that of the kindreds we studied. Regardless, the family histories of our *MSH6* germline mutation carriers underscore the uncertainty as to who should be screened for *MSH6* germline mutations. We note a heterogeneous spectrum of cancers and precancers (colonic adenomas). Two kindreds (1335 and 1401) could be classified as atypical HNPCC families (because of two, as opposed to three, first-degree relatives with endometrial/colon cancer in kindred 1335 and because of older age at diagnosis in kindred 1401). However, the other four kindreds may not have been identified as families with increased risk for inherited cancer susceptibility on the basis of family history alone.

The families we studied have many features in common with *MSH6* kindreds that have been described elsewhere. However, we detected only 1 case of colon carcinoma among 13 different types of cancers and precancers found in 11 affected mutation-carriers (excluding the probands). This finding confirms the suggestion that *MSH6* kindreds may have a lower penetrance of colorectal cancer (Wijnen et al. 1999) in comparison with that of *MSH2* and *MLH1* HNPCC kindreds. We did find an increased number of colonic adenomas in the mutation carriers compared with the number found in noncarriers (four vs. one), possibly suggesting increased potential for carcinogenesis with very slow progression. We found one case of endometrial cancer among the five female affected mutation-carriers with a mean age of 65 years, in addition to the six known endometrial cancers in the probands. However, many of the female family members in the older generations had hysterectomies at an early age for benign reasons. Other authors have reported a predominance of endometrial cancers (52%–73%) in female *MSH6* germline mutation carriers (Wijnen et al. 1999; Wagner et al. 2001; Berends et al. 2002). Including our six endometrial cancer probands, the incidence of endometrial cancer in female mutation carriers in this study (7 [64%] of 11) confirms this suggestion.

Although the comparison was limited by small numbers, there was no significant difference between the types of cancers found in mutation carriers and those found in nonmutation carriers (table 3), with the exception of an excess of colonic adenomas in the mutation carriers. This clinical heterogeneity (variable expressivity) underlines the limitations of screening patients for a family history suggestive of HNPCC or atypical HNPCC to detect those with germline *MSH6* defects.

Our data demonstrate that *MSH6* germline mutations

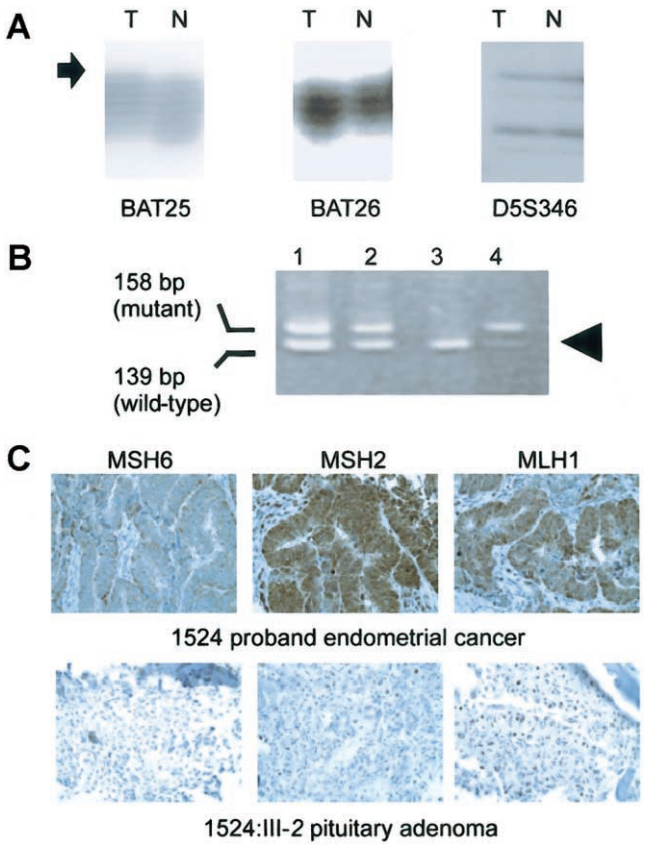

Figure 2 Molecular analysis of an atypical pituitary adenoma that arose in an *MSH6* mutation carrier. *A,* MSI analysis. Instability at the BAT25 marker is seen as a subtle increase in the size of the PCR products in the tumor DNA (T), compared with those in the normal DNA (N), and as a novel insertion (*arrow*). The tumor and matched normal specimens show identical patterns with the BAT26 and D5S346 markers. *B,* Mutation analysis revealing loss of the wild-type allele in the tumor (*arrowhead*). *C,* Immunohistochemistry revealing loss of expression of *MSH6* in the proband's endometrial cancer (*upper panel*) and in the atypical pituitary adenoma (*lower panel*). Note the positive staining with *MLH1* and *MSH2* in both tumors.

are, indeed, associated with increased cancer risk. Although the mouse studies by Edelmann et al. (1997) suggest that *MSH6* mutation is associated with highly penetrant, late-onset disease, studies of humans, to date, could not truly address onset and penetrance, as the majority of kindreds evaluated were chosen on the basis of increased familial risk. Screening patients for inherited cancer susceptibility associated with *MSH6* mutation perhaps should include an awareness of the overall increased numbers of relatives with any cancer at any age, rather than a specific focus on HNPCC-type disease. The exact tumor spectrum associated with *MSH6* defects will require further investigation.

Lastly, the results of our molecular correlative studies suggest the utility of immunohistochemistry in the assessment of the role of *MSH6* in tumorigenesis. We demonstrated a very consistent pattern of absent staining for *MSH6* in all endometrial tumors of our seven probands, whereas both *MSH2* and *MLH1* staining remained intact. These findings confirm a report by de Leeuw et al. (2000), in which the authors described consistent isolated absence of *MSH6* staining in tumors from *MSH6* germline mutation carriers, consistent absence of both *MSH2* and *MSH6* staining in *MSH2* mutation carriers, and inconsistent results in tumors from *MLH1* mutation carriers. De Leeuw et al.'s study (2000) showed more variability with regard to MSI than did ours. As noted, all of our index endometrial cancers were MSI-H, and the mutations we evaluated are all presumed loss-of-function defects. However, some of the other tumors found in *MSH6* mutation carriers with absent or questionable *MSH6* staining were MSI-L (the colonic adenoma in 1524:II-3 had MSI at D17S250, and the pituitary adenoma in 1524:III-2 had MSI at BAT25), and none were MSS (table 2). The combination of MSI and immunohistochemistry (IHC) analysis was able to demonstrate that three tumors arising in *MSH6* mutation carriers, (a SCC of the tongue in 1064:III-1, a prostate carcinoma in 1524:II-2, and a colonic adenoma in 1335:II-3) were unlikely to be associated with loss of *MSH6* function.

Our data provide only an estimate of the penetrance of *MSH6* germline mutations because we were unable to obtain tissue on all confirmed cancers in mutation carriers. Therefore, it is possible that we may have overestimated the penetrance of the mutations. However, our conservative estimate of 57.7% total penetrance (excluding the three affected mutation-carriers without any evidence of *MSH6* involvement in their tumors) is still higher than elsewhere appreciated. As expected, the mean age of cancer diagnosis among affected mutation-

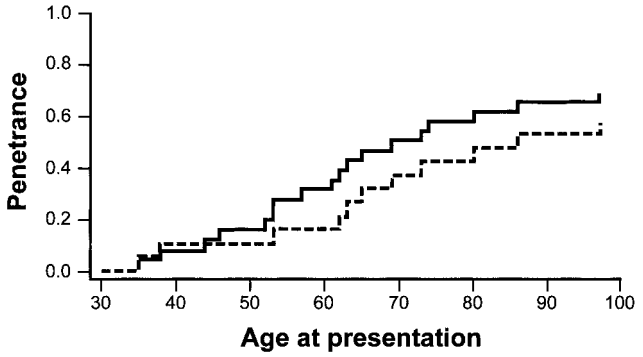

Figure 3 Penetrance of *MSH6* mutations in 26 mutation carriers. The seven endometrial cancer probands are included in one plot (*solid line*) and are excluded in the other plot (*dashed line*).

Table 3

Summary of Cancers and Precancers Found in *MSH6* Mutation Carriers Versus Noncarriers

Precancer/Cancer[a,b]	No. in *MSH6* Mutation Carriers	No. in Noncarriers
Colonic adenoma	4	1
Colon ca	1	1
Endometrial ca	1	0
Rectal ca	0	1
Hepatobiliary ca	1	0
Bladder ca	0	1
Brain ca	2	1
Pancreatic ca	0	1
Lung ca	0	1
Skin ca	1	1
Prostate ca	2	0
Breast ca	1	0
All HNPCC-associated	9 (of 13)	4 (of 8)

[a] ca = carcinoma.

[b] Tumors in italics are associated with HNPCC in accordance with the ICG definition of "Lynch Syndrome" (Vasen et al. 1999).

carriers (65.6 years) is significantly higher than the mean age reported for HNPCC patients with *MLH1* or *MSH2* mutations (41–48 years, depending on cancer type; see Wagner et al. [2001]).

In summary, our study represents an unbiased analysis of the natural history, including penetrance and expressivity, of *MSH6* germline mutations. We demonstrate an increased cancer risk associated with these mutations, although the exact tumor spectrum remains to be elucidated. Our data confirm other reports that some *MSH6* kindreds fall under the extended or atypical HNPCC spectrum and also show that other kindreds are characterized by tumors only recently implicated in HNPCC, such as prostate cancer (Soravia et al. 2003), or not previously associated with HNPCC, such as an atypical pituitary adenoma. The overall penetrance of *MSH6* germline mutations may be as high as 58%, and, as noted by other investigators, the age at onset of cancers is later than is seen in kindreds segregating *MSH2* and *MLH1* mutations. Neither tumor MSI status, young age at cancer diagnosis, nor a family history suggestive of atypical or classic HNPCC will identify all *MSH6* mutation carriers. A combination of MSI and IHC analyses for mismatch-repair–protein expression in tumor tissue may be helpful in screening for possible mutation carriers.

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Electronic-Database Information

URL for data presented herein is as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM

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