Phenotype Comparison of *MLH1* and *MSH2* Mutation Carriers in a Cohort of 1,914 Individuals Undergoing Clinical Genetic Testing in the United States

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Abstract

Background and Aims: **Lynch syndrome is caused by germ-line mismatch repair gene mutations. We examined the phenotypic differences between *MLH1* and *MSH2* gene mutation carriers and whether mutation type (point versus large rearrangement) affected phenotypic expression.**

Methods: **This is a cross-sectional prevalence study of 1,914 unrelated probands undergoing clinical genetic testing for *MLH1* and *MSH2* mutations at a commercial laboratory.**

Results: **Fifteen percent (285 of 1,914) of subjects had pathogenic mutations (112 *MLH1*, 173 *MSH2*). *MLH1* carriers had a higher prevalence of colorectal cancer (79% versus 69%, $P = 0.08$) and younger mean age at diagnosis (42.2 versus 44.8 years, $P = 0.03$) than *MSH2* carriers. Forty-one percent of female carriers had endometrial cancer and prevalence was similar in both groups. Other cancers were more frequent in *MSH2* carriers (24% versus 9%, $P = 0.001$) and their families ($P < 0.001$). Multivariable analyses confirmed these associations. Of the 1,016 subjects who underwent Southern blot analysis, 42 had large rearrangements (7 *MLH1*, 35 *MSH2*). There were no phenotypic differences between carriers with large rearrangements and point mutations.**

Conclusions: **In this large study of mismatch repair gene mutation carriers from the United States, *MLH1* carriers had more colorectal cancer than *MSH2* carriers whereas endometrial cancer prevalence was similar. Large genomic rearrangements were more frequent in the *MSH2* gene. *MSH2* carriers and their relatives have more extracolonic nonendometrial Lynch syndrome–associated cancers and may benefit from additional screening.** (Cancer Epidemiol Biomarkers Prev 2008;17(8):2044–51)

Introduction

Lynch syndrome, also known as hereditary nonpolyposis colorectal cancer (HNPCC), is the most common inherited colorectal cancer syndrome. It is estimated to account for up to 5% of all colorectal cancers and is caused predominantly by a mutation in one of four DNA mismatch repair genes: *MSH2, MLH1, MSH6*, and *PMS2*. Germ-line alterations in the associated cancer susceptibility genes seem to confer a 60% to 80% lifetime risk of developing colorectal cancer in the absence of medical intervention (1-4).

Mismatch repair gene mutations are also associated with a significantly increased risk for certain types of extracolonic malignancies. Lifetime endometrial cancer risk is estimated to be 40% to 60%, and risks for ovarian cancer and tumors of the urinary bladder and renal collecting system are believed to range from 10% to 20% (5-7). Other gastrointestinal tumors such as stomach, hepatobiliary, and pancreatic carcinomas, although overrepresented in Lynch syndrome compared with their prevalence in the general population, seem to occur less commonly. The possibility that cancer risks may vary depending on the type of mismatch repair gene mutation may have significant implications on cancer screening recommendations.

Despite the major advances made in molecular genetics, there remains a limited awareness of the syndrome in the general medical community. Deficiencies in family information and incomplete gene penetrance have contributed to the underrecognition of affected patients and families. Clinicians are challenged by the heterogeneity that exists when assessing the Lynch syndrome phenotype (8). Multiple predictive models have been recently developed to aid in the recognition of mutation carriers (9-11). However, whether there is a difference in genotype-phenotype expression between carriers of the two most common mismatch repair gene mutations, *MLH1* and *MSH2*, has not been resolved. The existing data are limited and derived predominantly from European family registries that invariably are subject to ascertainment bias.

In the present study, we examine the genotype-phenotype differences in Lynch syndrome mutation carriers in a large United States population undergoing clinical genetic testing for mutations in the *MLH1* and *MSH2* genes. Because different mutation types (point

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mutations versus large genomic rearrangements) may have different functional consequences on *MLH1* and *MSH2* genes, correlations between mutation type and phenotypic expression were also explored.

Materials and Methods

Study Population. A total of 2,276 unrelated probands submitted blood samples for full gene sequencing of *MLH1* and *MSH2* to Myriad Genetic Laboratories, Inc. (Salt Lake City, UT), starting in 2000. Information on the proband demographic profile, including age, gender, and ethnicity, as well as personal and family cancer history was obtained from the test order form that was completed by the health care professional ordering the genetic testing. Because of missing information, 362 probands were excluded. Data from 1,914 probands were available for analysis (9).

The total number of relatives per proband included those who were (*a*) first-degree relatives (FDR) or second-degree relatives (SDR) of the probands; (*b*) affected with the following Lynch syndrome–related cancers: colorectum, endometrium, stomach, small intestine, pancreas, bile ducts, ovaries, urinary tract (kidney, ureter, bladder), brain (glioblastoma multiforme), or sebaceous glands; and (*c*) on the affected side of the family. Overall, there were a total of 4,361 relatives with Lynch syndrome–related cancers in the final study sample.

Data collection and analysis occurred independently. Collection of clinical information and molecular analyses occurred at Myriad Genetic Laboratories and an anonymized, electronic data set was provided to investigators at Dana-Farber/Harvard Cancer Center for further statistical analyses. This study was reviewed and approved by the Dana-Farber/Harvard Cancer Center Institutional Review Board.

Laboratory Methods. Mutation analysis was done using methods previously described (9). Full gene sequencing of *MLH1* and *MSH2* was done on 898 unrelated probands that submitted blood samples to Myriad Genetics starting in 2000. Starting in August 2004, full gene sequencing and large genomic rearrangement analysis of *MLH1* and *MSH2* genes were done on 1,016 additional blood samples from unrelated probands. Southern blot analysis was used to search for large genomic alterations in the *MLH1* and *MSH2* genes. Methylation analyses to evaluate the presence of *MLH1* or *MSH2* germ-line epimutations were not done on the samples provided by the subjects in this study.

Individuals with deleterious mutations or ''suspected deleterious'' mutations were defined as mutation positive. Mutations leading to truncating or unstable proteins were considered deleterious and included frameshift, nonsense, and splice site mutations, as well as large deletions and rearrangements. All exon deletions and nonsense and frameshift mutations occurring at or before amino acids 733 and 888 of *MLH1* and *MSH2,* respectively, were defined as mutation-positive in this study. ''Suspected deleterious'' mutations were those genetic variants for which the available evidence indicated a likelihood (but no proof) that the mutation was deleterious. Examples include splice site mutations that occur at the conserved locations of splice acceptors and splice donors. In this study, missense mutations and noncoding intervening sequence mutations were defined as deleterious based on data derived from linkage analysis of high-risk families, functional assays, biochemical evidence, and/or demonstration of abnormal mRNA transcript processing. Mutation-negative probands were individuals with identified missense mutations within intronic regions whose clinical significance is not yet established, as well as those with polymorphisms and unclassified variants.

Statistical Methods. Statistical analysis was done using SAS statistical software (version 9.1; SAS Institute, Inc.). The variables related to the proband included presence and age(s) of colorectal cancer (none, one, two, or more), endometrial cancer, and/or other Lynch syndrome–related cancer [stomach, small intestine, pancreas, bile ducts, ovaries, urinary tract (kidney, ureter, bladder), brain (glioblastoma multiforme), or sebaceous glands]. Age was treated as a continuous variable and thereafter dichotomized to <50 or ≥ 50 years. The variables related to the proband family members included number of colorectal cancers, endometrial cancers, and/or other Lynch syndrome–related cancers and corresponding age(s) of diagnosis, as well as relationship to proband (FDR or SDR). To incorporate the effect of genetic distance between FDR and SDR, a weighted sum of cancer diagnoses among relatives was used. Cancer diagnoses in SDR were weighted half that in FDR (12). In probands and family members diagnosed as having the same cancer more than once, the age at diagnosis was defined as the youngest age.

Univariate analyses were used to assess the relationship between mismatch repair genes mutation status or mutation type and potential predictive variables related to the carriers' personal and family history of cancer. Categorical outcome data were reported as frequencies. Comparisons between mismatch repair mutation status groups (*MLH1* versus *MSH2*) and mutation types (point mutations versus large rearrangements) were assessed using χ^2 tests. Continuous data were reported in mean values and compared between groups using Student's *t* test. Comparisons are reported as odds ratios (OR) with 95% confidence intervals (95% CI). A two-sided *P* value of <0.05 was considered statistically significant.

Multivariable logistic regression analysis was used to further assess the associations of clinical features with mutation status and type. The multivariable analysis of type of mutation controlled for the presence of a mutation in the *MLH1* or *MSH2* gene. To account for all Lynch syndrome–related cancers, it was determined *a priori* that the presence of endometrial cancer and any other Lynch syndrome–related cancer(s) would be included in the model irrespective of statistical significance in the univariate analysis.

Results

Univariate Analysis. Two hundred eighty-five (15%) patients had detectable deleterious mismatch repair mutations in the *MLH1* or *MSH2* genes (112 *MLH1* and 173 *MSH2*). The clinical characteristics of the identified mutation carriers appear in Table 1. Additional

demographic data pertaining to ancestry and country of origin have been previously reported (9). Ninety-eight percent of the samples and data provided were by individuals residing in the United States. Only 63% (179 of 285) of all mutation carriers fulfilled the Modified Amsterdam Criteria. Conversely, the mismatch repair mutation detection rate was 34% (179 of 539) among all subjects fulfilling the Modified Amsterdam Criteria in the entire study population. *MSH2* mutation carriers were more likely to have family histories fulfilling the Amsterdam criteria than *MLH1* carriers (OR, 1.7; 95% CI, 1.04-2.8). Mutation carriers reported a total of 936 family members with colorectal cancer or other Lynch syndrome–related cancers.

Colorectal Cancer. The prevalence of colorectal cancer was higher in *MLH1* than *MSH2* mutation carriers (79% versus 69%, $P = 0.08$), but the percentage of mutation carriers with two or more colorectal cancer diagnoses was similar (14% versus 12% for *MLH1* and *MSH2* carriers, respectively; Table 1). Among *MLH1* carriers, 55 of 112 (50%) reported colorectal cancer as their only cancer compared with 74 of 173 (43%) of *MSH2* carriers ($P = 0.3$). *MLH1* carriers were significantly more likely to be diagnosed with colorectal cancer at age less than 50 years compared with *MSH2* carriers ($P = 0.01$). The mean age of colorectal cancer diagnosis in the entire sample was 42.2 years for *MLH1* carriers compared with 44.8 years for *MSH2* carriers (Table 2) where male *MLH1* carriers had a significantly younger mean age at colorectal cancer diagnosis compared with *MSH2* carriers (38 versus 44 years; $P < 0.01$). This age differential was not appreciated among female carriers.

The mean number of colorectal cancer tumors per family was significantly higher in families with *MLH1* than *MSH2* mutations (1.8 versus 1.5, respectively; $P = 0.04$, Table 1). In addition, relatives (FDR and SDR) of *MLH1* carriers reported earlier age at colorectal cancer diagnosis compared with relatives of *MSH2* carriers (mean age at colorectal cancer diagnosis: 40.5 versus 44.6 years; $P = 0.01$; Table 2).

Endometrial Cancer. Endometrial cancer was reported in 41% of all mutation carriers (68 of 167) and its prevalence did not statistically differ among females with *MLH1* or *MSH2* mutations (36% versus 44%, respectively). Of all females undergoing predictive genetic testing, 9% (119 of 1,308) reported endometrial cancer as their only cancer diagnosis; 16% (19 of 119) of these women were found to be *MSH2* mutation carriers and 6% (7 of 119) were *MLH1* mutation carriers ($P = 0.2$). The mean age at the time of endometrial cancer diagnosis

Table 1. Clinical characteristics of mutation carriers: *MLH1* versus *MSH2*

	Gene mutation		*P*	OR (95% CI)*
	MLH1 ($n = 112$)	*MSH2* ($n = 173$)		
	Frequency (%)	Frequency (%)		
Gender			0.39	0.8 (0.5-1.3)
Male	50 (45)	68 (39)		
Female	62 (55)	105 (61)		
Modified Amsterdam	62 (55)	117 (68)	0.03	1.7 (1.04-2.8)
Personal cancer history				
CRC				
Yes	88 (79)	119 (69)	0.08	0.6 (0.3-1.0)
No	24 (21)	54 (31)		
None	25 (21)	54 (31)	0.25	—
One	72 (64)	99 (57)		
Two or more	16 (14)	20 (12)		
CRC diagnosed at age <50 y	72 (83)	80 (67)	0.01	0.4 (0.2-0.8)
Endometrial cancer (among females only)	22/62 (36)	46/105 (44)	0.33	1.4 (0.7-2.7)
Other HNPCC†	10 (9)	41 (24)	0.001	3.2 (1.5-6.6)
Multiple HNPCC	33 (29)	51 (29)	1.00	1.0 (0.6-1.7)
Family cancer history				
Mean number of tumors				
FDR				
CRC	1.33	1.19	0.30	
Endometrial cancer	0.24	0.30	0.43	
Other HNPCC	0.14	0.39	<0.001	
SDR				
CRC	0.99	0.67	0.01	
Endometrial cancer	0.23	0.13	0.12	
Other HNPCC	0.13	0.16	0.53	
FDR + SDR‡				
CRC	1.82	1.53	0.04	
Endometrial cancer	0.36	0.36	0.99	
Other HNPCC	0.20	0.46	<0.001	

Abbreviation: CRC, colorectal cancer.

*Reference group: *MLH1* carriers (OR, 1.0).

†HNPCC includes cancers in the colorectum, kidney, ureter, bladder, brain, biliary tract, stomach, small intestine, ovary, pancreas, and sebaceous neoplasms.

‡A weighted sum of cancer diagnoses among FDR + SDR is used to incorporate the effect of genetic distance. Cancer diagnoses in SDR were weighted half that in FDR (12).

Table 2. Age of diagnosis among mutation carriers

	Gene mutation		*P*
	MLH1	*MSH2*	
Mean youngest age of diagnosis (±SE), y			
Carrier			
CRC	42.2 (±0.9)	44.8 (±0.7)	0.03
Endometrial cancer	45.8 (±0.5)	45.5 (±0.4)	0.69
Other HNPCC* cancer	49.1 (±3.2)	49.6 (±2.0)	0.92
Relatives			
FDR			
CRC	41.1 (±1.3)	44.8 (±1.1)	0.03
Endometrial cancer	48.0 (±1.2)	46.2 (±1.5)	0.38
Other HNPCC	49.9 (±3.1)	50.0 (±2.0)	1.00
SDR			
CRC	43.6 (±1.7)	52.2 (±1.8)	<0.01
Endometrial cancer	48.3 (±2.2)	48.4 (±3.0)	1.00
Other HNPCC	48.8 (±3.7)	50.9 (±3.9)	0.72
FDR + SDR †			
CRC	40.5 (±1.2)	44.6 (±1.1)	0.01
Endometrial cancer	46.8 (±1.1)	45.8 (±1.3)	0.55
Other HNPCC	49.2 (±2.4)	50.2 (±1.9)	0.77

NOTE: Carriers of *MLH1* and *MSH2* gene mutations.
*HNPCC includes cancers in the colorectum, kidney, ureter, bladder, brain, biliary tract, stomach, small intestine, ovary, pancreas, and sebaceous neoplasms.
†A weighted sum of cancer diagnoses among FDR + SDR is used to incorporate the effect of genetic distance. Cancer diagnoses in SDR were weighted half that in FDR (12).

was also similar for both *MLH1* and *MSH2* carriers, 45.8 versus 45.5 years, respectively (Table 2). There was no difference in the mean number of endometrial cancers or the mean age of cancer diagnosis among family members.

Other Lynch syndrome–associated cancers. Eighteen percent (51 of 285) of mutation carriers reported other Lynch syndrome–associated cancers. The prevalence of such cancers was significantly higher among *MSH2* carriers compared with *MLH1* carriers: 24% versus 9%, respectively (OR, 3.2; 95% CI: 1.5-6.6; Tables 1 and 3).

The proportion of carriers with history of sebaceous skin tumors differed significantly between *MLH1* and *MSH2* mutation carriers, 2% versus 8% ($P = 0.05$). Eighty-seven percent (13 of 15) of mutation carriers with sebaceous skin tumors had a *MSH2* mutation. Most of the urinary tract cancers (11 of 15) and ovarian cancers (11 of 14) were found in subjects with *MSH2* mutations. All other cancer types associated with Lynch syndrome were uncommon (Table 3). The mean number of other Lynch syndrome–related tumors per family (FDR + SDR) was lower in *MLH1* compared with *MSH2* carriers ($P < 0.001$; Table 1).

Type of Deleterious Mutation: Large Genomic Rearrangements versus Point Mutations. A total of 42 patients had large genomic rearrangements among 1,016 probands who underwent both full gene sequencing and large rearrangement analysis, with 7 in the *MLH1* and 35 in the *MSH2* genes. The age at diagnosis of colorectal cancer was the most striking difference in phenotype among carriers (48.3 years in those with large rearrangements versus 43.0 years for those with point mutations,

Table 3. Lynch syndrome-associated cancers among mutation carriers

Type of tumor	*MLH1* (*n* = 112)	*MSH2* (*n* = 173)	*P*
	Frequency (%)	Frequency (%)	
Colorectal	88 (79)	119 (69)	0.08
Endometrial	22 (20)	46 (27)	0.33
Other*	10 (9)	41 (24)	0.001
Urinary tract tumors	4	11	
Renal/kidney	1	6	
Ureter	2	3	
Bladder	1	4	
Brain	0	2	
Biliary	1	0	
Stomach	0	3	
Small intestine/duodenum	0	3	
Ovary	3	11	
Sebaceous †	2	13	
Pancreas	0	0	

*Four *MSH2* mutation carriers reported more than one Lynch syndrome–related cancer other than colorectal or endometrial cancer.
†$P = 0.05$.

Table 4. Clinical characteristics of carriers by type of mutation: point mutations versus large rearrangements

	Type of mutation		*P*	OR (95% CI)*
	Point mutations (*n* = 243)	Large rearrangements (*n* = 42)		
	Frequency (column %)	Frequency (column %)		
Gender			0.24	1.5 (0.8-2.9)
Male	97 (40)	21 (50)		
Female	146 (60)	21 (50)		
Modified Amsterdam	148 (61)	31 (76)	0.08	2.0 (0.9-4.2)
Personal cancer history				
CRC				
Yes	173 (71)	34 (81)	0.26	1.7 (0.8-3.9)
No	70 (29)	8 (19)		
None	71 (29)	8 (19)	0.14	—
One	139 (57)	31 (74)		
Two or more	33 (14)	3 (7)		
CRC diagnosed at age <50 y	135 (78)	17 (50)	0.001	0.3 (0.1-0.6)
Endometrial cancer (among females only)	58/146 (40)	10/21 (48)	0.49	1.4 (0.6-3.5)
Other HNPCC†	40 (16)	11 (26)	0.13	1.8 (0.8-3.9)
Multiple HNPCC	70 (29)	14 (33)	0.58	1.2 (0.6-2.5)
Family cancer history				
Mean number of tumors:				
FDR				
CRC	1.23	1.33	0.47	
Endometrial cancer	0.28	0.26	0.86	
Other HNPCC	0.26	0.45	0.11	
SDR				
CRC	0.79	0.81	0.92	
Endometrial cancer	0.15	0.24	0.47	
Other HNPCC	0.13	0.21	0.20	
FDR + SDR‡				
CRC	1.63	1.74	0.45	
Endometrial cancer	0.35	0.38	0.79	
Other HNPCC	0.33	0.56	0.07	

NOTE: Carriers of *MLH1* and *MSH2* gene mutations.
*Reference group: *MLH1* carriers (OR, 1.0).
†HNPCC includes cancers in the colorectum, kidney, ureter, bladder, brain, biliary tract, stomach, small intestine, ovary, pancreas, and sebaceous neoplasms.
‡A weighted sum of cancer diagnoses among FDR + SDR is used to incorporate the effect of genetic distance. Cancer diagnoses in SDR were weighted half that in FDR (12).

P = 0.001; Table 5). Individuals with point mutations were more likely to have colorectal cancer diagnosed at age less than 50 years (Table 4).

The mean number of colorectal and endometrial tumors among FDRs and SDRs was similar between the two mutation types. However, family members of mutation carriers with large rearrangement mutations had a slightly higher mean number of other Lynch syndrome–related cancers (not statistically significant; Table 4). Overall, the mean youngest age of any Lynch syndrome cancer (including colorectal cancer) among FDRs and SDRs did not differ between carriers with point mutations or large rearrangements (Table 5).

Multivariable Analysis. Multivariable logistic regression analysis identified three clinical features predictive of *MLH1* versus *MSH2* mutation: (*a*) *MLH1* mutation carriers had a slightly higher likelihood of having more colorectal cancer diagnosed among family members than *MSH2* carriers (OR, 1.3; 95% CI, 1.0-1.7); (*b*) *MSH2* mutation carriers were more likely to have a personal history of nonendometrial extracolonic cancers compared with *MLH1* mutation carriers (OR, 3.2; 95% CI, 1.4-7.3); and (*c*) *MSH2* mutation carriers were more likely to have an increased number of other cancers diagnosed among relatives than *MLH1* mutation carriers (OR, 2.1; 95% CI, 1.2-3.7). Personal history of endometrial cancer and colorectal cancer diagnosed at a young age were not predictive of which mismatch repair gene was mutated (Table 6). No significant interactions were found between the independent predictors and age or gender. There were also no clinical variables predictive of the type of mutation (Table 6).

Discussion

Our study provides data on genotype-phenotype relationships associated with germ-line mutations in the mismatch repair genes *MLH1* and *MSH2* from a large, diverse population of mutation carriers from the United States. The results shed light on important similarities and differences between other studies of mismatch repair gene mutation carriers, which have been conducted primarily in European registry–based populations (Table 7). Because of the large number of mutation carriers, we were also able to evaluate whether the type of mutation affected phenotypic expression and found results contrary to our expectations.

Compared with *MSH2* carriers, *MLH1* mutation carriers were more likely to have colorectal cancer as

Table 5. Age of diagnosis among carriers with point mutations versus large rearrangements

	Type of mutation		*P*
	Point mutations	Large rearrangements	
Mean youngest age of diagnosis (±SE), y			
Carrier			
CRC	43.0	48.3	0.001
Endometrial cancer	45.7	44.7	0.12
Other HNPCC* cancer	49.7 (±2.1)	48.7 (±2.8)	0.82
Relatives			
FDR			
CRC	42.8 (±0.9)	45.9 (±2.3)	0.14
Endometrial cancer	46.8 (±1.2)	47.3 (±1.6)	0.85
Other HNPCC	50.9 (±1.9)	46.0 (±3.4)	0.24
SDR			
CRC	47.6 (±1.4)	52.7 (±3.6)	0.17
Endometrial cancer	48.7 (±2.1)	46.0 (±5.3)	0.64
Other HNPCC	48.5 (±2.8)	57.2 (±8.7)	0.23
FDR + SDR†			
CRC	42.8 (±0.9)	44.1 (±2.1)	0.54
Endometrial cancer	46.1 (±1.0)	46.3 (±1.8)	0.94
Other HNPCC	50.0 (±1.6)	49.5 (±3.6)	0.89

NOTE: Carriers of *MLH1* and *MSH2* gene mutations.
*HNPCC includes cancers in the colorectum, kidney, ureter, bladder, brain, biliary tract, stomach, small intestine, ovary, pancreas, and sebaceous neoplasms.
†A weighted sum of cancer diagnoses among FDR + SDR is used to incorporate the effect of genetic distance. Cancer diagnoses in SDR were weighted half that in FDR (12).

the only type of cancer diagnosed, an increased number of colorectal cancer among relatives, and a younger age of cancer diagnosis. The younger age at diagnosis of colorectal cancer was more notable among *MLH1* mutation–positive male carriers than in females. Contrary to prior findings in other studies, we found no appreciable difference in endometrial cancer prevalence or age of diagnosis between *MLH1* and *MSH2* mutation carriers. Similar to our previously reported experience (13) and studies from European registry–based populations, other Lynch syndrome–associated cancers were more prevalent among both *MSH2* mutation carriers and their relatives. Overall, we found that a personal history or an increased number of extracolonic cancers other than endometrial among family members were clinical features associated with the presence of a *MSH2* gene mutation.

We evaluated the effect of the type of gene alteration on phenotype, expecting that large rearrangements and deletions would lead to a more severe phenotype with more multiple cancers and earlier ages of onset (14). The results unexpectedly showed that individuals who had a rearrangement had older ages at cancer diagnosis but age was not found to be a significant independent predictor of mutation type. Overall, there were few differences between the cancer histories of individuals with point mutations versus large rearrangements, which suggest that the specific mismatch repair gene involved is more important than the type of mutation in determining phenotype.

Our results illustrate some important similarities and differences among reports of genotype-phenotype associations in Lynch syndrome. Reviewing the data from relatively large cohorts of Dutch, Finnish, French, and German *MSH2* and *MLH1* mutation carriers (7, 15-18), most studies have reported that extracolonic nonendometrial cancers are more prevalent in *MSH2* carriers than in *MLH1*. Although the small numbers of these cancers in this study limited statistical power, ovarian and upper urinary tract cancers were slightly more prevalent among *MSH2* mutation carriers, corroborating previous reports by Vasen et al. (7). Our finding that sebaceous skin tumors were significantly associated with *MSH2* mutations also supports data from Mangold et al. (19)

Table 6. Multivariable analysis for factors predicting gene mutation status and mutation type

Characteristics	Odds ratio (95% CI)	
	*MLH1** vs *MSH2*	Point mutations* versus large rearrangements
CRC age of diagnosis less than 50 y	0.6 (0.3-1.3)	0.7 (0.3-1.5)
Endometrial cancer in carriers	1.7 (0.8-3.4)	0.8 (0.4-1.9)
Extracolonic cancers† in carriers	3.2 (1.4-7.4)	1.3 (0.6-2.9)
Increased number of CRC tumors in FDR+SDR‡	0.8 (0.6-0.9)	1.1 (0.9-1.6)
Increased number of extracolonic tumors in FDR+SDR	2.1 (1.2-3.6)	1.4 (0.9-2.2)

*Reference group.
†Extracolonic cancers include kidney, ureter, bladder, brain, biliary tract, stomach, small intestine, ovary, pancreas, and sebaceous neoplasms.
‡A weighted sum of cancer diagnoses among FDR + SDR is used to incorporate the effect of genetic distance. Cancer diagnoses in SDR were weighted half that in FDR (12).

Table 7. Summary of previous studies comparing genotype and phenotype in Lynch syndrome

Study	Population	No. MMR mutations	No. subjects (families/ mutation carriers)	Summary findings of phenotypic comparison
Vasen et al. (7)	Dutch	34 *MLH1* 40 *MSH2*	79 families 1,842 related carriers	CRC: higher lifetime risk in *MSH2* (NS) Endometrial cancer: higher lifetime risk in *MSH2* (NS) Other cancers: brain, stomach, ovarian, urinary tract* more prevalent in *MSH2*
Peltomaki et al. (16)	Finnish	51 *MLH1* 4 *MSH2*	55 families 295 related carriers	CRC: more prevalent in *MLH1* Endometrial cancer: more prevalent in *MSH2* (NS) Other cancers: more prevalent in *MSH2* (NS)
Parc et al. (17)	French	65 *MLH1* 79 *MSH2*	163 index cases 348 related carriers	CRC: no difference Endometrial cancer: higher risk in *MSH2* (NS) Other cancers: no difference
Goecke et al. (18)	German	124 *MLH1* 157 *MSH2*	281 families 988 related carriers	CRC: more prevalent in *MLH1**; younger age of diagnosis in *MLH1* males Endometrial cancer: no difference Other cancers: sebaceous skin tumors,* prostate, bladder more prevalent in *MSH2*
Kastrinos et al.	United States	112 *MLH1* 173 *MSH2*	285 unrelated carriers 936 affected family members	CRC: more prevalent in *MLH1* (NS); younger age of diagnosis in *MLH1* males Endometrial cancer: no difference Other cancers: urinary tract, ovarian, sebaceous skin tumors* more prevalent in *MSH2*

Abbreviations: MMR, mismatch repair; NS, statistically nonsignificant.
*$P < 0.05$.

regarding the increased prevalence of *MSH2* mutations among individuals with the Muir-Torre variant of Lynch syndrome.

The endometrial cancer prevalence results have shown more variability. A majority of prior studies, some of which did not distinguish endometrial cancer from other extracolonic cancers, report an increased prevalence of endometrial cancer in *MSH2* mutation carriers. Most recently, this association has not been supported (18). Therefore, in combination with our results, it is becoming apparent that endometrial cancer rates and ages of onset do not differ between *MLH1* and *MSH2* mutation carriers.

Finally, the earlier age of onset of colon cancer that is more prevalent in male *MLH1* mutation carriers is an interesting finding. The French study suggested this gender difference although it was not statistically significant (17). Only recently have Goecke and the German HNPCC Consortium reported a similar association in their analysis of 988 related subjects in 281 mutation-positive families (18). The biological basis for this gender difference is not clear and warrants further study.

An important strength of our study is that it represents a diverse population of unrelated individuals who were not selected from family registries. It represents the largest number of unrelated mutation carriers studied to date. The majority of subjects did not meet the strict clinical criteria that have previously been used to select persons suitable for mutation analysis. Previous studies have specifically analyzed mismatch repair gene mutations in selected kindreds with familial clustering of colorectal cancer. Therefore, the ascertainment of the families not only leads to an overestimation of colorectal cancer risk but also hampers the ability to define a reliable phenotypic association to particular germ-line mismatch repair gene mutations. Ethnic homogeneity among certain European cohorts may highlight a founder mutation effect leading to an overrepresentation of certain extracolonic cancers. In addition, environmental factors may contribute to the differences seen in the prevalence of extracolonic cancers. For example, the higher incidence of gastric cancer in the general populations of Finland compared with the Dutch population may suggest an environmental contribution to the differences seen in the gastric cancer incidence between the Dutch and the Finnish families (6, 7).

Nevertheless, potential limitations of the current analysis must be acknowledged. This study is not truly ''population-based.'' The least biased of all possible samples would involve population screening of patients with sporadic cancer, which represents an enormous, expensive undertaking that may provide very low-yield results with respect to the number of mismatch repair gene mutations detected. In this study, subjects were selected to undergo testing based on some personal or family clinical history that triggered a heightened suspicion for Lynch syndrome. This population is typical of one that is referred to cancer genetics clinics for consultation, counseling, and predictive genetic testing, and best represents a mutation frequency more reflective of the general population at risk.

In addition, the cross-sectional prevalence study design does not afford us the ability to determine whether a prognostic advantage exists between the two mismatch repair gene mutation carriers. Analyzing data provided by surviving mutation carriers at one distinct point in time may influence estimates of the overall prevalence of disease and the comparisons made between the two mutation carrier groups. In turn, the conclusions drawn from this cross-sectional prevalence study may be vulnerable to inferential reasoning. Prospective, population-based studies of a large number of mutation carriers would be necessary to completely resolve such a survival bias and such studies are difficult to undertake due to the rarity of Lynch syndrome.

A third limitation to this study is the inability to confirm the cancer diagnoses reported in probands and family members. Although previous evidence shows that family history reported by probands in FDRs is reliable, the same cannot be said for SDRs. Reporting errors are possible in this study. However, because health care professionals were responsible for the clinical data provided, it is less likely that erroneous diagnoses were documented. Despite these shortcomings, the strong predictive effects as reported in our previous work using this study group (9) that have recently been validated in a population-based sample (20) are indicative that the information is likely to be reliable.

In conclusion, the phenotypic expression of disease in individuals and within families with Lynch syndrome is influenced by whether the genetic mutation is present in *MLH1* or *MSH2*. In the United States, a preponderance of colorectal cancer is more often associated with a mutation in the *MLH1* gene with an increased number of colorectal tumors seen among relatives of *MLH1* mutation carriers. Extracolonic Lynch syndrome–associated tumors, other than endometrial cancer, predominate in *MSH2* carriers with a higher tumor burden noted among family members. Testing for a specific mismatch repair gene based on family history features may provide an efficient approach to predictive genetic testing. The clinical phenotypes described in this study could be used to tailor recommendations regarding cancer screening, surveillance, and prevention for patients and families with Lynch syndrome.

Disclosure of Potential Conflicts of Interest

Dr. Syngal reports having received lecture honoraria from Myriad Genetics Laboratories, Inc. No other conflicts of interest were disclosed.

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References

1. Lynch HT, de la Chapelle A. Hereditary colorectal cancer. N Engl J Med 2003;348:919–32.
2. Grady WM. Genetic testing for high-risk colon cancer patients. Gastroenterology 2003;124:1574–94.
3. Chung DC, Rustgi AK. The hereditary nonpolyposis colorectal cancer syndrome: genetic and clinical implications. Ann Intern Med 2003;138:560–70.
4. Aarnio M, Sankila R, Pukkala E, et al. Cancer risk in mutation carriers of DNA-mismatch-repair genes. Int J Cancer 1999;81:214–8.
5. Watson P, Lynch HT. Extracolonic cancer in hereditary nonpolyposis colorectal cancer. Cancer 1993;71:677–85.
6. Aarnio M, Mecklin JP, Aaltonen LA, Nystrom-Lahti M, Jarvinen HJ. Life-time risk of different cancers in hereditary non-polyposis colorectal cancer (HNPCC) syndrome. Int J Cancer 1995; 64:430–3.
7. Vasen HFA, Stormorken A, Menko FH, et al. *MSH2* mutation carriers are at higher risk of cancer than *MLH1* mutation carriers: a study of hereditary nonpolyposis colorectal cancer families. J Clin Oncol 2001; 19:4074–80.
8. Lynch HT, Boland CR, Gong G, et al. Phenotypic and genotypic heterogeneity in the Lynch syndrome: diagnostic, surveillance and management implications. Eur J Hum Genet 2006;14:390–402.
9. Balmaña J, Stockwell DH, Steyerberg EW, et al. Prediction of *MLH1* and *MSH2* mutations in Lynch Syndrome. JAMA 2006; 296:1469–78.
10. Barnetson RA, Tenesa A, Farrington SM, et al. Identification and survival of carriers of mutations in DNA mismatch-repair genes in colon cancer. N Engl J Med 2006;354:2751–63.
11. Chen S, Wang W, Lee S, et al. Prediction of germline mutations and cancer risk in the Lynch Syndrome. JAMA 2006;296:1469–78.
12. Steyerberg EW, Balmaña J, Stockwell DH, Syngal S. Data reduction for prediction: a case study on robust coding of age and family history for the risk of having a genetic mutation. Stat Med 2007;26: 5545–56.
13. Pandipalliam B, Garber J, Kolodner RD, Syngal S. Clinical presentation correlates with the type of mismatch repair gene involved in hereditary nonpolyposis colon cancer. Gastroenterology 2004;126: 936–7.
14. Baudhuin LM, Ferber MJ, Winters JL, et al. Characterization of h*MLH1* and h*MLH2* gene dosage alterations in Lynch Syndrome patients. Gastroenterology 2005;129:846–54.
15. Vasen HF, Wijnen JT, Menko FH, et al. Cancer risk in families with hereditary nonpolyposis colorectal cancer diagnosed by mutation analysis. Gastroenterology 1996;111:1020–7.
16. Peltomaki P, Gao X, Mecklin JP. Genotype and phenotype in hereditary nonpolyposis colon cancer: a study of families with different vs. shared predisposing mutations. Fam Cancer 2001;1: 9–15.
17. Parc Y, Boisson C, Thomas G, Olschwang S. Cancer Risk in 348 French *MSH2* or *MLH1* gene carriers. J Med Genet 2003;40: 208–13.
18. Goecke T, Schulmann K, Engel C, et al. Genotype-phenotype comparison of German *MLH1* and *MSH2* mutation carriers clinically affected with Lynch Syndrome: a report by the German HNPCC Consortium. J Clin Oncol 2006;24:4285–92.
19. Mangold E, Pagenstecher C, Leister M, et al. A genotype-phenotype correlation in HNPCC: strong predominance of *MSH2* mutations in 41 patients with Muir-Torre syndrome. J Med Genet 2004;41: 567–72.
20. Balaguer F, Balmaña J, Castellví-Bel S, et al. Validation and extension of the $PREMM_{1,2}$ model in a population-based cohort of colorectal cancer patients. Gastroenterology 2008;134:39–46.

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