

# Multigene Panel Testing Provides a New Perspective on Lynch Syndrome

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## A B S T R A C T

### Purpose

Most existing literature describes Lynch syndrome (LS) as a hereditary syndrome leading to high risks of colorectal cancer (CRC) and endometrial cancer mainly as a result of mutations in *MLH1* and *MSH2*. Most of these studies were performed on cohorts with disease suggestive of hereditary CRC and population-based CRC and endometrial cancer cohorts, possibly biasing results. We aimed to describe a large cohort of mismatch repair (MMR) mutation carriers ascertained through multigene panel testing, evaluate their phenotype, and compare the results with those of previous studies.

### Methods

We retrospectively reviewed clinical histories of patients who had multigene panel testing, including the MMR and *EPCAM* genes, between March 2012 and June 2015 (N = 34,981) and performed a series of statistical comparisons.

### Results

Overall, *MSH6* mutations were most frequent, followed by *PMS2*, *MSH2*, *MLH1*, and *EPCAM* mutations, respectively. Of 528 patients who had MMR mutations, 63 (11.9%) had breast cancer only and 144 (27.3%) had CRC only. When comparing those with breast cancer only to those with CRC only, *MSH6* and *PMS2* mutations were more frequent than *MLH1* and *MSH2* mutations ( $P = 2.3 \times 10^{-5}$ ). Of the 528 patients, 22.2% met *BRCA1* and *BRCA2* (*BRCA1/2*) testing criteria and not LS criteria, and 5.1% met neither *BRCA1/2* nor LS testing criteria. *MSH6* and *PMS2* mutations were more frequent than *MLH1* and *MSH2* mutations among patients who met *BRCA1/2* testing criteria but did not meet LS testing criteria ( $P = 4.3 \times 10^{-7}$ ).

### Conclusion

These results provide a new perspective on LS and suggest that individuals with *MSH6* and *PMS2* mutations may present with a hereditary breast and ovarian cancer phenotype. These data also highlight the limitations of current testing criteria in identifying these patients, as well as the need for further investigation of cancer risks in patients with MMR mutations.

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## INTRODUCTION

Lynch syndrome (LS) is a well-described hereditary cancer syndrome caused by mutations in the mismatch repair (MMR) genes (*MLH1*, *MSH2*, *MSH6*, and *PMS2*) and large deletions, including the 3' end of *EPCAM*. LS has been known as one of the most common hereditary cancer syndromes for some time, and a recent study showed that the population frequency may be as high as 1 in 279, which would make it the most common hereditary cancer syndrome.<sup>1</sup> Individuals with LS have high risks for colorectal cancer (CRC) and endometrial cancer (EC), as well as many other cancers. A large

body of literature has suggested that most cases of LS are a result of mutations in *MLH1* and *MSH2* and that the highest cancer risks are for CRC and EC, followed by gastric and ovarian cancers (OCs).<sup>2-7</sup> However, many of these studies included individuals with a personal and/or family history of CRC and/or EC or were cohorts of patients suggestive of LS because they met the Amsterdam criteria or Bethesda guidelines. In addition, clinical testing for *MSH6*, *PMS2*, and *EPCAM* became available well after testing for *MLH1* and *MSH2* became available, so many early studies did not assess for mutations in those genes. An association between breast cancer (BC) and LS has been suggested by a few studies, but it remains

## ASSOCIATED CONTENT



Appendix  
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controversial, and there are currently no guidelines recommending increased BC screening for women with LS.<sup>8-10</sup>

Initially, candidates for LS genetic testing were identified on the basis of meeting the Amsterdam criteria or Bethesda guidelines, but more recently, these algorithms have been shown to miss as many as 72% and 27% of individuals with LS, respectively.<sup>5</sup> In addition, these criteria are focused on CRC and EC diagnoses. In more recent years, universal screening for LS using microsatellite instability (MSI) and/or immunohistochemistry (IHC) for the MMR proteins has been implemented in a growing number of institutions, but this screening is mostly performed on CRC and, in some cases, EC tumors.<sup>2,3,5,11-14</sup> Although they are effective screening tools, MSI and IHC may miss approximately 13% to 23% of patients with LS and may have lower sensitivity for detecting *MSH6* and *PMS2* mutation carriers in particular.<sup>5</sup>

The use of multigene panel testing (MGPT) with next-generation sequencing (NGS) for the diagnosis of hereditary cancer has increased dramatically over the past 4 years; however, limited data have been published describing individuals with LS identified through MGPT.<sup>15,16</sup> Because more patients are being assessed with MGPT, individuals are being identified who have mutations in what are believed to be well-described, highly penetrant genes, but those patients do not meet established clinical and/or testing criteria for the respective syndrome.<sup>15,16</sup>

In this study, we aimed to describe the characteristics of individuals with LS identified through MGPT at a single laboratory and compare them to characteristics of patients in the literature with MMR mutations found through traditional means.

## METHODS

### Study Population

Deidentified clinical histories and molecular results were reviewed for all patients who underwent MGPT, including the MMR and *EPCAM* genes between March 2012 and June 2015 at Ambry Genetics (Aliso Viejo, CA). The study was reviewed by Solutions Institutional Review Board and found to be exempt.

### MGPT

Patients underwent comprehensive analysis of nine to 49 hereditary cancer predisposition genes, depending on which test was ordered (Appendix Table A1, online only). The test ordered was determined by the ordering clinician. The specific indication for testing was not available in our data set but may be inferred in some cases from the personal and/or family history of cancer and the testing criteria met. Gene analysis is described in detail in the Appendix (online only).

All variants, with the exception of previously characterized benign alterations, underwent thorough assessment and review of available evidence (eg, population frequency information, published case reports, case/control and functional studies, locus-specific databases [such as the InSIGHT database from International Society of Gastrointestinal Hereditary Tumors],<sup>17</sup> internal co-occurrence and cosegregation data, evolutionary conservation, and in silico predictions). Variants were classified per the Ambry five-tier variant classification protocol (pathogenic mutation; variant, likely pathogenic; variant of unknown significance [VUS]; variant, likely benign; and benign) which is based on guidelines published by the American College of Medical Genetics and Genomics, the Association for Molecular Pathology, and the International Agency for Research on Cancer.<sup>18-20</sup>

### Data Analysis

Information on demographic characteristics and personal and family cancer history was extracted from clinician-completed test requisition forms (TRFs) and, when available, clinic notes and pedigrees. Individuals known to be family members of a proband previously tested at our laboratory, as indicated by the ordering provider, and those who were likely to be related because of a shared mutation and similar personal and family histories, were excluded. Patients were eligible for inclusion in phenotype comparisons if the personal and family history sections were completed on the TRF and they had one MMR or *EPCAM* pathogenic mutation or likely pathogenic variant. Individuals with two or more mutations or likely pathogenic variants were excluded. Tumor-specific cohorts used for genotype-phenotype comparisons were designed to reduce bias by focusing on isolated tumor types as follows: patients with BC only who had BC but not OC, CRC, or EC; patients with CRC only who had CRC, but not BC, EC, or OC; patients with OC only who had OC, but not BC, CRC, or EC; and patients with EC only who had EC but not CRC, BC, or OC. Comparisons of gene-specific mutation frequencies were performed between these groups by using multivariable logistic regression analysis and controlling for age at testing, sex, race/ethnicity, and family history of CRC and/or EC among first- and second-degree relatives. Clinical histories were further evaluated to determine whether National Comprehensive Cancer Network (NCCN) LS and/or *BRCA1* and *BRCA2* (*BRCA1/2*) testing criteria, two of the most commonly used testing criteria in the United States, were met.<sup>21,22</sup> Gene-specific mutation frequencies among those who met each set of criteria were compared by using Fisher's exact test. *P* values from the multivariable analysis and Fisher's exact test were adjusted for multiple comparisons via false discovery rate with a cutoff of 5%. The  $\chi^2$  and binomial proportion tests were used to compare our mutation frequencies with those from previous studies.

## RESULTS

### Demographic Characteristics

Of 34,980 individuals who, to the best of our knowledge, were unrelated and who underwent MGPT for the MMR genes and *EPCAM*, 618 MMR and *EPCAM* mutations were identified in 612 (1.7%) individuals. Thirty-three individuals had a second pathogenic mutation in an LS ( $n = 6$ ) or another cancer predisposition gene ( $n = 27$ ) and were excluded from further analyses, leaving a cohort of 579 probands. Of these, 120 (20.7%) also had a VUS in one of the genes analyzed. This cohort was mostly female (73.4%), white (66.7%), and affected with cancer (85.8%, excluding non-melanoma skin cancer; Table 1). Mutations in *MSH6* were most frequent (29.3%), followed by *PMS2* (24.2%), *MSH2* (23.7%), *MLH1* (21.6%), and *EPCAM* (1.2%; Fig 1). This distribution is statistically significantly different from those in the largest previous studies (Fig 2).<sup>23</sup> *EPCAM* mutation carriers were combined with *MSH2* carriers for further analyses. *MLH1* carriers were diagnosed with their first primary cancer at the youngest average age (42.2 years), followed by *MSH2/EPCAM*, *PMS2*, and *MSH6* carriers (Table 1).

### Mutation Spectrum

All unique variants classified as pathogenic or likely pathogenic ( $n = 346$ ) are listed in Appendix Table A2 (online only). Five mutations were seen 10 or more times each. *PMS2* c.137G>T p.S46I ( $n = 21$ ) is reported as a European founder mutation, and *MSH2* c.1906G>C p.A636P ( $n = 13$ ) is a known Ashkenazi Jewish founder mutation.<sup>24,25</sup> *MSH2* c.942+3A>T ( $n = 18$ ) and *MSH6*

**Table 1.** Demographic and Clinical Characteristics

Characteristic	No.	%	Average Age at Diagnosis (years)	Range (years)	No.*
<b>Sex</b>					
Female	423	73.1	47.2	11-83	339†
Male	156	26.9	45.0	18-78	117†
<b>Cancer diagnosis‡</b>					
Unaffected	72	13.6	—		
Affected	456	86.4	46.6	11§-83	456†
Colorectal	186	35.2	45.6	18-89	182
Endometrial	136	25.8	50.4	23-86	135
Breast	124	23.5	52.9	22-84	116
Ovarian	74	14.0	47.9	11§-77	74
Kidney	16	3.0	55.6	23-75	16
Melanoma	14	2.7	47.0	27-68	14
Small bowel	13	2.5	51.2	26-74	13
Pancreatic	11	2.1	59.4	46-73	11
Prostate	10	1.9	58.5	44-70	10
Other	85	16.1	—		
<b>Mutated gene</b>					
<i>MLH1</i>	125	21.6	42.2	18-71	96
<i>MSH2/EPCAM</i>	144	24.9	44.9	20-78	114
<i>MSH6</i>	170	29.3	50.3	18-83	142
<i>PMS2</i>	140	24.2	47.7	11§-72	104
<b>Adenomatous polyps</b>					
Had polyps	102	19.3			
1	22	4.2			
2-5	41	7.8			
6-9	6	1.1			
10-19¶	16	3.0			
20-99¶	7	1.3			
≥100¶	1	0.2			
No. not specified	9	1.7			
No polyps	103	19.5			
Data not provided	323	61.2			

\*Individuals without cancer and those for whom age at diagnosis was not specified were excluded.

†If individuals were diagnosed with more than one primary cancer, only the age at the first primary cancer was considered.

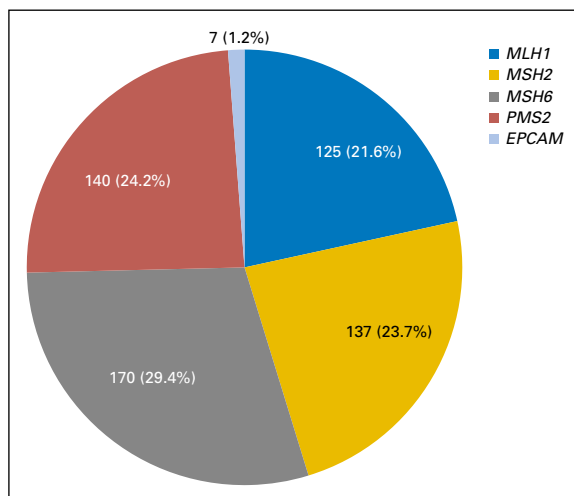
‡Patients for whom the presence or absence of personal and/or family history of cancer was not indicated were excluded from phenotype analyses (n = 51).

§One individual was reported to have an ovarian cancer diagnosed at age 11. No histology or other information was provided.

||Other cancers seen in < 10 probands: gastric, sarcoma, thyroid, cervical, lung, sebaceous, brain, ureter, ampulla of vater, renal pelvis, adrenocortical, appendiceal, clear cell, tonsil, liver, abdominal, neuroendocrine, urethral, and vulvar cancers, pancreatic neuroendocrine tumor, leukemia, lymphoma, multiple myeloma, and unknown.

¶All probands with 10 or more polyps had a panel that included *APC*, *BMPR1A*, *MUTYH*, *PTEN*, *SMAD4*, and *STK11*.

c.3261dupC p.F1088Lfs\*5 (n = 16) are recurrent mutations occurring in microsatellite tracts,<sup>26-28</sup> and *PMS2* c.1831dupA (n = 10) was previously reported in three patients.<sup>24</sup>

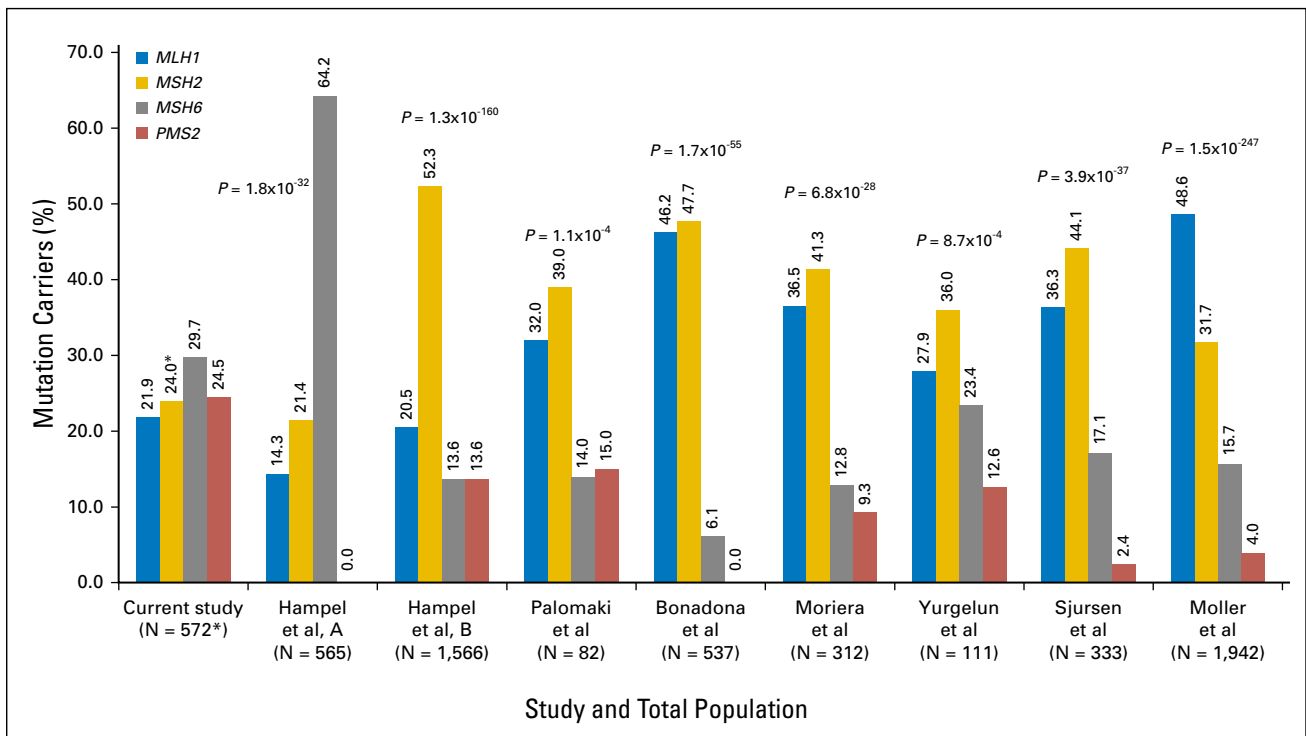


**Fig 1.** Overall mismatch repair gene and *EPCAM* mutation distribution (n = 579).

## Phenotype

Individuals for whom personal and/or family history of cancer was not provided (n = 51) were excluded from phenotype analyses, leaving a cohort of 528. The most common cancers in MMR mutation carriers were CRC (n = 186 [35.2%]), EC (n = 136 [25.8%]), BC (n = 124 [23.5%]), and OC (n = 74 [14.0%]; Table 1). In the remainder of the cohort tested for MMR gene mutations for whom personal and family cancer history information was available (n = 31,545), BC was most common (n = 12,173 [38.6%]), followed by OC (n = 4,416 [14.0%]), CRC (n = 3,378 [10.7%]), and EC (n = 1,831 [5.8%]). The average age of first primary cancer diagnosis was 46.6 years. In addition, 19.3% of LS probands reported one or more adenomatous polyps and 4.5% had 10 or more polyps (Table 1).

BC was the first primary cancer for 84 (15.9%) of the MMR mutation carriers, and OC was the first primary cancer for 61 (11.6%) probands. Overall, 145 patients (27.5%) presented with BC or OC, 73.1% of whom had not been diagnosed with CRC or EC at the time of genetic testing. Nine MMR mutation carriers had both BC and OC without CRC or EC, seven of whom had *MSH6* (n = 4) or *PMS2* (n = 3) mutations.



**Fig 2.** Percentage of mutation carriers per mismatch repair gene compared with previous studies. The  $\chi^2$  test was used to compare mutation frequencies from this study with those from previous studies. Hampel et al, A<sup>2,14</sup> is a population-based endometrial cancer cohort. Hampel et al, B<sup>3,13</sup> is a population-based colorectal cancer cohort. Palomaki et al<sup>5</sup> calculated a weighted proportion from four previous studies on colorectal cancer cohorts. Moreira et al<sup>6</sup> includes patients from four large registries of population-based and/or high-risk families with colorectal cancer. Yurgelun et al<sup>16</sup> is a cohort of patients with histories suggestive of LS. Sjrursen et al<sup>23</sup> is a cohort of individuals with a clinical diagnosis of hereditary nonpolyposis colorectal cancer and/or a molecular diagnosis of LS. The cohort in Møller et al<sup>7</sup> is a pooled prospective database of individuals with LS from several collaborative groups. (\*) *EPCAM* mutations were excluded for all comparisons except those with Møller et al. Most previous studies did not analyze *EPCAM*, but in Møller et al, *EPCAM* mutations were combined with *MSH2* and could not be excluded. As a result, the number of patients for the comparison with Møller et al in this study is 579, and the *MSH2* percentage for this study is 24.9 with *EPCAM* mutations included.

When comparing those with BC only (n = 63; Fig 3A) with those who had CRC only (n = 144; Fig 3B), *MSH6* and *PMS2* mutations were more frequent than *MLH1* and *MSH2* mutations ( $P = 2.3 \times 10^{-5}$ ). When comparing those with OC only (n = 40; Fig 3C) with those who had CRC only, there was a trend toward *MSH6* mutations being more frequent than *MLH1* and *MSH2* mutations, but it did not reach statistical significance. When comparing probands with EC only (n = 71; Fig 3D) with those who had CRC only, *MSH6* mutations were more frequent than *MLH1* and *PMS2* mutations ( $P = 1.1 \times 10^{-3}$  and  $P = 1.7 \times 10^{-2}$ , respectively), and *MSH2* mutations were more frequent than *MLH1* mutations ( $P = 1.2 \times 10^{-2}$ ). Detailed results of statistical comparisons are listed in Table 2.

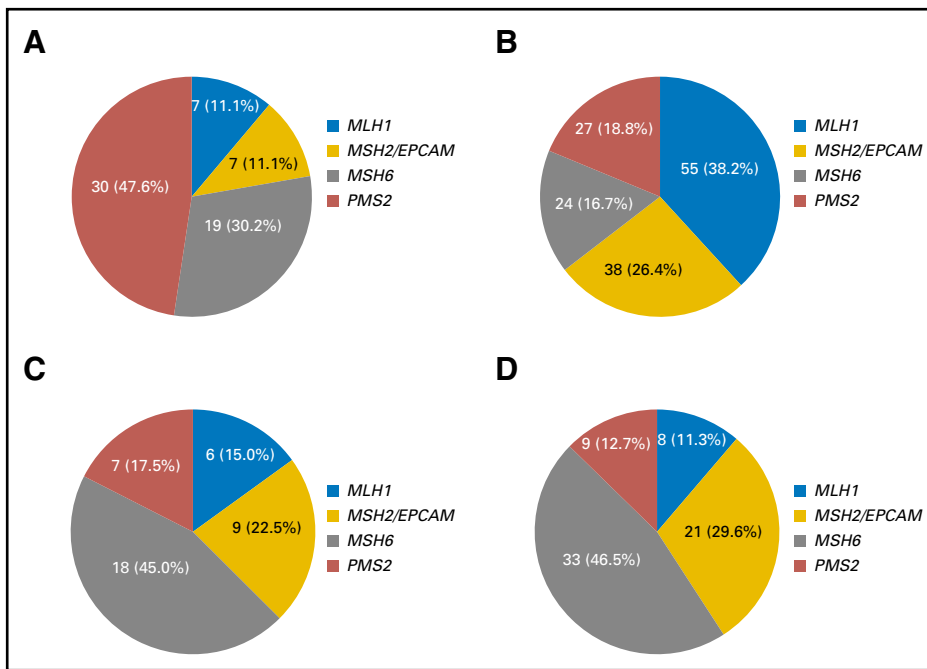
In our study, mutation frequencies for patients with CRC only and EC only were significantly higher than recently published population frequencies for all four MMR genes (Appendix Table A3, online only).<sup>1</sup> For patients with BC only, the frequency of *PMS2* mutations was significantly higher than the population estimates, but there was no significant difference in *MLH1*, *MSH2*, or *MSH6* mutations. Conversely, among patients with OC only, the frequencies of *MLH1*, *MSH2*, and *MSH6* mutations were significantly higher than the population estimates, but there was no significant increase in the frequency of *PMS2* mutations (Appendix Table A3).

### Testing Criteria

In our cohort, 18.2% of MMR mutation carriers met the Amsterdam criteria and 37.3% met the revised Bethesda guidelines, although 68.6% had a PREMM<sub>1,2,6</sub> score  $\geq 5\%$  and 72.7% met the NCCN guidelines for LS testing (not mutually exclusive).<sup>21,29</sup> Interestingly, 58.9% met the NCCN guidelines for *BRCA1/2* testing and 22.2% met only these guidelines, although 36.7% met both guidelines. An additional 5.1% did not meet any testing criteria for LS or *BRCA1/2*. Altogether, 27.3% of the patients in this cohort who had MMR mutations would not have been identified by current LS testing criteria. *MSH6* and *PMS2* carriers were more likely to meet only the NCCN *BRCA1/2* testing criteria than *MLH1* and *MSH2* carriers ( $P = 4.3 \times 10^{-7}$ ). Conversely, *MLH1* and *MSH2* mutation carriers were more likely to meet the NCCN guidelines for LS testing than *MSH6* and *PMS2* carriers ( $P = 7.5 \times 10^{-8}$ ; Table 3).

### Tumor Testing

Results of MSI and/or IHC analysis (tumor testing) were provided on the TRF for 99 MMR mutation carriers (18.8%); 10 (1.9%) had MSI only, 65 (12.3%) had IHC only, and 24 (4.5%) had both MSI and IHC results. Of those with tumor testing results, eight (8.1%) were discordant with the mutated gene (Appendix



**Fig 3.** Mismatch repair gene mutation distributions among tumor-specific subgroups. (A) Patients with breast cancer (BC) without ovarian cancer (OC), colorectal cancer (CRC), or endometrial cancer (EC; n = 63). (B) Patients with CRC without BC, EC, or OC (n = 144). (C) Patients with OC without BC, CRC, or EC (n = 40). (D) Patients with EC without CRC, BC, or OC (n = 71).

Table A4, online only). Seven of the eight had normal tumor testing and one had loss of proteins, which indicated a mutation in a different gene. Of *MLH1* and *MSH2/EPCAM* mutation carriers who had tumor testing results (n = 23 and n = 30, respectively), the results were discordant for one *MSH2* carrier (3.3%). Of *MSH6* and *PMS2* mutation carriers who had MSI and/or IHC results (n = 24 and n = 22, respectively), results were discordant for three *MSH6* carriers (12.5%) and four *PMS2* carriers (18.2%).

## DISCUSSION

Traditionally, LS has been described as a hereditary CRC and EC syndrome caused primarily by mutations in *MLH1* and *MSH2*<sup>4-6,29,30</sup>; however, our data suggest that this may not be a complete description of the syndrome. Although CRC and EC were the most common cancers among MMR mutation carriers in this cohort, affecting 35.2% and 25.8% of probands, respectively, BC was nearly as common (23.5% of probands) and 14.0% had OC. In fact, 27.5% (n = 145) of MMR mutation carriers presented with BC (15.9%) or OC (11.6%) as their first primary cancer, the majority of which (n = 106 [73.1%]) had not been diagnosed with CRC or EC at the time of genetic testing. In addition, there was an approximately equal distribution of mutations in *MLH1*, *MSH2*, *MSH6*, and *PMS2* in this study, with the greatest percentages of mutations in *MSH6* and *PMS2*.

Several previous studies have suggested a higher risk for EC in *MSH6* mutation carriers than in carriers of other MMR mutations.<sup>2,30-32</sup> More recently, *MSH6* and *PMS2* mutations have been reported at higher frequencies than *MLH1* and *MSH2* in hereditary breast and/or ovarian cancer (HBOC) MGPT cohorts, but in small numbers.<sup>33-36</sup> Another recent MGPT study assessing patients with disease suggestive of LS found that of 114 patients

with LS, 35% had a mutation in *MSH2*, 27% in *MLH1*, 23% in *MSH6*, 12% in *PMS2*, and 3% in *EPCAM*. This is more consistent with previous LS studies and significantly different from the distribution in our cohort ( $P = 8.7 \times 10^{-4}$ ; Fig 2).<sup>16</sup> To the best of our knowledge, this study reports on the largest cohort of individuals tested for and with mutations in the MMR genes identified through MGPT to date.

Previous studies have shown that the Amsterdam criteria will identify approximately 13% to 58% of families with LS, and the revised Bethesda criteria will identify approximately 65% to 91% of patients with LS compared with 18.2% and 37.3%, respectively, in this study.<sup>2,5,12,23</sup> We found that 27.3% of patients did not meet any current criteria for LS testing, and *MSH6* and *PMS2* mutation carriers were significantly more likely to meet only *BRCA1/2* testing criteria (and not LS criteria) than *MLH1* and *MSH2* mutation carriers. In addition, 15.2% of available MSI and/or IHC results for patients with *MSH6* or *PMS2* mutations were discordant, which could have led to the mutation being missed in the absence of MGPT. It is known that MSI and IHC do not have 100% sensitivity for LS, and many of the discordant *MSH6* mutations may be explained by known issues with IHC for the *MSH6* protein.<sup>5,37,38</sup>

Most recently, data from a population-based cohort of patients with CRC who were screened for MMR gene mutations were used to estimate population frequencies of MMR mutations.<sup>1</sup> Results of that study demonstrated that of the MMR genes, mutations in *PMS2* are the most frequent in the general population, followed by *MSH6*, *MLH1*, and *MSH2*. It is not surprising that the mutation frequencies for all MMR genes are significantly higher in our CRC and EC only probands because these are the cancers with the highest risks in LS. The fact that *PMS2* mutations were significantly more frequent in probands with BC only suggests an excess of BC in our cohort of MMR mutation carriers, particularly among *PMS2* carriers, beyond what would be expected in the

**Table 2.** Comparison of Genotypes and Phenotypes

Comparison (v patients with CRC who did not have BC, OC, or EC)	OR	95% CI	P	FDR-Adjusted P
<b>BC without OC, CRC, or EC</b>				
<i>MSH6</i> and <i>PMS2</i> v <i>MLH1</i> and <i>MSH2</i>	6.1	2.9 to 13.6	$3.3 \times 10^{-6}$	<b><math>2.3 \times 10^{-5}</math></b>
<i>MSH6</i> v <i>MLH1</i>	7.2	2.2 to 26.2	$1.4 \times 10^{-3}$	<b><math>2.5 \times 10^{-3}</math></b>
<i>MSH6</i> v <i>MSH2</i>	3.6	1.2 to 11.7	$2.4 \times 10^{-2}$	<b><math>3.3 \times 10^{-2}</math></b>
<i>PMS2</i> v <i>MLH1</i>	9.1	3.2 to 30.5	$1.1 \times 10^{-4}$	<b><math>3.8 \times 10^{-4}</math></b>
<i>PMS2</i> v <i>MSH2</i>	6.1	2.2 to 19.3	$8.9 \times 10^{-4}$	<b><math>2.1 \times 10^{-3}</math></b>
<i>MLH1</i> v <i>MSH2</i>	0.6	0.1 to 2.0	$3.6 \times 10^{-1}$	$3.9 \times 10^{-1}$
<i>MSH6</i> v <i>PMS2</i>	0.7	0.3 to 1.6	$3.9 \times 10^{-1}$	$3.9 \times 10^{-1}$
<b>OC without BC, CRC, or EC</b>				
<i>MSH6</i> and <i>PMS2</i> v <i>MLH1</i> and <i>MSH2</i>	2.4	1.0 to 5.9	$5.9 \times 10^{-2}$	$2.1 \times 10^{-1}$
<i>MSH6</i> v <i>MLH1</i>	3.7	1.0 to 15.2	$5.5 \times 10^{-2}$	$2.1 \times 10^{-1}$
<i>MSH6</i> v <i>MSH2</i>	2.8	0.9 to 9.9	$9.1 \times 10^{-2}$	$2.1 \times 10^{-1}$
<i>PMS2</i> v <i>MLH1</i>	1.3	0.3 to 5.5	$7.5 \times 10^{-1}$	$7.5 \times 10^{-1}$
<i>PMS2</i> v <i>MSH2</i>	1.3	0.4 to 4.6	$7.0 \times 10^{-1}$	$7.5 \times 10^{-1}$
<i>MLH1</i> v <i>MSH2</i>	0.5	0.1 to 1.6	$2.3 \times 10^{-1}$	$3.2 \times 10^{-1}$
<i>MSH6</i> v <i>PMS2</i>	2.7	0.8 to 10.1	$1.2 \times 10^{-1}$	$2.1 \times 10^{-1}$
<b>EC without OC, BC, or CRC</b>				
<i>MSH6</i> and <i>MSH2</i> v <i>MLH1</i> and <i>PMS2</i>	4.2	2.1 to 8.8	$9.7 \times 10^{-5}$	<b><math>6.8 \times 10^{-4}</math></b>
<i>MSH6</i> v <i>MLH1</i>	6.8	2.5 to 20.7	$3.2 \times 10^{-4}$	<b><math>1.1 \times 10^{-3}</math></b>
<i>MSH6</i> v <i>PMS2</i>	3.8	1.4 to 10.8	$9.8 \times 10^{-3}$	<b><math>1.7 \times 10^{-2}</math></b>
<i>MSH2</i> v <i>MLH1</i>	4.7	1.6 to 15.3	$5.3 \times 10^{-3}$	<b><math>1.2 \times 10^{-2}</math></b>
<i>MSH2</i> v <i>PMS2</i>	1.9	0.6 to 6.3	$2.5 \times 10^{-1}$	$2.9 \times 10^{-1}$
<i>MLH1</i> v <i>PMS2</i>	0.7	0.2 to 2.6	$6.0 \times 10^{-1}$	$6.0 \times 10^{-1}$
<i>MSH6</i> v <i>MSH2</i>	2.2	0.9 to 5.3	$8.3 \times 10^{-2}$	$1.2 \times 10^{-1}$

NOTE. Statistical comparisons were performed with multivariable logistic regression analysis, controlling for age, ethnicity, sex, and family history of colorectal cancer (CRC) and endometrial cancer (EC) in first- and second-degree relatives. P values were adjusted for multiple comparisons via false discovery rate (FDR) with a cutoff of 5% (shown in **bold**).  
Abbreviations: BC, breast cancer; OC, ovarian cancer; OR, odds ratio.

general population. Although the study by Win et al<sup>1</sup> is population based, our cohort represents a high-risk cohort, with most probands submitted for testing as a result of disease suggestive of some

kind of hereditary cancer but not necessarily LS. This may explain why *MSH6* mutations, which have been published in association with higher risk for gynecologic cancers and more moderate risk

**Table 3.** Genetic Testing Criteria Gene by Gene Comparisons

Comparison	OR	95% CI	P	FDR-Adjusted P
<b>Met NCCN BRCA1/2 criteria only</b>				
<i>MSH6</i> and <i>PMS2</i> v <i>MLH1</i> and <i>MSH2</i>	3.4	2.1 to 5.6	$6.1 \times 10^{-8}$	<b><math>4.3 \times 10^{-7}</math></b>
<i>MSH6</i> v <i>MLH1</i>	2.4	1.2 to 5.3	$1.1 \times 10^{-2}$	<b><math>1.5 \times 10^{-2}</math></b>
<i>MSH6</i> v <i>MSH2</i>	2.1	1.2 to 4.2	$2.3 \times 10^{-2}$	<b><math>2.7 \times 10^{-2}</math></b>
<i>PMS2</i> v <i>MLH1</i>	5.3	2.6 to 11.4	$2.2 \times 10^{-7}$	<b><math>7.6 \times 10^{-7}</math></b>
<i>PMS2</i> v <i>MSH2</i>	4.6	2.4 to 9.1	$5.1 \times 10^{-7}$	<b><math>1.2 \times 10^{-6}</math></b>
<i>MLH1</i> v <i>MSH2</i>	0.9	0.4 to 2.0	$8.5 \times 10^{-1}$	$8.5 \times 10^{-1}$
<i>PMS2</i> v <i>MSH6</i>	2.2	1.3 to 3.8	$3.0 \times 10^{-3}$	<b><math>5.2 \times 10^{-3}</math></b>
<b>Met NCCN Lynch criteria only</b>				
<i>MLH1</i> and <i>MSH2</i> v <i>MSH6</i> and <i>PMS2</i>	1.6	1.1 to 2.3	$1.1 \times 10^{-2}$	<b><math>2.6 \times 10^{-2}</math></b>
<i>MLH1</i> v <i>MSH6</i>	1.9	1.2 to 3.3	$8.5 \times 10^{-3}$	<b><math>2.6 \times 10^{-2}</math></b>
<i>MLH1</i> v <i>PMS2</i>	2.3	1.3 to 4.2	$1.5 \times 10^{-3}$	<b><math>1.1 \times 10^{-2}</math></b>
<i>MSH2</i> v <i>MSH6</i>	1.1	0.7 to 1.9	$6.2 \times 10^{-1}$	$6.2 \times 10^{-1}$
<i>MSH2</i> v <i>PMS2</i>	1.4	0.8 to 2.4	$2.3 \times 10^{-1}$	$3.2 \times 10^{-1}$
<i>MLH1</i> v <i>MSH2</i>	1.7	1.0 to 3.0	$4.0 \times 10^{-2}$	$7.0 \times 10^{-2}$
<i>MSH6</i> v <i>PMS2</i>	1.2	0.7 to 2.1	$5.2 \times 10^{-1}$	$6.1 \times 10^{-1}$
<b>Met NCCN Lynch criteria</b>				
<i>MLH1</i> and <i>MSH2</i> v <i>MSH6</i> and <i>PMS2</i>	3.1	2.0 to 4.9	$3.2 \times 10^{-8}$	<b><math>7.5 \times 10^{-8}</math></b>
<i>MLH1</i> v <i>MSH6</i>	2.0	1.1 to 4.0	$2.7 \times 10^{-2}$	<b><math>3.8 \times 10^{-2}</math></b>
<i>MLH1</i> v <i>PMS2</i>	5.2	2.7 to 10.2	$2.8 \times 10^{-8}$	<b><math>7.5 \times 10^{-8}</math></b>
<i>MSH2</i> v <i>MSH6</i>	1.9	1.0 to 3.6	$3.4 \times 10^{-2}$	<b><math>4.0 \times 10^{-2}</math></b>
<i>MSH2</i> v <i>PMS2</i>	4.8	2.6 to 9.0	$3.1 \times 10^{-8}$	<b><math>7.5 \times 10^{-8}</math></b>
<i>MLH1</i> v <i>MSH2</i>	1.1	0.5 to 2.3	$8.6 \times 10^{-1}$	$8.6 \times 10^{-1}$
<i>MSH6</i> v <i>PMS2</i>	2.5	1.5 to 4.3	$2.1 \times 10^{-4}$	<b><math>3.7 \times 10^{-4}</math></b>

NOTE. Statistical comparisons were performed with Fisher's exact test. P values were adjusted for multiple comparisons via false discovery rate (FDR) with a cutoff of 5% (shown in **bold**).  
Abbreviations: NCCN, National Comprehensive Cancer Network; OR, odds ratio.

for CRC,<sup>7,32</sup> were most frequent in our cohort, while *PMS2* mutations, which are believed to have the lowest penetrance of MMR mutations, were most frequent in the population-based estimates.<sup>1</sup>

Although this cohort did not have the bias of being selected for patients with a personal and/or family history suggestive of LS, the majority of patients submitted for MGPT at this laboratory were females with disease suggestive of HBOC, which may have biased our results and explain some of the BC and OC in our patients with MMR mutations. We do not know whether the BCs seen in this cohort were sporadic or if they were caused by LS, so it is possible at least in part, that *MSH6* and *PMS2* carriers were more likely to have an HBOC phenotype because they had a lower risk for CRC and EC, while BC and OC were common in our testing population. *MSH6* and *PMS2* mutations are not commonly seen in HBOC overall. Among all 32,103 patients tested who had data on personal and family history, *MSH6* and *PMS2* mutations were 4.4% (49 of 1,120) of mutations found in patients with BC only and in 5.5% (25 of 453) of mutations in patients with OC only.

Although we do not know the indication for genetic testing, we found that 72.7% of individuals with MMR mutations met NCCN guidelines for LS testing, 58.9% met NCCN guidelines for *BRCA1/2* testing, and 36.7% met both criteria, so it is likely that these syndromes were high on the ordering clinician's differential diagnosis for these patients. Regarding the possibility of other genetic causes for the BC and OC in our MMR mutation carriers, the *BRCA1/2* genes were analyzed in 92.3% of patients with BC and/or OC (167 of 181), and no pathogenic or likely pathogenic variants were found. Of the 14 patients who had a panel that did not include *BRCA1/2* and did not indicate previously completed and negative *BRCA1/2* testing, only five met current NCCN guidelines for this testing.<sup>22</sup> In addition, 76.8% of patients with BC and/or OC (139 of 181) had a panel that included several other genes associated with increased risk for BC and/or OC (Appendix Table A1), and no pathogenic or likely pathogenic variants were found. Regarding the exclusion of hereditary polyposis genes,

a panel that included *APC*, *BMPRIA*, *MUTYH*, *PTEN*, *SMAD4*, and *STK11* was performed for all patients with 10 or more polyps, and no pathogenic or likely pathogenic variants were found in those genes. Finally, most of the clinical information analyzed in this study was ascertained from TRFs and some from clinical notes and pedigrees submitted with the TRFs. Additional studies are needed to confirm our results, assess the causative role of MMR mutations in BC, and clarify gene-specific cancer risks for individuals with LS.

In summary, these data from an MGPT cohort provide a different perspective of LS than previous reports and add to the literature describing genotype-phenotype correlations within LS. Results from this study suggest that carriers of *MSH6* and *PMS2* mutations may present with an HBOC phenotype and are more likely to be missed by current LS screening methods and testing guidelines. This may explain why *MSH6* and *PMS2* mutations have been underrepresented in previous cohorts and suggests they may be underidentified in general.

#### AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at [jco.org](http://jco.org).

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#### Prior Presentation

Portions of this study have been presented at the Annual Meeting of the Western Association of Gynecologic Oncologists, June 8-11, 2016, Sedona, AZ; 35th Annual Conference of the National Society of Genetic Counselors, September 28-October 1, 2016, Seattle, WA; and the 20th Annual Meeting of the Collaborative Group of the Americas on Inherited Colorectal Cancer, October 2-3, 2016, Seattle, WA.



**AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST**

**Multigene Panel Testing Provides a New Perspective on Lynch Syndrome**

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## Appendix

### Methods

Genomic DNA (gDNA) was isolated from blood or saliva by using a QIASymphony DNA kit (Qiagen, Valencia, CA) and quantified. Sequence enrichment was performed by incorporating the gDNA onto a microfluidics chip or into microdroplets with primer pairs or by a bait-capture methodology by using long biotinylated oligonucleotide probes (RainDance Technologies, Billerica, MA or Integrated DNA Technologies, San Diego, CA), followed by polymerase chain reaction (PCR) and next-generation sequencing (NGS; Illumina, San Diego, CA) of all coding regions plus at least five bases into the 5' and 3' ends of all introns and untranslated regions (5'UTR and 3'UTR). NGS of the promoter region was also performed for *PTEN* (c.-1300 to c.-745), *MLH1* (c.-337 to c.-194), and *MSH2* (c.-318 to c.-65). Sanger sequencing was performed for regions with insufficient depth of coverage and for verification of all variant calls other than known nonpathogenic alterations. Analysis for the *MSH2* inversion of coding exons 1-7 was performed on select patients per the ordering clinician, by NGS and/or PCR and agarose gel electrophoresis.

A targeted chromosomal microarray was used for the detection of gross deletions and duplications for all genes except *PMS2* (Agilent, Santa Clara, CA). Gross deletion and duplication analysis of *PMS2* was performed by using MLPA kit P008-B1 (MRC-Holland, Amsterdam, Netherlands). If a deletion was detected in exons 13, 14, or 15 of *PMS2*, double-stranded sequencing of the appropriate exon(s) of the pseudogene *PMS2CL* was performed to determine whether the deletion was located in the *PMS2* gene or the pseudogene.

Initial data processing and base calling, including extraction of cluster intensities, was performed by using RTA 1.12.4 (HiSeq Control Software 1.4.5; Illumina, Hayward, CA). Sequence quality filtering was executed with the CASAVA software (version 1.8.2; Illumina). Sequence fragments were aligned to the reference human genome (GRCh37), and variant calls were generated by using CASAVA. A minimum quality threshold of Q30 was applied, translating to an accuracy of > 99.9% for called bases.

**Table A1.** Genes Tested for MMR Mutation Carriers

Variable	Test Name						
	GYNplus	PancNext	ColoNext	RenalNext	OvaNext	CancerNext	CancerNext-Expanded
No. of MMR mutation carriers	77	6	158	7	113	145	22
Genes tested	<i>BRCA1</i> <i>BRCA2</i> <i>EPCAM</i> <i>MLH1</i> <i>MSH2</i> <i>MSH6</i> <i>PMS2</i> <i>PTEN</i> <i>TP53</i>	<i>APC</i> <i>ATM</i> <i>BRCA1</i> <i>BRCA2</i> <i>CKDN2A</i> <i>EPCAM</i> <i>MLH1</i> <i>MSH2</i> <i>MSH6</i> <i>PALB2</i> <i>PMS2</i> <i>SKT11</i> <i>TP53</i>	<i>APC</i> <i>BMPR1A</i> <i>CDH1</i> <i>CHEK2</i> <i>EPCAM</i> <i>GREM1</i> <i>MLH1</i> <i>MLH1</i> <i>MSH2</i> <i>MSH6</i> <i>MUTYH</i> <i>PMS2</i> <i>PMS2</i> <i>POLD1*</i> <i>POLE *</i> <i>PTEN</i> <i>SMAD4</i> <i>STK11</i> <i>TP53</i>	<i>BAP1*</i> <i>EPCAM</i> <i>FH</i> <i>FLCN</i> <i>MET</i> <i>MIT</i> <i>MLH1</i> <i>MLH1</i> <i>MSH2</i> <i>MSH6</i> <i>PMS2</i> <i>PTEN</i> <i>SDHA</i> <i>SDHB</i> <i>SDHC</i> <i>SDHD</i> <i>TP53</i> <i>TSC1</i> <i>TSC2</i> <i>VHL</i>	<i>ATM</i> <i>BARD1</i> <i>BRCA1†</i> <i>BRCA2†</i> <i>BRIP1</i> <i>CDH1</i> <i>CHEK2</i> <i>EPCAM</i> <i>MLH1</i> <i>MRE11A</i> <i>MSH2</i> <i>MSH6</i> <i>MUTYH</i> <i>NBN</i> <i>NF1‡</i> <i>PALB2</i> <i>PMS2</i> <i>PTEN</i> <i>RAD50</i> <i>RAD51C</i> <i>RAD51D‡</i> <i>SMARCA4*</i> <i>TP53</i>	<i>APC</i> <i>ATM</i> <i>BARD1</i> <i>BMPR1A</i> <i>BRCA1†</i> <i>BRCA2†</i> <i>BRIP1</i> <i>CDH1</i> <i>CDK4‡</i> <i>CDKN2A‡</i> <i>CHEK2</i> <i>EPCAM</i> <i>GREM1*</i> <i>MLH1</i> <i>MRE11A</i> <i>MSH2</i> <i>MSH6</i> <i>MUTYH</i> <i>NBN</i> <i>NF1‡</i> <i>PALB2</i> <i>PMS2</i> <i>POLD1*</i> <i>POLE*</i> <i>PTEN</i> <i>RAD50</i> <i>RAD51C</i> <i>RAD51D‡</i> <i>SMAD4</i> <i>SMARCA4*</i> <i>STK11</i> <i>TP53</i>	<i>APC</i> <i>ATM</i> <i>BARD1</i> <i>BMPR1A</i> <i>BRCA1</i> <i>BRCA2</i> <i>BRIP1</i> <i>CDH1</i> <i>CDK4</i> <i>CDKN2A</i> <i>CHEK2</i> <i>EPCAM</i> <i>GREM1*</i> <i>MLH1</i> <i>MRE11A</i> <i>MSH2</i> <i>MSH6</i> <i>MUTYH</i> <i>NBN</i> <i>NF1‡</i> <i>PALB2</i> <i>PMS2</i> <i>POLD1*</i> <i>POLE*</i> <i>PTEN</i> <i>RAD50</i> <i>RAD51C</i> <i>RAD51D</i> <i>SMAD4</i> <i>SMARCA4*</i> <i>STK11</i> <i>TP53</i>

+17 kidney cancer, PGL,  
and PCC genes

Abbreviations: MMR, mismatch repair; PCC, pheochromocytoma; PGL, paraganglioma.

\**BAP1*, *GREM1*, *POLD1*, *POLE*, and *SMARCA4* were added to these panels, as applicable, in May 2015. ColoNext (n = 7), OvaNext (n = 2), CancerNext (n = 6), and CancerNext-Expanded (n = 2) patients have been tested since then.

†*BRCA1* and *BRCA2* were added to these panels in June 2013. OvaNext (n = 5) and CancerNext (n = 13) patients were tested before then.

‡*CDK4*, *CDKN2A*, *NF1*, and *RAD51D* were added to these panels, as applicable, in October of 2013. OvaNext (n = 18), and CancerNext (n = 21) patients were tested before then.

Multigene Panel Testing: A New Perspective on Lynch Syndrome

Table A2. Mutation Data

Gene	Coding DNA Sequence (c.)	Protein Sequence (p.)	No. of Times Seen
<i>PMS2</i>	c.137G>T	p.S46I	21
<i>MSH2</i>	c.942+3A>T		18
<i>MSH6</i>	c.3261dupC	p.F1088Lfs*5	16
<i>MSH2</i>	c.1906G>C	p.A636P	13
<i>PMS2</i>	c.1831dupA	p.I611Nfs*2	10
<i>MSH2</i>	5'UTR_Ex6del		9
<i>MSH6</i>	c.10C>T	p.Q4*	9
<i>MSH6</i>	c.3939_3957dupTCAAAAGGGACATAGAAAA	p.A1320Sfs*5	9
<i>PMS2</i>	Ex10del		9
<i>MSH6</i>	c.2731C>T	p.R911*	7
<i>PMS2</i>	c.736_741delCCCCCTinsTGTGTGTGAAG	p.P246Cfs*3	7
<i>MLH1</i>	c.1975C>T	p.R659*	6
<i>MLH1</i>	c.199G>A	p.G67R	6
<i>MSH6</i>	c.1135_1139delAGAGA	p.R379*	6
<i>MLH1</i>	c.1381A>T	p.K461*	5
<i>MLH1</i>	c.1517T>C	p.V506A	5
<i>MLH1</i>	c.1731G>A	p.S577S	5
<i>MSH2</i>	c.2038C>T	p.R680*	5
<i>MSH6</i>	c.2150_2153delTCAG	p.V717Afs*18	5
<i>MSH6</i>	c.3439-2A>G		5
<i>PMS2</i>	c.736_741del6ins11		5
<i>MLH1</i>	Ex6_12dup		4
<i>MSH6</i>	c.3261delC	p.F1088Sfs*2	4
<i>MSH6</i>	c.3699_3702delAGAA	p.K1233Nfs*6	4
<i>PMS2</i>	c.1927C>T	p.Q643*	4
<i>PMS2</i>	c.765C>A	p.Y255*	4
<i>MLH1</i>	c.1845_1847del	p.K616DEL	3
<i>MLH1</i>	c.200G>A	p.G67E	3
<i>MLH1</i>	c.589-2A>G		3
<i>MSH2</i>	c.1226_1227delAG	p.Q409Rfs*7	3
<i>MSH2</i>	c.1786_1788delAAT	p.N596del	3
<i>MSH2</i>	c.2131C>T	p.R711*	3
<i>MSH2</i>	Ex7del		3
<i>MSH6</i>	c.2230dupG	p.E744Gfs*12	3
<i>MSH6</i>	c.2314C>T	p.R772W	3
<i>MSH6</i>	c.3226C>T	p.R1076C	3
<i>MSH6</i>	c.3725G>A	p.R1242H	3
<i>MSH6</i>	c.3984_3987dupGTCA	p.L1330Vfs*12	3
<i>PMS2</i>	c.1874delT	p.L625*	3
<i>PMS2</i>	c.1A>G	p.M1?	3
<i>PMS2</i>	c.2007-2A>C		3
<i>PMS2</i>	c.2117delA	p.K706Sfs*19	3
<i>PMS2</i>	c.2500_2501delATinsG	p.M834Gfs*17	3
<i>PMS2</i>	c.2T>A	p.M1?	3
<i>PMS2</i>	c.943C>T	p.R315*	3
<i>PMS2</i>	Ex13_14del		3
<i>PMS2</i>	Ex14_15del		3
<i>EPCAM</i>	5'UTR_3'UTRdel + <i>MSH2</i> 5'UTR_3'UTRdel		2
<i>MLH1</i>	5'UTR_Ex13del		2
<i>MLH1</i>	c.117-2A>G		2
<i>MLH1</i>	c.1190delT	p.L397Rfs*4	2
<i>MLH1</i>	c.1459C>T	p.R487*	2
<i>MLH1</i>	c.1667G>C	p.S556T	2
<i>MLH1</i>	c.207+1G>A		2
<i>MLH1</i>	c.676C>T	p.R226*	2
<i>MLH1</i>	c.677+3A>G		2
<i>MLH1</i>	c.790+1G>T		2
<i>MLH1</i>	c.83C>T	p.P28L	2
<i>MLH1</i>	Ex16del		2
<i>MLH1</i>	Ex3_5dup		2
<i>MLH1</i>	Ex3_6del		2
<i>MSH2</i>	c.1076+1G>A		2
<i>MSH2</i>	c.1165C>T	p.R389*	2
<i>MSH2</i>	c.2005+3_2005+14del12		2
<i>MSH2</i>	c.2060T>C	p.L687P	2
<i>MSH2</i>	c.2785C>T	p.R929*	2
<i>MSH2</i>	c.301_306delGAAGTT	p.E101_V102del	2

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Table A2. Mutation Data (continued)

Gene	Coding DNA Sequence (c.)	Protein Sequence (p.)	No. of Times Seen
<i>MSH2</i>	Ex2del		2
<i>MSH2</i>	Ex5_6del		2
<i>MSH6</i>	c.1483C>T	p.R495*	2
<i>MSH6</i>	c.1634_1635delAA	p.K545Rfs*17	2
<i>MSH6</i>	c.2550C>A	p.Y850*	2
<i>MSH6</i>	c.2906_2907delAT	p.Y969Lfs*5	2
<i>MSH6</i>	c.3037_3041delAAGAA	p.K1013Vfs*3	2
<i>MSH6</i>	c.3103C>T	p.R1035*	2
<i>MSH6</i>	c.3312dupT	p.G1105Wfs*3	2
<i>MSH6</i>	c.3332_3335dupATGA	p.D1112Efs*2	2
<i>MSH6</i>	c.3514dupA	p.R1172Kfs*5	2
<i>MSH6</i>	c.3516_3517delAG	p.R1172Sfs*4	2
<i>MSH6</i>	c.3562_3565dupAGTA	p.T1189Kfs*6	2
<i>MSH6</i>	c.3577G>A	p.E1193K	2
<i>MSH6</i>	c.3743_3744insT	p.H1248Hfs*27	2
<i>MSH6</i>	c.3882delT	p.P1295Lfs*32	2
<i>MSH6</i>	c.3959_3962delCAAG	p.A1320Efs*6	2
<i>MSH6</i>	c.3991C>T	p.R1331*	2
<i>MSH6</i>	c.578delIT	p.L193Wfs*18	2
<i>MSH6</i>	c.742delC	p.R248Efs*31	2
<i>MSH6</i>	c.892C>T	p.R298*	2
<i>PMS2</i>	c.1939A>T	p.K647*	2
<i>PMS2</i>	c.2095G>C	p.D699H	2
<i>PMS2</i>	c.2113G>A	p.E705K	2
<i>PMS2</i>	c.2156delA	p.Q719Rfs*6	2
<i>PMS2</i>	c.251-2A>T		2
<i>PMS2</i>	c.354-1G>A		2
<i>PMS2</i>	c.400C>T	p.R134*	2
<i>PMS2</i>	c.746_753delACTCCGTG	p.D249Vfs*2	2
<i>PMS2</i>	c.809C>G	p.S270*	2
<i>PMS2</i>	c.862_863delCA	p.Q288Vfs*10	2
<i>PMS2</i>	c.904-2A>G		2
<i>PMS2</i>	Ex11_in12dup		2
<i>PMS2</i>	Ex12_15del		2
<i>PMS2</i>	Ex14del		2
<i>PMS2</i>	Ex6_8del		2
<i>PMS2</i>	Ex7_14del		2
<i>PMS2</i>	Ex8del		2
<i>EPCAM</i>	5'UTR_3'UTRdel + MSH2 5'UTR_Ex6del		1
<i>EPCAM</i>	5'UTR_3'UTRdel + MSH2 5'UTR_Ex7del		1
<i>EPCAM</i>	Ex5_3'UTRdel + MSH2 5'UTR_Ex3del		1
<i>EPCAM</i>	Ex8_3'UTRdel		1
<i>EPCAM</i>	Ex8_9del		1
<i>MLH1</i>	5'UTR_Ex19del		1
<i>MLH1</i>	5'UTR_Ex1del		1
<i>MLH1</i>	c.1036delC	p.Q346Rfs*21	1
<i>MLH1</i>	c.1046dupT	p.L349Lfs*13	1
<i>MLH1</i>	c.1090delA	p.T364Qfs*3	1
<i>MLH1</i>	c.112A>C	p.N38H	1
<i>MLH1</i>	c.1257_1273del17	p.I419Mfs*5	1
<i>MLH1</i>	c.1380_1381delGA	p.E460Efs*18	1
<i>MLH1</i>	c.1401delC	p.S467Rfs*24	1
<i>MLH1</i>	c.1409+1G>A		1
<i>MLH1</i>	c.1411_1414delAAGA	p.K471Dfs*19	1
<i>MLH1</i>	c.1489dupC	p.R497Pfs*6	1
<i>MLH1</i>	c.1522_1523delIAG	p.L509Pfs*5	1
<i>MLH1</i>	c.1558+1G>A		1
<i>MLH1</i>	c.156delA	p.E53Rfs*4	1
<i>MLH1</i>	c.1588_1590delTTC	p.F530del	1
<i>MLH1</i>	c.1746delC	p.F583Lfs*8	1
<i>MLH1</i>	c.1790_1791delIGGinsATCTGGACC	p.W597Yfs*15	1
<i>MLH1</i>	c.184C>T	p.Q62*	1
<i>MLH1</i>	c.1855G>C	p.A619P	1
<i>MLH1</i>	c.1858delG	p.E620Rfs*17	1
<i>MLH1</i>	c.187G>A	p.D63N	1
<i>MLH1</i>	c.1890dupT	p.I630Lfs*2	1
<i>MLH1</i>	c.191A>G	p.N64S	1

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Table A2. Mutation Data (continued)

Gene	Coding DNA Sequence (c.)	Protein Sequence (p.)	No. of Times Seen
MLH1	c.1942C>T	p.P648S	1
MLH1	c.2006_2010delAAAAG	p.E669Gfs*4	1
MLH1	c.2041G>A	p.A681T	1
MLH1	c.2059C>T	p.R687W	1
MLH1	c.2070_2071insTT	p.Y690Yfs	1
MLH1	c.208-2A>C		1
MLH1	c.2141G>A	p.W714*	1
MLH1	c.2148_2168del21ins5		1
MLH1	c.2179_2182delCACA	p.H727Ffs	1
MLH1	c.218T>G	p.L73R	1
MLH1	c.2206G>T	p.E736*	1
MLH1	c.229T>C	p.C77R	1
MLH1	c.244A>G	p.T82A	1
MLH1	c.245_247delCTA	p.T82del	1
MLH1	c.245C>T	p.T82I	1
MLH1	c.-27C>A + c.85G>T	N/A + p.A29S	1
MLH1	c.304G>A	p.E102K	1
MLH1	c.306G>T	p.E102D	1
MLH1	c.37G>T	p.E13*	1
MLH1	c.3G>A	p.M1?	1
MLH1	c.47_53delTGAACCG	p.V16Afs*18	1
MLH1	c.55A>T	p.I19F	1
MLH1	c.677G>A	p.R226Q	1
MLH1	c.691delA	p.I231Lfs*8	1
MLH1	c.727_730delAATG	p.N243Vfs*10	1
MLH1	c.755C>A	p.S252*	1
MLH1	c.791-2A>G		1
MLH1	c.793C>T	p.R265C	1
MLH1	c.86C>G	p.A29G	1
MLH1	c.885-2A>G		1
MLH1	c.970delG	p.E324Sfs*43	1
MLH1	c.971dupA	p.R325Afs*37	1
MLH1	c.982C>T	p.Q328*	1
MLH1	c.984_997delGCACATCGAGAGCA	p.H329Afs*28	1
MLH1	Ex14del		1
MLH1	Ex16_3'UTRdel		1
MLH1	Ex4_8del		1
MLH1	in12_3'UTRdel		1
MSH2	c.1009C>T	p.Q337*	1
MSH2	c.1023delT	p.L341Lfs*16	1
MSH2	c.1076G>C	p.R359T	1
MSH2	c.1120C>T	p.Q374*	1
MSH2	c.1125_1126insAT	p.L376Lfs*37	1
MSH2	c.1147C>T	p.R383*	1
MSH2	c.1210delT	p.C404Vfs*8	1
MSH2	c.1216C>T	p.R406*	1
MSH2	c.123_124insTTCT		1
MSH2	c.1344delC	p.K449Sfs*5	1
MSH2	c.1351C>T	p.Q451*	1
MSH2	c.1390G>T	p.E464*	1
MSH2	c.1442T>G	p.L481*	1
MSH2	c.1457_1460delATGA	p.N486Tfs*10	1
MSH2	c.1462_1463delTT	p.L488Gfs*24	1
MSH2	c.1477C>T	p.Q493*	1
MSH2	c.1571G>C	p.R524P	1
MSH2	c.1697delA	p.N566Ifs*24	1
MSH2	c.1759+2T>A		1
MSH2	c.1782dupA	p.L595Tfs*3	1
MSH2	c.1784T>G	p.L595R	1
MSH2	c.1793delT	p.V598Gfs*2	1
MSH2	c.1803dupG	p.L602Afs*42	1
MSH2	c.181C>T	p.Q61*	1
MSH2	c.1861C>T	p.R621*	1
MSH2	c.2004delTinsCA	p.G669Rfs*7	1
MSH2	c.2047G>A	p.G683R	1
MSH2	c.2090G>A	p.C697Y	1
MSH2	c.20delA	p.E7Gfs*57	1

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Table A2. Mutation Data (continued)

Gene	Coding DNA Sequence (c.)	Protein Sequence (p.)	No. of Times Seen
<i>MSH2</i>	c.211G>C	p.G71R	1
<i>MSH2</i>	c.2152C>T	p.Q718*	1
<i>MSH2</i>	c.2245G>A	p.E749K	1
<i>MSH2</i>	c.2275G>T	p.G759*	1
<i>MSH2</i>	c.229_230delAG	p.S77Cfs*4	1
<i>MSH2</i>	c.2388delT	p.V797Lfs*15	1
<i>MSH2</i>	c.2502_2508delTAATTC	p.N835Lfs*4	1
<i>MSH2</i>	c.251delA	p.N84Ifs*6	1
<i>MSH2</i>	c.2633_2634delAG	p.E878Afs*3	1
<i>MSH2</i>	c.2680dupA	p.M894Nfs*5	1
<i>MSH2</i>	c.34dupG	p.E12Gfs*70	1
<i>MSH2</i>	c.388_389delCA	p.Q130Vfs*2	1
<i>MSH2</i>	c.645+2T>G		1
<i>MSH2</i>	c.675_678del	p.R227Kfs*18	1
<i>MSH2</i>	c.746delA	p.K249Rfs*5	1
<i>MSH2</i>	c.792+2T>C		1
<i>MSH2</i>	c.830T>G	p.L277*	1
<i>MSH2</i>	c.860dupG	p.Q288Tfs*3	1
<i>MSH2</i>	c.868G>T	p.E290*	1
<i>MSH2</i>	c.913dupG	p.A305Gfs*7	1
<i>MSH2</i>	c.942+2T>C		1
<i>MSH2</i>	c.998G>A	p.C333Y	1
<i>MSH2</i>	Ex1_7inv		1
<i>MSH2</i>	Ex2_4del		1
<i>MSH2</i>	Ex3_8del		1
<i>MSH2</i>	Ex4_3'UTRdel		1
<i>MSH2</i>	Ex6del		1
<i>MSH2</i>	Ex7_10del		1
<i>MSH2</i>	Ex7_3'UTRdel		1
<i>MSH2</i>	Ex7_9del		1
<i>MSH2</i>	Ex8_10del		1
<i>MSH2</i>	Ex8_15del		1
<i>MSH2</i>	Ex8_3'UTRdel		1
<i>MSH2</i>	Ex8del		1
<i>MSH2</i>	Ex9del		1
<i>MSH2</i>	Ex9_10del		1
<i>MSH2</i>	Ex9_15del		1
<i>MSH2</i>	Ex9_3'UTRdel		1
<i>MSH2</i>	In6_Ex8del		1
<i>MSH6</i>	c.1059_1063delITGGAG	p.S353Rfs*3	1
<i>MSH6</i>	c.1059dupT	p.G354Wfs*4	1
<i>MSH6</i>	c.1238G>A	p.W413*	1
<i>MSH6</i>	c.1290delG	p.G430Gfs*23	1
<i>MSH6</i>	c.1295T>C	p.F432S	1
<i>MSH6</i>	c.1304T>C	p.L435P	1
<i>MSH6</i>	c.1407T>A	p.Y469*	1
<i>MSH6</i>	c.1421_1422dupTG	p.Q475Cfs*7	1
<i>MSH6</i>	c.1519dupA	p.R507Kfs*9	1
<i>MSH6</i>	c.1610_1613delAGTA	p.K537Ifs*33	1
<i>MSH6</i>	c.1634_1637delAAGA	p.K545Rfs*25	1
<i>MSH6</i>	c.170_182del13	p.P57Rfs*20	1
<i>MSH6</i>	c.1705_1706delITT	p.F569Hfs*7	1
<i>MSH6</i>	c.1772delC	p.P591Qfs*19	1
<i>MSH6</i>	c.1898delC	p.T633Ifs*2	1
<i>MSH6</i>	c.1900_1901delITT	p.L634Efs*5	1
<i>MSH6</i>	c.2036_2042delTGGCCCTinsGG		1
<i>MSH6</i>	c.2057G>A	p.G686D	1
<i>MSH6</i>	c.2061T>A	p.C687*	1
<i>MSH6</i>	c.2062_2063delGT	p.V688Lfs*9	1
<i>MSH6</i>	c.2079dupA	p.C694Mfs*4	1
<i>MSH6</i>	c.2125dupT	p.Y709Lfs*7	1
<i>MSH6</i>	c.2127T>A	p.Y709*	1
<i>MSH6</i>	c.2147_2150del	p.V717Afs*18	1
<i>MSH6</i>	c.2194C>T	p.R732*	1
<i>MSH6</i>	c.2212_2222del11	p.V738Qfs*14	1
<i>MSH6</i>	c.2653A>T	p.K885*	1
<i>MSH6</i>	c.2778_2779insA		1

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Table A2. Mutation Data (continued)

Gene	Coding DNA Sequence (c.)	Protein Sequence (p.)	No. of Times Seen
<i>MSH6</i>	c.2805_2806insTT	p.D936Lfs*10	1
<i>MSH6</i>	c.2832_2833delAA	p.I944Mfs*4	1
<i>MSH6</i>	c.2927G>A	p.R976H	1
<i>MSH6</i>	c.2932C>T	p.Q978*	1
<i>MSH6</i>	c.2964dupC	p.N989Qfs*16	1
<i>MSH6</i>	c.3020delG		1
<i>MSH6</i>	c.3155_3156delAG	p.E1052Vfs*13	1
<i>MSH6</i>	c.3173-1G>C		1
<i>MSH6</i>	c.3198_3199delTA	p.Y1066*	1
<i>MSH6</i>	c.3202C>T	p.R1068*	1
<i>MSH6</i>	c.3253_3254insC		1
<i>MSH6</i>	c.3267_3268insC	p.E1090Rfs*3	1
<i>MSH6</i>	c.3311_3312delTT	p.F1104Wfs*3	1
<i>MSH6</i>	c.3330_3331insAATG		1
<i>MSH6</i>	c.3416delG	p.G1139Afs*6	1
<i>MSH6</i>	c.3416dupG	p.K1140Qfs*24	1
<i>MSH6</i>	c.3476dupA	p.Y1159*	1
<i>MSH6</i>	c.3485_3487delCTG	p.A1162del	1
<i>MSH6</i>	c.3513_3514delTA	p.D1171Efs*5	1
<i>MSH6</i>	c.3722_3724del	p.R1242del	1
<i>MSH6</i>	c.3724_3726delCGT	p.R1242del	1
<i>MSH6</i>	c.3804dupA	p.C1269Mfs*6	1
<i>MSH6</i>	c.3846dupT	p.I1283Yfs*6	1
<i>MSH6</i>	c.3847_3850dupATTA	p.T1284Nfs*6	1
<i>MSH6</i>	c.3939_3940dupTC	p.Q1314Lfs*14	1
<i>MSH6</i>	c.397_410del14	p.F133Pfs*7	1
<i>MSH6</i>	c.3978_3979insA		1
<i>MSH6</i>	c.3980_3983dupATCA	p.Q1328Qfs*14	1
<i>MSH6</i>	c.4004_4007dupAAGT	p.C1337Sfs*5	1
<i>MSH6</i>	c.467C>G	p.S156*	1
<i>MSH6</i>	c.565A>T	p.K189*	1
<i>MSH6</i>	c.577del	p.L193Wfs*18	1
<i>MSH6</i>	c.626_627+15delAGGTGGGACACGGCAAG		1
<i>MSH6</i>	c.651dupT	p.K218*	1
<i>MSH6</i>	c.718C>T	p.R240*	1
<i>MSH6</i>	c.900dupG	p.K301Efs*11	1
<i>MSH6</i>	c.989C>A	p.S330*	1
<i>MSH6</i>	Ex2_6del		1
<i>MSH6</i>	Ex2del		1
<i>MSH6</i>	Ex4del		1
<i>PMS2</i>	5'UTR_Ex15del		1
<i>PMS2</i>	c.1067delA	p.K356Rfs*4	1
<i>PMS2</i>	c.1198C>T	p.Q400*	1
<i>PMS2</i>	c.1261C>T	p.R421*	1
<i>PMS2</i>	c.1411C>T	p.Q471*	1
<i>PMS2</i>	c.164-1G>C		1
<i>PMS2</i>	c.1687C>T	p.R563*	1
<i>PMS2</i>	c.1738A>T	p.K580*	1
<i>PMS2</i>	c.1840A>T	p.K614*	1
<i>PMS2</i>	c.1981G>T	p.E661*	1
<i>PMS2</i>	c.2155C>T	p.Q719*	1
<i>PMS2</i>	c.2174+1G>A		1
<i>PMS2</i>	c.2182_2187delACTCTCinsGCTC	p.T728Afs*7	1
<i>PMS2</i>	c.2192_2196delTAACT	p.L731Cfs*3	1
<i>PMS2</i>	c.2249G>A	p.G750D	1
<i>PMS2</i>	c.23+1G>T		1
<i>PMS2</i>	c.2404C>T	p.R802*	1
<i>PMS2</i>	c.2446-1G>T		1
<i>PMS2</i>	c.2506G>T	p.E836*	1
<i>PMS2</i>	c.325delG	p.E109Kfs*3	1
<i>PMS2</i>	c.697C>T	p.Q233*	1
<i>PMS2</i>	c.703C>T	p.Q235*	1
<i>PMS2</i>	c.706-2A>C		1
<i>PMS2</i>	c.823C>T	p.Q275*	1
<i>PMS2</i>	c.825A>G	p.Q275Q	1
<i>PMS2</i>	c.851delC	p.S284*	1
<i>PMS2</i>	c.857_864del8	p.D286Vfs*10	1

(continued on following page)



**Table A2.** Mutation Data (continued)

Gene	Coding DNA Sequence (c.)	Protein Sequence (p.)	No. of Times Seen
<i>PMS2</i>	c.861_864delACAG	p.R287Sfs*19	1
<i>PMS2</i>	c.88C>T	p.Q30*	1
<i>PMS2</i>	c.989-2A>G		1
<i>PMS2</i>	Ex12_14del		1
<i>PMS2</i>	Ex15del		1
<i>PMS2</i>	Ex6del		1
<i>PMS2</i>	Ex7del		1
<i>PMS2</i>	In1_ex9del		1
<i>PMS2</i>	In2_Ex11del		1
<i>PMS2</i>	In8_Ex10del		1
<i>PMS2</i>	In8_Ex11del		1

**Table A3.** Gene-Specific Mutation Frequencies by Proband Tumor Type Compared with Population Frequencies

Proband Tumor Type	<i>MLH1</i>		<i>MSH2</i>		<i>MSH6</i>		<i>PMS2</i>	
	%	<i>P</i>	%	<i>P</i>	%	<i>P</i>	%	<i>P</i>
CRC only* (n = 2,795)	1.968	<b><math>3.4 \times 10^{-66}</math></b>	1.324	<b><math>9.9 \times 10^{-45}</math></b>	0.859	<b><math>1.8 \times 10^{-12}</math></b>	0.966	<b><math>1.9 \times 10^{-14}</math></b>
EC only† (n = 1,001)	0.799	<b><math>7.1 \times 10^{-8}</math></b>	2.098	<b><math>3.1 \times 10^{-30}</math></b>	3.297	<b><math>1.9 \times 10^{-34}</math></b>	0.899	<b><math>1.6 \times 10^{-5}</math></b>
BC only‡ (n = 10,689)	0.065	$5.1 \times 10^{-1}$	0.056	$2.9 \times 10^{-1}$	0.178	$1.8 \times 10^{-1}$	0.281	<b><math>6.1 \times 10^{-4}</math></b>
OC only§ (n = 3,597)	0.167	<b><math>1.1 \times 10^{-2}</math></b>	0.195	<b><math>3.3 \times 10^{-4}</math></b>	0.500	<b><math>2.6 \times 10^{-6}</math></b>	0.195	$3.6 \times 10^{-1}$
Population estimates from Win et al <sup>1</sup>	0.051	—	0.035	—	0.132	—	0.140	—

NOTE. Mutation frequencies from this study were compared with the population estimates from Win et al<sup>1</sup> by using the binomial proportion test. Bold *P* values indicate statistical significance.

\*Probands with colorectal cancer (CRC) only, but not endometrial cancer (EC), breast cancer (BC), or ovarian cancer (OC).

†Probands with EC only, but not CRC, BC, or OC.

‡Probands with BC only, but not OC, CRC, or EC.

§Probands with OC only, but not BC, CRC, or EC.

**Table A4.** Patients with Discordant MSI and/or IHC Results

MSI and/or IHC Results	No. of Patients	Mutated Gene
Normal MSI and IHC	3	<i>PMS2</i> × 2 <i>MSH6</i>
Normal MSI, no IHC	1	<i>MSH6</i>
No MSI, normal IHC	3	<i>PMS2</i> × 2 <i>MSH2</i>
Loss of MLH1 and PMS2	1	<i>MSH6</i>

Abbreviations: IHC, immunohistochemistry; MSI, microsatellite instability.