Associations of Pathogenic Variants in $\text{MLH1}$, $\text{MSH2}$, and $\text{MSH6}$ With Risk of Colorectal Adenomas and Tumors and With Somatic Mutations in Patients With Lynch Syndrome

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Associations of Pathogenic Variants in MLH1, MSH2, and MSH6 With Risk of Colorectal Adenomas and Tumors and With Somatic Mutations in Patients With Lynch Syndrome

Short Title: Colorectal cancer development in Lynch syndrome


on behalf of the German HNPCC Consortium, the Dutch Lynch Syndrome Collaborative Group, and the Finnish Lynch Syndrome Registry

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ABSTRACT

Background & Aims: Lynch syndrome is caused by variants in DNA mismatch repair (MMR) genes and associated with an increased risk of colorectal cancer (CRC). In patients with Lynch syndrome, CRCs can develop via different pathways. We studied associations between Lynch syndrome-associated variants in MMR genes and risks of adenoma and CRC and somatic mutations in APC and CTNNB1 in tumors in an international cohort of patients.

Methods: We combined clinical and molecular data from 3 studies. We obtained clinical data from 2747 patients with Lynch syndrome associated with variants in MLH1, MSH2, or MSH6 from Germany, the Netherlands, and Finland who received at least 2 surveillance colonoscopies and were followed for a median time of 7.8 years for development of adenomas or CRC. We performed DNA sequence analyses of 48 colorectal tumors (from 16 patients with mutations in MLH1, 29 patients with mutations in MSH2, and 3 with mutations in MSH6) for somatic mutations in APC and CTNNB1.

Results: Risk of advanced adenoma in 10 y was 17.8% in patients with pathogenic variants in MSH2 vs 7.7% in MLH1 (P<.001). Higher proportions of patients with pathogenic variants in MLH1 or MSH2 developed CRC in 10 y (11.3% and 11.4%) than patients with pathogenic variants in MSH6 (4.7%) (P=.001 and P=.003 for MLH1 and MSH2 vs MSH6, respectively). Somatic mutations in APC were found in 75% of tumors from patients with pathogenic variants in MSH2 vs 11% in MLH1 (P=.015). Somatic mutations in CTNNB1 were found in 50% of tumors from patients with pathogenic variants in MLH1 vs 7% in MSH2 (P=.002). None of the 3 tumors with pathogenic variants in MSH6 had a mutation in CTNNB1, but all had mutations in APC.
Conclusions: In an analysis of clinical and DNA sequence data from patients with Lynch syndrome from 3 countries, we associated pathogenic variants in MMR genes with risk of adenoma and CRC, and somatic mutations in APC and CTNNB1 in colorectal tumors. If these findings are confirmed, surveillance guidelines might be adjusted based on MMR gene variants.

Keywords: prognostic factor, genetic analysis, outcome, cancer risk
Lynch syndrome (LS) is the most common hereditary colorectal cancer (CRC) syndrome, accounting for approximately 3% of all cases of CRC.\(^1\) LS is an autosomal dominant inherited disorder caused by pathogenic germline variants in mismatch repair (MMR) genes, including \(MLH1\), \(MSH2\) (\(EPCAM\)), \(MSH6\) and \(PMS2\). Carriers of such gene defects are at high risk of developing primarily CRC and endometrial cancer, but also other malignancies.\(^2\) The main features of LS include an early age of cancer onset, a high risk of developing multiple cancers, and microsatellite instability and loss of MMR expression in the tumors.\(^3\) The risk of developing a specific cancer type in LS depends on the underlying germline MMR defect.\(^4\)-\(^6\)

Carriers of pathogenic MMR gene variants show an increased frequency of adenoma development compared to non-carriers undergoing intensive colonoscopic surveillance.\(^7\) In addition, a Finnish study demonstrated that colonoscopy with polypectomy reduces CRC incidence and CRC mortality by >50%.\(^8\) Together these observations confirmed the role of adenomas in the development of CRC in LS and constitute the basis for colonoscopic surveillance programs.\(^4, 9\)

Nevertheless, a substantial number of carriers develop CRC despite colonoscopic surveillance.\(^10\)-\(^12\) Recent publications report risks of CRC of up to 46% in patients under surveillance, with much higher risks found for \(MLH1\) and \(MSH2\) carriers (43-46%) than for \(MSH6\) (15%) and \(PMS2\) carriers (0%).\(^13\) Another recent prospective study evaluated the risk of incident CRC in a large series of \(MLH1\), \(MSH2\), or \(MSH6\) carriers from Germany, Finland and the Netherlands.\(^14\) Despite intensive surveillance, 4-18% of the carriers developed CRC after 10 years of follow-up, independently of the screening interval.

In 2016, Ahadova et al. hypothesized that LS-CRC might develop through a pathway characterized by a lack of adenomatous tissue and by immediate invasive growth under the mucosal surface.\(^15\) The authors suggested that these LS-CRCs may emerge from MMR-deficient crypt foci that grow under the mucosal surface and cannot be detected by colonoscopy at a pre-invasive stage,
potentially explaining CRC development despite colonoscopic surveillance and polypectomies. It was subsequently demonstrated that LS-CRCs are heterogeneous and can develop via different molecular pathways with distinct initiating events: whereas some LS-CRCs develop from MMR-proficient adenomas, the majority develop from MMR-deficient lesions, either via an adenomatous phase or in the absence of a detectable precursor lesion. Nonetheless, the impact of certain MMR gene mutations on the development of particular CRC subtypes remains unclear.

The development of CRC despite surveillance is an important problem in the clinical management of LS and deserves closer examination to clarify the underlying mechanisms. A better understanding of carcinogenesis in each specific group of pathogenic MMR variant carriers will have important consequences for decision-making on appropriate surveillance intervals.

The purpose of this study was to assess possible differences in the pathways of CRC development between MLH1, MSH2 and MSH6 carriers. Our specific aims were (1) to compare risks for (advanced) adenoma and CRC, and (2) to compare the frequencies of CTNNB1 and APC mutations in CRCs between MLH1, MSH2 and MSH6 carriers.
METHODS

Study population

This report combines clinical and molecular data obtained in the course of three earlier studies. The clinical part is based on data from a prospective cohort study conducted to compare CRC incidence and stage in LS patients from three different countries (Germany, the Netherlands, Finland) recommending different colonoscopy intervals.\textsuperscript{14} The study population has been described in detail elsewhere.\textsuperscript{14} Briefly, the cohort consisted of 2,747 LS patients included in the LS registries of Germany, the Netherlands, and Finland. In all three registries, LS patients were followed prospectively within a framework of intensive colonoscopic surveillance programs. Written informed consent was obtained from all LS patients enrolled in the registries and who participated in prospective surveillance studies. Patients were eligible for the present analysis if they i) were carriers of a pathogenic germline variant in either the MLH1, MSH2 or MSH6 gene, and ii) had completed at least two surveillance colonoscopies after registry inclusion. For each colonoscopy, age at examination and worst finding (normal, adenoma, advanced adenoma, CRC) were noted, and for each CRC the age at diagnosis was recorded. An advanced adenoma was defined by a size of $\geq 1cm$ or the presence of either villous histology or high-grade dysplasia. In the present analysis, we used this study population to compare the cumulative incidences of (advanced) adenomas and CRC between the MLH1, MSH2, and MSH6 carriers.

Molecular analysis and histology assessment

Separately from the clinical part, the molecular part of the present report represents a re-analysis of data from two studies reported previously.\textsuperscript{15, 17} Briefly, formalin-fixed paraffin-embedded (FFPE) tissue blocks from LS-CRCs were collected within the Department of Applied Tumor Biology, Institute of Pathology, University Hospital Heidelberg. Tumor tissue was microdissected from FFPE tissue sections and DNA was isolated for the downstream analyses. Histopathology review
revealed a tumor cell content of more than 50% in all studied samples. Mutational analysis was performed either by targeted Sanger sequencing (for determination of \textit{CTNNB1} mutation status) or by Illumina panel sequencing of mutation HotSpot regions in 30 genes, including \textit{CTNNB1} and \textit{APC}.\textsuperscript{18} The data were analyzed by Sequencing Analysis Software and Ion Torrent Suite Software, respectively. Only variants with an allele frequency >5% and minimum coverage >100 reads were taken into account. For the purposes of the present study, molecular data obtained from CRCs were sorted depending on the underlying MMR defect. All patients provided informed, written consent and the study was approved by the relevant Institutional Ethics Committee.

\textbf{Statistical analysis}

In the clinical cohort, prospective observation started with the first colonoscopy conducted after enrollment in the LS register (index colonoscopy) and ended with the last colonoscopy or the occurrence of a primary CRC diagnosis. CRCs detected at the index colonoscopy were defined as prevalent CRCs. All other CRCs detected during prospective observation were defined as incident cancers. The occurrence of incident extra-colonic tumors was ignored if surveillance colonoscopies were continued after such an event. Time to incident (advanced) adenoma or CRC was analyzed using the Kaplan-Meier method, with time zero at the index colonoscopy and group comparisons made using the log-rank test. Additionally, we performed multivariate Cox regression analyses adjusting for age at index colonoscopy and country as confounders, the latter reflecting the differences in colonoscopy intervals and the differences in the proportions of patients with prior CRC. Comparisons of categorical data between groups were performed using the chi-square test, or Fisher's exact test where appropriate. P values below 0.05 were considered statistically significant. All analyses were carried out using IBM SPSS Statistics for Windows, Version 24.0 (IBM Corp., Armonk, NY, USA).
RESULTS

Risk of (advanced) adenomas and CRC

The clinical cohort comprised 2,747 LS patients in total (1,027 from Germany, 806 from the Netherlands, and 914 from Finland). Basic patient characteristics can be found in Table 1. A total of 1,038 patients (38%) already had a prior CRC before the index colonoscopy. Due to the presence of two MLH1 founder mutations in the Finnish population, the proportion of MLH1 carriers was higher in Finland (79%) compared to Germany (39%) and the Netherlands (35%). Patients had a median of five consecutive colonoscopies (16,327 colonoscopies in total). The median per-patient observation time was 7.8 years (interquartile range 4.2 to 12.0). The cumulative prospective observation time amounted to 23,309 person-years in total. At the index colonoscopy, the frequency of prevalent adenomas was 10.2% and the frequency of prevalent CRC was 2.3%.

Figure 1 shows the comparison of the cumulative risk of incident adenoma between MLH1, MSH2 and MSH6 carriers. Ten years after the index colonoscopy, the highest risk was observed in MSH2 carriers (44.2%, 95%CI 40.0-48.4%), followed by MSH6 carriers (38.4%, 95%CI 30.8-45.9%), and was lowest in MLH1 carriers (32.2%, 95%CI 29.2-35.2%). The differences in risk between MLH1 and MSH2 carriers (p<0.001) and between MLH1 and MSH6 carriers (p=0.029) were statistically significant, but not between MSH2 and MSH6 carriers (p=0.400). Figure 2 shows the comparison of the cumulative risk of incident advanced adenoma between the three groups. Ten years after the index colonoscopy, the risk of advanced adenoma was similar for MLH1 and MSH6 carriers (7.7%, 95%CI 6.0-9.4% and 9.4%, 95%CI 5.4-13.4%, respectively, p=0.543), but both had a significantly (p<0.001 and p=0.010, respectively) lower risk than MSH2 carriers (17.8%, 95%CI 14.6-21.0%).

Figure 3 shows the cumulative risks for incident CRC. Ten-year CRC risks were almost identical for MLH1 and MSH2 carriers (11.3%, 95%CI 9.4-13.2% and 11.4%, 95%CI 8.9-14.0%, respectively, p=0.468). In contrast, CRC risk in MSH6 carriers was significantly lower (4.7%, 95%CI 1.8-7.7%) compared to MLH1 (p=0.001) and MSH2 (p=0.003) carriers. Multivariate Cox regression analyses
adjusting for age at index colonoscopy and country revealed similar results regarding significant group differences except for adenoma risk between MLH1 and MSH6 carriers, which was not significant (p-adjusted=0.265) compared to the unadjusted analysis.

Molecular features of LS-CRC

Molecular analysis was performed on CRCs from 16 MLH1, 29 MSH2, and 3 MSH6 carriers. Allele frequencies of the observed somatic mutations ranged between 21% and 73%. The results are summarized in Table 2. Of the 16 MLH1-associated CRCs, eight displayed somatic CTNNB1 mutations (50%, 95%CI: 28.0-72.0%), whereas only two somatic CTNNB1 mutations were detected in the 29 MSH2-associated CRCs (7%, 95%CI: 0.9-23.0%), demonstrating a significantly higher proportion of somatic CTNNB1 mutations in MLH1- compared to MSH2-associated CRCs (p=0.002). In contrast, somatic APC mutations were detected in only one of nine MLH1-associated CRCs (11%, 95%CI: 0-45.7%), whereas MSH2-associated CRCs carried somatic APC mutations in six out of eight analyzed CRCs (75%, 95% CI: 40.1-93.7%, p=0.015). None of the three MSH6-associated CRCs analyzed carried a somatic CTNNB1 mutation, but all three presented with a somatic APC mutation.
DISCUSSION

Previous studies in LS have shown that up to 1 in 5 patients develop CRC despite intensive colonoscopic surveillance. 8, 10-12, 14 MLH1 and MSH2 carriers under surveillance are at high risk of CRC, whereas MSH6 carriers have a much lower risk, and PMS2 carriers may even have zero CRC risk under surveillance. 13 The main goal of the present study was to evaluate whether the molecular pathways of carcinogenesis are different between MLH1, MSH2 and MSH6 carriers and therefore explain the observed differences in adenoma and CRC risk and the effectiveness of screening programs.

The prospective clinical data of our study demonstrates that the risk of adenomas is significantly greater in MSH2 and MSH6 carriers compared to MLH1 carriers, and that the risk of advanced adenomas is higher in MSH2 carriers compared to both MLH1 and MSH6 carriers. However, incident CRC was more frequently observed in MLH1 and MSH2 carriers than in MSH6 carriers. This is in contrast to a recently published single center study involving 242 MLH1, MSH2 and MSH6 carriers over 1739 years of follow-up, which could not detect significant differences in (advanced) adenoma incidence between MMR genes, probably due to the low sample size. 19

Our study also demonstrates molecular differences in the carcinogenesis between MLH1- and MSH2-associated LS-CRCs. Whereas MSH2-associated CRCs presented with a higher frequency of somatic APC mutations compared to MLH1-associated CRCs, a significantly higher frequency of CTNNB1 mutations was observed in MLH1-associated CRCs compared to MSH2-associated ones. 20, 21

Interestingly, incident CRC risk in MLH1 carriers was as high as in MSH2 carriers, but MLH1 carriers presented with a substantially lower (advanced) adenoma incidence than MSH2 carriers. Moreover, the cumulative risk for advanced adenoma in MSH2 carriers was higher than the CRC risk, which agrees with the notion that not all adenomas develop into cancer. In MLH1 carriers, however, the risk for advanced adenoma was lower than the incident cancer risk. Assuming similar
progression rates for *MLH1* and *MSH2*-associated advanced adenomas into CRC, this observation might suggest different pathways of CRC development in *MLH1* versus *MSH2* carriers. Seen in the light of the enhanced CRC risk in both *MLH1* and *MSH2* carriers and their molecular characteristics, it is conceivable that somatic *CTNNB1* and *APC* mutations, in combination with MMR deficiency, may both contribute to tumor progression. Whereas *MSH2*-associated cancers may have an accelerated adenoma-carcinoma sequence with somatic *APC* mutations often following MMR deficiency, a substantial proportion of *MLH1*-associated cancers may progress without prior polyp formation through an immediate invasive pathway, presumably arising from MMR-deficient crypts due to acquired somatic *CTNNB1* mutations.

The higher frequency of somatic *APC* mutations in CRCs of *MSH2* vs. *MLH1* carriers is in line with the observed higher incidence of (advanced) adenomas in *MSH2* vs. *MLH1* carriers, as *APC* mutations are often associated with adenoma development in the colonic epithelium.\(^{22}\)

Both *MSH2*- and *MSH6*-associated CRCs presented with a high proportion of somatic *APC* mutations and a low proportion of somatic *CTNNB1* mutations. While this finding should be validated in a larger cohort in the case of *MSH6*-associated CRCs, this outcome is intriguing since *MSH6* carriers show a lower risk of advanced adenoma development compared to *MSH2* carriers. One explanation for this observation could be an incomplete MMR deficiency caused by MSH6 loss, with predominantly mononucleotide repeats affected, which potentially lowers the likelihood of driver mutations secondary to MMR deficiency.\(^{23}\) This is further corroborated by the observation of MSH6-deficient cancers that lack MSI\(^{24}\) and possibly indirectly by a lower proportion of MMR-deficient adenomas (approximately 27%) in *MSH6* carriers compared to *MSH2* or *MLH1* carriers (75-80%), as demonstrated previously.\(^{17, 20, 25, 26}\)

In view of the low frequency of MMR-deficient adenomas in *MSH6*, it is conceivable that MMR-deficient crypt foci are either less common or less likely to progress in *MSH6* carriers. Progression according to the classic adenoma-carcinoma sequence, with MMR deficiency occurring after
adenoma development, could therefore be the more frequent pathway of carcinogenesis in this group of carriers. This hypothesis is supported by the observation of low grade adenomas presenting with an MSS phenotype in MSH6 carriers, in contrast to MLH1 carriers.\textsuperscript{27, 28} As a consequence, surveillance colonoscopy with polypectomy might be more effective in MSH6 carriers, as described in a recent study.\textsuperscript{13}

A similar hypothesis might explain the high efficacy of colonoscopic screening in PMS2 carriers. Adenomas in these patients are usually MMR-proficient and PMS2-associated CRCs do not commonly present with somatic CTNNB1 mutations.\textsuperscript{21} Thus, the lower CRC risks observed in MSH6 and PMS2 carriers under surveillance might be explained by inherently lower risks of CRC development in these carriers, but may also be partly due to a different pathogenesis and thereby more effective prevention by polypectomy.

The current study had several strengths, as well as some limitations. A major strength of the clinical part was the large prospective cohort with long duration of follow-up. A limitation was the low number of CRCs from MSH6 carriers available for molecular studies, which does not allow drawing definitive conclusions. It is also important to note that the molecular data were not obtained from the CRCs of the clinical cohort, but from separate CRC samples. Since the selection of these samples did not differ from the selection of patients for the clinical part of the study, this should not have influenced the results of our study in a major way. However, prospective validation in a larger number of cancers is warranted.

What are the implications of our findings for clinical practice? Our results suggest that the high CRC risk in MLH1 and MSH2 carriers under surveillance might be largely attributed to the molecular characteristics of the CRCs, possibly contributing to a fast adenoma-carcinoma sequence or initial submucosal growth in the absence of adenomatous tissue. The development of CRC despite intensive surveillance colonoscopy in these groups of carriers should therefore be considered as part of the phenotype. Fortunately, most CRCs detected by screening are local,
without metastatic disease, and almost all patients have a favorable outcome.\textsuperscript{6, 29} These considerations should be discussed with the patients, and it should be emphasized that detection of a CRC during intensive colonoscopic surveillance is not surprising and does not necessarily indicate a failure of the surveillance program. In contrast, colonoscopy and polypectomy are effective in almost all \textit{MSH6} carriers, as also reported for \textit{PMS2} carriers.\textsuperscript{6}

In conclusion, this is the first study to compare clinical and molecular findings between carriers of alterations in the MMR genes \textit{MLH1}, \textit{MSH2} and \textit{MSH6}. Our results suggest that growth characteristics and accumulation of certain molecular alterations may explain the high CRC risk in both \textit{MLH1} and \textit{MSH2} carriers. Despite similar CRC risks there were remarkable differences in \textit{CTNNB1} and \textit{APC} mutation frequencies between \textit{MLH1} and \textit{MSH2} carriers. The previously reported low frequency of MMR deficiency in \textit{MSH6}-associated adenomas, in combination with the low frequency of \textit{CTNNB1} mutations and the higher frequency of \textit{APC} mutations, suggests that an MMR-deficient polypous pathway in \textit{MSH6} carriers typically arises after the development of adenomas. If these findings are confirmed in future studies, surveillance guidelines might be adjusted based on the underlying MMR gene variant.
REFERENCES


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FIGURES

Figure 1. Cumulative incidence of adenoma by affected gene (MLH1, MSH2, MSH6)

Figure 2. Cumulative incidence of advanced adenoma by affected gene (MLH1, MSH2, MSH6)

Figure 3. Cumulative incidence of incident CRC by affected gene (MLH1, MSH2, MSH6)
### Table 1: Clinical characteristics of patients in the clinical cohort

<table>
<thead>
<tr>
<th>Country, no (%)</th>
<th>MLH1</th>
<th>MSH2</th>
<th>MSH6</th>
<th>Total</th>
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<tr>
<td>Germany</td>
<td>400 (28.4)</td>
<td>507 (51.4)</td>
<td>120 (33.9)</td>
<td>1027 (37.4)</td>
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<tr>
<td>Netherlands</td>
<td>285 (20.3)</td>
<td>336 (34.1)</td>
<td>185 (52.3)</td>
<td>806 (29.3)</td>
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<tr>
<td>Finland</td>
<td>722 (51.3)</td>
<td>143 (14.5)</td>
<td>49 (13.8)</td>
<td>914 (33.3)</td>
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<table>
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<tr>
<th>Sex, no (%)</th>
<th>MLH1</th>
<th>MSH2</th>
<th>MSH6</th>
<th>Total</th>
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<tr>
<td>male</td>
<td>672 (47.8)</td>
<td>483 (49.0)</td>
<td>160 (45.2)</td>
<td>1315 (47.9)</td>
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<tr>
<td>female</td>
<td>735 (52.2)</td>
<td>503 (51.0)</td>
<td>194 (54.8)</td>
<td>1432 (52.1)</td>
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<tr>
<th>CRC before index colonoscopy, no (%)</th>
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<th>MSH6</th>
<th>Total</th>
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<tr>
<td>Germany</td>
<td>526 (37.4)</td>
<td>405 (41.1)</td>
<td>107 (30.2)</td>
<td>1038 (37.8)</td>
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<tr>
<td>Netherlands</td>
<td>42.7 (±13.5)</td>
<td>44.0 (±12.3)</td>
<td>48.7 (±13.7)</td>
<td>43.9 (±13.2)</td>
</tr>
<tr>
<td>Finland</td>
<td>2002 (±6)</td>
<td>2004 (±5)</td>
<td>2005 (±4)</td>
<td>2003 (±5)</td>
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<th>Number of colonoscopies</th>
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<th>MSH2</th>
<th>MSH6</th>
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<tr>
<td>per patient, median (IQR)</td>
<td>5 (3-8)</td>
<td>6 (4-8)</td>
<td>4 (3-6)</td>
<td>5 (3-8)</td>
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<tr>
<td>cumulative</td>
<td>8229</td>
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<td>1798</td>
<td>16327</td>
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<th>Observation time, years</th>
<th>MLH1</th>
<th>MSH2</th>
<th>MSH6</th>
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<td>per patient, median (IQR)</td>
<td>8.5 (4.2-13.2)</td>
<td>7.4 (4.4-11.3)</td>
<td>6.5 (4.1-9.4)</td>
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<th>MSH6</th>
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<tr>
<td>167 / 169</td>
<td>93 / 97</td>
<td>12 / 13</td>
<td>272 / 279</td>
<td></td>
</tr>
</tbody>
</table>

IQR, interquartile range; SD, standard deviation

### Table 2. Presence of somatic mutations in CTNNB1 and APC in tumors of MLH1, MSH2, and MSH6 carriers

<table>
<thead>
<tr>
<th>MLH1</th>
<th>MSH2</th>
<th>MSH6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis, median</td>
<td>46</td>
<td>45</td>
</tr>
<tr>
<td>CTNNB1 mutated</td>
<td>8 of 16 (50%)</td>
<td>2 of 29 (7%)</td>
</tr>
<tr>
<td>APC mutated</td>
<td>1 of 9 (11%)</td>
<td>6 of 8 (75%)</td>
</tr>
</tbody>
</table>

Not all tumors could be analyzed for APC mutations.
$P_{\text{overall}} < 0.001$

$P_{\text{MLH1, MSH2}} < 0.001$ (<0.001*)

$P_{\text{MLH1, MSH6}} = 0.029$ (≈0.265*)

$P_{\text{MSH2, MSH6}} = 0.400$ (≈0.126*)

* adjusted for age at index colonoscopy and country
Cumulative incidence

Years since index colonoscopy

\[ P_{\text{overall}} < 0.001 \]

\[ P_{\text{MLH1, MSH2}} < 0.001 \ (= 0.007^*) \]

\[ P_{\text{MLH1, MSH6}} = 0.543 \ (= 0.246^*) \]

\[ P_{\text{MSH2, MSH6}} = 0.010 \ (= 0.004^*) \]

* adjusted for age at index colonoscopy and country
$P_{\text{overall}} = 0.003$

$P_{MLH1, MSH2} = 0.468$ ($= 0.137^*$)
$P_{MLH1, MSH6} = 0.001$ ($< 0.001^*$)
$P_{MSH2, MSH6} = 0.003$ ($= 0.001^*$)

* adjusted for age at index colonoscopy and country
What you need to know:

BACKGROUND AND CONTEXT: Lynch syndrome is caused by variants in DNA mismatch repair (MMR) genes and is associated with an increased risk of colorectal cancer (CRC). Variants in MMR genes are likely to have different effects on CRC and adenoma risk.

NEW FINDINGS: In an analysis of adenomas and colorectal tumors from patients with Lynch syndrome from 3 countries, we associated pathogenic variants in MMR genes with risk of adenoma and CRC, and with somatic mutations in APC and CTNNB1.

LIMITATIONS: We analyzed DNA sequences of 48 tumor samples. Larger studies of CRC development, and features of tumors, are needed in patients with Lynch syndrome.

IMPACT: Surveillance guidelines for patients with Lynch syndrome might need to be adjusted based on MMR gene variants.

Lay Summary: Lynch syndrome is caused by alterations in a specific group of genes. Patients with different types of genetic alterations develop adenomas and CRC at different rates, and tumors have specific genetic features.