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## Identification of Lynch Syndrome Among Patients With Colorectal Cancer

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Drs Moreira and Castells had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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## Abstract

**Context**—Lynch syndrome is the most common form of hereditary colorectal cancer (CRC) and is caused by germline mutations in DNA mismatch repair (MMR) genes. Identification of gene carriers currently relies on germline analysis in patients with MMR-deficient tumors, but criteria to select individuals in whom tumor MMR testing should be performed are unclear.

**Objective**—To establish a highly sensitive and efficient strategy for the identification of MMR gene mutation carriers among CRC probands.

**Design, Setting, and Patients**—Pooled-data analysis of 4 large cohorts of newly diagnosed CRC probands recruited between 1994 and 2010 (n = 10 206) from the Colon Cancer Family Registry, the EPICOLON project, the Ohio State University, and the University of Helsinki examining personal, tumor-related, and family characteristics, as well as microsatellite instability, tumor MMR immunostaining, and germline MMR mutational status data.

**Main Outcome Measures**—Performance characteristics of selected strategies (Bethesda guidelines, Jerusalem recommendations, and those derived from a bivariate/multivariate analysis of variables associated with Lynch syndrome) were compared with tumor MMR testing of all CRC patients (universal screening).

**Results**—Of 10 206 informative, unrelated CRC probands, 312 (3.1%) were MMR gene mutation carriers. In the population-based cohorts (n=3671 probands), the universal screening approach (sensitivity, 100%; 95% CI, 99.3%–100%; specificity, 93.0%; 95% CI, 92.0%–93.7%; diagnostic yield, 2.2%; 95% CI, 1.7%–2.7%) was superior to the use of Bethesda guidelines (sensitivity, 87.8%; 95% CI, 78.9%–93.2%; specificity, 97.5%; 95% CI, 96.9%–98.0%; diagnostic yield, 2.0%; 95% CI, 1.5%–2.4%;  $P < .001$ ), Jerusalem recommendations (sensitivity, 85.4%; 95% CI, 77.1%–93.6%; specificity, 96.7%; 95% CI, 96.0%–97.2%; diagnostic yield, 1.9%; 95% CI, 1.4%–2.3%;  $P < .001$ ), and a selective strategy based on tumor MMR testing of cases with CRC diagnosed at age 70 years or younger and in older patients fulfilling the Bethesda guidelines (sensitivity, 95.1%; 95% CI, 89.8%–99.0%; specificity, 95.5%; 95% CI, 94.7%–96.1%; diagnostic yield, 2.1%; 95% CI, 1.6%–2.6%;  $P < .001$ ). This selective strategy missed 4.9% of Lynch syndrome cases but resulted in 34.8% fewer cases requiring tumor MMR testing and 28.6% fewer cases undergoing germline mutational analysis than the universal approach.

**Conclusion**—Universal tumor MMR testing among CRC probands had a greater sensitivity for the identification of Lynch syndrome compared with multiple alternative strategies, although the increase in the diagnostic yield was modest.

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Colorectal cancer (CRC) is the third most common cancer worldwide and the second leading cause of cancer-related death.<sup>1</sup> Lynch syndrome, also known as hereditary nonpolyposis colorectal cancer (HNPCC), is the most common form of hereditary CRC, accounting for 1% to 3% of all these tumors. It is an autosomaldominant disorder caused by germline mutations in DNA mismatch repair (MMR) genes (ie, *MSH2*, *MLH1*, *MSH6*, and *PMS2*).<sup>2</sup>

The abnormal function of these genes leads to accumulation of errors during DNA replication, especially in repetitive sequences known as microsatellites. As a result, tumors of patients with Lynch syndrome characteristically demonstrate MMR deficiency, defined as the presence of microsatellite instability (MSI) or loss of the MMR protein expression, which is the hallmark of this disorder.<sup>3,4</sup>

Identification of patients with Lynch syndrome needs to be improved because, unless there is strong clinical suspicion, the majority of cases remain undetected, leading to the lack of implementation of highly effective preventive measures. Indeed, intensive CRC screening by colonoscopy and prophylactic gynecological surgery have been demonstrated to reduce both the incidence and mortality of these tumors.<sup>5</sup>

In 1991, the International Collaborative Group on HNPCC proposed the Amsterdam criteria and subsequently the extended Amsterdam II criteria,<sup>6</sup> the first clinical definition of the syndrome and as a means to identify the genes responsible. However, these criteria were limited in clinical practice because of their low sensitivity. Consequently, the National Cancer Institute proposed the Bethesda guidelines, and more recently the revised Bethesda guidelines,<sup>7</sup> for identifying those individuals who should undergo tumor MSI testing. Although this strategy has been demonstrated to be both effective and cost-effective,<sup>8</sup> it is not fully accepted because some MMR gene mutation carriers do not fulfill these criteria and because they are difficult to apply in clinical practice.<sup>9</sup> Virtually all Lynch syndrome-associated CRC display MMR deficiency, so universal tumor MMR screening has been proposed using MSI testing or immunostaining of all CRC patients.<sup>2,4</sup> Recently, it was suggested that tumor MMR screening should be performed in, at a minimum, all CRC occurring in individuals younger than 70 years (ie, Jerusalem recommendations).<sup>10</sup> Nevertheless, while this strategy overcomes the limitations of using any selection based on clinical criteria, it might not represent the most effective approach.

The controversy reflects that, at present, tumor MMR testing is the cornerstone for identification of Lynch syndrome. However, it is still under debate which CRC patients should undergo these analyses. Most sets of recommendations are not empirically based<sup>6,7</sup> or derived from series in which patients were selected on the basis of their personal or family history.<sup>11-14</sup> To overcome these limitations, a pooled data analysis of population-based series with fully integrated, comprehensive, and reliable data seems the most appropriate approach to outline a highly sensitive, efficient, and widely accepted strategy for the identification of MMR gene mutation carriers among CRC probands.

## METHODS

The study sample came from the Colon Cancer Family Registry (CFR), the EPICOLON project,<sup>8</sup> the Clinical Cancer Genetics Program of the Ohio State University,<sup>4,9</sup> and the Department of Medical Genetics of the University of Helsinki, Finland<sup>15,16</sup> (Figure 1 and Figure 2). Overall, cases were recruited between 1994 and 2010. The Colon CFR, an international resource for studies on the etiology of CRC described in detail elsewhere,<sup>17</sup> recruited families through 6 administrative centers.<sup>18</sup> The EPICOLON, Ohio, and Helsinki cohorts are population based and represent the core of the comparative analyses of diagnostic strategies for identification of Lynch syndrome (Figure 2). The Colon CFR recruited from both population-based cancer registries and through cancer family and high-risk clinics and used an upper age limit of 75 years (except for the Australian site, which did not recruit participants older than 60 years).<sup>17</sup> Therefore, Colon CFR probands were used only in the analysis of variables associated with the presence of germline MMR gene mutations and not in ascertaining the performance characteristics of selected strategies for Lynch syndrome identification.

Exclusion criteria were polyposis syndromes and personal history of inflammatory bowel disease. Written informed consent was obtained from all study participants, and the study protocol was approved at each participating center.

Personal, tumor-related, and familial characteristics of probands were pooled from each series. Tumor MSI testing and immunostaining for the 4 MMR proteins were performed as previously described.<sup>4,8,9,15–17,19</sup> MSI testing was done at each center using different panels of microsatellite markers, and patients were classified as MSI high or MSI-low/microsatellite stable according to previously described criteria.<sup>20</sup> Overall, tumors were deemed MSI-high if instability was seen at 30% or more markers or instability was present at monomorphic mononucleotide markers. Tumors were considered MMR deficient if they were MSI high, exhibited loss of MMR protein expression, or both.

Germline MMR gene testing was performed by both multiple ligation probe amplification analysis and direct sequencing at each participating center. Whereas *MSH2*, *MLH1*, and *MSH6* genes were evaluated in all cohorts, evaluation of the *PMS2* gene was not included in the study design of the cohorts of EPICOLON, Helsinki, and the University of Southern California Consortium (part of the Colon CFR). Deletions, insertions, duplications, nonsense, and frame shift mutations were considered deleterious; missense mutations were considered deleterious based on published data and existing mutation databases. Tumor MMR status was not used to classify any variant of unknown significance. Germline MMR mutational analysis was usually driven by demonstration of tumor MMR deficiency, although in a subset of 187 patients (1.8%), direct germline MMR gene testing was performed without assessment of MMR status (Figure 1). These patients were used in the analysis of variables associated with presence of MMR gene mutation but not in ascertaining the performance characteristics of selected strategies for Lynch syndrome identification. Similarly, in a subset of 1395 Colon CFR probands, germline gene testing was done although they had an MMR-proficient tumor. On the other hand, germline MMR gene testing was not performed in 318 patients (3.1%) in spite of having an MMR deficient tumor (Figure 1), and consequently, they were excluded from all analyses.

## Statistical Analysis

The focus of the analysis was to establish, primarily, the most sensitive strategy and, secondarily, the most efficient one for identification of MMR gene mutation carriers among CRC patients. The presence of a germline mutation was considered the gold standard. Efficiency was defined as the capacity to detect a germline mutation with the minimum amount of diagnostic resources (ie, tumor MMR testing and germline MMR gene analysis).

Age at diagnosis was treated as a continuous variable. In probands diagnosed with the same type of cancer more than once, the age at diagnosis of cancer at which they were first identified as having Lynch syndrome was considered. In relatives diagnosed with the same type of cancer more than once, the age at diagnosis was defined as the earliest one. The number of relatives with CRC or other Lynch syndrome–related tumors were also treated as continuous variables. All other evaluated variables were considered dichotomous.

Logistic regression analysis was performed, adjusted by age, sex, and participating center, to identify individual variables associated with the presence of germline MMR gene mutations. Multivariate models based on regression tree analysis were explored to establish the most discriminative combination of variables to identify MMR gene mutation carriers. Recursive partitioning programs build classification or regression models of a very general structure using a 2-stage procedure; the resulting models can be represented as binary trees. Because the proportion of carriers was low, a high cost for misclassification was used to prime

sensitivity over specificity. These analyses were limited to probands with information on the mutational status of MMR genes. Results were expressed as odds ratios (ORs) with 95% CI.

Performance characteristics (ie, sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratio, diagnostic yield, and false-positive yield) of selected strategies for Lynch syndrome identification were calculated with respect to the presence of germline MMR gene mutations in the population-based cohorts. Selected strategies included germline testing of probands with an MMR deficient lesion (1) after tumor testing of any CRC (ie, universal screening strategy); (2) after tumor testing of patients fulfilling the revised Bethesda guidelines<sup>7</sup>; (3) after tumor testing of patients fulfilling the Jerusalem recommendations<sup>10</sup>; or (4) after tumor testing of patients fulfilling the model derived from the multivariate analysis. These analyses were performed overall (ie, mutation in any MMR gene) and for each specific MMR gene. Comparison among strategies was made using the Matthews correlation coefficient and its 95% CI, which appropriately weights sensitivity and specificity values, as a measure of the quality of binary classifications.<sup>21</sup>

All calculations were performed with SPSS version 18.0 (SPSS) and R package rpart version 3.1–50.<sup>22</sup> All tests were 2-sided, and a *P* value of less than .05 was considered statistically significant.

## RESULTS

A total of 13 151 unrelated CRC probands from the 4 cohorts were included (Figure 1). Of these, 2945 cases were excluded due to lack of reliable information on tumor MMR or germline MMR mutational status. Therefore, 10 206 informative, unrelated CRC probands constituted the basis of this pooled-data analysis. Demographic, clinical, and tumor-related characteristics of the patients are summarized in Table 1.

Tumor MMR testing was performed in 10 019 probands (98.1%), whereas in 187 patients (1.8%), germline MMR gene analysis was done without previous tumor MMR testing (Figure 1). The number of cases that were tested by MSI only was 2150; by immunostaining only, 2278; and by both MSI and immunostaining, 5591. In this latter group, concordance between MSI and immunostaining was 97.5% (94 cases [1.7%] showed MSI with retained protein expression and 49 [0.8%] exhibited loss of expression with microsatellite stability). A total of 1386 cases (13.8%) exhibited tumor MMR deficiency. Germline MMR mutational analysis was completed in 2650 probands and identified 312 gene mutation carriers in *MSH2* (n=129), *MLH1* (n = 114), *MSH6* (n = 40), or *PMS2* (n=29), representing 3.1% of the whole series (individual data available from the authors on request).

Among the 312 probands diagnosed with Lynch syndrome, mean (SD) age at CRC diagnosis was 48.1 (2.9) years; 131 (42.5%) had 1 or more first-degree relatives with CRC; 41 (14.0%) and 85 (27.2%) fulfilled Amsterdam I and II criteria, respectively; and 214 (68.6%) fulfilled at least 1 criterion of the revised Bethesda guidelines (eTable 1, available at <http://www.jama.com>). Moreover, 289 probands (92.6%) exhibited tumor MMR deficiency, whereas 12 (3.8%) (ie, 5 *MLH1*, 3 *MSH2*, 3 *MSH6*, and 1 *PMS2* gene carriers) showed MMR proficiency. Of those, 5 cases had a tumor retaining protein expression (MSI analysis not performed), 4 cases exhibited microsatellite stability (immunostaining not performed), and 3 cases retained protein expression and showed microsatellite stability. In the remaining 11 probands (3.5%), tumor MMR testing was not performed (eTable 1).

## MMR Gene Mutation Carriers

To identify variables associated with Lynch syndrome, a bivariate analysis was performed in those probands with information regarding germline MMR mutational status (n=2650) (Table 2). This analysis identified CRC diagnosed at age 70 years or younger (OR, 4.0; 95% CI, 2.2–7.1) and fulfillment of at least 1 criterion of the revised Bethesda guidelines (OR, 7.3; 95% CI, 4.6–11.0) as the variables with the highest sensitivity (94.2% and 88.1%, respectively) and negative predictive value (97.0% and 97.3%, respectively). All other evaluated characteristics showed sensitivities lower than 70% and negative predictive values lower than 95% (Table 2). Distribution of germline MMR gene mutations according to the age at CRC diagnosis is shown in eTable 2.

In the multivariate analysis, based on regression trees, the highest discrimination was achieved when MMR testing was done for probands with any of the following characteristics: CRC diagnosed at 60 years or younger, presence of at least 1 first-degree relative with CRC diagnosed at 50 years or younger, or personal history of metachronous Lynch syndrome–related tumors diagnosed at 50 years or younger (OR, 11.3; 95% CI, 6.70–19.0). The sensitivity of this model was 90.1%, with a negative predictive value of 97.5%.

## Performance of Selected Strategies

Strategies based on tumor MMR testing of probands fulfilling at least 1 criterion of the revised Bethesda guidelines, Jerusalem recommendations, the model resulting from the most sensitive variables in the bivariate analysis (ie, CRC diagnosis at 70 years and fulfillment of at least 1 criterion of the revised Bethesda guidelines, henceforth “selective strategy”), or the model resulting from the multivariate analysis, followed by germline MMR testing of individuals with an MMR-deficient tumor, were compared with the universal screening approach in which tumor MMR testing was performed in all CRC patients (Table 3 and Table 4). As expected, only the universal screening strategy achieved 100% sensitivity (95% CI, 99.3%–100%) and negative predictive value (95% CI, 99.9%–100%) in the identification of patients with Lynch syndrome, when the analysis was limited to population-based cohorts (n=3671) (Figure 2).

Universal tumor testing (sensitivity, 100%; 95% CI, 99.3%–100%; specificity, 93.0%; 95% CI, 92.0%–93.7%; diagnostic yield, 2.2%; 95% CI, 1.7%–2.7%) was superior to the selective strategy (sensitivity, 95.1%; 95% CI, 89.8%–99.0%; specificity, 95.5%; 95% CI, 94.7%–96.1%; diagnostic yield, 2.1%; 95% CI, 1.6%–2.6%; Matthews correlation coefficient, 0.54;  $P < .001$ ), Bethesda guidelines (sensitivity, 87.8%; 95% CI, 78.9%–93.2%; specificity, 97.5%; 95% CI, 96.9%–98.0%; diagnostic yield, 2.0%; 95% CI, 1.5%–2.4%; Matthews correlation coefficient, 0.61;  $P < .001$ ), and Jerusalem recommendations (sensitivity, 85.4%; 95% CI, 77.1%–93.6%; specificity, 96.7%; 95% CI, 96.0%–97.2%; diagnostic yield, 1.9%; 95% CI, 1.4%–2.3%; Matthews correlation coefficient, 0.55;  $P < .001$ ) (Table 3 and Table 4). However, differences in diagnostic yield from the universal approach were small, with a difference between universal screening and the next less intensive strategy (ie, selective strategy) of only 0.11% (Table 3 and Table 4) and accompanied by an increase in false-positive yield of 2.5%. Indeed, the selective strategy resulted in a 34.8% fewer CRC patients requiring tumor MMR testing and an additional 28.6% fewer cases undergoing germline MMR mutational analysis in comparison with universal screening (Table 3 and Table 4). All these results were similar to those obtained in the overall series (eTable 3).

When the analysis was conducted for each specific MMR gene, the selective strategy resulted in identical sensitivity and negative predictive value to those achieved with the universal tumor MMR testing approach but only for the identification of *MLH1* and *MSH2*

gene carriers, in a similar manner as the fulfillment of Bethesda guidelines for the identification of *MLH1* gene carriers (Table 5). Again, these results were similar to those obtained in the whole series (eTable 4).

## COMMENT

Results of this international, multicenter, pooled-data analysis demonstrate that unless a universal screening approach consisting of tumor MMR testing in all CRC patients is performed, a clinically meaningful proportion of MMR gene mutation carriers will remain undiagnosed. Specifically, use of the revised Bethesda guidelines will miss approximately 12%, use of the Jerusalem recommendations will miss approximately 15%, and use of a selective criteria (performing tumor MMR testing of CRC probands diagnosed at 70 years or younger or fulfilling 1 criterion of the revised Bethesda guidelines) will miss approximately 5%. Conversely, the specificity for these strategies ranged from 93.0% for the universal tumor MMR testing approach to 97.5% for the Bethesda guidelines. These data may be useful to more empirically inform discussions on the most efficient approaches for the identification of Lynch syndrome among CRC probands.

This study has several strengths. First, this is the largest series published so far in which fully characterized CRC patients were evaluated to ascertain the most effective and efficient strategy for the identification of Lynch syndrome, using personal and family history, tumor MMR testing, and germline MMR mutational data. This comprehensive approach overcomes previous attempts—Amsterdam criteria,<sup>6,23</sup> Bethesda guidelines,<sup>7</sup> and Jerusalem recommendations<sup>10</sup>—in which strategies were not empirical or were based on expert consensus. Second, this analysis was based on population-based cohorts,<sup>4,8,9,15,16</sup> its results being applicable to an unremarkable newly diagnosed CRC patient rather than in the subset of individuals usually referred to genetic counseling because of a high suspicion of an inherited disorder. Third, the methodological approach, which included an exploratory analysis of the most discriminative variables associated with presence of germline MMR mutations and evaluation of the performance characteristics of comprehensive strategies, allowed us not only to establish their accuracy for the identification of Lynch syndrome, but also to estimate the molecular resources needed.

We are aware of some limitations of the study. First, the results of this investigation have not been replicated in an independent set of CRC patients because the prevalence of Lynch syndrome is relatively low and, accordingly, it is difficult to find 2 database sets adequate for such analyses. Second, all probands were diagnosed with CRC, thus precluding our ability to extrapolate our results to patients presenting with other Lynch syndrome–related tumors. Nevertheless, CRC represents the most prevalent neoplasm in such patients, and in fact, it is the most common “red flag” to drive the subsequent molecular confirmation.<sup>24,25</sup> Third, germline MMR mutational analysis was not performed in all probands, although it was done in the vast majority of patients with MMR-deficient tumors and also in a notable proportion of those with proficient lesions. In that sense, it is important to note that 12 mutation carriers had an MMR-proficient neoplasm, thus indicating that a reduced number of patients with Lynch syndrome will remain undiagnosed if screening relies on MMR tumor testing. To overcome this limitation, sequencing all genes of concern in all CRC patients would represent the most sensitive approach. When high-throughput technology becomes more affordable, cost-effectiveness analysis of this approach will be warranted.

Fourth, no information was available regarding either tumor *BRAF* V600E mutation or tumor *MLH1* gene promoter methylation. Both molecular techniques are helpful in excluding epigenetically driven inactivation of the *MLH1* gene among patients with *MLH1*-deficient tumors.<sup>26</sup> This fact may explain the lower specificity of all evaluated strategies for

the identification of *MLH1* gene carriers with respect to the other 3 MMR genes, but it does not affect their sensitivity, which is the main goal of our analysis. Finally, in contrast to the other 3 MMR genes, the *PMS2* gene was not systematically analyzed in all evaluated cohorts. This limitation, however, has been addressed by analyzing the results separately for each specific gene.

Our analysis demonstrates that, although the revised Bethesda guidelines have been considered as the mainstay for selecting patients to undergo tumor MMR testing so far,<sup>7,8,27,28</sup> they have a low sensitivity for the identification of Lynch syndrome. The lack of sensitivity is mainly due to its poor performance in identifying *MSH6* gene carriers<sup>29</sup> and, to less extent, *PMS2* and *MSH2* gene carriers. On the other hand, the use of age at CRC diagnoses as a criterion to select patients requiring tumor MMR testing, as was suggested in the Lynch syndrome conference held in Jerusalem,<sup>10</sup> is also limited by a low sensitivity, because 15% of patients were older than 70 years at the diagnosis of Lynch syndrome.

Universal tumor screening has, as expected, the highest sensitivity. Although it is not sufficient to just consider sensitivity when comparing different strategies, this is the most important parameter clinically (ie, to minimize the number of patients with undiagnosed Lynch syndrome). Indeed, it is accepted that the whole Lynch syndrome screening process is cost-effective when the benefits to immediate relatives of identified patients are considered<sup>30</sup>; accordingly, the more patients who are diagnosed, the more atrisk relatives can undergo genetic evaluation and receive appropriate cancer surveillance and other preventive interventions.

On the other hand, any policy recommendation needs to consider the economic and psychosocial harms of false-positive results obtained in each strategy. It is notable that the selective strategy of performing tumor MMR testing of CRC probands diagnosed at 70 years or younger, and in older probands fulfilling at least 1 criterion of the revised Bethesda guidelines, achieved a similar diagnostic yield to the universal strategy, while reducing by about 35% and 30% the number of patients requiring tumor and germline MMR testing, respectively. Therefore, if resources are limited, this selective strategy may represent an alternative approach to universal tumor screening for the identification of Lynch syndrome, although it remains to be demonstrated that this strategy can be implemented consistently in a clinical setting. Whereas recent data suggest that universal tumor testing may yield substantial benefits at acceptable costs,<sup>31</sup> further studies assessing cost-effectiveness of those strategies evaluated in this study are still needed.

In addition to the pragmatic approach proposed in this study, a more precise characterization of probands exhibiting *MLH1*-deficient tumors is needed. Because tumor MMR deficiency in the vast majority of such patients is due to epigenetic *MLH1* inactivation, performance of tumor *BRAF* V600E mutation analysis,<sup>32,33</sup> or even better, methylation analysis of *MLH1* gene promoter,<sup>34,35</sup> may contribute to increasing the specificity of this strategy for the identification of *MLH1* gene carriers and consequently to further decreasing the cost associated with germline testing.

The strategies evaluated in this study rely heavily on tumor testing. However, they should not be in conflict with available mathematical algorithms to predict MMR gene mutation carriers based on personal and family history.<sup>11–14,36–38</sup> Indeed, both approaches must be viewed as complementary because it is not always feasible to obtain tumor tissue.<sup>36,39</sup> More importantly, these models may also encompass individuals affected by other non-CRC Lynch syndrome–related tumors.<sup>11–14,36–38</sup> In addition, it would be interesting to explore whether the use of predictive algorithms may contribute to identifying gene mutation carriers among patients with MMR-proficient tumors.



Finally, it is important to note that significant differences were observed among the 3 population-based cohorts evaluated in this study. Indeed, in the EPICOLON cohort, the prevalence of Lynch syndrome, as well as of tumor MMR deficiency, was roughly half that observed in the Ohio and Helsinki series. This finding, rather than being considered as a drawback of our analysis, should be regarded as an opportunity to generalize its results broadly. The geographical variation in Lynch syndrome genotypic and phenotypic characteristics<sup>4,8,9,15–17</sup> may reflect some specific gene-environment interactions and therefore deserves further investigation.

In conclusion, identification of patients with Lynch syndrome is critical to drive presymptomatic diagnosis of relatives at risk, as well as subsequent preventive measures for decreasing morbidity and mortality. Universal tumor MMR testing followed by germline testing offers the highest sensitivity and a somewhat lower specificity than alternative screening strategies for this purpose, although the increase in the diagnostic yield is modest. The empirical data from this large multinational study may help inform clinical recommendations for individuals diagnosed with CRC.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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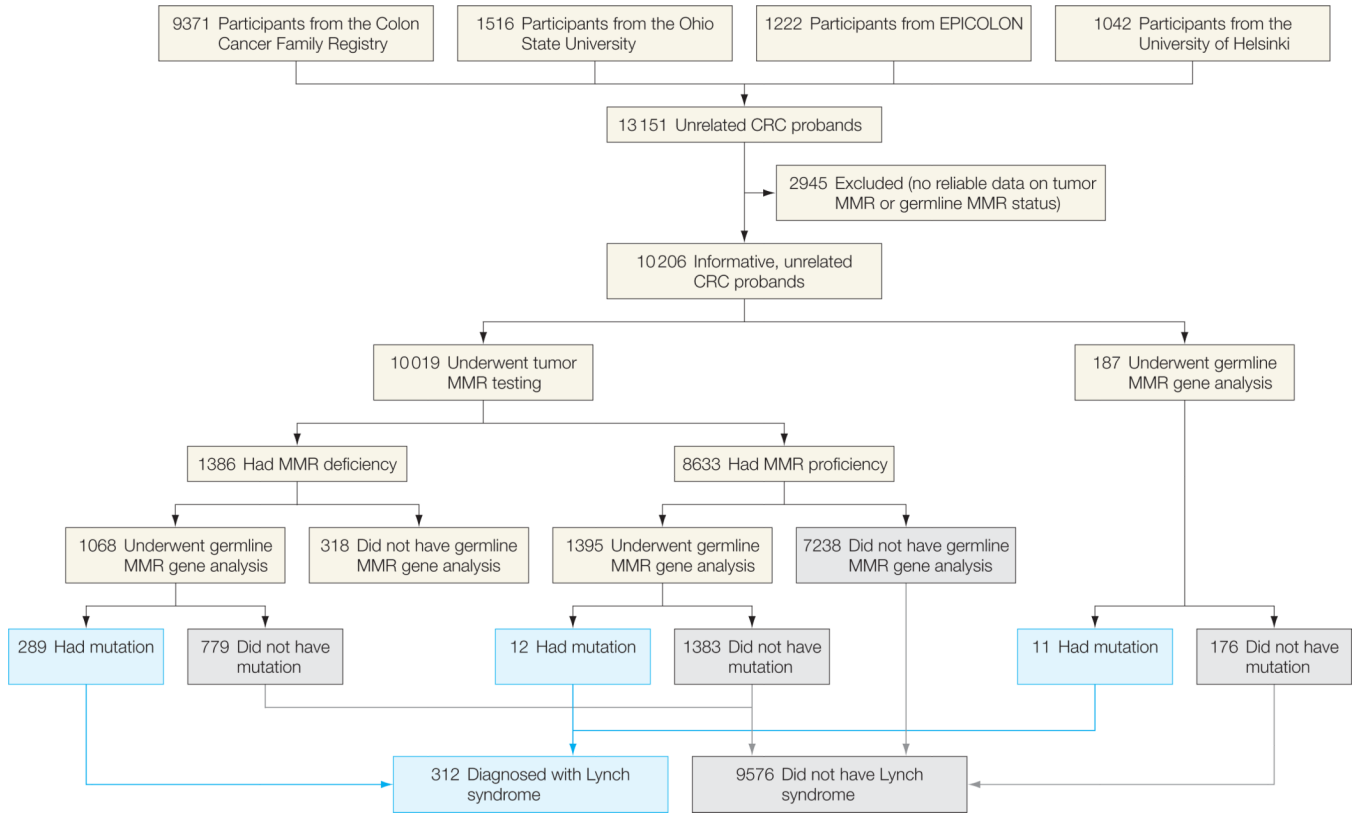
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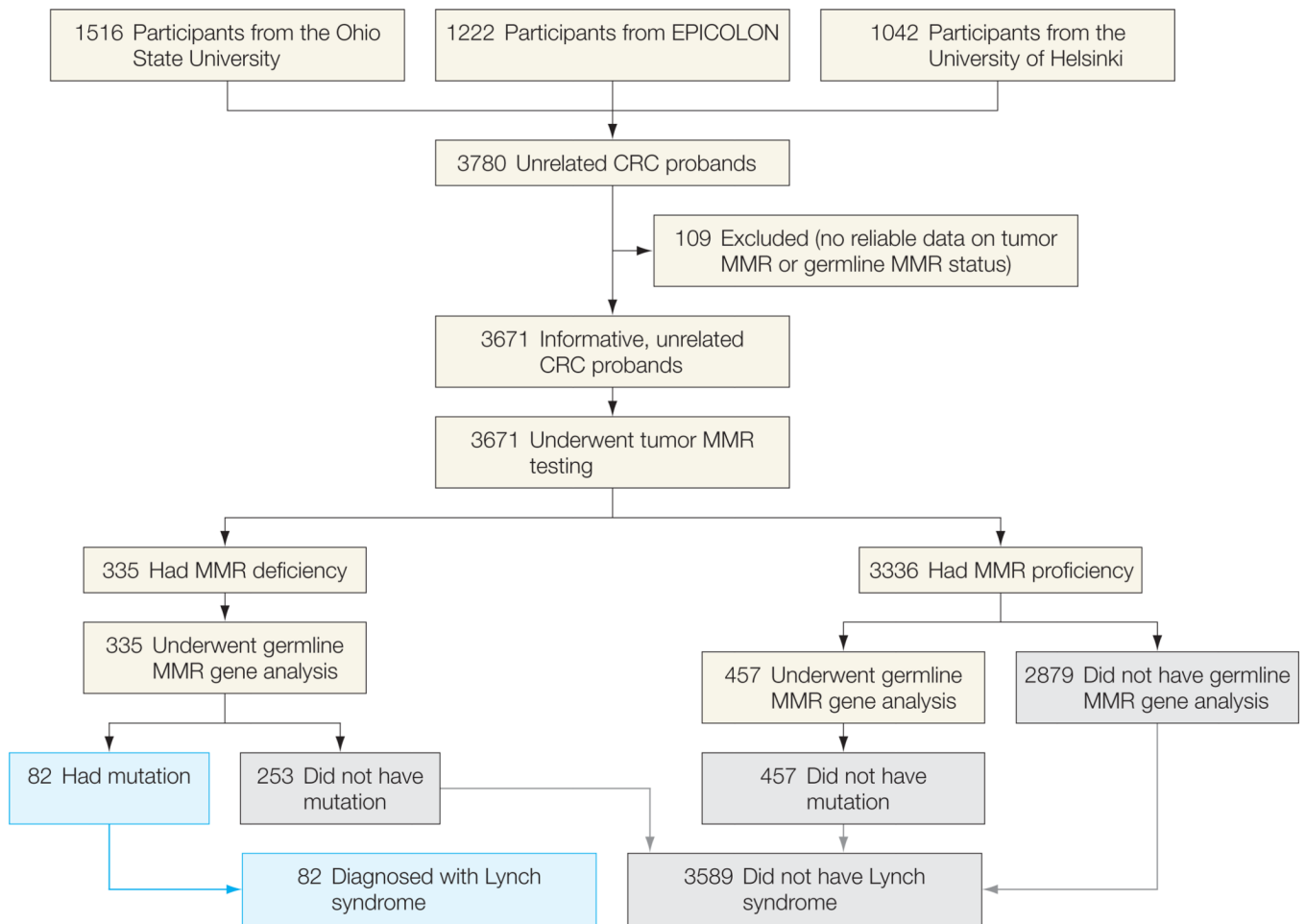
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**Figure 1.** Flowchart of the Study for the Overall Series  
A patient is assumed to not have Lynch syndrome if the tumor is mismatch repair (MMR) proficient; germline MMR gene analysis was not performed for most of these individuals. CRC indicates colorectal cancer.



**Figure 2.** Flowchart of the Study for the Population-Based Cohorts  
 A patient is assumed to not have Lynch syndrome if the tumor is mismatch repair (MMR) proficient; germline MMR gene analysis was not performed for most of these individuals. CRC indicates colorectal cancer.

**Table 1**  
 Characteristics of Probands From Each Colorectal Cancer Cohort Included in the Pooled-Data Analysis

Characteristics	No. (%)				Total
	Colon CFR <sup>17</sup>	OSU <sup>14,9</sup>	EPICOLON <sup>8</sup>	University of Helsinki <sup>15,16</sup>	
Probands included, No.	6535	1516	1222	933	10,206
Age, mean (SD), y	53.7 (11.4)	63.2 (13.0)	69.9 (11.3)	67.4 (11.8)	59.1 (13.4)
Male sex	3305 (50.5)	830 (54.9)	731 (59.8)	528 (56.6)	5394 (52.8)
First-degree relatives with CRC	1105 (16.9)	331 (21.9)	156 (12.8)	142 (15.2)	1736 (17.0)
Revised Bethesda guidelines	3056 (46.8)	561 (37.1)	287 (23.5)	156 (16.7)	4060 (39.8)
Amsterdam criteria					
I	85 (1.3)	15 (1.0)	18 (1.5)	2 (0.2)	120 (1.2)
II	156 (2.4)	35 (2.3)	22 (1.8)	31 (3.3)	246 (2.5)
Tumor MMR deficiency <sup>a</sup>	948 (14.5)	214 (14.2)	90 (7.4)	133 (14.2)	1386 (13.8)
Germline MMR gene mutation <sup>b</sup>	227 (3.5)	44 (2.9)	11 (0.9)	30 (3.2)	312 (3.1)

Abbreviations: CFR, Cancer Family Registry; CRC, colorectal cancer; MMR, mismatch repair; OSU, Ohio State University.

<sup>a</sup>Tumor MMR testing was performed in 10 019 patients. MMR deficiency was defined as loss of MMR protein expression (*MLH1*, *MSH2*, *MSH6*, and/or *PMS2*) and/or microsatellite instability-high.

<sup>b</sup>Germline MMR gene mutational analysis was done in 2650 probands.

**Table 2**  
Analyses of Variables Associated With Presence of a Germline Mismatch Repair Gene Mutation (Bivariate Analysis)<sup>a</sup>

Variable	Evaluable Probands, No. <sup>b</sup>	MMR Gene Carriers Fulfilling the Condition, No./Total No. <sup>c</sup>	% (95% CI)				
			OR (95% CI) <sup>d</sup>	Sensitivity	Specificity	NPV	
Proximal CRC <sup>e</sup>	1644	130/208	2.5 (1.7–3.7)	62.3 (55.6–69.3)	51.4 (48.7–53.9)	15.6 (13.1–18.2)	90.4 (88.3–92.5)
Mucinous CRC	1492	52/201	1.7 (1.2–2.4)	25.9 (19.5–32.1)	84.0 (82.0–86.0)	20.2 (15.0–25.2)	87.9 (86.0–89.7)
Poorly differentiated CRC	745	24/104	1.3 (0.8–2.1)	23.1 (14.5–31.6)	81.1 (78.0–84.2)	16.6 (10.1–22.9)	86.7 (83.8–89.4)
Crohn-like lymphocytic reaction	300	27/55	2.0 (1.1–3.7)	49.1 (34.9–63.2)	67.8 (61.7–73.8)	25.5 (16.7–34.2)	85.6 (80.3–90.7)
Tumor infiltrating lymphocytes	367	44/64	3.2 (1.7–5.9)	68.8 (56.6–80.8)	61.7 (56.0–67.3)	27.5 (20.2–34.7)	90.3 (86.0–94.6)
Synchronous or metachronous CRC	2160	50/244	5.0 (3.3–7.8)	20.7 (15.4–26.0)	94.3 (93.2–95.3)	31.4 (23.9–38.9)	90.5 (89.1–91.7)
Metachronous Lynch syndrome–related tumor <sup>f</sup>	2650	53/311	5.1 (3.4–7.7)	17.0 (12.7–21.3)	95.7 (94.8–96.5)	34.6 (26.7–42.5)	89.6 (88.4–90.8)
CRC excluded	2650	21/311	4.7 (2.6–8.4)	6.7 (3.8–9.7)	97.6 (97.0–98.2)	27.6 (16.9–38.3)	88.7 (87.4–89.9)
Diagnosed 50 y	2649	36/310	6.2 (3.7–10.4)	11.6 (7.8–15.3)	98.4 (97.8–98.9)	48.6 (36.5–60.7)	89.3 (88.1–90.5)
CRC excluded (diagnosed 50 y)	2650	12/311	4.2 (1.9–9.2)	3.8 (1.5–6.1)	99.1 (98.7–99.5)	37.5 (19.1–55.8)	88.5 (87.5–89.8)
FDR with CRC							
1	2644	131/308	3.0 (2.3–4.1)	42.5 (37.0–48.4)	81.9 (80.3–83.5)	23.7 (20.1–27.4)	91.5 (90.3–92.7)
2	2644	56/306	5.0 (3.3–7.6)	18.3 (13.8–22.8)	96.0 (95.1–96.8)	37.3 (29.2–45.4)	90.0 (88.7–91.1)
FDR with CRC diagnosed 50 y							
1	2610	79/289	8.0 (5.5–11.8)	27.3 (22.2–32.9)	96.3 (95.4–97.0)	47.6 (40.0–55.7)	91.5 (90.2–92.5)
2	2633	22/297	11.9 (5.4–26.0)	7.4 (4.2–10.5)	99.5 (99.1–99.8)	64.7 (47.1–82.2)	89.4 (88.2–90.6)
FDR with Lynch syndrome–related tumor <sup>f</sup>							
1	2640	154/303	2.8 (2.1–3.7)	50.8 (45.0–56.6)	74.0 (72.1–75.7)	20.2 (17.2–23.1)	92.1 (90.8–93.3)
2	2648	88/310	4.5 (3.1–6.4)	28.4 (23.1–33.5)	93.2 (92.1–94.2)	35.5 (29.3–41.6)	90.8 (89.5–91.9)
FDR with Lynch syndrome–related tumor diagnosed 50y <sup>f</sup>							
1	2589	95/281	5.7 (4.1–8.0)	33.8 (28.1–39.5)	92.9 (91.8–94.0)	36.8 (30.7–42.9)	92.0 (90.9–93.1)
2	2619	32/283	12.4 (6.6–23.0)	11.3 (7.9–15.8)	99.1 (98.7–99.5)	61.5 (49.1–76.7)	90.3 (89.0–91.3)

Variable	Evaluable Probands, No. <sup>b</sup>	MMR Gene Carriers Fulfilling the Condition, No./Total No. <sup>c</sup>	% (95% CI)				
			OR (95% CI) <sup>d</sup>	Sensitivity	Specificity	NPV	
CRC diagnosed 70 y (Jerusalem recommendations)	2112	226/240	4.0 (2.2–7.1)	94.2 (90.4–97.0)	26.3 (24.4–28.3)	13.4 (11.7–15.0)	97.0 (94.7–98.3)
Fulfillment of Amsterdam criteria							
I	2627	41/291	9.6 (5.7–16.2)	13.7 (9.5–17.7)	98.6 (98.1–99.1)	55.6 (43.3–67.7)	90.2 (88.9–91.2)
II	2650	85/312	11.4 (7.3–17.7)	27.2 (22.1–32.3)	97.9 (97.3–98.5)	63.4 (54.9–71.9)	91.0 (89.7–92.0)
Fulfillment of 1 criterion of revised Bethesda guidelines	2128	214/243	7.3 (4.6–11.0)	88.1 (83.7–92.3)	54.4 (52.1–56.6)	19.9 (17.4–22.3)	97.3 (96.2–98.2)

Abbreviations: CRC, colorectal cancer; FDR, first-degree relative; MMR, mismatch repair; NPV, negative predictive value; OR, odds ratio; PPV, positive predictive value.

<sup>a</sup>This analysis was limited to patients with information on the germline mutational status of MMR genes and without considering the result of tumor MMR testing.

<sup>b</sup>Probands in whom the corresponding variable could be assessed.

<sup>c</sup>MMR gene carriers fulfilling the condition with respect to those MMR gene carriers in whom the corresponding variable could be evaluated.

<sup>d</sup>Adjusted by age, sex, and participating center.

<sup>e</sup>With respect to the splenic flexure.

<sup>f</sup>Lynch syndrome–related tumors: colorectal, endometrial, ovarian, gastric, hepatobiliary, small bowel, urinary tract, pancreatic, and brain cancer.



**Table 3**  
Performance Characteristics of Selected Strategies for the Identification of Patients With Lynch Syndrome<sup>a</sup>

Tumor MMR Testing	Germline MMR Gene Mutation											
	No. (%)		Sensitivity		Specificity		Positive Predictive Value		Negative Predictive Value			
	Probands Requiring Tumor MMR Testing <sup>b</sup>	Probands Requiring Germline MMR Gene Analysis <sup>c</sup>	No./Total No	% (95% CI)	No./Total No	% (95% CI)	No./Total No	% (95% CI)	No./Total No	% (95% CI)	No./Total No	% (95% CI)
CRC patients fulfilling a condition Multivariate model <sup>d</sup>	1158 (31.5)	128 (3.5)	67/82	81.7 (72.7–90.6)	3528/3589	98.3 (97.8–98.7)	67/128	52.3 (43.3–61.3)	3528/3543	99.6 (99.3–99.8)		
Jerusalem recommendations <sup>e</sup>	2125 (57.8)	189 (5.1)	70/82	85.4 (77.1–93.6)	3470/3589	96.7 (96.0–97.2)	70/189	37.0 (29.9–44.2)	3470/3482	99.7 (99.4–99.8)		
1 Criterion of revised Bethesda guidelines	992 (27.0)	162 (4.4)	72/82	87.8 (78.9–93.2)	3499/3589	97.5 (96.9–98.0)	72/162	44.4 (36.4–52.4)	3499/3509	99.7 (99.5–99.9)		
Jerusalem recommendations <sup>e</sup> or 1 criterion of revised Bethesda guidelines	2394 (65.2)	239 (6.5)	78/82	95.1 (89.8–99.0)	3428/3589	95.5 (94.7–96.1)	78/239	32.6 (26.4–38.7)	3428/3432	99.9 (99.7–100)		
Any CRC patient (universal strategy)	3671 (100)	335 (9.1)	82/82	100 (99.3–100)	3336/3589	93.0 (92.0–93.7)	82/335	24.5 (19.7–29.2)	3336/3336	100 (99.9–100)		

Abbreviations: CRC, colorectal cancer; MMR, mismatch repair.

<sup>a</sup>This analysis was limited to population-based cohorts (n=3671 probands).

<sup>b</sup>Probands requiring tumor MMR testing in each strategy, with respect to those in whom it could be assessed.

<sup>c</sup>Probands requiring germline MMR gene analysis because of the demonstration of tumor MMR deficiency in each strategy, with respect to those in whom it could be assessed.

<sup>d</sup>Defined as fulfillment of 1 of the following characteristics: CRC diagnosed at 60 years, 1 first-degree relative with CRC diagnosed at 50 years, or personal history of metachronous Lynch syndrome-related tumors diagnosed at 50 years.

<sup>e</sup>Age at CRC diagnosis 70 years.

**Table 4**  
 Diagnostic and False-Positive Yields of Selected Strategies for the Identification of Patients With Lynch Syndrome<sup>a</sup>

	Germline MMR Gene Mutation					
	Diagnostic Yield <sup>b</sup>		P Value <sup>c</sup>	Incremental Diagnostic Yield, % <sup>d</sup>	False-Positive Yield <sup>e</sup>	
	No./Total No.	% (95% CI)			No./Total No.	% (95% CI)
<b>Tumor MMR Testing</b>						
CRC patients fulfilling a condition	67/3671	1.8 (1.3–2.2)	<.001		61/3671	1.7 (1.2–2.1)
Multivariate model <sup>f</sup>						
Jerusalem recommendations <sup>g</sup>	70/3671	1.9 (1.4–2.3)	<.001	0.08	119/3671	3.2 (2.6–3.8)
1 Criterion of revised Bethesda guidelines	72/3671	2.0 (1.5–2.4)	<.001	0.05	90/3671	2.5 (2.0–2.9)
Jerusalem recommendations <sup>g</sup> or 1 criterion of revised Bethesda guidelines	78/3671	2.1 (1.6–2.6)	<.001	0.16	161/3671	4.4 (3.7–5.0)
Any CRC patient (universal strategy)	82/3671	2.2 (1.7–2.7)	[Reference]	0.11	253/3671	6.9 (6.0–7.7)

Abbreviations: CRC, colorectal cancer; MMR, mismatch repair.

<sup>a</sup>This analysis was limited to population-based cohorts (n=3671 probands).

<sup>b</sup>Diagnostic yield refers to probands requiring germline MMR gene analysis in whom a mutation was found.

<sup>c</sup>Matthews correlation coefficient comparison of diagnostic yield with respect to the universal strategy.

<sup>d</sup>Compared with the next least intensive strategy.

<sup>e</sup>False-positive yield refers to probands requiring germline MMR gene analysis in whom no mutation was found.

<sup>f</sup>Defined as fulfillment of 1 of the following characteristics: CRC diagnosed at 60 years, 1 first-degree relative with CRC diagnosed at 50 years, or personal history of metachronous Lynch syndrome-related tumors diagnosed at 50 years.

<sup>g</sup>Age at CRC diagnosis 70 years.

**Table 5**  
 Performance Characteristics of Selected Strategies for the Identification of Patients With Lynch Syndrome, According to the Mismatch Repair Gene Mutated<sup>d</sup>

Tumor MMR Testing	Germline MMR Gene Mutation											
	Sensitivity			Specificity			Positive Predictive Value			Negative Predictive Value		
No./Total No.	% (95% CI)	No./Total No.	% (95% CI)	No./Total No.	% (95% CI)	No./Total No.	% (95% CI)	No./Total No.	% (95% CI)	No./Total No.	% (95% CI)	
<b>MLH1 (n = 3589)<sup>c</sup></b> CRC patients fulfilling a condition 1 Criterion of revised Bethesda guidelines	34/34	100 (98.5–100)	3481/3555	97.9 (97.4–98.4)	34/108	31.5 (22.2–40.7)	3481/3481	100 (99.9–100)				<.001
Jerusalem recommendations <sup>d</sup>	32/34	94.1 (84.7–100)	3464/3555	97.4 (96.9–97.9)	32/123	26.0 (17.8–34.1)	3464/3466	99.9 (99.8–100)				<.001
Jerusalem recommendations <sup>d</sup> or 1 criterion of revised Bethesda guidelines	34/34	100 (98.5–100)	3424/3555	96.3 (95.6–96.8)	34/165	20.6 (14.1–27.0)	3424/3424	100 (99.9–100)				<.001
Multivariate model <sup>e</sup>	28/34	82.4 (68–96.6)	3513/3555	98.8 (98.4–99.1)	28/70	40.0 (27.8–52.1)	3513/3519	99.8 (99.6–99.9)				<.001
Any CRC patient (universal strategy)	34/34	100 (98.5–100)	3336/3555	93.8 (93.0–94.6)	34/253	13.4 (9.0–17.8)	3336/3336	100 (99.9–100)				[Reference]
<b>MSH2 (n = 3422)<sup>c</sup></b> CRC patients fulfilling a condition 1 Criterion of revised Bethesda guidelines	31/33	93.9 (84.2–100)	3366/3389	99.3 (99.0–99.6)	31/54	57.4 (43.2–71.5)	3366/3368	99.9 (99.8–100)				<.001
Jerusalem recommendations <sup>d</sup>	29/33	87.9 (75.2–100)	3361/3389	99.2 (98.8–99.4)	29/57	50.9 (37.0–64.7)	3361/3365	99.9 (99.7–100)				<.001
Jerusalem recommendations <sup>d</sup> or 1 criterion of revised Bethesda guidelines	33/33	100 (98.4–100)	3353/3389	98.9 (98.5–99.3)	33/69	47.8 (35.3–60.3)	3353/3353	100 (99.9–100)				<.001
Multivariate model <sup>e</sup>	31/33	93.9 (84.2–100)	3370/3389	99.4 (99.1–99.6)	31/50	62.0 (47.5–76.4)	3370/3372	99.9 (99.8–100)				<.001
Any CRC patient	33/33	100 (98.4–100)	3336/3389	98.4 (98.0–98.8)	33/86	38.4 (27.5–49.2)	3336/3336	100 (99.9–100)				[Reference]
<b>MSH6 (n = 3391)<sup>c</sup></b> CRC patients fulfilling a condition 1 Criterion of revised Bethesda guidelines	3/9	33.3 (0–69.6)	3362/3382	99.4 (99.1–99.6)	3/23	13.0 (0–28.9)	3362/3368	99.8 (99.6–99.9)				<.001

	Germline MMR Gene Mutation									
	Sensitivity			Specificity			Positive Predictive Value		Negative Predictive Value	
Tumor MMR Testing	No./Total No.	% (95% CI)	No./Total No.	% (95% CI)	No./Total No.	% (95% CI)	No./Total No.	% (95% CI)	No./Total No.	% (95% CI)
Jerusalem recommendations <sup>d</sup>	6/9	66.7 (30.3–100)	3359/3382	99.3 (99.0–99.6)	6/29	20.7 (4.22–37.1)	3359/3362	99.9 (99.7–100)		.11
Jerusalem recommendations <sup>d</sup> or 1 criterion of revised Bethesda guidelines	7/9	77.8 (45.0–100)	3350/3382	99.1 (98.6–99.3)	7/39	17.9 (4.62–31.2)	3350/3352	99.9 (99.8–100)		.13
Multivariate model <sup>e</sup>	5/9	55.6 (17.5–93.5)	3365/3382	99.5 (99.2–99.7)	5/22	22.7 (2.94–42.5)	3365/3369	99.9 (99.7–100)		.02
Any CRC patient	9/9	100 (94.4–100)	3336/3382	98.6 (98.2–99.0)	9/55	16.4 (5.6–27.0)	3336/3336	100 (99.9–100)		[Reference]
<b>PMS2 (n = 3351)<sup>c</sup></b> CRC patients fulfilling a condition 1 criterion of revised Bethesda guidelines	4/6	66.7 (20.6–100)	3342/3345	99.9 (99.7–99.9)	4/7	57.1 (13.3–100)	3342/3344	99.9 (99.8–100)		.30
Jerusalem recommendations <sup>d</sup>	3/6	50.0 (1.6–98.3)	3340/3345	99.9 (99.7–100)	3/8	37.5 (0–77.3)	3340/3343	99.9 (99.7–100)		<.001
Jerusalem recommendations <sup>d</sup> or 1 criterion of revised Bethesda guidelines	4/6	66.7 (20.6–100)	3339/3345	99.8 (99.6–99.9)	4/10	40.0 (4.64–75.3)	3339/3341	99.9 (99.8–100)		<.001
Multivariate model <sup>e</sup>	3/6	50.0 (1.6–98.3)	3341/3345	99.9 (99.7–100)	3/7	42.9 (0–86.6)	3341/3344	99.9 (99.7–100)		<.001
Any CRC patient	6/6	100 (91.6–100)	3336/3345	99.7 (99.5–99.9)	6/15	40.0 (11.8–68.1)	3336/3336	100 (99.9–100)		[Reference]

Abbreviations: CRC, colorectal cancer; MMR, mismatch repair.

<sup>a</sup>This analysis was limited to population-based cohorts.

<sup>b</sup>With respect to universal strategy (Mathews correlation coefficient comparison).

<sup>c</sup>Probands in whom strategies for the identification of germline mutations for each specific MMR gene could be assessed.

<sup>d</sup>Age at CRC diagnosis 70 years.

<sup>e</sup>Defined as fulfillment of 1 of the following characteristics: CRC diagnosed at 60 years, 1 first-degree relative with CRC diagnosed at 50 years, or personal history of metachronous Lynch syndrome-related tumors diagnosed at 50 years.