Development and Validation of the PREMM<sub>5</sub> Model for Comprehensive Risk Assessment of Lynch Syndrome


**Purpose:** Current Lynch syndrome (LS) prediction models quantify the risk to an individual of carrying a pathogenic germline mutation in three mismatch repair (MMR) genes: MLH1, MSH2, and MSH6. We developed a new prediction model, PREMM<sub>5</sub>, that incorporates the genes PMS2 and EPCAM to provide comprehensive LS risk assessment.

**Patients and methods:** PREMM<sub>5</sub> was developed to predict the likelihood of a mutation in any of the LS genes by using polytomous logistic regression analysis of clinical and germline data from 18,734 individuals who were tested for all five genes. Predictors of mutation status included sex, age at genetic testing, and proband and family cancer histories. Discrimination was evaluated by the area under the receiver operating characteristic curve (AUC), and clinical impact was determined by decision curve analysis; comparisons were made to the existing PREMM<sub>1,2,6</sub> model. External validation of PREMM<sub>5</sub> was performed in a clinic-based cohort of 1,058 patients with colorectal cancer.

**Results:** Pathogenic mutations were detected in 1,000 (5%) of 18,734 patients in the development cohort; mutations included MLH1 (n = 306), MSH2 (n = 354), MSH6 (n = 177), PMS2 (n = 141), and EPCAM (n = 22). PREMM<sub>5</sub> distinguished carriers from noncarriers with an AUC of 0.81 (95% CI, 0.79 to 0.82), and performance was similar in the validation cohort (AUC, 0.83; 95% CI, 0.75 to 0.92). Prediction was more difficult for PMS2 mutations (AUC, 0.64; 95% CI, 0.60 to 0.68) than for other genes. Performance characteristics of PREMM<sub>5</sub> exceeded those of PREMM<sub>1,2,6</sub>.

Decision curve analysis supported germline LS testing for PREMM<sub>5</sub> scores ≥ 2.5%.

**Conclusion:** PREMM<sub>5</sub> provides comprehensive risk estimation of all five LS genes and supports LS genetic testing for individuals with scores ≥ 2.5%. At this threshold, PREMM<sub>5</sub> provides performance that is superior to the existing PREMM<sub>1,2,6</sub> model in the identification of carriers of LS, including those with weaker phenotypes and individuals unaffected by cancer.

**Introduction**

Nearly 1 million individuals in the United States have Lynch syndrome (LS) but most are unaware of their diagnosis. LS is caused by germline alterations in DNA mismatch repair (MMR) genes (MLH1, MSH2, MSH6, or PMS2) or EPCAM (which causes epigenetic silencing of MSH2) and confers a 40% to 80% lifetime risk of colorectal cancer (CRC). In addition to CRC, mutation carriers are at increased risk for cancers of the endometrium, ovaries, stomach, small intestine, pancreas, urinary tract, brain, and cutaneous sebaceous glands. Personal and family histories of these component cancers can guide genetic testing to identify mutation carriers. If unidentified, these individuals miss the opportunity to pursue interventions known to effectively reduce the risk of Lynch-associated cancers, such as frequent colonoscopies, prophylactic surgeries, and chemoprevention.

Prediction models, such as PREMM<sub>1,2,6</sub>, are evidence-based tools that can help identify carriers of LS by using the personal and family history of Lynch-associated cancers.
Our aim was to develop and validate a new prediction model to quantify the risk of an individual carrying a pathogenic mutation in any of the five genes associated with LS (MLH1, MSH2, MSH6, PMS2, and EPCAM).

**Patients and methods**

**Patients**

**Development cohort.** We analyzed data from 18,734 patients (proband) who underwent germline testing of the MLH1, MSH2, MSH6, PMS2, and EPCAM genes by Myriad Genetic Laboratories (Salt Lake City, UT) after May 2011. Clinical data were obtained from the test order form completed by health care professionals who ordered germline testing, as previously described.

**Probands without personal history of these cancers were defined as unaffected. Family history was limited to first-degree relatives (FDRs) and second-degree relatives (SDRs) on the affected side.**

**Germline analysis was uniform for probands in both cohorts and was performed by Myriad Genetics. MLH1, MSH2, and MSH6 analyses were performed as previously described.**

malignancies in an individual to quantify the likelihood of carrying a germline mutation in the MLH1, MSH2, and MSH6 genes. Clinical practice guidelines from various national organizations recommend that individuals with ≥ 5% likelihood of LS by PREMM<sub>1,2,6</sub> undergo genetic testing.

However, current LS prediction models do not assess for PMS2 or EPCAM mutations. Our aim was to develop and validate a new prediction model to quantify the risk of an individual carrying a pathogenic mutation in any of the five genes associated with LS (MLH1, MSH2, MSH6, PMS2, and EPCAM).

**Laboratory methods**

Germline analysis was uniform for probands in both cohorts and was performed by Myriad Genetics. MLH1, MSH2, and MSH6 analyses were performed as previously described. PMS2 testing involved sequencing of all exons and adjacent intronic regions as well as large rearrangement testing by multiplex ligation-dependent probe amplification. EPCAM analysis involved large rearrangement testing by microarray comparative genomic hybridization for the 3-prime region. Individuals with deleterious/suspected deleterious mutations were named mutation positive. Patients with polymorphisms, unclassified variants, no alterations, or missense mutations for which clinical significance is not established were named mutation-negative.

**Statistical methods**

Variables in the previous PREMM<sub>1,2,6</sub> and PREMM<sub>1,2</sub> models were considered for the new PREMM<sub>5</sub> model. We examined the association of proband sex with mutation presence, because male sex was a predictor of mutations in previous analyses. Proband age at testing was considered for inclusion because of the high proportion of unaffected individuals who underwent testing. Summary statistics for each variable were catalogued by gene type for probands and relatives, and ages at diagnosis of CRC and endometrial cancer were truncated at the lower and upper one percentile to stabilize the resulting model. Patients were excluded if all cancer-related data were missing, if sex was unreported (n = 11), or if two pathogenic mutations (other than EPCAM and MSH2) were detected (n = 5). Multiple imputation was applied for missing values; five completed data sets were analyzed, and results were combined by Rubin’s rules. The imputation model included personal and family cancer characteristics, and the outcome...
was presence of a specific gene mutation. Among the 18,734 probands, missing data were imputed for ages at CRC (n = 28), endometrial cancer (n = 28), other cancers (n = 50), and genetic testing (n = 14). Among the 49,237 relatives, missing ages of CRC (n = 1,995), endometrial cancer (n = 407), and other cancers (n = 4,581) was imputed. Associations of overall and gene-specific mutation statuses were analyzed with F tests (continuous variables) and \( \chi^2 \) tests (categoric variables). A two-sided \( P \) value of less than .05 indicated statistical significance.

**Development of the PREMM\(_5\) model.**

PREMM\(_5\) predictions of any pathogenic mutation were based on a logistic regression model derived from the full cohort. The associations of predictors with mutation status were reported as odds ratios (ORs) with 95% CIs. Interaction terms were added to test for sex or age-specific effects. Different models were explored and adjusted for unaffected individuals and age at testing. MSH2 and EPCAM were combined into one category (MSH2), because there were few EPCAM mutations and because these mutations induce epigenetic inactivation of MSH2. Polytomous logistic regression was used to assess the associations of clinical features with mutation status by the specific gene, in which the response variable was a categoric variable with five levels: (1) MLH1, (2) MSH2 or EPCAM, (3) MSH6, (4) PMS2, and (5) mutation-negative status. For each individual, the PREMM\(_5\) score was calculated as the predicted probability of a mutation within the resulting multivariable polytomous logistic regression model. Internal validation was performed by bootstrap resampling that used 200 random samples drawn with replacement. Predictive models were developed in each bootstrap sample and evaluated in the entire cohort to quantify the optimism in the estimated apparent performance.

**Assessment of Model Performance.** We quantified the overall ability of the PREMM\(_5\) model to discriminate carriers from non-carriers by the area under the receiver operating characteristic curve (AUC), which also was used for each specific gene in the development cohort. Decision curve analysis was used to determine the clinical usefulness of the model, and the true-positive (TP) and false-positive (FP) classifications were considered at increasing decision thresholds. This methodology evaluates prediction models for their potential to improve clinical decision making and, in this case, to refer individuals for genetic testing. A decision curve shows the net benefit (NB) of using a model at different thresholds. The NB sums the TPs minus a weighted number of FPs: \( \text{NB} = (\text{TP} - w\text{FP})/n \), in which \( n \) is the total sample size and \( w \) is the relative weight of the harm of unnecessary testing versus the benefit of identification of a carrier. The relative weight—\( w \)—is defined by the threshold probability to define at-risk patients who need genetic testing. The NB of PREMM\(_5\) and two reference strategies—test none or test all—was calculated. Thresholds between 0% (test all) and 10% (test high-risk probands) were considered, and 5% was the focus, as recommended by national guidelines. The number needed to test (NNT), which represents the number of patients who have PREMM\(_5\) scores greater than a given threshold who should undergo testing to identify one mutation carrier, was calculated. The same performance metrics were analyzed with the validation cohort.

**Comparison of the PREMM\(_5\) and PREMM\(_{1,2,6}\) Models.** PREMM\(_{1,2,6}\) predictions were calculated for all patients and were compared with PREMM\(_5\) predictions by using receiver operating characteristic curves and reclassification analysis. Under- or overprediction of each model was quantified by the calibration intercept and was converted to a ratio of observed to expected (O/E) results: \( O/E = \exp(\text{intercept}) \). Statistical analysis was performed using SAS (version 9.4) for data management and univariable analysis, and R software (version 3.1.2) was used.
Results

Pathogenic mutations were found in 1,000 (5%) of 18,734 probands in the development cohort: MLH1 (n = 306), MSH2 (n = 354), MSH6 (n = 177), PMS2 (n = 141), and EPCAM. A total of 15,363 (82%) of 18,734 participants were women. Pathogenic mutations were more frequent among men (370 [11%] of 3,371) than women (630 [4%] of 15,363; P < .001).

Forty-six percent of probands (8,590 of 18,734) were unaffected by cancer, including 200 (20%) of 1,000 mutation carriers.

Multiple CRCs and other LS-associated malignancies were more frequent among carriers of MLH1, MSH2, and EPCAM than carriers of MSH6 and PMS2.

Carriers of MSH6 and PMS2 were older than other carriers diagnosed with CRC (P < .001) or endometrial cancer (P = .034).

Carriers of PMS2 had fewer relatives with CRC (P < .001) and fewer FDRs with endometrial cancer (P < .001) compared with other carriers.

In the validation cohort, the mean CRC age was 55.7 years (standard deviation, 12.6 years), and 587 (55.5%) of 1,058 were men. Pathogenic gene mutations were detected in 33 (3.1%) of 1,058 individuals: 13 with MLH1, seven with MSH2, six with MSH6, seven with PMS2, and none with EPCAM.

Development, validation, and performance of PREMM<sub>5</sub>

In multivariable analysis (Table 2), younger age at testing was associated with the presence of any gene mutation (OR, 0.69 per decade; 95% CI, 0.66 to 0.73) and added into PREMM<sub>5</sub> as a new predictor. After analysis was adjusted for all other predictors, male sex was associated with the presence of any mutation (OR, 2.22; 95% CI, 1.88 to 2.61); other predictors included personal history of CRC with one occurrence (OR, 6.18, 95% CI, 5.22 to 7.32), or multiple occurrences (OR, 8.54; 95% CI, 5.65 to 12.93), and relatives with CRC (OR, 3.02; 95% CI, 2.76 to 3.31). Personal and family CRC diagnoses and corresponding ages were less predictive of PMS2 mutations (OR, 2.69; 95% CI, 1.78 to 4.07 and OR, 1.00; 95% CI, 0.76 to 1.32, respectively) compared with other genes. Personal history of endometrial cancer was associated with any mutation (OR, 5.42; 95% CI, 4.39 to 6.68), but age was only predictive of MSH6 mutations. Personal history of other LS-associated cancers had an OR of 3.16 (95% CI, 2.49 to 4.02) for any mutation but was not predictive of MSH6 or PMS2 mutations. Family history of endometrial cancer was predictive of all gene mutations except PMS2. Family history of other LS-associated cancers was weakly predictive of any mutation (OR, 1.50; 95% CI, 1.29 to 1.74) but not of MSH6 or PMS2.

PREMM<sub>5</sub> performed well in discriminating carriers from noncarriers; the optimism-corrected AUC was 0.81 (95% CI, 0.79...
## TABLE 1 - Clinical characteristics of gene mutation carriers stratified by gene

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. (%) of patients</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carrier status</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Noncarrier (n = 17,734)</td>
<td>Carrier (n = 1,000)</td>
</tr>
<tr>
<td></td>
<td>MLH1 (n = 306)</td>
<td>MSH2 (n = 354)</td>
</tr>
<tr>
<td>Carrier status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>3,001 (17)</td>
<td>370 (37)</td>
</tr>
<tr>
<td>Female</td>
<td>14,733 (83)</td>
<td>630 (63)</td>
</tr>
<tr>
<td>Patient age at testing, years ± SD</td>
<td>49.4 ± 12.9</td>
<td>47.0 ± 11.7</td>
</tr>
<tr>
<td>Personal cancer history</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No cancer history (unaffected)</td>
<td>8,390 (47)</td>
<td>200 (20)</td>
</tr>
<tr>
<td>CRC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>12,030 (68)</td>
<td>412 (41)</td>
</tr>
<tr>
<td>1</td>
<td>5,419 (31)</td>
<td>550 (55)</td>
</tr>
<tr>
<td>≥ 2</td>
<td>285 (2)</td>
<td>36 (4)</td>
</tr>
<tr>
<td>CRC diagnosed &lt; 50 years of age</td>
<td>3,415 (60)</td>
<td>443 (75)</td>
</tr>
<tr>
<td>Endometrial cancer (among women only)</td>
<td>2,064 (14)</td>
<td>189 (19)</td>
</tr>
<tr>
<td>Other LS cancers*</td>
<td>1,071 (6)</td>
<td>115 (12)</td>
</tr>
<tr>
<td>Multiple LS cancers†</td>
<td>1,027 (6)</td>
<td>143 (14)</td>
</tr>
<tr>
<td>Family history of cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of FDRs with a history of cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>10,379 (58)</td>
<td>420 (42)</td>
</tr>
<tr>
<td>1</td>
<td>5,863 (33)</td>
<td>423 (42)</td>
</tr>
<tr>
<td>≥ 2</td>
<td>1,492 (8)</td>
<td>157 (16)</td>
</tr>
<tr>
<td>Endometrial cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>15,419 (87)</td>
<td>821 (82)</td>
</tr>
<tr>
<td>1</td>
<td>2,077 (12)</td>
<td>168 (17)</td>
</tr>
<tr>
<td>≥ 2</td>
<td>238 (1)</td>
<td>11 (1)</td>
</tr>
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</table>
### TABLE 1 - Clinical characteristics of gene mutation carriers stratified by gene (continued)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. (%) of patients</th>
<th>Carrier status</th>
<th>Gene mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Noncarrier (n = 17,734)</td>
<td>Carrier (n = 1,000)</td>
</tr>
<tr>
<td>Other LS</td>
<td></td>
<td>14,085 (79)</td>
<td>771 (77)</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>3,143 (18)</td>
<td>191 (19)</td>
</tr>
<tr>
<td>≥ 2</td>
<td></td>
<td>506 (3)</td>
<td>38 (4)</td>
</tr>
<tr>
<td>No. of SDRs with a history of cancer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRC</td>
<td></td>
<td>10,568 (60)</td>
<td>528 (53)</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>4,919 (28)</td>
<td>287 (29)</td>
</tr>
<tr>
<td>≥ 2</td>
<td></td>
<td>2,247 (13)</td>
<td>185 (19)</td>
</tr>
<tr>
<td>Endometrial cancer</td>
<td></td>
<td>13,626 (77)</td>
<td>821 (82)</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>3,250 (18)</td>
<td>145 (15)</td>
</tr>
<tr>
<td>≥ 2</td>
<td></td>
<td>858 (5)</td>
<td>34 (3)</td>
</tr>
<tr>
<td>Other LS</td>
<td></td>
<td>15,840 (89)</td>
<td>925 (93)</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>1,614 (9)</td>
<td>65 (7)</td>
</tr>
<tr>
<td>≥ 2</td>
<td></td>
<td>280 (2)</td>
<td>10 (1)</td>
</tr>
</tbody>
</table>

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

†Includes CRC and endometrial cancer.

CRC, colorectal cancer; FDR, first-degree relative; LS, Lynch syndrome; N/A, not applicable; SD, standard deviation; SDR, second-degree relative.
# TABLE 2 - Multivariable logistic regression analyses for the presence of Lynch syndrome gene mutations

<table>
<thead>
<tr>
<th>Predictor</th>
<th>OR (95% CI) by mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Any mutation* (n = 1,000)</td>
</tr>
<tr>
<td>Personal characteristic</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>2.22 (1.88 to 2.61)</td>
</tr>
<tr>
<td>Age at testing, by decade</td>
<td>0.68 (0.63 to 0.73)</td>
</tr>
<tr>
<td>Personal history</td>
<td></td>
</tr>
<tr>
<td>1 CRC</td>
<td>6.18 (5.22 to 7.32)</td>
</tr>
<tr>
<td>≥ 2 CRC</td>
<td>8.54 (5.65 to 12.93)</td>
</tr>
<tr>
<td>Endometrial cancer</td>
<td>5.42 (4.39 to 6.68)</td>
</tr>
<tr>
<td>Other LS cancers†</td>
<td>3.16 (2.49 to 4.02)</td>
</tr>
<tr>
<td>Family history</td>
<td></td>
</tr>
<tr>
<td>CRC</td>
<td></td>
</tr>
<tr>
<td>No family history of CRC</td>
<td>1.0</td>
</tr>
<tr>
<td>Presence of CRC in FDR/SDR†</td>
<td>3.02 (2.76 to 3.31)</td>
</tr>
<tr>
<td>Endometrial cancer</td>
<td></td>
</tr>
<tr>
<td>No family history of endometrial cancer</td>
<td>1.0</td>
</tr>
<tr>
<td>Presence of endometrial cancer in FDR/SDR†</td>
<td>1.98 (1.70 to 2.31)</td>
</tr>
<tr>
<td>Other LS cancers</td>
<td></td>
</tr>
<tr>
<td>No family history of other LS cancers</td>
<td>1.50 (1.29 to 1.74)</td>
</tr>
<tr>
<td>Presence of other LS cancer in FDR/SDR†</td>
<td>1.50 (1.29 to 1.74)</td>
</tr>
<tr>
<td>Age at diagnosis, years (by decade)</td>
<td></td>
</tr>
<tr>
<td>CRC</td>
<td>0.69 (0.66 to 0.73)</td>
</tr>
<tr>
<td>Endometrial cancer</td>
<td>1.15 (1.05 to 1.25)</td>
</tr>
</tbody>
</table>

*The any-mutation column includes the results of the logistic regression analysis of the binary response variable (mutation vs. no mutation); the remaining columns are the results of the polytomous logistic regression for the nominal categorical variable with the five levels (MLH1, MSH2/EPCAM, MSH6, PMS2, and no mutation).

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

‡Family history was coded as 1 × (No. of FDRs) + 0.5 × (No. of SDRs). For CRC and endometrial cancer, the number of FDRs was coded as 0, 1, or 2 for no, one, or at least two affected FDRs, respectively, and the number of SDRs was coded as 0, 1, or 2 for no, one, or at least two affected SDRs, respectively. Family history could have values of 0 (for no affected relatives) to 3 (for two or more affected FDRs and SDRs). For other LS cancers, the number of FDRs was coded as 0 or 1 for no or at least one affected FDR, and the number of SDRs was coded as 0 or 1 for no or at least one affected SDR. Family history could have values of 0 (for no affected relatives) to 1.5 (for one or more affected FDR and SDR). CRC, colorectal cancer; FDR, first-degree relatives; LS, Lynch syndrome; OR, odds ratio; SDR, second-degree relatives.
to 0.82; Figure 1). The polytomous multivariable model showed good discrimination for MLH1 (AUC, 0.89; 95% CI, 0.87 to 0.91), MSH2/EPCAM (AUC, 0.84; 95% CI, 0.82 to 0.86), and MSH6 (AUC, 0.76; 95% CI, 0.73 to 0.79) but less discrimination for PMS2 (AUC, 0.64; 95% CI, 0.60 to 0.68;). PREMM5 had similar discrimination on external validation (AUC, 0.83; 95% CI, 0.75 to 0.92; Figure 1).

The median PREMM score for carriers was 9.8% versus 2.6% for noncarriers. At the recommended ≥ of 5% threshold, 721 of 1,000 carriers were identified (Figure 2) with variability by specific gene (Figure 3). A threshold ≥ 2.5% identified 894 of 1,000 carriers, but with lower specificity. The NNT to identify one carrier at 5% and 2.5% was seven and 11 individuals, respectively; a decrease from 19 needed to test if PREMM5 was not used.

- Between 2.5% and 10%, the negative predictive value was 97% to 99% of individuals correctly identified as mutation negative.
- PREMM had an AUC of 0.83 (95% CI, 0.82 to 0.84) for the identification of carriers of MLH1, MSH2, or MSH6 among the 18,734 probands.
- Extending prediction to all five genes decreased discrimination to 0.79 (95% CI, 0.78 to 0.81), whereas PREMM prediction yielded an AUC of 0.81 (95% CI, 0.79 to 0.82).
- PREMM overpredicted mutation-positive status (ie, the observed mutation fraction for carriers of MLH1, MSH2, and MSH6 was 4.5% but the average PREMM prediction was 8.0% [O/E ratio, 0.557]).

By decision curve analysis, the clinical impact of PREMM5 to identify individuals for germline testing was observed at thresholds ≥ 2.5%; maximal utility occurred at 5%. A threshold ≥ 2.5% to guide testing was superior compared with testing of all patients (Figure 4). PREMM5 had minimal clinical impact at less than 2.5%, because few individuals would be excluded from genetic testing at this threshold, so it was similar to a test-all approach.

- Performance comparison of PREMM5 and PREMM1,2,6
  PREMM1,2,6 had an AUC of 0.83 (95% CI, 0.82 to 0.84) for the identification of carriers of MLH1, MSH2, or MSH6 among the 18,734 probands. Extending prediction to all five genes decreased discrimination to 0.79 (95% CI, 0.78 to 0.81), whereas PREMM5 prediction yielded an AUC of 0.81 (95% CI, 0.79 to 0.82). PREMM1,2,6 overpredicted mutation-positive status (ie, the observed mutation fraction for carriers of MLH1, MSH2, and MSH6 was 4.5% but the average PREMM1,2,6 prediction was 8.0% [O/E ratio, 0.557]). Reclassification plots confirmed overprediction by

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**Key points**

- A threshold ≥ 2.5% identified 894 of 1,000 carriers, but with lower specificity.
- The NNT to identify one carrier at 5% and 2.5% was seven and 11 individuals, respectively; a decrease from 19 needed to test if PREMM5 was not used.
- Between 2.5% and 10%, the negative predictive value was 97% to 99% of individuals correctly identified as mutation negative.
- PREMM had an AUC of 0.83 (95% CI, 0.82 to 0.84) for the identification of carriers of MLH1, MSH2, or MSH6 among the 18,734 probands.
- Extending prediction to all five genes decreased discrimination to 0.79 (95% CI, 0.78 to 0.81), whereas PREMM prediction yielded an AUC of 0.81 (95% CI, 0.79 to 0.82).
- PREMM overpredicted mutation-positive status (ie, the observed mutation fraction for carriers of MLH1, MSH2, and MSH6 was 4.5% but the average PREMM prediction was 8.0% [O/E ratio, 0.557]).
Development and Validation of the PREMM\textsubscript{5} Model for Comprehensive Risk

To improve the identification of carriers of LS mutations, we developed and validated the PREMM\textsubscript{5} model, a risk assessment tool that uses readily ascertained clinical features (age, sex, and personal and family cancer history) to provide accurate and comprehensive risk estimation for all five associated genes. PREMM\textsubscript{5} is a simple and efficient online tool for a diverse array of health care providers (eg, oncologists, gynecologists, gastroenterologists, and primary care practitioners) to rapidly identify individuals for germline testing for LS in routine clinical practice. The performance of the model is robust in quantifying an individual’s overall risk of carrying any pathogenic gene mutation, and its performance exceeds that of the previous model, PREMM\textsubscript{1,2,6}, which only predicted MLH1, MSH2, and MSH6 gene mutation status. We propose that PREMM\textsubscript{5} should replace PREMM\textsubscript{1,2,6} and that individuals with a $\geq 2.5\%$ likelihood of LS undergo genetic testing.

Beyond the use of prediction models such as PREMM\textsubscript{5}, the primary strategy to identify individuals with LS involves screening CRC and endometrial cancer tumor specimens for microsatellite instability or deficient MMR protein expression, followed by germline testing of individuals who have suggestive tumor testing results.\textsuperscript{2,3} The limitation of tumor testing is that it is only relevant to patients with cancer, so the

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Performance characteristics of PREMM\textsubscript{5}. Decreasing sensitivity and increasing specificity are shown for increasing risk thresholds for the PREMM\textsubscript{5} score, with a histogram for the distribution of predicted risks.}
\end{figure}

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|c|}
\hline
Risk Threshold & 0.00 & 0.25 & 0.50 & 0.75 & 1.00 \\
\hline
Sensitivity (true positive rate) & 0.894 & 0.721 & 0.593 & 0.484 & 0.000 \\
Specificity (true negative rate) & 0.492 & 0.751 & 0.854 & 0.905 & 1.000 \\
\hline
NNT = (TP + FP)/TP & 18.734 & 11.073 & 7.133 & 5.363 & 4.401 \\
NPV (negative predictive value) & 0.963 & 0.909 & 0.866 & 0.827 & NaN \\
PPV (positive predictive value) & 0.988 & 0.979 & 0.974 & 0.968 & 0.947 \\
\hline
\end{tabular}
\end{table}

\begin{tabular}{|c|c|c|c|c|}
\hline
PREMM\textsubscript{1,2,6} & 1,000 & 894 & 721 & 592 \\
TN (true negative) & 0 & 8,729 & 13,312 & 15,151 \\
FP (false positive) & 17,734 & 9,005 & 4,422 & 2,583 \\
FN (false negative) & 0 & 106 & 279 & 408 \\
Sensitivity (true positive rate) & 1 & 0.894 & 0.721 & 0.593 \\
Specificity (true negative rate) & 0 & 0.492 & 0.751 & 0.854 \\
\hline
NNT = (TP + FP)/TP & 18.734 & 11.073 & 7.133 & 5.363 \\
NPV (negative predictive value) & 0.963 & 0.909 & 0.866 & 0.827 \\
PPV (positive predictive value) & 0.988 & 0.979 & 0.974 & 0.968 \\
\hline
\end{tabular}

Key points

- PREMM is a simple and efficient online tool for a diverse array of health care providers (eg, oncologists, gynecologists, gastroenterologists, and primary care practitioners) to rapidly identify individuals for germline testing for LS in routine clinical practice.

- The primary strategy to identify individuals with LS involves screening CRC and endometrial cancer tumor specimens for microsatellite instability or deficient MMR protein expression, followed by germline testing of individuals who have suggestive tumor testing results.

PREMM\textsubscript{1,2,6} and showed considerable differences between PREMM\textsubscript{5} and PREMM\textsubscript{1,2,6} predictions.

\section*{Discussion}

To improve the identification of carriers of LS mutations, we developed and validated the PREMM\textsubscript{5} model, a risk assessment tool that uses readily ascertained clinical features (age, sex, and personal and family cancer history) to provide accurate and comprehensive risk estimation for all five associated genes. PREMM\textsubscript{5} is a simple and efficient online tool for a diverse array of health care providers (eg, oncologists, gynecologists, gastroenterologists, and primary care practitioners) to rapidly identify individuals for germline testing for LS in routine clinical practice. The performance of the model is robust in quantifying an individual’s overall risk of carrying any pathogenic gene mutation, and its performance exceeds that of the previous model, PREMM\textsubscript{1,2,6}, which only predicted MLH1, MSH2, and MSH6 gene mutation status. We propose that PREMM\textsubscript{5} should replace PREMM\textsubscript{1,2,6} and that individuals with a $\geq 2.5\%$ likelihood of LS undergo genetic testing.

Beyond the use of prediction models such as PREMM\textsubscript{5}, the primary strategy to identify individuals with LS involves screening CRC and endometrial cancer tumor specimens for microsatellite instability or deficient MMR protein expression, followed by germline testing of individuals who have suggestive tumor testing results.\textsuperscript{2,3} The limitation of tumor testing is that it is only relevant to patients with cancer, so the
FIGURE 4 ■ Net benefit curves for PREMM, compared with PREMM\textsubscript{1,3,6}. The y-axis measures net benefit, which is calculated by summing the benefits (true positives) and subtracting the harms (false positives), in which the latter are weighted by a factor related to the relative harm of a missed mutation carrier compared with the harm of unnecessary genetic testing. A model is considered of clinical value if it has the highest net benefit compared with other models and simple strategies, such as performing genetic testing in all patients (gold line) or no patients (horizontal blue line) across the full range of threshold probabilities at which a patient would undergo genetic testing. For example, the net benefit of using PREMM, to selectively test for mutation carriers exceeds that of PREMM\textsubscript{1,3,6} and testing all at risk threshold \( \geq 2.5\% \).
Development and Validation of the PREMM$_5$ Model for Comprehensive Risk

opportunity for cancer prevention in the proband has inherently been lost. Prior versions of PREMM and other LS models (eg, MMRpro, MMpred) were developed and validated in cohorts predominated by patients with cancer, and the performance of these models in unaffected patients is poorly understood. In this study, 46% of the development cohort (including 20% of LS mutation carriers) were unaffected but had a family history of LS-associated cancer, which supports the potential use of PREMM$_5$ as a risk assessment tool for unaffected individuals.

Despite existing recommendations for universal molecular tumor testing of all colorectal and endometrial cancers to screen for LS, the majority of the nearly 1 million carriers in the United States remain unidentified. Use of the PREMM$_5$ model can ameliorate the gap that exists between those identified through tumor testing and the vast majority of undiagnosed carriers of LS, many of whom are unaffected by cancer. Therefore, systematic implementation of PREMM$_5$ can be considered by providers in routine clinical care, including primary and preventive health care, for individuals with a personal or family history of LS-associated cancers. Also, the PREMM$_5$ model can be used in individuals who have colorectal and endometrial cancer when molecular tumor testing is unavailable or when resources are limited and universal tumor testing cannot be adopted.

The cohort used to develop PREMM$_5$ was far larger than those used for older prediction models, including PREMM$_{1,2,6}$. We examined data on 1,000 mutation carriers, in which 32% carried pathogenic PMS2 and MSH6 mutations, the less penetrant but more prevalent MMR genes associated with LS. Although PREMM$_5$ performs well estimating individuals’ overall risk of carrying any MMR gene mutation, variability exists in estimates for each individual gene. Reliable prediction of PMS2 was more difficult than other genes because of a weaker phenotype—carriers of PMS2 were older at CRC diagnoses and had fewer cancers among relatives compared with other families with LS. This is in line with studies of carriers of the PMS2 gene mutation, in which retention of mRNA expression resulted in a milder phenotype in patients who were older at the time of their CRC diagnosis and/or had no family history of CRC. The performance measures associated with PREMM$_5$ were comparable in the cohort used for external validation. A strength of the validation data set is that it minimized selection bias; patients had CRC but were not selected for genetic testing for LS because of features suggestive of the condition, such as young age of diagnosis, fulfillment of clinical criteria, or results of MMR tumor testing.

The large size of the development cohort allowed us to reassess previous predictors of mutation carrier status, such as sex of the individual tested. The frequency of pathogenic mutations differed between men and women (11% vs. 4% mutation prevalence, respectively), despite the presence of more women (73%) in the data set. This is consistent with prior reports and may be due to unmeasured selection bias in the development cohort. The sex distribution was more even in the validation cohort and did not alter the ability of the model to discern mutation carriers from noncarriers.

Incorporation of age at genetic testing improved risk estimation with PREMM$_5$; with every decade increase in age, the likelihood an individual would carry a pathogenic mutation decreased by 32%. This refinement compared with PREMM$_{1,2,6}$ is relevant to individuals unaffected by cancer, because the lack of such history in a 75-year-old patient who undergoes genetic evaluation is strong evidence against LS, whereas the absence of personal cancer history is less reassuring in a 25-year-old patient.

At the currently recommended threshold of 5%, the PREMM$_5$ model identifies fewer individuals than PREMM$_{1,2,6}$ for

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**Key points**

- In this study, 46% of the development cohort (including 20% of LS mutation carriers) were unaffected but had a family history of LS-associated cancer, which supports the potential use of PREMM$_5$ as a risk assessment tool for unaffected individuals.
- Despite existing recommendations for universal molecular tumor testing of all colorectal and endometrial cancers to screen for LS, the majority of the nearly 1 million carriers in the United States remain unidentified.
- PREMM$_5$ model can be used in individuals who have colorectal and endometrial cancer when molecular tumor testing is unavailable or when resources are limited and universal tumor testing cannot be adopted.
- The cohort used to develop PREMM$_5$ was far larger than those used for older prediction models, including PREMM.
- The performance measures associated with PREMM$_5$ were comparable in the cohort used for external validation.
Hot Topics

Key points

• Compared with PREMM, PREMM overpredicts an individual’s need to obtain genetic evaluation. With PREMM, at a ≥ 5% threshold, seven patients would need to be tested to identify one mutation carrier, with a negative predictive value of 98%.

• PREMM provides accurate and comprehensive risk assessment specifically for LS, so its performance must be examined when genetic testing includes an expanded panel of genes.

• In conclusion, PREMM provides comprehensive LS risk estimation for all five genes, including PMS2 and EPCAM.

With ongoing advances in next-generation DNA sequencing technologies, many commercial laboratories currently offer multigene hereditary cancer panels that provide simultaneous germline analysis of dozens of cancer risk genes. PREMM provides accurate and comprehensive risk assessment specifically for LS, so its performance must be examined when genetic testing includes an expanded panel of genes. Recent data to examine such panels have shown that individuals with PREMM scores ≥ 5% often have mutations in cancer susceptibility genes beyond those linked to LS, including APC, MUTYH, BRCA1, and BRCA2. These results suggest that prediction models such as PREMM may identify individuals who may have underlying mutations in a wide spectrum of syndromes, rather than just LS.

In conclusion, PREMM provides comprehensive LS risk estimation for all five genes, including PMS2 and EPCAM. PREMM identifies fewer high-risk individuals for genetic evaluation and testing who have a higher likelihood of carrying a pathogenic mutation compared with PREMM. These analyses support the use of a ≥ 2.5% threshold to identify individuals suitable for genetic evaluation for LS; this threshold optimizes identification of carriers of gene mutations, including those who carry MMR genes associated with a weaker phenotype or who are unaffected by cancer but have a family history suggestive of an inherited CRC syndrome.

References


