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ORIGINAL REPORT

Development and Validation of the PREMM$_5$ Model for Comprehensive Risk Assessment of Lynch Syndrome

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ABSTRACT

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$_5$, that incorporates the genes *PMS2* and *EPCAM* to provide comprehensive LS risk assessment.

Patients and Methods

PREMM$_5$ 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$_{1,2,6}$ model. External validation of PREMM$_5$ 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$_5$ 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$_5$ exceeded those of PREMM$_{1,2,6}$. Decision curve analysis supported germline LS testing for PREMM$_5$ scores $\geq$ 2.5%.

Conclusion

PREMM$_5$ provides comprehensive risk estimation of all five LS genes and supports LS genetic testing for individuals with scores $\geq$ 2.5%. At this threshold, PREMM$_5$ provides performance that is superior to the existing PREMM$_{1,2,6}$ model in the identification of carriers of LS, including those with weaker phenotypes and individuals unaffected by cancer.

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INTRODUCTION

Nearly 1 million individuals in the United States have Lynch syndrome (LS) but most are unaware of their diagnosis.[1] 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).[2-4] 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.[5-8] 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.[9-14]

Prediction models, such as PREMM$_{1,2,6}$, are evidence-based tools that can help identify carriers of LS by using the personal and family history of Lynch-associated malignancies in an individual to quantify the likelihood of carrying a germline mutation in the *MLH1*, *MSH2*, and

ASSOCIATED CONTENT

Appendix
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MSH6 genes.[15] Clinical practice guidelines from various national organizations recommend that individuals with ≥5% likelihood of LS by $PREMM_{1,2,6}$ undergo genetic testing.[2,3,16] However, current LS prediction models do not assess for *PMS2* or *EPCAM* mutations.[17,18] 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 (probands) 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,[15,19] including proband age at genetic testing, sex, personal and family cancer histories (including ages at diagnoses), and germline testing results. Cancer history included Lynch-associated malignancies of the colorectum, endometrium, stomach, ovaries, urinary tract, small intestine, pancreas, bile ducts, brain, or sebaceous glands. 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.

Validation cohort. Validation of the model was performed in an independent cohort of 1,058 patients with CRC who were recruited to an institutional sample registry at Dana-Farber Cancer Institute from 2008 to 2014, without preselection for high-risk features of LS (eg, age at diagnosis, personal or family cancer histories, or tumor microsatellite instability/MMR deficiency). All participants underwent germline testing for multiple genes, including *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.[15] *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.[15]

Statistical Methods

Variables in the previous $PREMM_{1,2,6}$ and $PREMM_{1,2}$ models were considered for the new $PREMM_5$ model.[15,19,20] We examined the association of proband sex with mutation presence, because male sex was a predictor of mutations in previous analyses.[15] 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.[21] 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 χ^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*.[22] 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.[23]

Assessment of Model Performance. We quantified the overall ability of the $PREMM_5$ model to discriminate carriers from noncarriers by the area under the receiver operating characteristic curve (AUC), which also was used for each specific gene in the development cohort.[24] 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[24-28] 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: $NB = (TP - wFP) / n$, 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.[2,3,16] 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 (intercept).[29] Statistical analysis was performed using SAS (version 9.4) for data management and univariable analysis, and R software (version 3.1.2) was used for multivariable analysis, bootstrap and external validation, and decision curve and reclassification analyses.

The study was investigator-initiated for model development; data collection and germline analyses occurred at Myriad Genetics, and anonymized data sets were provided to investigators at Dana-Farber Cancer Institute and Columbia University. Statistical analyses were conducted by researchers and independent statisticians, all of whom were unaffiliated with Myriad. Investigational review board approval was obtained for this study.

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* (n = 2; Table 1). 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; $P < .001$).

Carriers of gene mutations were younger at the time of genetic testing than noncarriers (47.0 *v* 49.4 years of age), and carriers of *MLH1*, *MSH2*, and *EPCAM* were younger at the time of testing (45.9 *v* 46.2 *v* 46.3 years of age, respectively) than carriers of *MSH6* and *PMS2* (49 years of age for both; $P < .001$). 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 ($P = .034$) (Appendix Table A3, online only). 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 (Appendix Table A4, online only). Pathogenic gene mutations were detected in 33 (3.1%) of 1,058 individuals: 13 with

Table 1. Clinical Characteristics of Gene Mutation Carriers Stratified by Gene

Characteristic	No. (%) of Patients							
	Carrier Status		Gene Mutation					
	Noncarrier (n = 17,734)	Carrier (n = 1,000)	*MLH1* (n = 306)	*MSH2* (n = 354)	*MSH6* (n = 177)	*PMS2* (n = 141)	*EPCAM* (n = 22)	*P*
Sex								
Male	3,001 (17)	370 (37)	140 (46)	137 (39)	48 (27)	39 (28)	6 (27)	< .001
Female	14,733 (83)	630 (63)	166 (54)	217 (61)	129 (73)	102 (72)	16 (73)	
Patient age at testing, years ± SD	49.4 ± 12.9	47.0 ± 11.7	45.9 ± 11.0	46.2 ± 11.4	49.1 ± 11.3	49.2 ± 14.0	46.3 ± 10.9	< .001
Personal cancer history								
No cancer history (unaffected)	8,390 (47)	200 (20)	46 (15)	68 (19)	45 (25)	34 (24)	7 (32)	.02
CRC								
None	12,030 (68)	412 (41)	84 (27)	161 (45)	96 (54)	63 (45)	8 (36)	< .001
1	5,419 (31)	550 (55)	205 (67)	174 (49)	81 (46)	77 (55)	13 (59)	
≥ 2	285 (2)	36 (4)	16 (5)	18 (5)	N/A	1 (0)	1 (5)	
CRC diagnosed < 50 years of age	3,415 (60)	443 (75)	180 (81)	153 (79)	53 (65)	46 (59)	11 (79)	< .001
Endometrial cancer (among women only)	2,064 (14)	189 (19)	38 (23)	76 (35)	52 (40)	23 (23)	N/A	< .001
Other LS cancers*	1,071 (6)	115 (12)	30 (10)	57 (16)	15 (8)	11 (8)	2 (9)	< .001
Multiple LS cancers†	1,027 (6)	143 (14)	45 (15)	64 (18)	21 (12)	11 (8)	2 (9)	< .001
Family history of cancer								
No. of FDRs with a history of cancer								
CRC								< .001
0	10,379 (58)	420 (42)	98 (32)	122 (34)	97 (55)	94 (67)	9 (41)	
1	5,863 (33)	423 (42)	142 (46)	164 (46)	64 (36)	41 (29)	12 (55)	
≥ 2	1,492 (8)	157 (16)	66 (22)	68 (19)	16 (9)	6 (4)	1 (5)	
Endometrial cancer								< .001
0	15,419 (87)	821 (82)	257 (84)	294 (83)	122 (69)	131 (93)	17 (77)	
1	2,077 (12)	168 (17)	48 (16)	55 (16)	51 (29)	9 (6)	5 (23)	
≥ 2	238 (1)	11 (1)	1 (0.3)	5 (1)	4 (2)	1 (0.7)	N/A	
Other LS								.27
0	14,085 (79)	771 (77)	246 (80)	257 (73)	136 (77)	115 (82)	17 (77)	
1	3,143 (18)	191 (19)	49 (16)	81 (23)	36 (20)	22 (16)	3 (14)	
≥ 2	506 (3)	38 (4)	11 (4)	16 (5)	5 (3)	4 (3)	2 (9)	
No. of SDRs with a history of cancer								
CRC								.04
0	10,568 (60)	528 (53)	138 (45)	178 (50)	109 (62)	90 (64)	13 (59)	
1	4,919 (28)	287 (29)	88 (29)	109 (31)	49 (28)	34 (24)	7 (32)	
≥ 2	2,247 (13)	185 (19)	80 (26)	67 (19)	19 (11)	17 (12)	2 (9)	
Endometrial cancer								.08
0	13,626 (77)	821 (82)	250 (82)	290 (82)	143 (81)	121 (86)	17 (77)	
1	3,250 (18)	145 (15)	49 (16)	50 (14)	26 (15)	15 (11)	5 (23)	
≥ 2	858 (5)	34 (3)	7 (2)	14 (4)	8 (5)	5 (4)	N/A	
Other LS								.81
0	15,840 (89)	925 (93)	282 (92)	331 (94)	157 (89)	133 (94)	22 (100)	
1	1,614 (9)	65 (7)	24 (8)	19 (5)	14 (8)	8 (6)	N/A	
≥ 2	280 (2)	10 (1)	N/A	4 (1)	6 (3)	N/A	N/A	

Abbreviations: CRC, colorectal cancer; FDR, first-degree relative; LS, Lynch syndrome; N/A, not applicable; SD, standard deviation; SDR, second-degree relative.
*LS includes cancers in the kidney, ureter, bladder, brain, biliary tract, stomach, small intestine, ovary, pancreas, and sebaceous neoplasms.
†Includes CRC and endometrial cancer.

MLH1, seven with *MSH2*, six with *MSH6*, seven with *PMS2*, and none with *EPCAM*.

Development, Validation, and Performance of PREMM$_5$

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$_5$ 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*. The PREMM$_5$ prediction model is available online[30] and in Appendix Tables A1 and A2 (online only).

PREMM$_5$ performed well in discriminating carriers from noncarriers; the optimism-corrected AUC was 0.81 (95% CI, 0.79 to 0.82; Fig 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; Appendix Table A5, online only). PREMM$_5$ had similar discrimination on external validation (AUC, 0.83; 95% CI, 0.75 to 0.92; Fig 1).

The median PREMM$_5$ score for carriers was 9.8% versus 2.6% for noncarriers. At the recommended ≥ of 5% threshold, 721 of 1,000 carriers were identified (Fig 2) with variability by specific gene (Fig 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 PREMM$_5$ was not used. Between 2.5% and 10%, the negative predictive value was 97% to 99% of individuals correctly identified as mutation negative. Similar sensitivity, specificity, and NNT data were observed at external validation with 2.5% and 5.0% thresholds (Appendix Fig A1, online only).

By decision curve analysis, the clinical impact of PREMM$_5$ to identify individuals for germline testing was observed at thresholds ≥ 2.5%; maximal utility occurred at 5%. A threshold

Table 2. Multivariable Logistic Regression Analyses for the Presence of Lynch Syndrome Gene Mutations

Predictor	OR (95% CI) by Mutation				
	Any Mutation* (n = 1,000)	*MLH1* (n = 306)	*MSH2*/*EPCAM* (n = 376)	*MSH6* (n = 177)	*PMS2* (n = 141)
Personal characteristic					
Male	2.22 (1.88 to 2.61)	2.47 (1.88 to 3.25)	2.55 (1.98 to 3.30)	2.26 (1.52 to 3.36)	1.34 (0.89 to 2.02)
Age at testing, by decade	0.68 (0.63 to 0.73)	0.62 (0.55 to 0.71)	0.62 (0.55 to 0.69)	0.70 (0.60 to 0.81)	0.93 (0.79 to 1.10)
Personal history					
1 CRC	6.18 (5.22 to7.32)	13.28 (9.74 to 18.10)	6.04 (4.65 to 7.85)	3.54 (2.45 to 5.12)	2.69 (1.78 to 4.07)
≥ 2 CRC	8.54 (5.65 to 12.93)	24.12 (12.87 to 45.19)	13.38 (7.66 to 23.37)	0	0.70 (0.09 to 5.21)
Endometrial cancer	5.42 (4.39 to 6.68)	5.06 (3.32 to 7.71)	6.85 (4.98 to 9.42)	5.81 (3.86 to 8.73)	2.09 (1.25 to 3.50)
Other LS cancer†	3.16 (2.49 to 4.02)	3.58 (2.30 to 5.58)	4.88 (3.51 to 6.78)	1.71 (0.98 to 2.98)	1.48 (0.78 to 2.83)
Family history					
CRC					
No family history of CRC	1.0	1.0	1.0	1.0	1.0
Presence of CRC in FDR/SDR‡	3.02 (2.76 to 3.31)	4.76 (4.09 to 5.53)	3.81 (3.33 to 4.36)	1.73 (1.39 to 2.14)	1.00 (0.76 to 1.32)
Endometrial cancer					
No family history of endometrial cancer	1.0	1.0	1.0	1.0	1.0
Presence of endometrial cancer in FDR/SDR‡	1.98 (1.70 to 2.31)	2.24 (1.68 to 2.98)	1.95 (1.54 to 2.47)	2.52 (1.92 to 3.29)	0.65 (0.38 to 1.13)
Other LS cancers					
No family history of Other LS cancers	1.0	1.0	1.0	1.0	1.0
Presence of other LS cancer in FDR/SDR‡	1.50 (1.29 to 1.74)	1.49 (1.13 to 1.96)	1.84 (1.47 to 2.30)	1.37 (0.99 to 1.89)	0.90 (0.60 to 1.34)
Age at diagnosis, years (by decade)					
CRC	0.69 (0.66 to 0.73)	0.57 (0.52 to 0.63)	0.64 (0.59 to 0.70)	0.91 (0.82 to 1.01)	0.92 (0.81 to 1.04)
Endometrial cancer	1.15 (1.05 to 1.25)	1.12 (0.94 to 1.34)	1.00 (0.87 to 1.16)	1.41 (1.22 to 1.64)	1.01 (0.78 to 1.30)

Abbreviations: CRC, colorectal cancer; FDR, first-degree relatives; LS, Lynch syndrome; OR, odds ratio; SDR, second-degree relatives.

*The any-mutation column includes the results of the logistic regression analysis of the binary response variable (mutation *v* no mutation); the remaining columns are the results of the polytomous logistic regression for the nominal categoric 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).

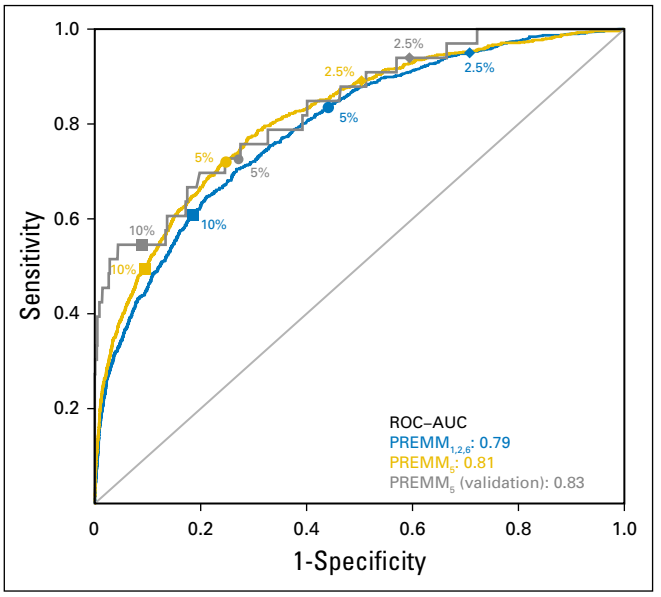


Fig 1. Receiver operating characteristic (ROC) curves to discriminate mismatch repair mutation carriers from noncarriers for the PREMM$_5$ and the PREMM$_{1,2,6}$ models. AUC, area under the ROC curve.

≥ 2.5% to guide testing was superior compared with testing of all patients (Fig 4). PREMM$_5$ 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 PREMM$_5$ and PREMM$_{1,2,6}$

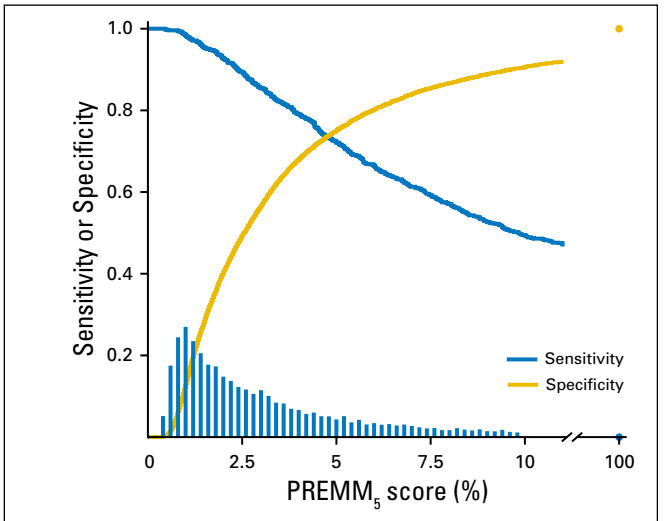
PREMM$_{1,2,6}$ had an AUC of 0.83 (95% CI, 0.82 to 0.84) for the identification of carriers of *MLH1*, *MSH2*, or *MSH6* among the 18,734 probands. Extending prediction to all five genes decreased discrimination to 0.79 (95% CI, 0.78 to 0.81), whereas PREMM$_5$ prediction yielded an AUC of 0.81 (95% CI, 0.79 to 0.82). PREMM$_{1,2,6}$ overpredicted mutation-positive status (ie, the observed mutation fraction for carriers of *MLH1*, *MSH2*, and *MSH6* was 4.5% but the average PREMM$_{1,2,6}$ prediction was 8.0% [O/E ratio, 0.557]). Reclassification plots confirmed overprediction by PREMM$_{1,2,6}$ and showed considerable differences between PREMM$_5$ and PREMM$_{1,2,6}$ predictions (Appendix Fig A2, online only).

DISCUSSION

To improve the identification of carriers of LS mutations, we developed and validated the PREMM$_5$ 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$_5$ 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$_{1,2,6}$, which only predicted *MLH1*, *MSH2*, and *MSH6* gene mutation status. We propose that PREMM$_5$ should replace PREMM$_{1,2,6}$ and that individuals with a ≥ 2.5% likelihood of LS undergo genetic testing.

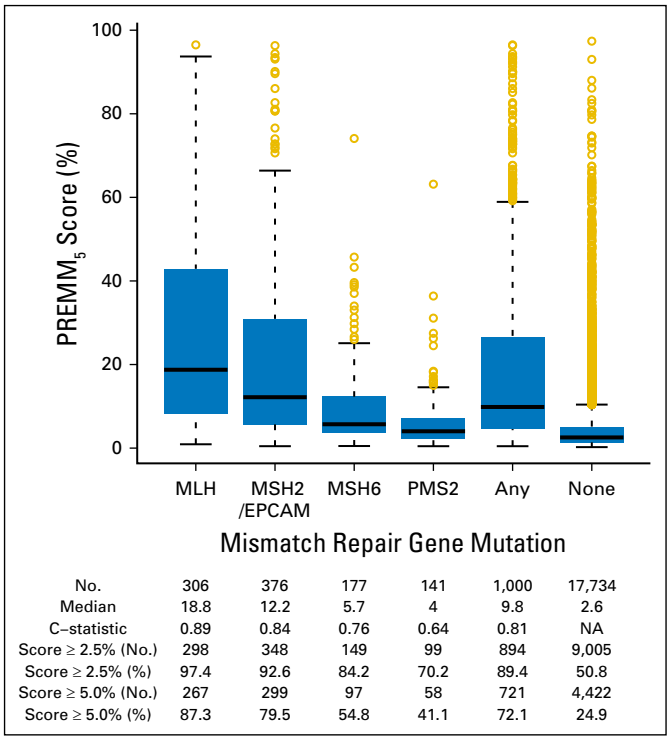
Beyond the use of prediction models such as PREMM$_5$, the primary strategy to identify individuals with LS involves screening CRC and endometrial cancer tumor specimens for microsatellite instability or deficient MMR protein expression, followed by germline testing of individuals who have suggestive tumor testing results.[2,3] The limitation of tumor testing is that it is only relevant to patients with cancer, so the opportunity for cancer prevention in the proband has inherently been lost. Prior versions of PREMM and other LS models (eg, MMRpro, MMRpredict) were developed and validated in cohorts predominated by patients with cancer,[17,18,31-35] 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.[1] 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,



TP	1,000	894	721	592	494	0
TN	0	8,729	13,312	15,151	16,054	17,734
FP	17,734	9,005	4,422	2,583	1,680	0
FN	0	106	279	408	506	1,000
Sensitivity	1	0.894	0.721	0.592	0.494	0
Specificity	0	0.492	0.751	0.854	0.905	1
NNT	18.734	11.073	7.133	5.363	4.401	
PPV	0.053	0.09	0.14	0.186	0.227	NaN
NPV	NaN	0.988	0.979	0.974	0.969	0.947

Fig 2. Performance characteristics of PREMM$_5$. Decreasing sensitivity and increasing specificity are shown for increasing risk thresholds for the PREMM$_5$ score, with a histogram for the distribution of predicted risks. FN, false negative; FP, false positive; NaN, not a number; NNT, number needed to test; NPV, negative predictive value; PPV, positive predictive value; TN, true negative; TP, true positive. FN, FP, TN, and TP are reported for a given cutoff value on the PREMM$_5$ score. NNT= (TP + FP) / TP.



	MLH	MSH2 /EPCAM	MSH6	PMS2	Any	None
No.	306	376	177	141	1,000	17,734
Median	18.8	12.2	5.7	4	9.8	2.6
C–statistic	0.89	0.84	0.76	0.64	0.81	NA
Score ≥ 2.5% (No.)	298	348	149	99	894	9,005
Score ≥ 2.5% (%)	97.4	92.6	84.2	70.2	89.4	50.8
Score ≥ 5.0% (No.)	267	299	97	58	721	4,422
Score ≥ 5.0% (%)	87.3	79.5	54.8	41.1	72.1	24.9

Fig 3. Box plots of $PREMM_5$ scores by specific gene mutation at model development. C-statistic: area under the receiver operating characteristic curve. Median values are represented by the horizontal lines in the boxes; 25th and 75th percentiles are represented by the lower and upper lines of the boxes, respectively. NA, not available.

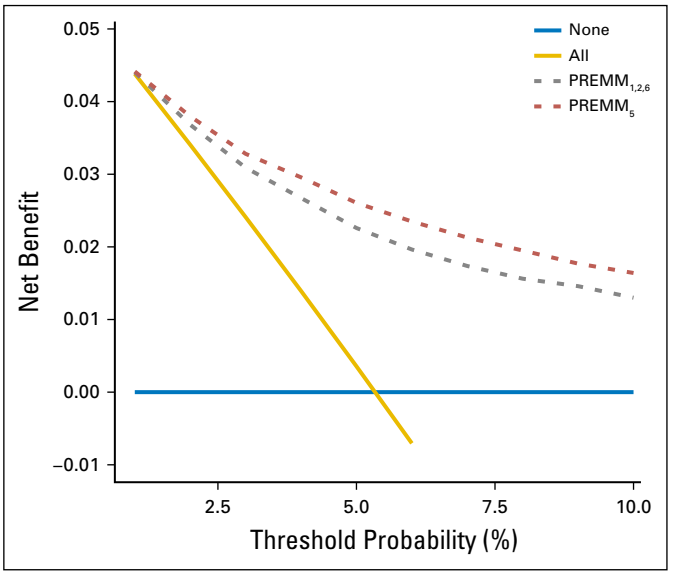


Fig 4. Net benefit curves for $PREMM_5$ compared with $PREMM_{1,2,6}$. The y-axis measures net benefit, which is calculated by summing the benefits (true positives) and subtracting the harms (false positives), in which the latter are weighted by a factor related to the relative harm of a missed mutation 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 $PREMM_5$ to selectively test for mutation carriers exceeds that of $PREMM_{1,2,6}$, and testing all at risk threshold ≥ 2.5%.

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.[36] 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.[37] 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.[38] 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% *v* 4% mutation prevalence, respectively), despite the presence of more women (73%) in the data set. This is consistent with prior reports[6] 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 genetic evaluation and has a higher likelihood than $PREMM_{1,2,6}$ of mutation carrier detection. Compared with $PREMM_5$, $PREMM_{1,2,6}$ overpredicts an individual's need to obtain genetic evaluation. With $PREMM_5$ at a ≥ 5% threshold, seven patients would need to be tested to identify one mutation carrier, with a negative predictive value of 98% (Fig 1). However, a ≥ 2.5% $PREMM_5$ score cutoff to guide LS genetic testing markedly increased the number of identified mutation carriers and preserved a high negative predictive value of 99% compared with the ≥ 5% cutoff endorsed by national guidelines for $PREMM_{1,2,6}$.

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$_5$ 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$_{1,2,6}$ scores $\geq$ 5% often have mutations in cancer susceptibility genes beyond those linked to LS, including *APC*, *MUTYH*, *BRCA1*, and *BRCA2*.[39] These results suggest that prediction models such as PREMM$_5$ may identify individuals who may have underlying mutations in a wide spectrum of syndromes, rather than just LS.

In conclusion, PREMM$_5$ provides comprehensive LS risk estimation for all five genes, including *PMS2* and *EPCAM*. PREMM$_5$ identifies fewer high-risk individuals for genetic evaluation and testing who have a higher likelihood of carrying a pathogenic mutation compared with PREMM $_{1,2,6}$. These analyses support the use of a $\geq$ 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.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at jco.org.

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Development and Validation of the PREMM$_5$ Model for Comprehensive Risk Assessment of Lynch Syndrome

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Appendix

Equation for the PREMM$_5$ Model

We encourage researchers who use this equation to contact the PREMM investigators for guidance on the appropriate application. Variables are explained in detail in Appendix Table A1. The use of the term "PREMM" is protected by a servicemark.

Predicted probability of any mismatch repair gene mutation: p(any) = predicted probability of *MLH1* mutation + predicted probability of *MSH2/EPCAM* mutation + predicted probability of *MSH6* mutation + predicted probability of *PMS2* mutation

Predicted probability of a mutation in *MLH1*: p(*MLH1*)
Predicted probability of a mutation in *MSH2 or EPCAM*: p(*MSH2/EPCAM*)
Predicted probability of a mutation in *MSH6:* p(*MSH6*)
Predicted probability of a mutation in *PMS2:* p(*PMS2*)
Predicted probability of no mutation: p(none)

$$p(\text{none}) = 1 - [p(MLH1) + p(MSH2/EPCAM) + p(MSH6) + p(PMS2)]$$

$$p(MLH1) = \exp(lp(MLH1)) / [(1 + \exp(lp(MLH1)) + \exp(lp(MSH2/EPCAM)) + \exp(lp(MSH6)) + \exp(lp(PMS2))]$$

$$p(MSH2/EPCAM) = \exp(lp(MSH2)) / [(1 + \exp(lp(MLH1)) + \exp(lp(MSH2)) + \exp(lp(MSH6) + \exp(lp(PMS2))]$$

$$p(MSH6) = \exp(lp(MSH6)) / [(1 + \exp(lp(MLH1)) + \exp(lp(MSH2)) + \exp(lp(MSH6)) + \exp(lp(PMS2))]$$

$$p(PMS2) = \exp(lp(PMS2)) / [(1 + \exp(lp(MLH1)) + \exp(lp(MSH2)) + \exp(lp(MSH6)) + \exp(lp(PMS2))]$$

$$lp(MLH1) = -5.325 + (0.904 \times V0) + (2.586 \times V1) + (3.183 \times V2) + (1.621 \times V3) + (1.276 \times V4) + (1.560 \times V5) + (0.804 \times V6) + (0.397 \times V7) + (-0.0557 \times V8) + (0.0115 \times V9) + (-0.0476 \times V10);$$

$$lp(MSH2/EPCAM) = -4.427 + (0.937 \times V0) + (1.799 \times V1) + (2.593 \times V2) + (1.924 \times V3) + (1.585 \times V4) + (1.337 \times V5) + (0.670 \times V6) + (0.607 \times V7) + (-0.0441 \times V8) + (0.0002 \times V9) + (-0.0482 \times V10);$$

$$lp(MSH6) = -4.675 + (0.816 \times V0) + (1.265 \times V1) + (-53.205 \times V2) + (1.759 \times V3) + (0.538 \times V4) + (0.545 \times V5) + (0.923 \times V6) + (0.313 \times V7) + (-0.0095 \times V8) + (0.0344 \times V9) + (-0.0363 \times V10);$$

$$lp(PMS2) = -4.913 + (0.294 \times V0) + (0.989 \times V1) + (-0.354 \times V2) + (0.739 \times V3) + (0.395 \times V4) + (-0.002 \times V5) + (-0.426 \times V6) + (-0.105 \times V7) + (-0.0086 \times V8) + (0.0008 \times V9) + (-0.0074 \times V10);$$

Table A1. Definitions for Variables of the PREMM$_5$ Model Equation

Variable	Remark
V0	Sex of patient; V0 = 1 if male, 0 if female
V1 and V2	V1 = patient has presence of one CRC V2 = patient has presence of two or more CRCs
V3	Patient has presence of EC
V4	Patient has presence of other LS-related cancer*
V5	Presence of CRC in FDR and/or SDR = 1 × presence of CRC in 1 FDR (A) + 2 × presence of CRC in 2 or more FDR (B) + 0.5 × presence of CRC in 1 SDR (C) + 1 × presence of CRC in 2 or more SDR (D) Note: Possible values for A through D: 0 and 1; 0 = absent, 1 = present A and B are mutually exclusive; if A is 1, then B must be 0. C and D are mutually exclusive; if C is 1, then D must be 0.
V6	Presence of EC in FDR and/or SDR = 1 × presence of EC in 1 FDR (A) + 2 × presence of EC in 2 or more FDR (B) + 0.5 × presence of EC in 1 SDR (C) + 1 × presence of EC in 2 or more SDR (D) Note: Possible values for A through D: 0 and 1; 0 = absent, 1 = present A and B are mutually exclusive; if A is 1, then B must be 0. C and D are mutually exclusive; if C is 1, then D must be 0.
V7	Presence of other LS-related cancer in FDR or SDR = 1 × presence of other LS related cancer in FDR (E) + 0.5 × presence of other LS related cancer in SDR (F) Note: Possible values for E and F: 0 and 1; 0 = absent, 1 = present. E and F are not mutually exclusive
V8 and V9	Ages at diagnosis refers to the youngest age at diagnosis (in years) for the patient and/or relatives with diagnosis. For V8, three ages at CRC diagnosis are summed, such that: V8 = (minimum age of CRC in the proband − 45) + (minimum age of CRC in a FDR − 45) + (minimum age of CRC in an SDR − 45) For V9, three ages at EC diagnosis are summed, such that V9 = (minimum age of EC in the proband − 45) + (minimum age of EC in a FDR − 45) + (minimum age of EC in an SDR − 45) If a patient or relative had a given diagnosis but the age at diagnosis was unknown, then the age at diagnosis should be estimated. NOTE: 1. If no age is entered, the model defaults to age at diagnosis of 45 years. This will happen in the following cases: a) If the patient and/or relatives are unaffected, the model defaults to age at diagnosis of 45 years, and b) If no age is entered for an affected proband and/or relative, the model defaults to age at diagnosis of 45 years 2. The following age limits (upper and lower) must be applied, when necessary, before the composite V8 and V9 variables, described earlier in this table, are created. If the reported ages for the patient, FDRs, and/or SDRs are less than or greater than the following age limits, the ages must be corrected to the values shown in Appendix Table A2.
V10	Current age, in years, of proband: Minimum, 22 Maximum, 83

Abbreviations: CRC, colorectal cancer; EC, endometrial cancer; FDR, first-degree relative; LS, Lynch syndrome; SDR, second-degree relative.
*Other LS-related cancers include cancer of the stomach, ovary, urinary tract/bladder/kidney, small intestine, pancreas, bile ducts, brain, and sebaceous gland.

Table A2. Ages at Diagnoses of CRC and EC in Calculation of Overall Risk Estimate

Diagnosis by Type and Relation	Lower and Upper Age Limits (years) for Overall Risk Estimate
CRC	
Patient	27, 77
FDR	28, 81
SDR	30, 85
EC	
Patient	31, 67
FDR	28, 69
SDR	34, 70

NOTE. If the reported age at diagnosis of CRC or EC is less than or greater than the lower and upper age limits, respectively, then the age of diagnosis should be corrected to that age given in the table. After any age adjustments are made to incorporate the upper and lower age limits, the corrected age(s) for the patient, FDR, and/or SDR can be applied to derive the V8 and V9 summation variables.
Abbreviations: CRC, colorectal cancer; EC, endometrial cancer; FDR, first-degree relative; SDR, second-degree relative.

Table A3. Age of Diagnosis Among Gene Mutation Carriers and Noncarriers in the Development Cohort

Cancer Type	Mean ± SD Youngest Age (years) of Diagnosis by Gene Mutation						*P*
	Noncarrier (n=17,734)	*MLH1* (n = 306)	*MSH2* (n = 354)	*MSH6* (n = 177)	*PMS2* (n = 141)	*EPCAM* (n = 22)	
Proband							
CRC	49.3 ± 12.9	40.7 ± 9.5	42.1 ± 9.1	46.7 ± 9.9	47.5 ± 13.7	44.4 ± 9.4	< .001
Endometrial cancer	48.9 ± 13.3	47.0 ± 7.6	45.7 ± 8.1	52.9 ± 7.3	51.4 ± 11.7	N/A	.034
Other LS cancers*	51.2 ± 13.7	49.5 ± 10.1	47.0 ± 9.4	51.9 ± 10.3	49.0 ± 14.9	38.0 ± 2.8	.94
Relatives							
FDR							
CRC	53.7 ± 13.4	42.9 ± 10.2	44.6 ± 11.2	53.1 ± 13.8	50.4 ± 13.3	47.2 ± 7.2	< .001
Endometrial cancer	47.1 ± 14.2	51.3 ± 12.8	50.4 ± 11.0	53.1 ± 11.1	44.0 ± 11.2	45.5 ± 6.4	.097
Other LS cancers*	54.6 ± 15.2	51.1 ± 12.0	51.6 ± 13.6	59.8 ± 13.2	60.1 ± 13.6	53.5 ± 17.6	< .001
SDR							
CRC	57.4 ± 13.8	46.3 ± 13.5	47.3 ± 13.7	54.7 ± 13.0	56.2 ± 11.0	48.7 ± 9.7	< .001
Endometrial cancer	50.2 ± 14.1	48.6 ± 8.3	45.6 ± 10.7	53.9 ± 12.7	50.8 ± 13.2	N/A	1.0
Other LS cancers*	57.0 ± 14.6	52.5 ± 13.1	52.5 ± 13.6	60.8 ± 12.1	57.3 ± 13.5	59.8 ± 1.8	< .001
FDR + SDR							
CRC	53.4 ± 13.3	41.6 ± 11.6	43.6 ± 12.7	51.8 ± 13.0	52.0 ± 12.6	44.3 ± 7.3	< .001
Endometrial cancer	48.0 ± 14.2	50.0 ± 12.0	49.5 ± 11.3	52.3 ± 10.6	47.1 ± 13.0	45.5 ± 6.4	.86
Other LS cancers*	54.7 ± 5.0	50.8 ± 12.6	51.3 ± 13.7	59.5 ± 12.6	58.1 ± 14.1	50.6 ± 11.5	< .001

Abbreviations: CRC, colorectal cancer; LS, Lynch syndrome; FDR, first-degree relative; N/A, not applicable; SD, standard deviation; SDR, second-degree relative.
*Other LS cancers include cancers in the kidney, ureter, bladder, brain, biliary tract, stomach, small intestine, ovary, pancreas, and sebaceous neoplasms.

Table A4. Clinical Characteristics of Individuals With Colorectal Cancer in the External Validation Cohort

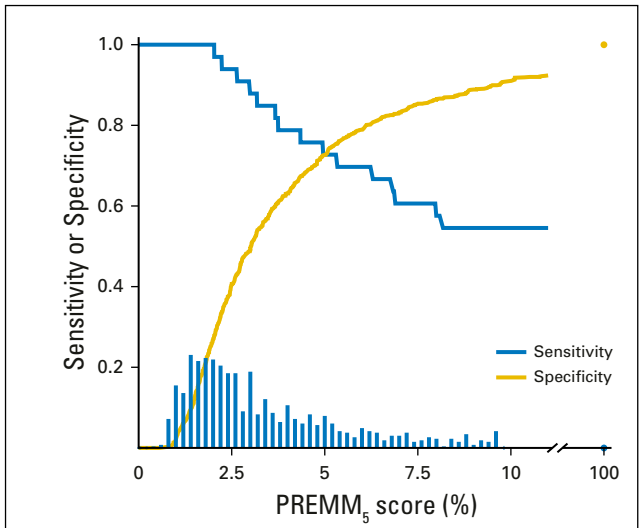
Characteristic	No. of Patients	% of Patients
Sex		
Male	587	55.5
Female	471	44.5
Race/ethnicity		
Non-Hispanic white	939	88.8
Non-Hispanic black	50	4.7
Hispanic/Latino	27	2.6
Asian	22	2.1
Other/multiple	8	0.8
Not reported	12	1.1
Age at first CRC diagnosis, years		
Mean (SD)	55.7	12.6
< 30	14	1.3
30-39	90	8.5
40-49	232	21.9
50-59	327	30.9
60-69	252	23.8
70-79	103	9.7
≥ 80	40	3.8
MSI/MMR IHC status (n = 572 available)		
MSI-H/MMR-D	70	12.2
MSI-L/MMR-P	13	2.3
MSS/MMR-P	489	85.5
Personal cancer history		
> 1 CRC	29	2.7
EC (among women only)	3	0.3
Other LS cancers	30	2.8
Family cancer history		
≥ 1 FDR with CRC	135	12.8
≥ 1 SDR with CRC	202	19.1
≥ 1 FDR with EC	25	2.4
≥ 1 SDR with EC	23	2.2
≥ 1 FDR with other Lynch cancer	137	12.9
≥ 1 SDR with other Lynch cancer	141	13.3
No family history	3	0.3

Abbreviations: CRC, colorectal cancer; EC, endometrial cancer; FDR, first-degree relative; IHC, immunohistochemistry; LS, Lynch syndrome; MMR-D, mismatch repair deficient; MMR-P, mismatch repair proficient; MSI, microsatellite instability; MSS, microsatellite stability; SD, standard deviation SDR, second-degree relative.

Table A5. Discrimination of PREMM$_5$ for Any MMR Gene and by Specific Gene

Gene by Cohort	AUC	95% CI
Development cohort*		
Any MMR gene	0.81	0.79 to 0.83
MLH1	0.89	0.87 to 0.91
MSH2/EPCAM	0.84	0.82 to 0.86
MSH6	0.76	0.73 to 0.79
PMS2	0.64	0.60 to 0.68
Validation cohort†		
Any MMR gene	0.83	0.75 to 0.92

Abbreviations: AUC, area under the receiver operating characteristic curve, corrected for optimism by bootstrap resampling; MMR, mismatch repair.
*Performance evaluated on the cohort used to develop the final model (n = 18,734). AUC estimates with the development cohort were corrected for optimism by bootstrap with 200 iterations.
†Performance evaluated on the independent validation cohort (n = 1,058).



TP	33	31	24	20	18	0
TN	0	416	745	875	933	1,025
FP	1,025	609	280	150	92	0
FN	0	2	9	13	15	33
Sensitivity	1	0.939	0.727	0.606	0.545	0
Specificity	0	0.406	0.727	0.854	0.91	1
NNT	32.061	20.645	12.667	8.5	6.111	
PPV	0.031	0.048	0.079	0.118	0.164	NaN
NPV	NaN	0.995	0.988	0.985	0.984	0.969

Fig A1. Performance characteristics of $PREMM_5$: validation cohort. FN, false negative; FP, false positive; NNT, number needed to test; NPV, negative predictive value; PPV, positive predictive value; TN, true negative; TP, true positive.

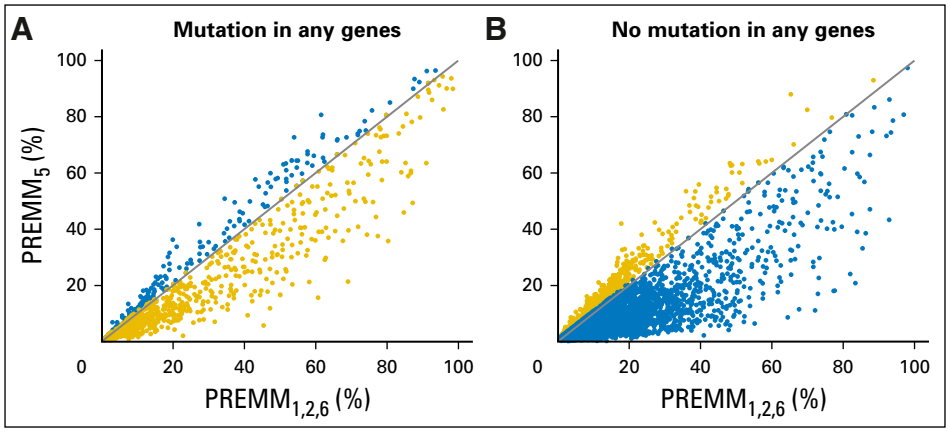


Fig A2. Reclassification plots for the comparison of $PREMM_{1,2,6}$ with $PREMM_5$. The scatterplot diagrams show the added predictive value provided by the $PREMM_5$ model in identification of (A) mutation carriers (mutation in any gene) and (B) noncarriers (no mutation in any gene) on the basis of the data from this study. (A) $PREMM_5$ predicts mutation carriers, as shown by patients (blue circles) above the line. This is regardless of the mutation carrier status of the patient. (B) Compared with $PREMM_{1,2,6}$, the extended $PREMM_5$ model better predicts noncarriers, as shown by more patients (blue circles) below the line of identity (diagonal line). Gold circles indicate reclassification by $PREMM_5$, in which, on average, the extended model provided lower risk estimates than $PREMM_{1,2,6}$.