

Supplementary Materials

Description of Lynch syndrome Prediction Models

MMRPredict: The MMRPredict model (13) was developed using data from unselected patients with colorectal cancer (CRC) diagnosed under the age of 55 years and used logistic regression to estimate the overall probability of having an *MLH1*, *MSH2*, or *MSH6* mutation. Of the 875 subjects included, 38 gene mutation carriers were identified (15 *MLH1*, 16 *MSH2*, 7 *MSH6*). The model was validated by the investigators in an independent, retrospective series of patients diagnosed with CRC before the age of 45 years. The model provides an overall likelihood of carrying any one of the three MMR genes but not gene-specific estimates. Predictors in the model include the patient's age at CRC diagnosis, gender, location of tumor (proximal versus distal), multiple CRCs (synchronous or metachronous), and presence and age(s) of CRC and/or endometrial cancer diagnosis limited to FDRs. No extracolonic Lynch Syndrome related cancers are evaluated for in the proband and CRC in the proband is necessary to obtain a risk estimate. Molecular tumor testing results are included in the prediction estimates. The model calculates the overall risk estimate in two stages; the first stage pertains to clinical information and the second stage incorporates MSI and IHC testing results to refine the risk estimate obtained from the first stage.

PREMM_{1,2,6}: The PREMM_{1,2,6} model (14) was developed using genotype and phenotypic data from 4539 individuals who underwent genetic testing based on either personal or family history of cancer. Using multivariable polytomous logistic regression, the PREMM_{1,2,6} model provides estimates on the overall probability and gene-specific probability of having an *MLH1*, *MSH2*, or *MSH6* gene mutation and included 525 mutation carriers (204 *MLH1*, 250 *MSH2*, 71 *MSH6*). The model also provides gene-specific mutation probabilities. The model has been externally validated in 1827 clinic and population-based CRC cases enrolled through the Colon Cancer Family Registries. Proband specific variables include gender, the occurrence and age at CRC diagnosis, endometrial cancer and other Lynch syndrome-associated cancers, including cancers of the ovary, stomach, kidney, ureter, bile duct, small bowel, brain (glioblastoma multiforme), pancreas, or sebaceous gland. Variables related to the relatives were limited to FDR and SDR cancer histories and include the number of relatives with CRC, endometrial cancer, or other Lynch syndrome-associated cancers (coded as 0, 1, 2+) as well as the minimum age at diagnosis of each cancer among relatives. Molecular tumor testing results are not included in the prediction estimates.

MMRPro: The MMRPro model (12) was developed using published values of prevalence and penetrance of *MLH1*, *MSH2*, and *MSH6* gene mutations. Through a sophisticated Bayesian approach, MMRPro provides estimates on the overall probability of carrying any MMR gene mutation, as well as gene-specific risk

estimates. Independent, external validation was performed by the investigators involved in its development using data from 279 patients enrolled through familial cancer registries where the mutation prevalence was 43% with 51 *MLH1*, 63 *MSH2*, and 7 *MSH6* gene mutation carriers. Data for the proband and for each FDR and SDR include age at diagnosis of CRC, endometrial cancer, and current age or age at last follow-up for those relatives unaffected by these cancers. The MMRPro model does not include the presence of multiple CRCs in the proband and other than endometrial cancer it does not include other Lynch Syndrome associated malignancies. Molecular tumor testing results are included in the prediction estimates. The model calculates the overall risk of carry any one of the three MMR genes and provides gene-specific risk estimates as well.

Evaluation for Lynch syndrome by participating sites in the Clinic and Population-based cohorts

All participants provided informed consent for inclusion through their respective sites/registries which were approved by the Institutional Review Boards at each of the institutions. Approval for the validation study to compare the three prediction models was obtained by the IRBs at Columbia University Medical Center and Dana-Farber Cancer Institute.

a. CLINIC-BASED COHORTS

Colon Cancer Family Registries

Detailed information about the CCFR can be found at <http://epi.grants.cancer.gov/CFR/> (16). Three centers recruited families with multiple or early-onset cases of CRC through clinical settings: Mayo Clinic, USC (Cleveland Clinic subcenter), and Australasia (seven family cancer clinics across Australia and New Zealand). Clinic-based probands, defined as the first family member enrolled in the CCFR, may or may not have had a personal history of CRC. Instead, eligibility was based on one or more of the following criteria: two or more relatives with a personal history of CRC or Lynch syndrome cancer, a proband diagnosed with CRC at a young age, or a proband presenting at a clinic with Lynch syndrome or Lynch-like syndrome. MMR testing was performed in all probands recruited through any of the clinic-based Colon CFR registries and was not dependent upon molecular tumor testing results.

Dana Farber Cancer Institute

Subjects were referred to the Dana Farber Cancer Institute's Gastrointestinal Cancer Genetics and Prevention Program for genetic evaluation based on personal or family history of cancer suggestive of an inherited CRC syndrome. DNA mutational analysis was based on either the personal and family cancer history, fulfillment of clinical criteria, and/or molecular tumor testing, including microsatellite instability (MSI) or immunohistochemical (IHC) testing for MMR protein expression. Tumor testing results were not used exclusively to select patients for MMR mutational analyses.

Milan, Italy

Subjects were referred to the Fondazione IRCCS Istituto Nazionale dei Tumori for genetic evaluation based on a personal or family history of CRC suggestive of an inherited cancer syndrome. Individuals who met clinical criteria for Lynch syndrome including Bethesda Guidelines or Amsterdam or Amsterdam II criteria underwent DNA mutational analysis. Molecular tumor testing was not used to select patients for germline testing.

Newfoundland, Canada

All subjects with CRC who were referred for genetic evaluation had tumors analyzed for MSI and IHC testing for MMR protein expression, irrespective of whether or not clinical criteria for Lynch syndrome were met. Tumors with MSI-High and/or MLH1 deficiency were tested for evidence of MLH1 promoter methylation and BRAF mutation status. DNA mutational analysis on patients with MLH1-deficient tumors was restricted to those without evidence of MLH1 methylation in their tumor. DNA mutational analysis was otherwise directed by which MMR protein was deficient on IHC, including MSH2, MSH6, and PMS2. Patients with microsatellite stable (MSS), IHC-intact tumors were not tested for MMR gene mutations and classified as non-carriers.

Rotterdam, the Netherlands

All probands with CRC referred for genetic evaluation based on age of CRC diagnosis or family cancer history suggestive of Lynch syndrome, underwent molecular tumor testing including MSI and IHC testing for MMR protein expression at Erasmus MC, Rotterdam, the Netherlands (17). Additional tumor testing, including both BRAF and MLH1 hypermethylation testing, was conducted in cases with loss of MLH1 protein expression on immunostaining or when tumor was MSI-high but IHC-intact. If the tumor was found to be MSS or with intact IHC, DNA mutational analysis was not performed and the subject was classified as a non-carrier. Germline testing was conducted in cases with loss of IHC expression and was guided by which MMR protein was deficient by IHC, and in the absence of suspected MLH1 hypermethylation (10).

Hereditary Cancer Group of the Spanish Medical Oncology Society (SEOM)

A clinic-based cohort was comprised of data collected from 12 hospitals in Spain that offer genetic evaluation for inherited CRC syndromes. All subjects with CRC had molecular tumor testing which included both MSI and IHC testing for MLH1, MSH2 and MSH6 protein expression. Subsequent germline genetic testing was conducted based on the results of molecular tumor testing. If tumors were MSS and IHC-intact, genetic testing was not conducted and patients were classified as non-carriers. If there was evidence of MSI or loss of protein expression on IHC testing for any of the MMR genes, germline genetic testing was performed and directed by the results from IHC testing.

b. POPULATION-BASED COHORTS

Colon Cancer Family Registries

Molecular tumor testing was conducted for all patients with CRC, including MSI and IHC testing (16). Germline testing was conducted in all probands with abnormal molecular tumor testing including high or low levels of MSI or loss of normal protein expression of MLH1, MSH2, MSH6, and PMS2 on IHC. All subjects with MSI-H

or MSI-low tumours had IHC testing. Because of the low frequency of absent protein staining in MSS cases, some CCFR centers did not perform IHC testing on all MSS cases. Subjects with MSS and/or IHC-intact tumors were classified as non-carriers.

Ohio State University Comprehensive Cancer Center

Subjects with newly diagnosed CRC, regardless of age or the presence or absence of a family history of cancer, were recruited at one of six participating hospitals in the Columbus, Ohio, metropolitan area (1). All tumors were analyzed for MSI and those individuals with MSI tumors underwent IHC testing for MLH1, MSH2, MSH6, and PMS2 protein expression, and methylation analysis of the *MLH1* promoter region with subsequent DNA mutation analysis for MLH1, MSH2, and MSH6 genes, when indicated. Selected tumors that showed the presence of the MLH1 protein and the absence of the PMS2 protein were analyzed for mutations in the *PMS2* gene. For subjects who were at high risk for Lynch syndromes but had MSS tumors, IHC testing was conducted for expression of the MLH1, MSH2, and MSH6 proteins. These patients were deemed high risk on the basis of one or more of the following criteria: a diagnosis of CRC before the age of 50 years, a diagnosis of synchronous or metachronous colorectal or endometrial cancer, and the presence of a first-degree relative with colorectal or endometrial cancer diagnosed at any age.

Newfoundland Colorectal Cancer Registry (NFCCR)

All consecutive subjects with CRC diagnosed at age 75 years or less underwent molecular tumor testing including MSI analysis, IHC testing for MMR protein expression, and when indicated, MLH1 hypermethylation and BRAF testing (18). A similar approach to that described for the clinic-based cases recruited through the Newfoundland genetics clinics was taken but without selection based on family history, fulfilment of clinical criteria for Lynch syndrome or young age of CRC diagnosis. DNA mutational analysis was conducted for cases with MSH2, MSH6 or PMS2 deficient tumors on IHC testing and cases whose tumors were MLH1 deficient but lacked *MLH1* promoter methylation, and all of which were MSI-H. Those subjects whose tumors did not display MSI or loss of IHC MMR protein expression were classified as non-carriers.

Rotterdam, the Netherlands

Molecular tumor testing was conducted for all population-based cases recruited through the LIMO study, a prospective, multicenter study which enrolled consecutive subjects with newly diagnosed CRC at age ≤ 70 years from one of 11 Dutch hospitals (10). MSI analysis and IHC testing for MMR protein expression of MLH1, MSH2, MSH6, and PMS2, were performed in all patients. In tumors with MSI and absent MLH1 protein expression, hypermethylation of the MLH1 promoter and BRAF alterations was evaluated. DNA mutational

analysis was performed in cases where tumor diagnostic testing revealed MMR deficiency; subjects whose tumors were without MMR deficiency were classified as non-carriers.

Spanish Consortium: EpiCOLON

Subjects with newly diagnosed CRC presenting to any one of 25 hospitals participating in the EpiCOLON study underwent molecular tumor testing for the evaluation of Lynch syndrome (19). Participating sites prospectively recruited consecutive, unselected CRC cases, to determine the incidence of Lynch syndrome in Spain. MSI and IHC testing for DNA mismatch repair proteins associated with Lynch syndrome were performed in all patients regardless of age, or personal or family history. Subjects whose tumors displayed MSI and/or lack of protein expression of any MMR genes on IHC underwent germline genetic testing which was often directed by IHC testing results. Those with MSS and IHC-intact tumors did not undergo DNA mutational analysis and were classified as non-carriers.

Description of Net Benefit Analysis

The mathematical background to the concept and calculation of net benefit analysis dates back to a publication in *Science* by Peirce in 1884 (Peirce CS). More recent work has expanded on Pierce's idea of how to summarize the quality of predictions in a single metric (Baker SG, et al., 23). In addition, net benefit and related measures such as relative utility are gaining in popularity in the medical literature; a landmark paper by Vickers in 2006 has near 400 citations and a review on statistical and decision analytic approaches has over 500 (23, 25). A more recent, very readable Editorial in the *Annals of Internal Medicine* discusses why net benefit type of measures should more widely be used, beyond measures such as AUC in the assessment of prediction models (26).

Net benefit analyses take into account the clinical implications related to model predictions. This methodology incorporates the harms and benefits of clinical decisions based on the model predictions, i.e. the relative weight of a false-positive classification (harm of unnecessary testing) versus a true-positive classification (benefit of finding a mutation carrier). This relative weight is reflected in the risk threshold that is used to make classifications as high risk versus low risk. The risk threshold is the absolute risk for carrying a germline MMR gene mutation at which one might choose to undergo genetic testing. Discrimination, calibration, sensitivity, and specificity alone are insensitive to clinical consequences. These predictive measures focus purely on the mathematical accuracy of the models. Net benefit is larger for more discriminating models, but is decreased by poor calibration of risk predictions (Van Calster B, et al).

Decision curves plot the net benefit of the risk prediction model versus risk thresholds. The net benefit is the total number of true-positive classifications minus the total number of false-positive classifications. The latter are weighted by the odds of the risk threshold for a proper calculation of net benefit. The weighting converts a false-positive classification into the same units as the true-positive classification; therefore the net benefit is interpreted as the number of true-positive classifications adjusted for the detrimental effect of false-positive classifications.

We emphasize that the decision threshold is logically determined by the relative weight of false-positive vs true-positive classifications (Peirce CS, 23). In our case, we consider thresholds between 5% and 20% as a plausible range to consider for the selection of subjects for mutation analysis.

The decision curve compares the net benefit of using a prediction model with two alternative strategies. The first is testing no one, which has a net benefit of zero, since no true or false positives arise. The second natural alternative is testing all. This is optimal for a threshold of 0%, which implies we do not care about false-positive

classifications (harm to benefit ratio is 0). The net benefit is then equal to the event rate (the mutation prevalence). If we use a higher threshold, e.g. 5%, we weight false positives as 1/19 of true positives. Since we still classify all as positive, the sum of $TP - 1/19*FP$ is necessary smaller than $TP - 0*FP$. At the event rate, the sum is zero: $TP - (TP/FP)*FP = 0$. This behavior of the test all reference line is hence mathematically defined.

The net benefit of a prediction model depends on the combination of discrimination and calibration, and should be higher than any of the reference lines (test none, test all). The difference between the net benefit of a model and the highest reference line can be interpreted as the extra number of true positives (mutations detected) without increasing the number of false-positives (unnecessary testing). This increase in net benefit will be higher for better discriminating models, and for decision thresholds closer to the event rate (mutation prevalence). The impact of poor calibration on the clinical utility of a prediction model depends on the type of miscalibration, the level of discrimination, and the adopted risk threshold (Van Calster B, et al). Miscalibration may result in a clinically harmful model, i.e. when net benefit drops below one of the default strategies of classifying all patients as positive or as negative mutation carriers. There are two situations in which this undesirable result can be observed:

1. when models underestimate risk at a threshold below the event rate, and
2. when models overestimate risk at a threshold above the event rate.

1. Peirce CS. The Numerical Measure of the Success of Predictions. *Science*. 1884; 4: 453-454.
2. Baker SG, Cook NR, Vickers A, Kramer BS. Using relative utility curves to evaluate risk prediction. *J R Stat Soc A*. 2009; 172:729–748.
3. Van Calster B, Vickers AJ. Calibration of Risk Prediction Models: Impact on Decision-Analytic Performance. *Med Decis Making* 2015;35:162–169.

Supplementary Table 1a & 1b. Site-specific performance characteristics of MMRPro, PREMM_{1,2,6}, and MMRPredict for prediction of any MMR gene mutation among colorectal cancer cases

Supplementary Table 1a. Clinic-based cohort

	MMRPro	PREMM_{1,2,6}	MMRPredict
<i>DISCRIMINATION</i>			
AUC (95% CL)			
CCFR	0.85 (0.82, 0.89)	0.84 (0.81, 0.88)	0.78 (0.73, 0.82)
DFCI	0.83 (0.77, 0.89)	0.85 (0.79, 0.90)	0.78 (0.71, 0.85)
Milan, Italy	0.72 (0.65, 0.78)	0.79 (0.73, 0.85)	0.72 (0.65, 0.78)
Newfoundland, Canada	0.87 (0.76, 0.98)	0.91 (0.84, 0.98)	0.86 (0.76, 0.96)
Rotterdam, Netherlands	0.77 (0.70, 0.84)	0.79 (0.73, 0.86)	0.65 (0.57, 0.73)
Spanish Consortium	0.85 (0.81, 0.89)	0.85 (0.82, 0.89)	0.84 (0.79, 0.88)
<i>CALIBRATION</i>			
O/E ratio (95% CL)			
CCFR	0.61 (0.43, 0.86)	1.0 (0.79, 1.3)	0.29 (0.21, 0.39)
DFCI	0.57 (0.33, 1.0)	1.1 (0.73, 1.6)	0.30 (0.18, 0.49)
Milan, Italy	1.8 (1.3, 2.5)	1.2 (0.82, 1.7)	0.51 (0.31, 0.84)
Newfoundland, Canada	0.35 (0.14, 0.86)	0.74 (0.40, 1.4)	0.13 (0.07, 0.26)
Rotterdam, Netherlands	0.51 (0.35, 0.76)	0.69 (0.50, 0.94)	0.20 (0.14, 0.29)
Spanish Consortium	0.48 (0.35, 0.67)	1.2 (0.96, 1.5)	0.83 (0.63, 1.1)
Slope (95% CL)			
CCFR	0.39 (0.32, 0.46)	0.71 (0.57, 0.84)	0.28 (0.22, 0.34)
DFCI	0.39 (0.28, 0.50)	0.73 (0.52, 0.94)	0.28 (0.19, 0.38)
Milan, Italy	0.38 (0.23, 0.52)	0.52 (0.36, 0.68)	0.20 (0.12, 2.7)
Newfoundland, Canada	0.50 (0.29, 0.71)	1.35 (0.79, 1.9)	0.68 (0.35, 1.0)
Rotterdam, Netherlands	0.42 (0.31, 0.54)	0.91 (0.67, 1.1)	0.17 (0.09, 0.26)
Spanish Consortium	0.53 (0.44, 0.62)	1.0 (0.86, 1.2)	0.61 (0.5, 0.72)

AUC: area under the receiver operating characteristic curve; CL: confidence limits;
O/E: observed/expected; CCFR=Colon Cancer Family Registries; DFCI=Dana Farber Cancer Institute.

Supplementary Table 1b. Population-based cohort

	MMRPro	PREMM_{1,2,6}	MMRPredict
<i>DISCRIMINATION</i>			
AUC (95% CL)			
CCFR	0.83 (0.78, 0.88)	0.87 (0.82, 0.92)	0.86 (0.81, 0.90)
OSU	0.97 (0.93, 1.00)	0.95 (0.87, 1.00)	0.94 (0.87, 1.00)
Newfoundland, Canada	0.86 (0.80, 0.92)	0.88 (0.81, 0.94)	0.76 (0.66, 0.85)
Rotterdam, Netherlands	0.75 (0.62, 0.88)	0.80 (0.69, 0.91)	0.75 (0.64, 0.85)
Spanish Consortium	-	0.74 (0.53, 0.95)	0.78 (0.60, 0.95)
<i>CALIBRATION</i>			
O/E ratio (95% CL)			
CCFR	0.26 (0.18, 0.37)	0.79 (0.60, 1.04)	1.01 (0.73, 1.40)
OSU	0.29 (0.12, 0.68)	0.32 (0.17, 0.59)	0.09 (0.05, 0.17)
Newfoundland, Canada	1.09 (0.50, 2.36)	1.51 (0.91, 2.49)	0.56 (0.26, 1.21)
Rotterdam, Netherlands	0.71 (0.36, 1.42)	1.13 (0.67, 1.93)	0.52 (0.23, 1.19)
Spanish Consortium	-	0.28 (0.15, 0.54)	0.06 (0.03, 0.12)
<i>SLOPE</i>			
Slope (95% CL)			
CCFR	0.45 (0.36, 0.53)	0.99 (0.82, 1.16)	0.55 (0.45, 0.65)
OSU	0.78 (0.52, 1.04)	1.73 (1.14, 2.32)	0.78 (0.48, 1.09)
Newfoundland, Canada	0.30 (0.19, 0.41)	0.73 (0.46, 1.01)	0.23 (0.14, 0.33)
Rotterdam, Netherlands	0.44 (0.24, 0.64)	1.01 (0.55, 1.47)	0.21 (0.09, 0.34)
Spanish Consortium	-	0.97 (0.55, 1.39)	0.50 (0.27, 0.74)

AUC: area under the receiver operating characteristic curve; CL: confidence limits;
O/E: observed/expected; CCFR=Colon Cancer Family Registries; OSU=Ohio State University.

Supplementary Table 2a & 2b. Site-specific performance characteristics of MMRPro and PREMM_{1,2,6} for prediction of gene-specific MMR gene mutations associated with Lynch syndrome among colorectal cancer cases

Supplementary Table 2a. Clinic-based cohort

	MMRPro			PREMM _{1,2,6}		
	<i>MLH1</i>	<i>MSH2</i>	<i>MSH6</i>	<i>MLH1</i>	<i>MSH2</i>	<i>MSH6</i>
DISCRIMINATION						
AUC (95% CL)						
CCFR	0.88 (0.84, 0.91)	0.87 (0.83, 0.90)	0.60 (0.42, 0.78)	0.87 (0.83, 0.91)	0.86	0.74 (0.59, 0.88)
DFCI	0.88 (0.82, 0.95)	0.85 (0.78, 0.91)	0.44 (0.18, 0.71)	0.92 (0.87, 0.97)	0.85 (0.78, 0.92)	0.63 (0.44, 0.82)
Milan, Italy	0.75 (0.68, 0.83)	0.71 (0.63, 0.80)	0.56 (0.40, 0.72)	0.84 (0.78, 0.91)	0.79 (0.71, 0.87)	0.52 (0.31, 0.73)
Newfoundland, Canada	0.84 (0.69, 0.99)	0.88 (0.75, 1.0)	0.82 (0.75, 0.90)	0.99 (0.96, 1.0)	0.91 (0.83, 0.99)	0.51 (0.41, 0.60)
Rotterdam, Netherlands	0.90 (0.82, 0.98)	0.79 (0.69, 0.89)	0.59 (0.45, 0.73)	0.82 (0.73, 0.91)	0.83 (0.74, 0.92)	0.71 (0.57, 0.84)
Spanish Consortium	0.88 (0.84, 0.93)	0.83 (0.77, 0.90)	0.69 (0.49, 0.88)	0.87 (0.82, 0.91)	0.87 (0.81, 0.93)	0.65 (0.49, 0.82)
CALIBRATION						
O/E ratio (95% CL)						
CCFR	0.46 (0.30, 0.71)	0.51 (0.34, 0.76)	0.17 (0.08, 0.37)	0.89 (0.63, 1.3)	0.97 (0.70, 1.3)	1.1 (0.59, 2.1)
DFCI	0.44 (0.23, 0.87)	0.45 (0.23, 0.88)	0.24 (0.08, 0.77)	0.95 (0.56, 1.6)	0.97 (0.56, 1.7)	1.5 (0.61, 3.5)
Milan, Italy	1.5 (0.93, 2.4)	1.6 (1.0, 2.5)	0.92 (0.38, 2.3)	1.0 (0.63, 1.7)	0.97 (0.60, 1.6)	1.5 (0.72, 3.3)
Newfoundland, Canada	0.13 (0.03, 0.52)	0.38 (0.14, 1.0)	0.07 (0.01, 0.60)	0.36 (0.11, 1.2)	1.0 (0.51, 2.0)	0.52 (0.07, 3.8)
Rotterdam, Netherlands	0.33 (0.17, 0.62)	0.38 (0.22, 0.66)	0.65 (0.36, 1.2)	0.61 (0.37, 1.0)	0.51 (0.31, 0.83)	1.4 (0.84, 2.2)
Spanish Consortium	0.45 (0.31, 0.67)	0.38 (0.25, 0.60)	0.11 (0.04, 0.29)	1.4 (1.0, 1.9)	1.1 (0.82, 1.6)	0.65 (0.30, 1.4)
Slope (95% CL)						
CCFR	0.41 (0.31, 0.50)	0.39 (0.31, 0.48)	0.13 (-0.05, 0.29)	0.7 (0.54, 0.86)	0.69 (0.53, 0.84)	0.63 (0.13, 1.14)
DFCI	0.49 (0.33, 0.65)	0.38 (0.23, 0.52)	-0.15 (-0.52, 0.21)	0.90 (0.61, 1.2)	0.62 (0.38, 0.86)	0.40 (-0.33, 1.1)
Milan, Italy	0.41 (0.23, 0.58)	0.33 (0.17, 0.48)	0.10 (-0.25, 0.45)	0.6 (0.41, 0.81)	0.48 (0.31, 0.65)	0.05 (-0.66, 0.76)
Newfoundland, Canada	0.42 (0, 0.84)	0.56 (0.31, 0.80)	0.28 (-0.42, 0.98)	4.7 (-0.93, 10)	1.30 (0.70, 1.9)	-0.27 (-3.9, 3.3)
Rotterdam, Netherlands	0.69 (0.48, 0.90)	0.42 (0.24, 0.61)	0.17 (-0.03, 0.37)	0.92 (0.60, 1.2)	0.91 (0.58, 1.2)	1.2 (0.70, 1.7)
Spanish Consortium	0.58 (0.47, 0.70)	0.51 (0.40, 0.62)	0.23 (-0.05, 0.51)	0.96 (0.76, 1.2)	1.0 (0.80, 1.2)	0.53 (-0.11, 0.82)

AUC: area under the receiver operating characteristic curve; CL: confidence limits; O/E: observed/expected; CCFR=Colon Cancer Family Registries; DFCI=Dana Farber Cancer Institute; NF=Newfoundland.

Supplementary Table 2b. Population-based cohort

	MMRPro			PREMM _{1,2,6}		
	<i>MLH1</i>	<i>MSH2</i>	<i>MSH6</i>	<i>MLH1</i>	<i>MSH2</i>	<i>MSH6</i>
DISCRIMINATION						
AUC (95% CL)						
CCFR	0.84 (0.75, 0.92)	0.91 (0.86, 0.95)	0.58 (0.45, 0.70)	0.90 (0.83, 0.97)	0.91 (0.85, 0.97)	0.69 (0.54, 0.84)
OSU	0.98 (0.94, 1.00)	0.99 (0.97, 1.00)	0.86 (0.71, 1.00)	1.00 (0.99, 1.00)	0.99 (0.96, 1.00)	0.44 (0.05, 0.83)
Newfoundland, Canada	0.86 (0.74, 0.97)	0.91 (0.86, 0.96)	0.74 (0.61, 0.87)	0.91 (0.85, 0.98)	0.93 (0.89, 0.98)	0.70 (0.50, 0.90)
Rotterdam, Netherlands	0.62 (0.36, 0.87)	0.97 (0.93, 1.00)	0.71 (0.54, 0.89)	0.77 (0.57, 0.96)	0.90 (0.82, 0.98)	0.76 (0.57, 0.95)
Spanish Consortium	-	-	-	0.74 (0.53, 0.95)	0.76 (0.55, 0.97)	0.52 (0.30, 0.73)
CALIBRATION						
O/E ratio (95% CL)						
CCFR	0.17 (0.11, 0.29)	0.19 (0.12, 0.30)	0.15 (0.07, 0.30)	0.74 (0.48, 1.14)	0.78 (0.53, 1.15)	0.74 (0.42, 1.32)
OSU	0.09 (0.02, 0.50)	0.33 (0.12, 0.92)	0.17 (0.04, 0.79)	0.15 (0.04, 0.61)	0.46 (0.21, 0.97)	0.21 (0.05, 0.84)
Newfoundland, Canada	0.57 (0.12, 2.82)	0.77 (0.26, 2.30)	0.79 (0.17, 3.59)	1.03 (0.40, 2.65)	1.41 (0.69, 2.89)	2.27 (1.01, 5.13)
Rotterdam, Netherlands	0.43 (0.12, 1.54)	0.34 (0.09, 1.22)	1.08 (0.44, 2.62)	0.89 (0.31, 2.52)	0.57 (0.20, 1.66)	2.55 (1.32, 4.90)
Spanish Consortium	-	-	-	0.99 (0.52, 1.89)	0.80 (0.42, 1.51)	0.83 (0.44, 1.55)
Slope (95% CL)						
CCFR	0.47 (0.35, 0.59)	0.53 (0.42, 0.65)	0.07 (-0.14, 0.28)	0.91 (0.69, 1.12)	0.97 (0.76, 1.18)	0.87 (0.0.30, 1.43)
OSU	0.81 (0.27, 1.36)	0.92 (0.54, 1.29)	0.44 (-0.03, 0.92)	6.85 (-)	1.95 (1.06, 2.83)	-0.92 (-4.52, 2.68)
Newfoundland, Canada	0.25 (0.11, 0.39)	0.32 (0.19, 0.45)	0.17 (0.00, 0.34)	0.59 (0.27, 0.92)	0.70 (0.40, 1.0)	0.83 (0.22, 1.45)
Rotterdam, Netherlands	0.29 (-0.10, 0.69)	0.90 (0.36, 1.44)	0.37 (0.14, 0.61)	0.68 (-0.05, 1.41)	1.02 (0.35, 1.68)	1.54 (0.68, 2.41)
Spanish Consortium	-	-	-	0.95 (0.52, 1.38)	1.07 (0.62, 1.51)	0.68 (-0.27, 1.63)

AUC: area under the receiver operating characteristic curve; CL: confidence limits; O/E: observed/expected; CCFR=Colon Cancer Family Registries; OSU=Ohio State Univeristy; NF=Newfoundland.

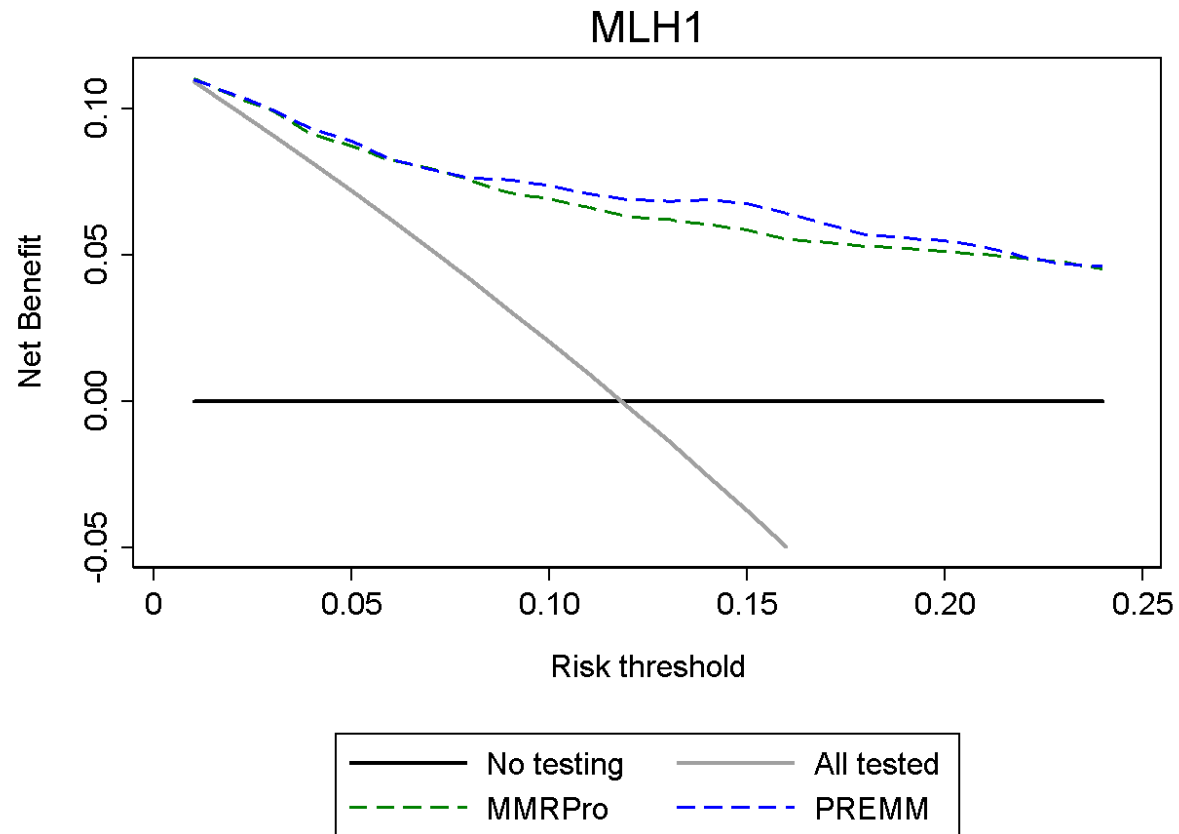
SUPPLEMENTARY FIGURES

Supplementary Figures 1a-c. Net benefit analysis for gene-specific risk estimates comparing MMRPro and PREMM_{1,2,6} among Clinic-based cohort

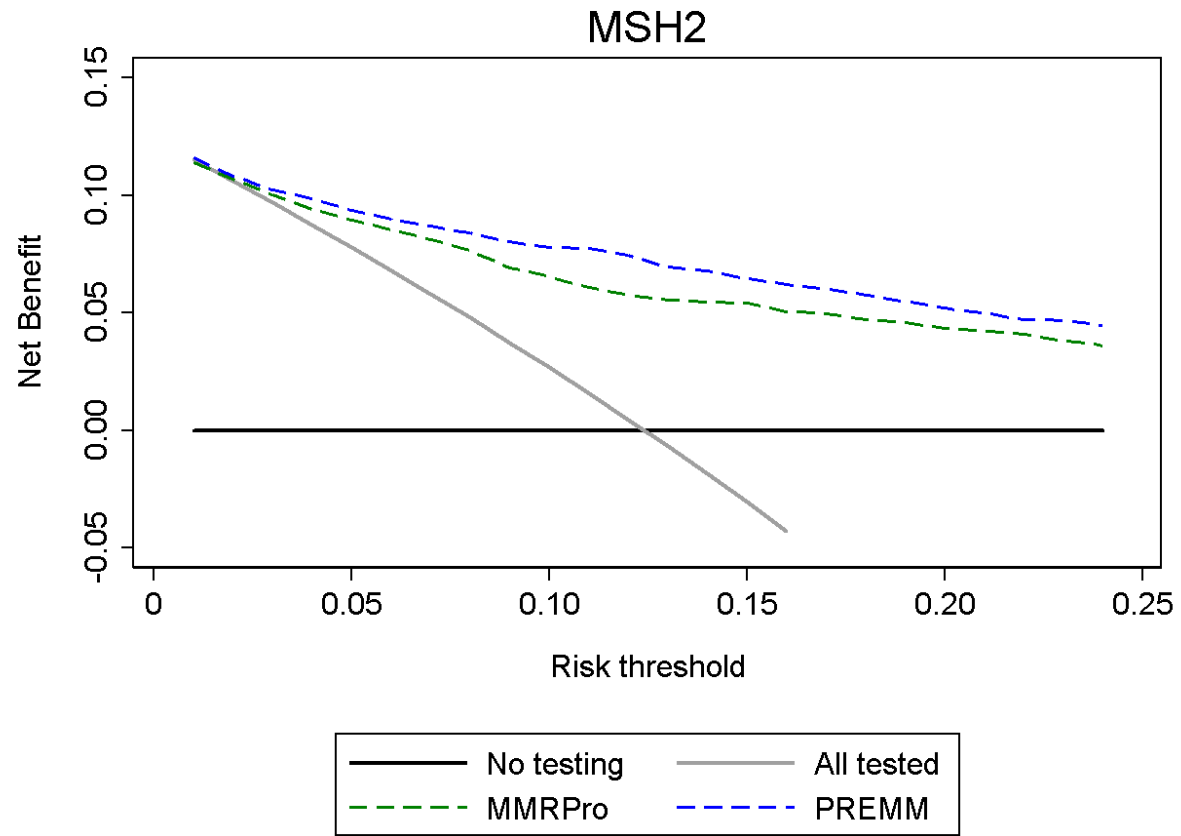
Figures 1a-c display the net benefit curves comparing MMRPro and PREMM_{1,2,6} for each MMR gene (*MLH1*, *MSH2*, and *MSH6*) among the clinic-based cohort. The y-axis measures net benefit which is calculated by summing the benefits (true positives) and subtracting the harms (false positives), where 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 (dashed black line) or no patients (horizontal black line) across the full range of threshold probabilities at which a patient would choose to undergo genetic testing.

Supplementary Figure 1a-c. Net benefit analysis for gene-specific risk estimates comparing MMRPro and PREMM_{1,2,6} among clinic-based cohorts

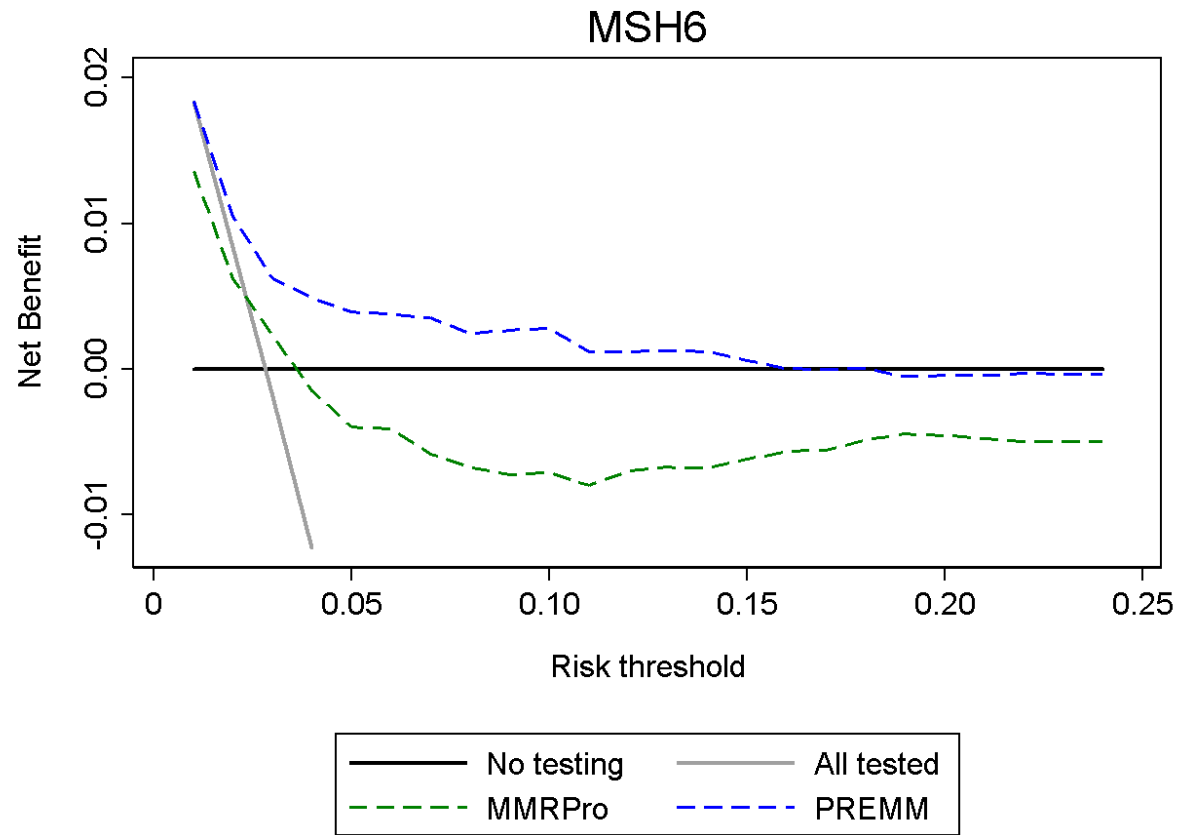
1a.



1b.



1c.

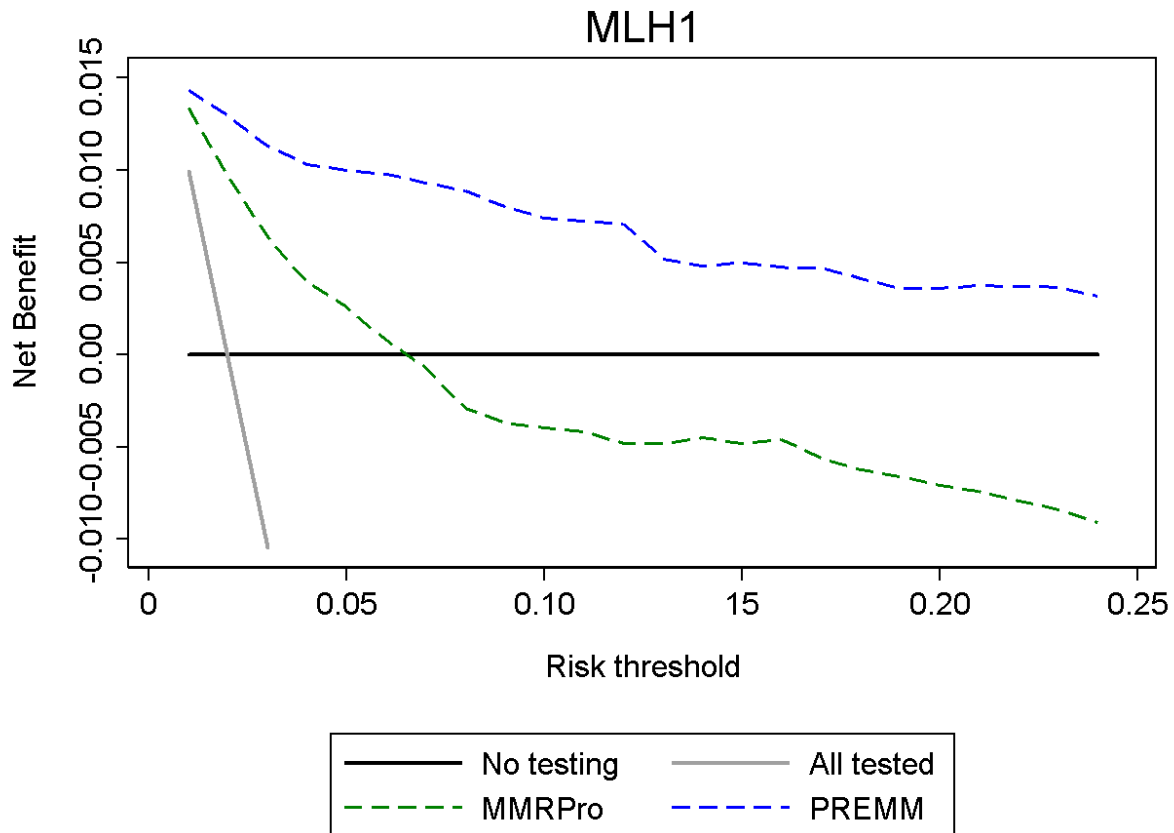


Supplementary Figures 2a-c. Net benefit analysis for gene-specific risk estimates comparing MMRPro and PREMM_{1,2,6} among Population-based cohort

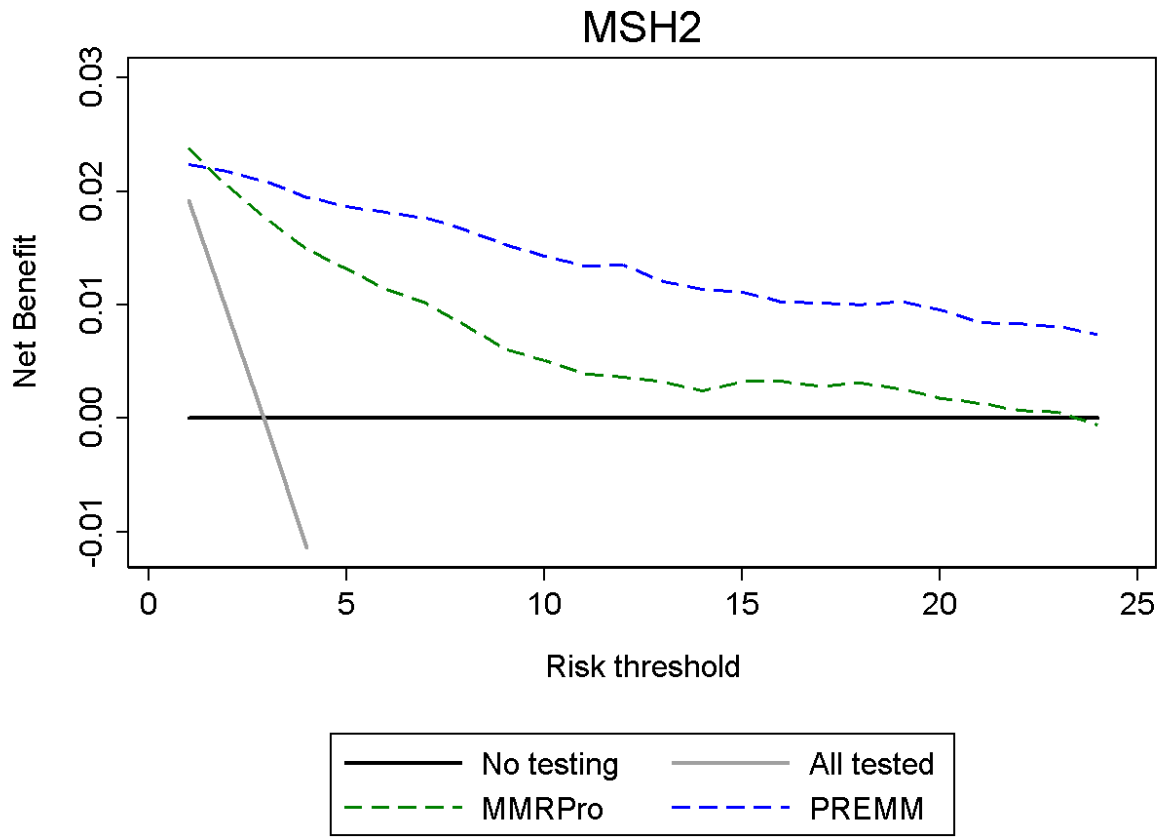
Figures 2a-c display the net benefit curves comparing MMRPro and PREMM_{1,2,6} for each MMR gene (*MLH1*, *MSH2*, and *MSH6*) among the clinic-based cohort. The y-axis measures net benefit which is calculated by summing the benefits (true positives) and subtracting the harms (false positives), where 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 (dashed black line) or no patients (horizontal black line) across the full range of threshold probabilities at which a patient would choose to undergo genetic testing.

Supplementary Figure 2a-c. Net benefit analysis for gene-specific risk estimates comparing MMRPro and PREMM_{1,2,6} among population-based cohorts

2a.



2b.



2c.

