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Prediction of Germline Mutations and Cancer Risk in the Lynch Syndrome

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Abstract

Context—Identifying families at high risk for the Lynch syndrome (ie, hereditary non-polyposis colorectal cancer) is critical for both genetic counseling and cancer prevention. Current clinical guidelines are effective but limited by applicability and cost.

Objective—To develop and validate a genetic counseling and risk prediction tool that estimates the probability of carrying a deleterious mutation in mismatch repair genes *MLH1*, *MSH2*, or *MSH6* and the probability of developing colorectal or endometrial cancer.

Design, Setting, and Patients—External validation of the MMRpro model was conducted on 279 individuals from 226 clinic-based families in the United States, Canada, and Australia (referred between 1993–2005) by comparing model predictions with results of highly sensitive germline

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mutation detection techniques. MMRpro models the autosomal dominant inheritance of mismatch repair mutations, with parameters based on meta-analyses of the penetrance and prevalence of mutations and of the predictive values of tumor characteristics. The model's prediction is tailored to each individual's detailed family history information on colorectal and endometrial cancer and to tumor characteristics including microsatellite instability.

Main Outcome Measure—Ability of MMRpro to correctly predict mutation carrier status, as measured by operating characteristics, calibration, and overall accuracy.

Results—In the independent validation, MMRpro provided a concordance index of 0.83 (95% confidence interval, 0.78–0.88) and a ratio of observed to predicted cases of 0.94 (95% confidence interval, 0.84–1.05). This results in higher accuracy than existing alternatives and current clinical guidelines.

Conclusions—MMRpro is a broadly applicable, accurate prediction model that can contribute to current screening and genetic counseling practices in a high-risk population. It is more sensitive and more specific than existing clinical guidelines for identifying individuals who may benefit from MMR germline testing. It is applicable to individuals for whom tumor samples are not available and to individuals in whom germline testing finds no mutation.

The lynch syndrome (ie, hereditary nonpolyposis colorectal cancer [HNPCC]) is the most common familial colorectal cancer (CRC).[1,2] It can be caused by germline deleterious mutations of DNA mismatch repair (MMR) genes, including *MLH1*,[3,4] *MSH2*,[5] *MSH6*,[6] and several others.[7] Screening for individuals likely to carry a deleterious mutation of these genes has traditionally relied on examination of family history, as per the Amsterdam Criteria,[8–10] and has recently moved toward multistep algorithms combining family history with molecular tumor characteristics such as microsatellite instability (MSI),[11] as per the Bethesda Guidelines.[2,12]

The latter were developed to help recognize those individuals who would potentially benefit from a more detailed molecular diagnostic workup, including MSI and subsequent germline testing. This approach is useful, but not without important limitations. First, MSI tests can only be performed on affected patients for whom a tumor block is available. This limits the preventive usefulness of the approach. Second, the algorithm calls for MSI testing on a large population with colorectal cancer, which increases the cost per mutation carrier detected. Third, for individuals tested with commercial germline testing techniques that have imperfect sensitivity, this approach cannot offer guidance for decision making when no mutation is found.

Extensive knowledge is now available about the Lynch syndrome: the mode of inheritance of the genes is autosomal dominant,[13] prevalence and penetrance have been studied, and their clinical/molecular manifestation in tumors is well characterized. The translation of such knowledge into clinically useful algorithms can benefit greatly from a systematic, quantitative, and objective approach. Application of such an approach to a specific family should use as much clinical and biological information about the pedigree as possible, to provide an individualized assessment of risks. Insurance companies may demand an objective assessment of mutation probability when considering reimbursement for genetic testing, and they often accept a statistical estimate of mutation probability as a justification.

A step in this direction is the Leiden model,[14] which estimates the combined probability of carrying a mutation of an MMR gene using a logistic regression based on 3 variables: fulfillment of the Amsterdam Criteria, mean age of CRC diagnoses, and presence of any endometrial cancers in the family. This model is useful but does not use all available biological knowledge and does not provide risk predictions.

The translational goals described above are more fully achieved by formulating an explicit genetic model,[15] as was done successfully for the *BRCA* genes.[16–19] To this end, we developed MMRpro, a model that estimates the probability that an individual carries a deleterious mutation in an MMR gene. This probability is evaluated on the basis of detailed family history of colorectal and endometrial cancer, including information for the individual being counseled and each of his or her first- and second-degree relatives, as described in the BOX. In addition, MMRpro provides estimates of future cancer risks for unaffected individuals, including known mutation carriers, untested individuals, and individuals in whom no mutation is found. MMRpro is appropriate for prediction in both population-based and clinic-based families. It is useful to clinical geneticists in parallel with clinical criteria, especially when current guidelines are inadequate to address the particularity of a given family and when the possibility of a heritable defect cannot be directly addressed in the laboratory.

Box. Family History as Inputs to the MMRpro Model and Resulting Output

Input (for the counselee and each first- or second-degree relative)*

- Exact relation to the counselee
- Colorectal cancer status (affected or unaffected)
  - Age at diagnosis (in years) of colorectal cancer if affected
- Endometrial cancer status (affected or unaffected)
  - Age at diagnosis (in years) of endometrial cancer if affected
  - Current age or age at last follow-up (in years) if unaffected
- Result of microsatellite instability testing (instability present or not present) or immunohistochemical staining (loss of expression or present) if tumor available
- Result of previous germline testing of MLH1, MSH2, or MSH6 (positive or not found)

Output (for the counselee)†

- Probability, by gene, that the counselee carries a deleterious mutation of MLH1, MSH2, and MSH6
- Probability, in yearly intervals, that the counselee, if asymptomatic, will develop colorectal or endometrial cancers in the future

*Any input can be left unspecified if not available, with the exception of relation to the counselee.

†Prediction can be made for any member in the family by designating that member as the counselee.

METHODS

This study included the development and validation of MMRpro. Development consisted of estimating genetic parameters from published studies and specifying a mutation-prediction algorithm as well as a cancer-risk prediction algorithm. External validation consisted of comparing model predictions with germline testing results in a sample of families.

Parameter Estimation

MMRpro relies on estimates of mutation prevalence and penetrance of MMR genes and on sensitivity and specificity of MSI and germline testing. We obtained these via comprehensive

literature reviews summarized here and described in detail at http://astor.som.jhmi.edu/~sining/documents/MMRpro_Supplement.pdf.

Penetrance refers to the age-specific risks of developing colorectal and endometrial tumors among mutation carriers, by gene and by sex. We restricted our meta-analysis to 5 studies[20–24] that used design and analysis plans giving unbiased risk estimates. Among these, 4 are population-based; the fifth used an adjustment for ascertainment bias. We estimated prevalence of MMR mutations indirectly, from penetrance estimates obtained from the literature as described above; CRC incidence from the Surveillance, Epidemiology, and End Results registry; and carrier prevalence among cases reported in the literature.

Testing for MSI is not perfectly sensitive in predicting MMR mutations because of uncertainty in the classification of the instability and its lower prevalence in tumors with certain mutations, such as mutations on *MSH6*. It is also not perfectly specific because of *MLH1* promoter hypermethylation. Mutation analysis is also not always sensitive because of the complex spectrum of MMR mutations. To accurately account for the predictive ability of these tests in predicting a true germline mutation, these sensitivities and specificities were derived based on a comprehensive published meta-analysis[25] of 16 studies.[26–41] The sensitivity and specificity of immunohistochemical testing was set to equal that of MSI testing because its predictive value of loss of protein expression is similar.[42–45]

To represent a specific population (different mutation prevalence/penetrance) or assay (different sensitivity/specificity), it is straightforward for users to replace the default values described above with preferred ones.

Model Specification

MMRpro translates estimates of mutation prevalence and penetrance of MMR genes into mutation predictions based on a general mendelian modeling approach, described in detail elsewhere.[46] In summary, the model involves the application of the Bayes rule[15,16,47–49] and mendelian laws as follows: $Pr_{genotype|history} = (Pr_{genotype} \times Pr_{history|genotype})/Pr_{history}$, where *Pr* denotes probability, *genotype* denotes counselee's genotype (deleterious mutations of *MLH1*, *MSH2*, or *MSH6* by gene), *history* denotes family history (refers to the information presented in the Box), and *Pr(A|B)* denotes the probability of A given that B is observed.

In the numerator, the probability of the counselee's genotype is derived from the mutations' prevalences, while the probability of family history given the counselee's genotype is evaluated as a weighted average of the probabilities of family history conditional on all possible relatives' genotypes, where the weights are the mendelian autosomal dominant probabilities of each genotype configuration. The probability of the family history under each genotype configuration can be broken down into the product of each relative's probability of phenotype given his or her own genotype. Each term is calculated based on the penetrance for affected relatives or the probability of censoring for unaffected relatives. When a family member undergoes MSI, immunohistochemical, or germline testing, the corresponding probabilities of test results given the genotype come from the sensitivity and specificity of that test in detecting true genotype. To evaluate the denominator, we sum the probabilities at the numerator over all possible genotypes for the counselee. Finally, risks of developing colorectal and endometrial cancers for unaffected individuals are estimated based on averages of the carriers' and noncarriers' risks, weighted by carrier probability.[46]

Validation

Patients—Our validation sample included 279 germline-tested individuals from 226 families in 3 clinic-based groups. All study patients provided written informed consent for this study, and the study was approved by the institutional review board at each participating institution.

The first group included 81 individuals from 59 families from the Johns Hopkins Colorectal Cancer Risk Assessment Clinic and Hereditary Colorectal Cancer Registry, Baltimore, Md. These individuals were either self-referred or referred to the clinic by health care workers between 1993–1996 because of a personal and/or family history of colorectal cancer. Patients and families were entered in the registry if at least 2 family members had colon or rectal cancer or if an individual developed colorectal or uterine cancer before age 50 years. Most patients came from Maryland. All individuals were screened extensively for *MLH1* and *MSH2* mutations using techniques including direct sequencing, protein truncation, conversion, and, in some individuals, more than 1 of the above.[50–52] Testing for MSI was performed on a subset of cases.

The second group of patients was recruited since 1997 through 4 centers (the Mayo Clinic and the University of Southern California Consortium in the United States, Cancer Care Ontario in Canada, and the University of Queensland in Australia) belonging to the National Cancer Institute Colon Cancer Family Registry (Colon CFR). The purpose was studying the clinical usefulness of conversion analysis.[53] Patients were included if they had a prior diagnosis of CRC, had an available EpsteinBarr virus–transformed cell line, and met any of 3 criteria: (1) were members of a family meeting the Amsterdam Criteria I; (2) had at least 2 first-or second-degree relatives with CRC, or 1 relative with endometrial cancer and at least 1 other with CRC; and (3) were otherwise diagnosed with CRC before age 50 years. In addition to meeting these criteria, all patients had prior evidence of a defect in MMR due to having either a tumor classified as MSI-high or loss of expression of an MMR protein demonstrated by immunohistochemistry. Individuals from the Colon Cancer Family Registry were uniformly tested by both DNA sequencing and conversion. Southern blotting was performed to detect large genomic deletions. Microsatellite instability testing and immunohistochemical analysis was performed on all cases.[53]

The third group consisted of 144 individuals from the Memorial Sloan-Kettering Cancer Center. These individuals were referred from within the center and by outside physicians to the center's Clinical Genetics Service between November 1994 and December 2005 for the possible presence of an MMR mutation. Criteria for referral varied, but generally included either early age at onset of colon cancer or multiple cases of colon cancer in multiple generations. Among all patients who underwent MMR gene testing, a randomly selected subset of 144 was used in the validation. This group included approximately equal numbers of patients referred for clinical testing and enrolled into 3 clinical protocols using different ascertainment criteria. Testing for MSI was performed when tumors were available. *MLH1* and *MSH2* mutational analysis was carried out on all index CRC patients from whom a blood sample could be obtained. *MSH6* mutation analysis was performed on all patients enrolled in one of the clinical protocols. MMR mutations were tested using a combination of denaturing high-performance liquid chromatography and semiquantitative fluorescent multiplex–polymerase chain reaction analysis.[11,54–56]

Family history and demographic information from all 3 groups are summarized by source in TABLE 1. The families include those at high risk (eg, Amsterdam Criteria), moderate risk (eg, HNPCC-like or Bethesda Guidelines), and others who do not fulfill any of these criteria. Overall, this mix reflects the population presenting for clinical and genetic counseling and for whom mutation prediction models are most useful. All individuals across the 3 clinic groups

were searched extensively for germline mutations with highly sensitive techniques that are capable of detecting large-scale deletions, exon duplications, and monoallelic mutations.

Data Analysis—We computed MMR-pro and Leiden mutation predictions on all germline-tested individuals in the validation set. MMRpro predicts risks for mutations on *MLH1*, *MSH2*, and *MSH6*. For individuals screened only for *MLH1* and *MSH2* mutations, we used the predictions on these 2 genes for comparability with the mutation analysis performed. We calculated MMRpro probabilities both with and without MSI testing. In both cases we used the full set of individuals and only incorporated MSI information when available. We evaluated the models' refinement, calibration, and overall performance.[57,58] Refinement is the ability to discriminate between mutation-positive and -negative individuals and is measured by the receiver operating characteristic (ROC) curve, which is summarized by the area under the curve (AUC), ie, the concordance index. Calibration is the correspondence between the number of mutation-positive individuals predicted and the number found and is quantified by the ratio of observed to expected positive results. Overall performance is quantified by mean squared error of prediction, a measure of distance between the predicted probabilities and the observed mutation carrier status. Confidence intervals are evaluated using the bootstrap method[59] with 95% coverage.

We also evaluated sensitivity and specificity of Amsterdam Criteria II[9] and revised Bethesda Guidelines[12] (hereafter, "II" and "revised" are implied), examining whether each patient's family history fulfilled the criteria. For evaluating Bethesda Guidelines followed by MSI testing, we defined positive individuals as those fulfilling the Bethesda Guidelines and showing MSI. Since we did not find individuals who fulfilled the Bethesda Guidelines but were not tested for MSI to be a biased subsample, we imputed their MSI status according to the proportions observed among Bethesda Guidelines–positive individuals whose MSI status is available.

RESULTS

MMRpro Software

Software for performing MMRpro calculations is open source and available free of charge via either the mendelian risk prediction package BayesMendel[46] at http://astor.som.jhmi.edu/BayesMendel/ or the genetic counseling package CancerGene[60] at http://www4.utsouthwestern.edu/breasthealth/cagene/.

Clinical Application

To demonstrate the application of the MMRpro model in a counseling setting, we consider 5 variations of the pedigree in FIGURE 1. The scenarios are hypothetical but realistic. In each, we give the MMR mutation probability for the counselee (arrow), as estimated by MMRpro, before and after MSI testing. As a reference, the mutation probability of a random individual for whom no information is available is 0.002. Meanwhile, the mutation probability for the counselee alone, with no information on relatives, is 0.05 prior to MSI testing and 0.34 after a positive result. This level at 0.34 may be sufficient to justify germline testing or increased colonoscopic screening, even though the counselee alone would not meet any clinical criteria.

In scenario 1, the counselee and her father were both diagnosed with CRC in their 50s. This family history does not fulfill the Bethesda Guidelines, but yields a significant mutation probability of 0.15. Should the counselee have MSI testing, her possible post-MSI mutation probabilities would differ substantially (0.65 if MSI and 0.04 if microsatellite stable), indicating that MSI testing is highly informative for her. MMRpro results can justify insurance coverage for her MSI test. Another important use of the MMR-pro estimate is to interpret an

inconclusive test result quantitatively. If the counselee has an MSI-positive tumor and no mutation was found by germline testing using sequencing, her postsequencing probability of an undetected germline mutation would be as high as 0.36. Counselees may be less likely to undertake screening when they are informed that no mutation was found. In such a case, immunohistochemical analysis for *MSH6* expression or conversion analysis, for example, would be additional considerations justified by this model. Also in this example, the counselee's 30-year-old son has a 0.18 mutation probability. This leads to a 15% chance of developing CRC by age 70 years, making him a likely candidate for intensive screening despite his mother's inconclusive test result.

In scenario 1, the counselee's paternal grandmother died at age 30 years without a cancer diagnosis. Had she lived longer, knowledge of whether she was affected or not would have a stronger effect on the counselee's mutation probability. For example, in scenario 2, she lived cancer-free until age 71 years, providing evidence against autosomal dominant transmission. This results in a reduction in mutation probability from 0.15 to 0.10 in the counselee.

In scenario 3, the paternal grandmother is affected with CRC at 71 years, and the diagnoses of the father and the paternal aunt are switched. Now the counselee has 2 relatives diagnosed with CRC, fulfilling the Bethesda Guidelines. However, the father is cancer-free until age 79 years, reducing the chance that the counselee's cancer is due to inherited mutation, and her mutation probability is one third of that in scenario 1.

Scenario 4 presents an additional case of endometrial cancer on the maternal side. Although endometrial cancer is highly predictive of a mutation, the maternal lineage of this tumor is independent of the mendelian dominant transmission pattern in the paternal lineage. So, while this additional diagnosis changes the counselee's Bethesda Guidelines status from scenario 1, her mutation probability does not increase significantly.

In scenario 5, endometrial cancer occurs in the counselee's sister. This is stronger evidence of a transmitted mutation, so the pretest probability increases to 0.82. For counselees at such high risk, the use of a prediction model can help them proceed directly to immunohistochemical analysis or germline testing. Even if the tumor does not show MSI, the posttest probability is still high. This suggests that the family may still be genetically susceptible.

External Validation

MMRpro predicted the presence of approximately 129 mutations. This shows a close correspondence (calibration) with the observed 121 mutations (ratio of observed to expected results, 0.94), as shown in TABLE 2. The slight overprediction by MMRpro may be an indication of its ability to predict germline mutations that are not detected by the mutation analysis performed here, such as mutations in *PMS2*. On the other hand, the Leiden model predicts a substantially lower number of cases than are observed (3 cases are observed for every 2 that are predicted). A possible explanation is that it was developed using results from conventional mutation analysis techniques, which may have missed a large fraction of MMR mutations.[50,53]

MMRpro provides better discriminatory ability than both the Leiden model and the Bethesda Guidelines. FIGURE 2 shows ROC curves for the MMRpro and Leiden models with and without MSI, as well as sensitivity and specificity of the Bethesda Guidelines with and without MSI testing. The corresponding AUCs are presented (as concordance indexes) in Table 2. The difference between the AUC of MMRpro with MSI testing and that of the Leiden model is 0.06 (95% confidence interval, −0.02 to 0.14), an important difference in the AUC scale. MMRpro with MSI testing discriminates better than the Leiden model in 93% of the bootstrap replicates. When calibration and discrimination are combined into a single evaluation using

the mean squared error of prediction, the MMR-pro model shows significantly improved performance compared with the Leiden model (Table 2).

The point corresponding to the Bethesda Guidelines without MSI testing lies below the ROC curve for MMR-pro without MSI testing (Figure 2), indicating that MMRpro performs better than the Bethesda Guidelines in selecting individuals who may carry a germline mutation. Similar results apply when comparing the point for the Bethesda Guidelines with MSI testing to the curve for MMRpro with MSI testing. Using a cutoff of 0.35 on the MMRpro probability, one can achieve the same specificity as the Bethesda Guidelines with MSI testing and higher sensitivity, while 0.62 will provide the same sensitivity and higher specificity. Cutoffs within this range provide both higher sensitivity and higher specificity. While the specific cutoffs may depend on the mix of families and the proportion of MSI-tested individuals in our sample, the positioning of the Bethesda Guidelines points below the corresponding MMRpro curves is likely to be robust.

The comparison of the ROC curves between MMRpro and MMRpro with MSI testing suggests that MMRpro correctly takes advantage of MSI test results, as predictions improve almost uniformly. However, the improvement is moderate. This may be due to the fact that not all cases have been tested for MSI. It may also indicate that in situations in which family history is very informative, MSI testing provides only limited additional information. In 99 of 120 MSI-tested individuals in our sample, MSI results changed probability by less than 0.10; in 88 individuals, by less than 0.05; and in 51 individuals, by less than 0.01. In such cases, MSI testing may not be the best course of action to determine the presence of a mutation. When used to assess the usefulness of MSI testing, MMRpro can lead to significant cost savings.

Finally, we considered how often MMRpro leads to a different classification compared with the Leiden model, Bethesda Guidelines, and Amsterdam Criteria. The results are shown in TABLE 3. MMRpro leads to reclassification of a significant fraction of individuals. In all 3 comparisons, correctly reclassified individuals outnumber those reclassified incorrectly. In the table, both MMRpro and Leiden predictions are dichotomized at 0.5. This threshold is chosen for illustrative purposes. In practice, thresholds should be chosen based on individual circumstances. However, MMRpro will always reclassify correctly more often than incorrectly, irrespective of the threshold chosen, based on ROC results.

COMMENT

This article introduces MMRpro, a model for prediction of genetic susceptibility in the Lynch syndrome, which makes efficient use of family history and tumor information and provides individualized evaluations. Because model-based prediction algorithms are increasingly used in genetic counseling and prevention activities, MMRpro is a timely tool for identifying and counseling families at risk for the Lynch syndrome and can improve current genetic counseling and early detection practice. In an independent validation, MMRpro demonstrated a better ability to predict mutation carriers than both the Leiden model and Bethesda Guidelines–based screening.

Current Bethesda Guidelines–based screening practice aims at identifying individuals likely to harbor tumors with MSI. An important limitation is that these criteria are not applicable when a tumor block is unavailable or to unaffected individuals concerned by family history and considering genetic testing and secondary prevention. Among individuals with tumors, the Bethesda Guidelines are sensitive but not highly specific and rely on MSI testing to improve specificity. However, even when tumor samples are available, MSI testing may not be the optimal course of action for all families. The high-resolution quantitative assessment obtained by setting a high threshold on MMRpro offers the option of performing germline testing

directly, without MSI testing, in selected families.[46] This strategy can both increase specificity and decrease costs. In our validation sample, MMRpro provides equal or better sensitivity and specificity with less MSI testing. We also estimated that a large fraction of families in the validation sample can reach an informed decision on whether to undertake germline testing without testing for MSI. For others, such as small families, families with older ages at diagnosis, and some families not meeting the Bethesda Guidelines, MSI testing is highly informative.

Some clinics also use immunohistochemical staining, because the loss of a protein product is highly predictive of the presence of mutations.[61] In our model we can account for either immunohistochemical or MSI results. Given the technical complexity of MSI analysis, which involves microdissection and DNA extraction, pathology departments may first perform immunohistochemical analysis and reserve MSI analysis for cases with a strong clinical suggestion. In that scenario, MMR-pro may also be useful, in that it would allow for setting a threshold for performing such additional analyses.

When germline mutation tests with relatively low sensitivity are used, MMRpro posttest probabilities are useful for individuals in whom, despite strong evidence of predisposition, no mutation is found. Before more sensitive techniques become available or new genetic factors are identified, the cancer risk predictions for such individuals provided by MMRpro help to guide subsequent clinical management. This feature is also valuable for counselees who do not wish to be genotyped but would still like to consider preventative measures.

Along with other screening approaches,[62,63] risk prediction based on family history is routinely used to identify individuals for CRC screening. Due to the imperfect sensitivity of sequencing, it remains likely that high-risk in dividuals who are untested or who receive “no mutation found” results will undergo routine screening.When asymptomatic individuals and their family members age without developing CRC, their chance of carrying deleterious mutations decreases. MMRpro can be used to help adaptively update their screening choices.

While useful, our model has limitations, some of which will be addressed in future updates as new studies provide the necessary information. Currently, MMRpro considers only endometrial cancer, the most common type of extracolonic tumor associated with the Lynch syndrome. The spectrum is wider, but at present, data on penetrance for these cancers are insufficient for modeling purposes. Colorectal adenomas and polyps and their histological features may also be predictive of MMR mutations, but their predictive value is difficult to quantify. The predictive value of MSI may vary with age because of the age-related increase in hypermethylation of the *MLH1* promoter region.[64,65] The MSI status of extracolonic cancers is likely to have different predictive abilities, depending on the site as well. Lifestyle risk factors also have yet to enter the model, as doprophylactic surgeries that reduce risks significantly.[66] In model-based risk counseling, variability in the estimates should be recognized. Uncertainty remains on the risk conferred by MMR mutation (as illustrated by the supplementary figure at http://astor.som.jhmi.edu/~sining/documents/MMRpro_Supplement.pdf), calling attention to the importance of more extensive investigations of penetrance.

Finally, in decision making for germline testing, a mathematical model can be informative for the reasons we have described. However, decision making regarding germline testing should reflect a broader range of factors, including the effectiveness and cost of genotyping; the available means and efficacy of measures for early detection and risk reduction; and possible psychological, social, and ethical implications. This should be done in concert with a health care professional experienced in cancer genetics[67–69] who can also advise on the choice of cutoffs appropriate for individual circumstances. The informed consent process will ensure that patients consider all of these issues prior to testing.[70]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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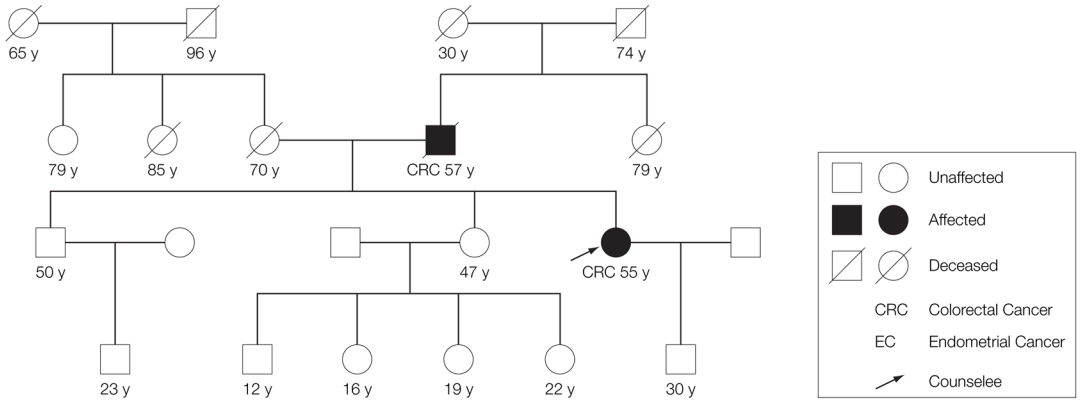

	Mutation Probability				
	MMRpro				
Scenario	No MS Test*	MSI*	MSS*	Leiden Model	Bethesda Guideline
1-As in Figure	0.15	0.65	0.04	0.015	No
2-As in Figure but Paternal Grandmother Deceased at Age 71 y	0.10	0.53	0.02	0.015	No
3-As in Figure but Father Healthy at 79 y, Paternal Aunt Had CRC at Age 57 y, and Paternal Grandmother Had CRC at Age 71 y	0.05	0.37	0.01	0.009	Yes
4-As in Figure but Maternal Aunt Had Endometrial Cancer at Age 50 y	0.19	0.70	0.05	0.075	Yes
5-As in Figure but Sister Had Endometrial Cancer at Age 50 y	0.82	0.98	0.49	0.075	Yes

Figure 1.

Comparisons of Carrier Probability Estimation Approaches on 5 Pedigree Variations

Numbers below each family member indicate current age, or age at death, for unaffected individuals and age at diagnosis for affected individual. The counselee is identified by the arrow. MS indicates microsatellite; MSI, microsatellite instability; MSS, microsatellite stable. *Information in these columns refers to MSI testing for the counselee.

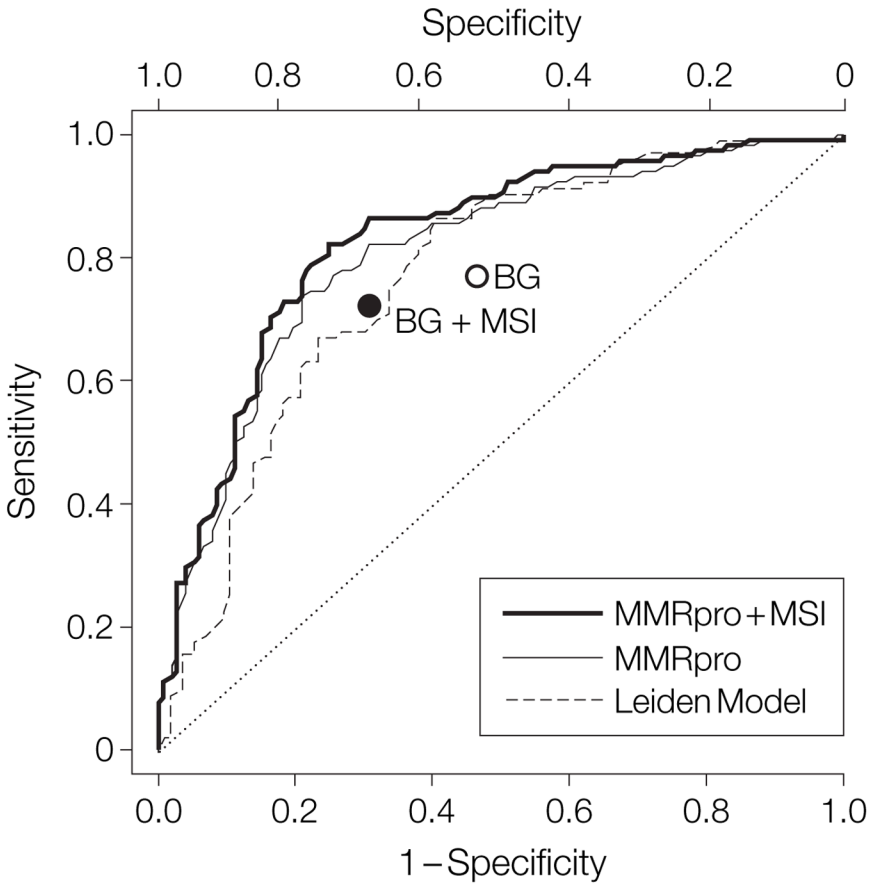

Figure 2.

Receiver Operating Characteristic Curves for the MMRpro and Leiden Models on the Validation Data Set

Also shown are the estimated true-positive (y-axis) and false-positive (x-axis) fractions associated with the Bethesda Guidelines (BG). MSI indicates microsatellite instability.

Table 1

Demographic Information for the Independent Validation Sample, by Source

Characteristic	No. JHU	Colon CFR	MSKCC	Total
Ascertainment	≥2 CRCs or age <50 y	Highly selected	Physician referred	
Genotyping method	≥1 of direct sequencing, PTT, conversion	Both direct sequencing and conversion	DHPLC or SQF-PCR	
Total germline-tested individuals	81	54	144	279
Total families	59	54	113	226
Total individuals in all families (counseled + first- and second-degree relatives)	1197	1548	3249	5994
Tested male individuals	39	33	62	134
Tested individuals with CRC	53	53	70	176
Tested individuals with EC	1	5	14	20
Mean CRC cases per family (first- and second-degree only)	3.7	4.9	2.7	3.4
Mean EC cases per family (first- and second-degree only)	0.34	0.61	0.60	0.54
Mean age at diagnoses of CRC in all affected members, y	50.9	43.6	51.4	48.6
Mean age at diagnoses of EC in all affected members, y	59.5	42.7	50.9	50.4
Families fulfilling Amsterdam Criteria II	32	48	65	145
Individuals fulfilling revised Bethesda Guidelines	52	53	61	166
Mutations found	14	27	10	51
MLH1				
MSH2	10	18	35	63
MSH6	0	0	7	7
MSI tests performed	15	46	59	120
MSI test results, mutations/tests	15/15	39/46	23/29	77/90
MSI-H				
MSI-L	−/0	−/0	1/2	1/2
MSS	−/0	−/0	1/21	1/21
Indeterminate	−/0	−/0	2/7	2/7

Abbreviations: Colon CFR, Colon Cancer Family Registry; CRC, colorectal cancer; DHPLC, denaturing high-performance liquid chromatography; EC, endometrial cancer; JHU, Johns Hopkins University; MSKCC, Memorial Sloan-Kettering Cancer Center; MSI, microsatellite instability; MSI-H, microsatellite instability–high; MSI-L, microsatellite instability–low; MSS, microsatellite stability; PTT, protein truncation testing; SQF-PCR, semiquantitative fluorescent multiplex–polymerase chain reaction.

Table 2

Summary of Validation Results

	Concordance Index 5% CI)*	O/E Ratio (95% CI)†	Mean Squared Error (95% CI)
MMRpro + MSI testing‡	0.83 (0.78 to 0.87)	0.94 (0.84 to 1.05)	0.18 (0.15 to 0.22)
MMRpro	0.79 (0.74 to 0.84)	0.97 (0.86 to 1.08)	0.19 (0.16 to 0.23)
Leiden	0.77 (0.71 to 0.83)	1.54 (1.35 to 1.80)	0.24 (0.20 to 0.28)
Difference between MMRpro + MSI and Leiden	0.06 (−0.02 to 0.14)	Not applicable§	0.06 (0.005 to 0.11)

	Sensitivity	Specificity
Revised Bethesda Guidelines + MSI‖	0.72	0.69
Revised Bethesda Guidelines	0.77	0.54
Amsterdam Criteria‖	0.75	0.62

Abbreviations: CI, confidence interval; MSI, microsatellite instability.

*Concordance index is equal to the area under the receiver operating characteristic curve.

†Ratio between the observed number of carriers and the total number of predicted carriers.

‡Represents MMRpro prediction after taking into account the MSI test results, when available.

§Computing the difference in this case is not an appropriate comparison, as each of the ratios should be compared directly with the reference value of 1.

‖Refers to applying the revised Bethesda Guidelines, testing the individuals who fulfill the guidelines for MSI, and referring those individuals testing positive for MSI to germline testing.

Table 3

Comparison of MMRpro-Based Probability With Leiden-Based Classification, Bethesda Guidelines, and Amsterdam Criteria*

	Carriers		Noncarriers		
	MMRpro Pr ≥0.5	MMRpro Pr <0.5	MMRpro Pr ≥0.5	MMRpro Pr <0.5	Reclassification Rate, %
Leiden					
≥0.5	54	3†	9	17‡	33
<0.5	39‡	22	23†	80	
Bethesda Guidelines					
+	83	10†	28	37‡	24
−	1‡	18	4†	63	
Amsterdam Criteria					
+	76	5†	26	11‡	15
−	17‡	23	6†	89	

Abbreviation: MMRpro Pr, MMRpro-based probability.

*MMRpro and Leiden predictions dichotomized at 0.5 for illustrative purposes.

†Patients classified differently using MMRpro, compared with the model in the corresponding row.

‡Patients reclassified correctly using MMRpro.