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Criteria and prediction models for mismatch repair gene mutations: a review

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ABSTRACT

One of the strongest predictors of colorectal cancer risk is carrying a germline mutation in a DNA mismatch repair (MMR) gene. Once identified, mutation carriers can be recommended for intensive screening that will substantially reduce their high colorectal cancer risk. Conversely, the relatives of carriers identified as non-carriers can be relieved of the burden of intensive screening. Criteria and prediction models that identify likely mutation carriers are needed for cost-effective, targeted, germline testing for MMR gene mutation. We reviewed 12 criteria/guidelines and 8 prediction models (Leiden, Amsterdam-plus, Amsterdam-alternative, MMRpro, PREMM_{1,2,6}, MMRpredict, Associazione Italiana per lo studio della Familiarità ed Ereditarietà dei tumori Gastrointestinali (AIFEG) and the Myriad Genetics Prevalence table) for identifying mutation carriers. While criteria are only used to identify individuals with colorectal cancer (yes/no for screening followed by germline testing), all prediction models except MMRpredict and Myriad tables can predict the probability of carrying mutations for individuals with or without colorectal cancer. We conducted a meta-analysis of the discrimination performance of 17 studies that validated the prediction models. The pooled estimate for the area under curve was 0.80 (95% CI 0.72 to 0.88) for MMRpro, 0.81 (95% CI 0.73 to 0.88) for MMRpredict, 0.84 (95% CI 0.81 to 0.88) for PREMM, and 0.85 (95% CI 0.78 to 0.91) for Leiden model. Given the high degree of overlap in the CIs, we cannot state that one model has a higher discrimination than any of the others. Overall, the existing statistical models have been shown to be sensitive and specific (at a 5% cut-off) in predicting MMR gene mutation carriers. Future models may need to: provide prediction of *PMS2* mutations, take into account a wider range of Lynch syndrome-associated cancers when assessing family history, and be applicable to all people irrespective of any cancer diagnosis.

INTRODUCTION

Lynch syndrome (OMIM 120435), previously termed Hereditary Non-Polyposis Colorectal Cancer (HNPCC),¹ is an autosomal dominantly inherited disorder of cancer susceptibility caused by germline mutations in one of the DNA mismatch repair (MMR) genes: *MLH1* (chromosome 3p21.3),^{2 3} *MSH2* (chromosome 2p22–21),⁴ *MSH6* (chromosome 2p16),^{5 6} and *PMS2* (chromosome 7p22.2)^{7 8}; or constitutional 3' end deletions of *EPCAM* (chromosome 2p21).^{9 10} These mutations cause 2–5% of all colorectal cancers^{11–16} and 10–15% of colorectal cancers diagnosed before age 50 years.^{11 12 17} They account for approximately 50% of the excess colorectal cancer cases observed in first-

degree relatives of a colorectal cancer case.¹⁸ Reported estimates of carrier frequency of germline mutations of these genes in the population vary depending on differences in assumptions; from approximately 1 in 300 to 1 in 3000.^{15 16 19–22} Mutation carriers are at substantially increased risk of cancers of the colon, rectum, endometrium, stomach, ovary, ureter, renal pelvis, brain, small bowel and hepatobiliary tract, and the diagnoses of these cancers occur at younger ages than for the general population on average.²³ Additionally, mutation carriers may also be at increased risk of cancer of the pancreas,^{24 25} prostate,²⁶ breast^{25 27–29} and cervix.³⁰

Screening colonoscopy,^{31 32} prophylactic hysterectomy and bilateral salpingo-oophorectomy³³ decrease the risk of colorectal, endometrial and ovarian cancer respectively for MMR gene mutation carriers. As a chemoprevention, 600 mg aspirin per day for an average two years has been shown to approximately halve the risk of colorectal cancer for MMR gene mutation carriers.³⁴ Given the substantial risk of cancers and the availability of effective interventions to reduce risk, identifying mutation carriers can prevent or minimise the impact of a substantial number of cancers. Once carriers are identified, testing of their relatives can also be performed, and this will identify additional carriers who can also benefit from screening, and non-carriers who can be spared the intensive screening and prophylactic surgery recommended for their mutation-carrying relatives.

As germline testing is required to confirm mutation carrier status, untargeted testing is not cost-effective given the cost of germline sequencing and the rarity of carriers. Attempts have been made to develop criteria to categorise people by their probability of carrying a mutation, and prediction models have been developed to estimate a person's probability of carrying a mutation. These criteria and models can be used to triage for germline sequencing. The aim of this review was to catalogue and describe the published criteria and prediction models, and to compare the performance of the prediction models for MMR gene mutation status.

CRITERIA AND GUIDELINES

Several criteria and guidelines have been developed for categorising families or individuals into those most likely to be carrying a MMR gene mutation so they can be triaged for germline testing. Given Lynch syndrome-associated colorectal cancers typically exhibit high level of DNA microsatellite instability (MSI) and/or loss of MMR protein expression that can be detected by immunohistochemistry (IHC), these techniques have been widely used as a screen for likely mutation carriers.

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Here, we categorise these criteria as 'clinical criteria' if they are only based on personal and family history of cancer, including ages and sites of diagnoses, and as 'clinicopathological criteria' if they are based on tumour pathology as well as the clinical features described above.

CLINICAL CRITERIA

Amsterdam criteria

Amsterdam Criteria-I developed by the International Collaborative Group on HNPCC (ICG-HNPCC) in 1990³⁵ is based only on family history of colorectal cancer (box 1). The strengths of the Amsterdam Criteria-I criteria are: that they are relatively simple to describe and use; and they are widely

recognised internationally. The limitations of the Amsterdam Criteria-I are: (1) they do not take into account extracolonic cancers that are recognised as Lynch syndrome spectrum tumours; (2) they have reduced sensitivity for small families³⁶ and (3) they require accurate recall and reporting of family history. Estimates of the Amsterdam Criteria-I sensitivity range between 47% and 91%, and specificity between 62% and 84%.^{37–42} MMR gene mutations are observed in approximately 50% (positive predictive value) of families that met the Amsterdam Criteria-I.^{37 39 43}

In 1998, the ICG-HNPCC devised the Amsterdam Criteria-II to produce the Amsterdam Criteria-II which broadened the definition of family history by including specified extracolonic

Box 1 Clinical criteria to identify mismatch repair gene mutations

Amsterdam Criteria-I (1990)³⁵

- ▶ At least three relatives affected with CRC; one of them should be a first-degree relative to the other two
- ▶ At least two successive generations affected
- ▶ At least one affected relative with CRC before age 50 years
- ▶ Familial adenomatous polyposis should be excluded
- ▶ Tumours should be verified by pathological examination.

Amsterdam Criteria-II (1998)⁴⁴

- ▶ At least three relatives affected with an HNPCC-associated cancer (large bowel, endometrium, small bowel, ureter, or renal pelvis); one of them should be a first-degree relative of the other two
- ▶ At least two successive generations affected
- ▶ At least one relative affected with CRC before age 50 years
- ▶ Familial adenomatous polyposis should be excluded in the CRC case(s) if any
- ▶ Tumours should be verified by pathological examination.

Modified Amsterdam Criteria (1993)^{47 48}

- ▶ 'Very small families', which cannot be further expanded, can be considered as HNPCC even if there are only two CRCs in first-degree relatives; CRC must be present in at least two generations, and one or more CRC cases must be diagnosed under age 55 years
- ▶ In families with two first-degree relatives affected by CRC, the presence of a third relative with an early onset (before age 55 years) 'unusual' neoplasm or endometrial cancer is sufficient.
- ▶ Neoplasms are considered as 'verified' when histological reports, clinical charts, or death certificates are available.

Mount Sinai Hospital Criteria (MC) (1995)⁴⁹

- ▶ Three individuals in at least two successive generations with at least one CRC, and two others with either gastrointestinal, genitourinary or gynaecological cancers with no age limit for the cancer diagnosis (MC-1)
- ▶ Any CRC patient diagnosed at <35 years of age irrespective of family history of cancer (MC-2)
- ▶ Any individual with multiple primary cancers of the sites associated with HNPCC irrespective of family history of cancer (MC-3).

Familial adenomatous polyposis should be excluded.

Japanese Criteria (1991)⁵⁰

- ▶ CRC patient with two or more first-degree relatives with CRC
- ▶ CRC patient with one first-degree relative with CRC and any of the following:
 1. Age at onset of CRC(s) of less than 50 years.
 2. Right colon involvement.
 3. Synchronous and/or metachronous multiple CRCs.
 4. Associated extracolorectal malignancy.

Familial adenomatous polyposis should be excluded.

Korean Criteria (1991)⁵¹

- ▶ Vertical transmission of CRC or at least two siblings in a family, affected with CRC
- ▶ Development of multiple colorectal tumours or at least one CRC case diagnosed before age 50 years.

Chinese Criteria (2003)⁵²

- ▶ At least two pathologically verified CRCs in a family; at least two of them first-degree relatives
- ▶ At least one of the following conditions has to be satisfied:
 1. At least one case with multiple colorectal cancers or adenomas
 2. At least one colorectal cancer diagnosed before age 50 years
 3. At least one case with an extracolonic cancer (gastric, endometrial, small bowel, ureter and renal pelvis, ovarian or hepatobiliary malignancies).

HNPCC, Hereditary Non-Polyposis Colorectal Cancer.

cancers.⁴⁴ Consequently, sensitivity increased (range between 77% and 81%) though specificity decreased (between 46% and 68%).^{38 42 45 46}

Other clinical criteria

Other clinical criteria have been developed, including: modified Amsterdam Criteria,^{47 48} the Mount Sinai Hospital Criteria,⁴⁹ Japanese Criteria,⁵⁰ Korean Criteria⁵¹ and Chinese Criteria⁵² (see detail in box 1). Note that all these clinical criteria are only used to identify Lynch syndrome *families* rather than *individuals*.

CLINICOPATHOLOGICAL CRITERIA

Bethesda guidelines

The Bethesda guidelines were developed in 1997⁵³ and revised in 2004²³ (box 2). MSI testing was recommended for any colorectal cancer case meeting at least one of the following criteria: Amsterdam-like family history of a range of cancers; particular pathological features of the tumour; and early age at diagnosis. Widening the inclusion criteria by adding indicators, resulted in increased sensitivity compared with the clinical criteria described above (89%, 95% CI 86% to 92%), and reduced specificity (53%, 95% CI 49% to 58%).⁴²

Age of diagnosis-only criteria

The Melbourne Criteria^{54 55} recommends that all colorectal cancer cases diagnosed before age 45 years should be tested for MMR-deficiency using IHC regardless of family history, and achieves a sensitivity of 100% (95% CI 82% to 100%) and a specificity of 91% (95% CI 83% to 96%). The Perth Criteria^{56 57} recommends to test MMR-deficiency using IHC and/or MSI for all colorectal cancer cases diagnosed before age 60 years regardless of family history, while the Jerusalem workshop²² recommends to test for cases before age 70 years.

Universal screening

Several groups recommend that all cases of colorectal cancer should be tested for MMR-deficiency regardless of their age at diagnosis or family history^{13 14 58–61} given that a proportion of colorectal cancers caused by MMR mutations do occur at old age. This so-called ‘universal screening’ has virtually complete sensitivity (100%; 95% CI 99.3% to 100%) as everyone with colorectal cancer is tested for MMR-deficiency by MSI and/or IHC.⁶⁰ To minimise loss of specificity due to substantial *MLH1* methylation-causing MMR-deficiency in the elderly, the proponents of universal testing recommend testing all *MLH1* and/or *PMS2*-deficient tumours for *MLH1* methylation (as well as the V600E mutation in the *BRAF* oncogene)^{62 63} prior to germline testing. Some claimed that universal screening is less

Box 2 Clinicopathological criteria to identify mismatch repair gene mutations

Bethesda Guidelines (1997)⁵³

Colorectal tumours should be tested for MSI in any of the following situations:

- ▶ Individuals with cancer in families that meet the Amsterdam Criteria
- ▶ Individuals with two HNPCC-related cancers, including synchronous and metachronous colorectal cancers or associated extracolonic cancers*
- ▶ Individuals with colorectal cancer, and a first-degree relative with colorectal cancer and/or HNPCC-related extracolonic cancer and/or a colorectal adenoma; one of the cancers diagnosed at age <45 years, and the adenoma diagnosed at age <40 years
- ▶ Individuals with colorectal cancer or endometrial cancer diagnosed at age <45 years
- ▶ Individuals with right-sided CRC with an undifferentiated pattern (solid/cirriiform) on histopathology diagnosed at age <45 years
- ▶ Individuals with CRC which was composed of >50% signet ring cells and diagnosed at age <45 years
- ▶ Individuals with adenomas diagnosed at age <40 years.

Revised Bethesda Guidelines (2004)²³

Colorectal tumours should be tested for MSI in any of the following situations:

- ▶ CRC diagnosed at age <50 years
- ▶ Presence of synchronous or metachronous HNPCC-related tumours,† regardless of age
- ▶ CRC with MSI-high histology‡ diagnosed in individuals aged <60 years
- ▶ CRC diagnosed in one or more first-degree relatives with an HNPCC-related tumour, with one of the cancers being diagnosed at age <50 years
- ▶ CRC diagnosed in two or more first-degree or second-degree relatives with HNPCC-related tumours†, regardless of age

Melbourne Criteria (2005)⁵⁴

- ▶ Individuals with CRC diagnosed at age <45 years irrespective of family history should be tested for mismatch repair deficiency using IHC.

Perth Criteria (2012)⁵⁷

- ▶ Individuals with CRC diagnosed at age <60 years irrespective of family history should be tested for MSI.

Jerusalem Recommendation (2010)²²

- ▶ Individuals with CRC diagnosed at age <70 years irrespective of family history should be tested for MSI or mismatch repair deficiency using IHC.

*Endometrial, ovarian, gastric, hepatobiliary, small bowel, ureter and renal pelvis tumours.

†colorectal, endometrial, stomach, small bowel, ovarian, pancreas, ureter and renal pelvis, biliary tract, and brain tumours, sebaceous gland adenomas and keratoacanthomas.

‡Presence of tumour-infiltrating lymphocytes, Crohn's-like lymphocytic reaction, mucinous/signet-ring differentiation, or medullary growth pattern.

HNPCC, Hereditary non-polyposis colorectal cancer; CRC, colorectal cancer; MSI, microsatellite instability; IHC, immunohistochemistry.

cost-effective compared with having an age of diagnosis cut-off before 50 years⁵⁵ or before 70 years,⁶⁴ while one study claimed that universal screening is more cost-effective than age-targeted testing.⁶⁵

None of these clinical and clinicopathological criteria provide the probability of being a MMR gene mutation carrier. They only indicate whether a colorectal cancer case should have their tumour tested for MSI and/or IHC testing, and depending on this result, to undergo germline testing for MMR gene mutations.⁶⁶ These MSI and IHC tests require a pathologist, at least to select sections for staining. MSI testing needs to be conducted in a molecular laboratory, is more expensive, and does not provide information on which MMR gene is mutated, if any. Also, mutations cannot be identified for about one-third of those with a MMR-deficient colorectal cancer (even after screening for *MLH1* methylation) tumour testing.^{14 54} The alternative of moving directly to germline testing, irrespective of tumour test results, would be too expensive and inefficient given only 2–5% of colorectal cancers are caused by germline MMR gene mutations.⁶⁷ Further, studies have also shown that only a fraction of individuals who should be referred for molecular evaluation are actually referred.⁶⁸

PREDICTION MODELS

Why are prediction models required for risk prediction of MMR gene mutations?

In addition to the limitations of all clinical and clinicopathological criteria that have been described above, they are not pertinent for: (1) individuals without colorectal cancer (except Amsterdam Criteria) and (2) individuals who have no relatives with colorectal cancer, who are able or willing to have their colorectal tumour tested for MMR-deficiency. For these individuals, statistical prediction models are needed to predict who are the most likely to be carriers based on their age and family history of colorectal and other Lynch syndrome spectrum cancers. Ideally, prediction models can quantitatively combine the complicated effects of many risk factors in a rational way; be easily updated when more accurate incidence data and population carrier frequencies become available; and are more widely applicable than clinical or clinicopathological criteria. For these reasons, risk prediction models for MMR gene mutations have been developed and used by physicians for their patients to help them decide whether to pursue germline testing or not.

Existing risk prediction models for MMR gene mutations

The currently available risk prediction models for MMR gene mutations are as summarised in table 1: Leiden by Wijnen *et al*,⁶⁹ Amsterdam-plus by Lipton *et al*,⁷⁰ Amsterdam-alternative by Lipton *et al*,⁷⁰ MMRpro (previously known as CRCAPRO) by Chen *et al*,⁷¹ PREMM_{1,2} by Balmana *et al*,⁷² and PREMM_{1,2,6} by Kastrinos *et al*,⁷³ MMRpredict by Barnetson *et al*,⁷⁴ AIFEG (Associazione Italiana per lo studio della Familiarità ed Ereditarietà dei tumori Gastrointestinali) by Marroni *et al*,⁷⁵ and the Myriad Genetics Prevalence table.⁷⁶

All these models except MMRpredict⁷⁴ and Myriad tables⁷⁶ can predict the probability of carrying mutations for individuals or families with or without colorectal cancer. Leiden,⁶⁹ Amsterdam-plus⁷⁰ and Amsterdam-alternative⁷⁰ models predict only at the family level while the other models predict at individual level. MMRpro⁷¹ and PREMM_{1,2,6}⁷³ predict the probability of a mutation for each of the genes *MLH1*, *MSH2* and *MSH6*, whereas the other models can only predict mutations in any MMR gene. None of the existing models take into account *PMS2* mutations, which account for 15% of all MMR gene

mutations⁷⁷ and have different cancer risk profile than other MMR gene mutations.⁷⁸ Apart from MMRpro,⁷¹ PREMM_{1,2}⁷² and PREMM_{1,2,6},⁷³ the models only take into account history of colorectal cancer and endometrial cancer, but not other Lynch syndrome-associated cancers. All models used multivariable logistic regression methods for their model development except AIFEG,⁷⁵ MMRpro⁷¹ and Myriad tables.⁷⁶ AIFEG⁷⁵ and MMRpro⁷¹ models require cancer data for full family pedigrees, and they applied Mendel's genetic laws to predict mutations within family. On the other hand, Myriad tables⁷⁶ just uses the prevalence of mutation among all tested colorectal cancer cases. Most of the models are easy to use as they all are web-based or based on a statistical formula (table 1).

Evaluation of existing prediction models for MMR gene mutations

Before a risk prediction model can be recommended as a useful tool for individualised decision making in a clinical setting, it needs to be validated using an independent sample than that used to develop the model.⁷⁹ The following characteristics were mainly evaluated by previous studies:

1. *Discrimination* (or *precision*): The concordance statistic (c-statistic) that corresponds to the area under a receiver operating characteristic curve (AUC) which plots sensitivity against one minus specificity.⁸⁰ A c-statistic of 0.5 indicates that there is no discrimination between individuals who have mutations and those who do not, whereas 1.0 indicates perfect discrimination;
2. *Calibration* (or *reliability*): The ratio of the expected number of events (E) with the observed number of events (O).⁸⁰ The ratio 1.0 indicates perfect calibration;
3. *Accuracy*: Sensitivity, specificity, positive and negative predictive values of a model for a given probability threshold.

We reviewed all previously published studies that evaluated the performance of MMR gene prediction models. Most of the studies evaluated discrimination (using AUC) and a few studies also evaluated calibration and accuracy. We conducted meta-analyses of the AUCs for the PREMM,^{72 73} MMRpro,⁷¹ MMRpredict⁷⁴ and Leiden⁶⁹ models to summarise their discrimination performance; and these meta-analyses were stratified by population-based or clinic-based samples that were used for validation. As a sensitivity analysis, we conducted the meta-analyses on just the studies that reported results for the three models (PREMM, MMRpro, MMRpredict) within the same study. Both random and fixed effects were fitted, and heterogeneity was tested using *I*-squared. All statistical analyses were performed using Stata V.11.0.⁸¹

We observed a total of 17 studies that evaluated the performance of prediction models for MMR gene mutations (see online supplementary table S1). Of them, four used population-based samples,^{74 82–84} 10 used clinic-based samples,^{71 72 75 85–91} and three used both clinic-based and population-based samples.^{73 92 93} Given there was significant evidence of heterogeneity between studies, we reported the pooled AUC values from random effect models (detail in table 2). The pooled AUC values from the combined analyses of population-based and clinic-based validation studies were 0.80 (95% CI 0.72 to 0.88) for MMRpro, 0.81 (95% CI 0.73 to 0.88) for MMRpredict, 0.84 (95% CI 0.81 to 0.88) for PREMM, and 0.85 (95% CI 0.78 to 0.91) for Leiden model (see online supplementary figures S1–4). When we restricted only to the seven studies (five clinic-based, one population-based and one both) validating three models within the same study, the AUCs were 0.81 (95% CI 0.72 to 0.89) for MMRpro, 0.78 (95% CI 0.68 to 0.89) for

Table 1 Summary of existing risk prediction models for mismatch repair (MMR) gene mutations

	Leiden ⁶⁹	Amsterdam-plus ⁷⁰	Amsterdam-alternative ⁷⁰	AIFEG ⁷⁵	MMRpro ⁷¹	MMRpredict ⁷⁴	PREMM _{1,2} ⁷²	PREMM _{1,2,6} ⁷³	Myriad ⁷⁶
Year published	1998	2004	2004	2006	2006	2006	2006	2011	–
CRC affected/unaffected	Both affected and unaffected	Both affected and unaffected	Both affected and unaffected	Both affected and unaffected	Both affected and unaffected	Affected only	Both affected and unaffected	Both affected and unaffected	Affected only
Genes	<i>MLH1, MSH2</i>	<i>MLH1, MSH2, MSH6</i>	<i>MLH1, MSH2, MSH6</i>	<i>MLH1, MSH2</i>	<i>MLH1, MSH2, MSH6</i>	<i>MLH1, MSH2, MSH6</i>	<i>MLH1, MSH2</i>	<i>MLH1, MSH2, MSH6</i>	<i>MLH1, MSH2</i>
Development dataset	184 CRC cases from 184 families; 47 (26%) mutation carriers (28 <i>MLH1</i> , 19 <i>MSH2</i>)	250 families recruited from family cancer clinics; 34 (14%) mutation carriers (25 <i>MLH1</i> , 8 <i>MSH2</i> , 1 <i>MSH6</i>)	250 families recruited from family cancer clinics; 34 (14%) mutation carriers (25 <i>MLH1</i> , 8 <i>MSH2</i> , 1 <i>MSH6</i>)	Literature review: published estimates of mutation frequencies and cancer penetrances in carriers and non-carriers	Literature review: meta-analyses of mutation frequencies and cancer penetrances and predictive value of MSI test	870 CRC cases diagnosed age <55 years, recruited regardless of family history; 38 (4%) mutation carriers (15 <i>MLH1</i> , 16 <i>MSH2</i> , 7 <i>MSH6</i>)	898 individuals (536 affected with CRC) with a personal or family history of Lynch syndrome; 130 (15%) mutation carriers (58 <i>MLH1</i> , 72 <i>MSH2</i>)	4539 individuals (2526 affected with CRC) with a personal or family history of Lynch syndrome; 525 (12%) mutation carriers (204 <i>MLH1</i> , 250 <i>MSH2</i> , 71 <i>MSH6</i>)	3410 individuals No further details given
Development method	Multivariable logistic regression	Multivariable logistic regression	Multivariable logistic regression	Application of the Mendelian laws	Application of the Bayes' rule and Mendelian laws	Multivariable logistic regression	Multivariable logistic regression	Multivariable logistic regression	Not stated
Input	<ul style="list-style-type: none"> ► Fulfilment of AC-II (yes/no) ► Mean age at diagnosis of CRC of affected relatives ► EC in family members (yes/no) 	<ul style="list-style-type: none"> ► Fulfilment of AC-II (yes/no) ► Number of relatives with CRC ► Number of relatives with >1 CRC and/or EC ► Number of relatives with EC ► Mean age at diagnosis of CRC and EC of affected relatives ► Number of relatives with >5 adenomas 	<ul style="list-style-type: none"> ► Number of relatives with CRC ► Number of relatives with >1 CRC and/or EC ► Number of relatives with EC ► Mean age at diagnosis of CRC and EC of affected relatives ► Number of relatives with >5 adenomas 	For the counselee and each relative: <ul style="list-style-type: none"> ► Exact relation to the counselee ► CRC (yes, no) ► Age at diagnosis of CRC if affected (<45, 45–60, >60 years) ► EC (yes, no) ► Result of MSI testing (instability present or not present) if tumour available 	For the counselee and each FDR or SDR: <ul style="list-style-type: none"> ► Exact relation to the counselee ► Type of cancer ► Age at diagnosis (years) if affected ► Current age or age at death or last follow-up (years) if unaffected ► Result of MSI (instability present or not) or IHC (loss of expression or present) if tumour available ► Result of previous germline testing (positive or negative) 	For the counselee: <ul style="list-style-type: none"> ► Age at diagnosis (years) ► Sex ► Tumour location (proximal, distal) ► Synchronous and/or metachronous (yes, no) For FDR: <ul style="list-style-type: none"> ► CRC (yes, no) ► Youngest age at diagnosis of CRC if affected (<50 or ≥50 years) ► EC (yes, no) 	For the counselee: <ul style="list-style-type: none"> ► CRC (none, one, ≥2); age at diagnosis if one, youngest age at diagnosis if ≥2 ► Colonic adenoma (yes, no); youngest age at diagnosis if affected ► EC (yes, no); youngest age at diagnosis ► HNPCC-associated cancer* (yes, no) For FDR and SDR (only from affected side of family): <ul style="list-style-type: none"> ► Number of relatives with CRC (none, one, ≥2); Age at diagnosis if one, youngest age at diagnosis if ≥2 ► Number of relatives with EC (none, one, ≥2); age at diagnosis if one, youngest age at diagnosis if ≥2 ► Any relatives with another HNPCC-associated cancer* (yes, no) 	For the counselee: <ul style="list-style-type: none"> ► Sex ► CRC (none, one, ≥2); age at diagnosis if one, youngest age at diagnosis if ≥2 ► EC (yes, no); youngest age at diagnosis ► HNPCC-associated cancer* (yes, no) For FDR and SDR (only from affected side of family): <ul style="list-style-type: none"> ► Number of relatives with CRC (none, one, ≥2); age at diagnosis if one, youngest age at diagnosis if ≥2 ► Number of relatives with EC (none, one, ≥2); age at diagnosis if one, youngest age at diagnosis if ≥2 ► Any relatives with another HNPCC-associated cancer* (yes, no) 	Not required

Continued

Table 1 Continued

	Leiden ⁶⁹	Amsterdam-plus ⁷⁰	Amsterdam-alternative ⁷⁰	AIFEG ⁷⁵	MMRpro ⁷¹	MMRpredict ⁷⁴	PREMM _{1,2} ⁷²	PREMM _{1,2,6} ⁷³	Myriad ⁷⁶
Gene-specific estimates given	No	No	No	No	Yes (MLH1, MSH2 and MSH6)	No	No	Yes (MLH1, MSH2 and MSH6)	No
Prediction level	Family	Family	Family	Individual	Individual	Individual	Individual	Individual	Individual
Calculation mode	Formula (by hand)	Formula (by hand)	Formula (by hand)	Software (MLINK of the FASTLINK package 99 100)	Software (CancerGene)	Web-based	Web-based	Web-based	Prevalence table
Source of calculation mode	—	—	—	AIFEG website	MMRpro website, CancerGene software website	MMRpredict website	PREMM _{1,2} website	PREMM _{1,2,6} website	Myriad website

MMRpro website: <http://astor.som.jhmi.edu/BayesMendel/mmrpro.html>.
CancerGene software website: <http://www4.utsouthwestern.edu/breasthealth/cagene/>.
MMRpredict website: <http://mpcpcpredict.hgu.mrc.ac.uk/>.
PREMM_{1,2} website: <http://dana-farber.prod.dfci.harvard.edu/pat/cancer/gastrointestinal/crc-calculator/old-calculator.asp>.
PREMM_{1,2,6} website: <http://dana-farber.prod.dfci.harvard.edu/pat/cancer/gastrointestinal/crc-calculator/default.asp>.
AIFEG website: <http://aifeg.dps.unipi.it/software.htm>.
Myriad website: <http://myriadpro.com/treating-diseases/hereditary-cancer-testing/lynch-syndrome-hnpcc/mlh1-and-msh2-prevalence-table>.
*Other HNPCC-associated cancers include ovary, stomach, small intestine, urinary tract/kidney, bile ducts, glioblastoma multiforme, sebaceous gland tumours, and pancreas.
AC-II, Amsterdam Criteria-II; CRC, colorectal cancer; EC, endometrial cancer; FDR, first-degree relative; AIFEG, Associazione Italiana per lo studio della Familiarità ed Ereditarietà dei tumori Gastrointestinali, IHC, immunohistochemistry; MSI, microsatellite instability; SDR, second-degree relative.

Table 2 Summary of meta-analyses of the area-under curves from studies that evaluated risk prediction models for mismatch repair (MMR) gene mutations

Model	Sample	I ² (%)	Heterogeneity p value	Pooled AUC (95% CI)
PREMM	Clinic-based	72.3	<0.001	0.82 (0.79 to 0.86)
	Population-based	73.8	0.004	0.88 (0.84 to 0.92)
	Combined	82.4	<0.001	0.84 (0.81 to 0.88)
MMRpro	Clinic-based	69.0	0.004	0.79 (0.73 to 0.84)
	Population-based	80.5	0.02	0.88 (0.70 to 1.05)
	Combined	92.5	<0.001	0.80 (0.72 to 0.88)
MMRpredict	Clinic-based	77.4	<0.001	0.77 (0.70 to 0.84)
	Population-based	74.8	0.008	0.89 (0.81 to 0.98)
	Combined	91.5	<0.001	0.81 (0.73 to 0.88)
Leiden	Clinic-based	47.5	0.11	0.82 (0.78 to 0.87)
	Population-based*	—	—	0.93 (0.91 to 0.95)
	Combined	88.4	<0.001	0.85 (0.78 to 0.91)

*Only one population-based study that validated Leiden model.
AUC, area under curve.

MMRpredict and 0.82 (95% CI 0.75 to 0.88) for PREMM (see online supplementary table S2). Given the high degree of overlap in the CIs, we cannot state that one model has a higher discrimination than any of the others.

SUMMARY

We have reviewed all major criteria and prediction models for MMR gene mutation status, which are currently available. This review is increasingly important, as consideration for who to screen and test for MMR gene mutations is now broader than it was in the past.

Overall, the existing prediction models are sensitive and specific with an AUC of approximately 90%. By contrast to the clinical or clinicopathological criteria that are dichotomous (yes/no for screening followed by germline testing), the prediction models provide a probability of having a MMR gene mutation for a family or an individual and, therefore, a cut-off point of probability needs to be set for when to test for the genetic mutation. Several levels have been suggested; for example, anyone with a 5% or greater probability of being carriers.⁹⁴ Dinh *et al*⁹⁵ observed that direct germline testing for MMR gene mutations in people aged 25–35 years with a 5% or greater risk of being carriers predicted by PREMM_{1,2,6} ‘... could improve health outcomes in a cost-effective manner relative to current practice’ (initial screening by IHC and/or MSI followed by germline testing for colorectal cancer-affected people with a strong family history). This recommendation is in line with the National Comprehensive Cancer Network,⁹⁶ which supports direct germline testing for MMR gene mutations for individuals with a 5% or greater risk of being carriers when a tumour sample is not readily available. For families in which there is no colorectal cancer tumour available for initial screening, this recommendation could result in numerous potential negative full gene screens and potentially increase costs compared within families where a colorectal tumour could be used for initial screening followed by germline testing.

Kastrinos *et al*^{92 97 98} proposed an algorithm for germline testing: IHC followed by MSI (and also *BRAF* testing for loss of *MLH1* protein expression) in people diagnosed with colorectal cancer who have a 5% or greater probability of mutation according to PREMM_{1,2,6}. For a fast implementation of IHC analyses for MMR-deficiencies in combination with molecular analysis of *BRAF* mutations and/or *MLH1* promoter methylation, new technology will be required as part of the diagnostic setting for all Lynch syndrome-associated cancers. Further, implementation of targeted sequencing of genes by next-generation sequencing will probably challenge the diagnostics of Lynch syndrome as it is expected to deliver lower costs and faster time of analysis.

Currently, all these models are free and publicly available. The cut-off level needs to be set by individual clinics depending on their resources and the proportion of mutation carriers they want to identify. Future models may need to: (1) provide prediction of *PMS2* mutations, (2) take into account a wider range of Lynch syndrome-associated cancers when assessing family history and (3) be applicable to all people irrespective of any cancer diagnosis.

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Supplementary Table 1 Summary of studies that evaluated risk prediction models for mismatch repair gene mutations

Study	Year	Sample	Mutation Carriers	Models	AUC (95% CI)	Sensitivity* (95% CI)	Specificity* (95% CI)	PPV* (95% CI)	E/O [#] (95% CI)
Population-based Sample (n = 7 studies)									
Barnetson [1]	2006	155 CRC cases diagnosed <45 years from the Scottish Cancer Registry	35 (23%) 19 <i>MLH1</i> 13 <i>MSH2</i> 3 <i>MSH6</i>	MMRpredict	0.82 (0.72-0.91)	94	27	27	-
Balaguer [2]	2008	1222 CRC cases recruited from 25 hospitals of the EPICOLON Study, Spain	8 (0.7%) 3 <i>MLH1</i> 5 <i>MSH2</i>	PREMM _{1,2}	-	100 (71-100)	68 (65-71)	2 (1-4)	-
				PREMM _{1,2} +MSI/IHC	-	100 (71-100)	97 (96-98)	21 (11-36)	-
Balmaña [3]	2008	1222 CRC cases recruited from 25 hospitals of the EPICOLON Study, Spain	8 (0.7%) 3 <i>MLH1</i> 5 <i>MSH2</i>	MMRpredict	0.92 (0.83-1.01)	75 (40-93)	94 (93-95)	8 (3-16)	-
				MMRpredict+MSI/IHC	-	75 (40-93)	99 (99-100)	35 (17-59)	-
				PREMM _{1,2}	0.93 (0.86-0.99)	100 (71-100)	68 (65-71)	2 (1-4)	-
				PREMM _{1,2} +MSI/IHC	-	100 (71-100)	97 (96-98)	21 (10-36)	-
				MSI/IHC	-	100 (71-100)	94 (92-95)	10 (5-18)	-
				Revised Bethesda	-	100 (71-100)	77 (74-79)	3 (1-5)	-
				Revised Bethesda +MSI/IHC	-	100 (99-100)	98 (97-98)	22 (11-38)	-
				Amsterdam-II	-	50 (22-78)	98 (97-99)	18 (7-39)	-
				Amsterdam-II +MSI/IHC	-	50 (22-78)	99 (99-100)	36 (15-65)	-
Green [4]	2009	725 CRC cases diagnosed age <75 years from 684 families, recruited through the Newfoundland Colorectal Cancer Registry	18 (2.5%) 1 <i>MLH1</i> 13 <i>MSH2</i> 2 <i>MSH6</i> 2 <i>PMS2</i>	Leiden	0.93 (0.91-0.95)	-	-	-	1.15 (0.72-1.83)
				MMRpredict	0.96 (0.94-0.97)	94 (73-99)	91 (88-93)	-	2.84 (1.79-4.51)
				PREMM _{1,2}	0.91 (0.89-0.93)	-	-	-	4.28 (2.70-6.80)
				MMRpro	0.95 (0.93-0.96)	-	-	-	2.09 (1.32-3.32)
				Revised Bethesda	-	94	51 (47-55)	-	-
Kastrinos [5]	2011	1214 CRC cases recruited from cancer registries of the Colon Cancer Family Registry [^]	82 (7%)	PREMM _{1,2,6}	0.88 (0.83-0.92)	90	67	-	-
Kastrinos [6]	2012	1181 CRC cases recruited from cancer registries of the Colon Cancer Family Registry [^]	80 (7%) 27 <i>MLH1</i> 43 <i>MSH2</i> 10 <i>MSH6</i>	PREMM _{1,2,6}	0.84 (0.81-0.88)	-	-	-	-
				MSI	0.82 (0.78-0.86)	-	-	-	-
				IHC	0.88 (0.85-0.90)	-	-	-	-
				MSI+IHC	0.90 (0.87-0.92)	-	-	-	-

				PREMM _{1,2,6} +MSI	0.92 (0.89-0.94)	-	-	-	-
				PREMM _{1,2,6} +IHC	0.94 (0.92-0.96)	-	-	-	-
				PREMM _{1,2,6} +MSI/IHC	0.94 (0.92-0.97)	-	-	-	-
Mercado [7]	2012	563 EC cases recruited from the Ohio State University Columbus area	14 (2.5%) 2 <i>MLH1</i> 3 <i>MSH2</i> 9 <i>MSH6</i>	MMRpredict	0.76 (0.54-0.97)	71	64	9	-
				PREMM _{1,2,6}	0.77 (0.60-0.93)	93 (66-100)	5 (4-7)	2 (1-4)	-
				MMRpro	0.77 (0.61-0.92)	57 (29-82)	85 (79-89)	20 (9-35)	-
				MMRpro+MSI/IHC	0.87 (0.77-0.97)	64 (35-87)	89 (84-93)	27 (13-46)	-
				MSI	-	92 (64-100)	78 (75-82)	9 (5-15)	-
				IHC	-	86 (57-98)	67 (62-72)	10 (5-16)	-
Clinic-based Sample (n = 13 studies)									
Balmaña [8]	2006	1016 individuals (550 affected with CRC) who underwent genetic testing in Myriad Genetic Laboratories Inc, Salt Lake City, Utah	155 (15%) 54 <i>MLH1</i> 101 <i>MSH2</i>	PREMM _{1,2}	0.80 (0.76-0.84)	-	-	-	0.85 (0.73-1.00)
Chen [9]	2006	279 individuals (176 affected with CRC) from 226 clinic-based families in the United States, Canada, and Australia [Johns Hopkins Colorectal Cancer Risk Assessment Clinic and Hereditary Colorectal Cancer Registry, Colon Cancer Family Registry ^A , and Memorial Sloan-Kettering Cancer Center]	121 (43%) 51 <i>MLH1</i> 63 <i>MSH2</i> 7 <i>MSH6</i>	Leiden	0.77 (0.71-0.83)	-	-	-	0.65 (0.56-0.74)
				MMRpro	0.79 (0.74-0.84)	-	-	-	1.03 (0.93-1.16)
				MMRpro+MSI	0.83 (0.78-0.87)	-	-	-	1.06 (0.95-1.19)
				Amsterdam-II	-	75	62	-	-
				Revised Bethesda	-	77	54	-	-
				Revised Bethesda+ MSI	-	72	69	-	-
Marroni [10]	2006	219 individuals (families) recruited at 5 clinical and molecular centers from Central and Northern Italy	68 (31%) 30 <i>MLH1</i> 38 <i>MSH2</i>	Leiden	0.81 (0.75-0.87)	-	-	-	0.64 (0.51-0.82)
				AIFEG	0.80 (0.73-0.85)	100 ^{**}	71 ^{**}	-	1.09 (0.86-1.39)
				Leiden+MSI	0.90 (0.86-0.94)	-	-	-	0.61 (0.47-0.79)
				AIFEG+MSI	0.90 (0.85-0.94)	-	-	-	1.04 (0.80-1.36)
				Amsterdam-I	-	62	78	-	-
				Revised Bethesda	-	100	65	-	-
Pouchet [11]	2009	81 individuals (75 affected with CRC) recruited from the	39 (48%) 20 <i>MLH1</i>	MMRpredict	0.73 (0.61-0.86)	-	-	-	1.00 (0.72-1.38)
				PREMM _{1,2}	0.77 (0.65-0.88)	-	-	-	1.00 (0.73-1.37)

		Jewish General Hospital and the Montreal General Hospital in Montreal, Canada	14 <i>MSH2</i> 4 <i>MSH6</i> 1 <i>PMS2</i>	MMRpro	0.73 (0.62-0.85)	-	-	-	1.00 (0.73-1.37)
Ramsoekh [12]	2009	321 CRC cases (321 families) recruited from the department of clinical genetics of the Erasmus Medical Center, the Netherlands	66 (21%) 25 <i>MLH1</i> 23 <i>MSH2</i> 18 <i>MSH6</i>	Amsterdam-plus	0.82 (0.75-0.89)	82	55	-	-
				Leiden	0.84 (0.78-0.90)	73	80	-	-
				MMRpredict	0.84 (0.78-0.90)	98	9	-	-
				PREMM _{1,2}	0.84 (0.79-0.90)	98	22	-	-
				Amsterdam-II	-	30	89	-	-
				Revised Bethesda	-	77	59	-	-
				MSI/IHC	-	100	89	-	-
Backes [13]	2009	562 EC cases recruited from three hospitals in Columbus, Ohio	13 (2.3%) 1 <i>MLH1</i> 3 <i>MSH2</i> 9 <i>MSH6</i>	MMRpredict	-	60-100	-	-	-
				PREMM _{1,2}	-	100	-	-	-
				MMRpro	-	84	-	-	-
Monzon [14]	2010	72 CRC cases recruited from the British Columbia Cancer Agency Hereditary Cancer Program	25 (35%) 11 <i>MLH1</i> 12 <i>MSH2</i> 2 <i>MSH6</i>	Myraid	0.75 (0.64-0.87)	-	-	-	-
				Leiden	0.90 (0.82-0.97)	-	-	-	-
				MMRpredict	0.86 (0.76-0.96)	-	-	-	-
				PREMM _{1,2}	0.93 (0.86-0.99)	-	-	-	-
				MMRpro	0.90 (0.82-0.98)	-	-	-	-
				Amsterdam-II	-	76	74	-	-
Khan [15]	2011	230 individuals (145 affected with CRC) recruited from the University of California at San Francisco's Colorectal Cancer Prevention Program and the University of Chicago's Cancer Risk Clinic	113 (49%) 47 <i>MLH1</i> 51 <i>MSH2</i> 15 <i>MSH6</i>	MMRpredict	0.76 (0.68-0.84)	85	35	-	-
				PREMM _{1,2,6}	0.78 (0.72-0.84)	90	37	-	-
				MMRpro	0.82 (0.74-0.86)	90 [¶]	36 [¶]	-	-
				Amsterdam-II	0.68	81	52	-	-
				Revised Bethesda	0.52	99	10	-	-
Kastrinos [5]	2011	613 CRC cases recruited from family cancer clinics of the Colon Cancer Family Registry [^]	198 (32%)	PREMM _{1,2,6}	0.81 (0.77-0.84)	94	56	-	-
Kastrinos [6]	2012	470 CRC cases recruited from family cancer clinics of the Colon Cancer Family Registry [^] (clinic-based)	159 (34%) 63 <i>MLH1</i> 82 <i>MSH2</i> 14 <i>MSH6</i>	PREMM _{1,2,6}	0.88 (0.84-0.93)	-	-	-	-
				MSI	0.79 (0.76-0.82)	-	-	-	-
				IHC	0.79 (0.76-0.82)	-	-	-	-
				MSI+IHC	0.80 (0.78-0.83)	-	-	-	-
				PREMM _{1,2,6} +MSI	0.93 (0.89-0.96)	-	-	-	-

				PREMM _{1,2,6} +IHC	0.92 (0.88-0.96)	-	-	-	-
				PREMM _{1,2,6} +MSI/IHC	0.94 (0.89-0.96)	-	-	-	-
Mercado [7]	2012	129 EC cases recruited through the familial cancer clinics in the Colon Cancer Family Registry	80 (62%) 31 <i>MLH1</i> 40 <i>MSH2</i> 9 <i>MSH6</i>	MMRpredict	0.54 (0.43-0.66)	91	0	67	-
				PREMM _{1,2,6}	0.67 (0.58-0.77)	99 (93-100)	2 (0.1-11)	62 (53-71)	-
				MMRpro	0.64 (0.54-0.73)	95 (88-99)	10 (4-22)	63 (54-72)	-
				MMRpro+MSI/IHC	0.73 (0.64-0.82)	93 (84-97)	22 (12-37)	66 (57-75)	-
				MSI	-	100 (79-100)	38 (9-76)	76 (53-92)	-
				IHC	-	94 (84-99)	48 (27-69)	80 (68-89)	-
Monteiro Santos [16]	2012	88 CRC cases recruited from two institutions in Sao Paulo State, Brazil	31 (35%) 15 <i>MLH1</i> 16 <i>MSH2</i>	Myriad	0.70 (0.60-0.80)	100	0	-	-
				Leiden	0.81 (0.71-0.88)	100	0	-	-
				MMRpredict	0.85 (0.76-0.92)	93	38	-	-
				PREMM _{1,2}	0.85 (0.75-0.91)	98	28	-	-
				MMRpro	0.82 (0.73-0.90)	100	3	-	-
Tresallet [17]	2012	214 CRC cases recruited from the Department of Surgery of Ambroise Paré Hospital, France	8 (4%) 2 <i>MLH1</i> 5 <i>MSH2</i> 1 <i>MSH6</i>	MMRpredict	0.76 (0.65-0.88)	63 (31-86)	88 (83-92)	-	-
				PREMM _{1,2}	0.76 (0.59-0.93)	75 (41-93)	50 (48-57)	-	-
				MMRpro	0.73 (0.49-0.98)	63 (31-86)	78 (71-83)	-	-
				Amsterdam-II	-	38 (14-69)	99 (97-100)	-	-
				Revised Bethesda	-	75 (41-93)	59 (52-66)	-	-

AUC, area under the curve; CRC, colorectal cancer; O, observed; E, expected

^ Colon Cancer Family Registry included six main recruitment centers: University of Hawaii, Fred Hutchinson Cancer Research Center, Mayo Clinic, University of Southern California Consortium, Cancer Care Ontario, and University of Melbourne.

*≥5% cut-off level

**10% cut-off level

¶>7% cut-off level

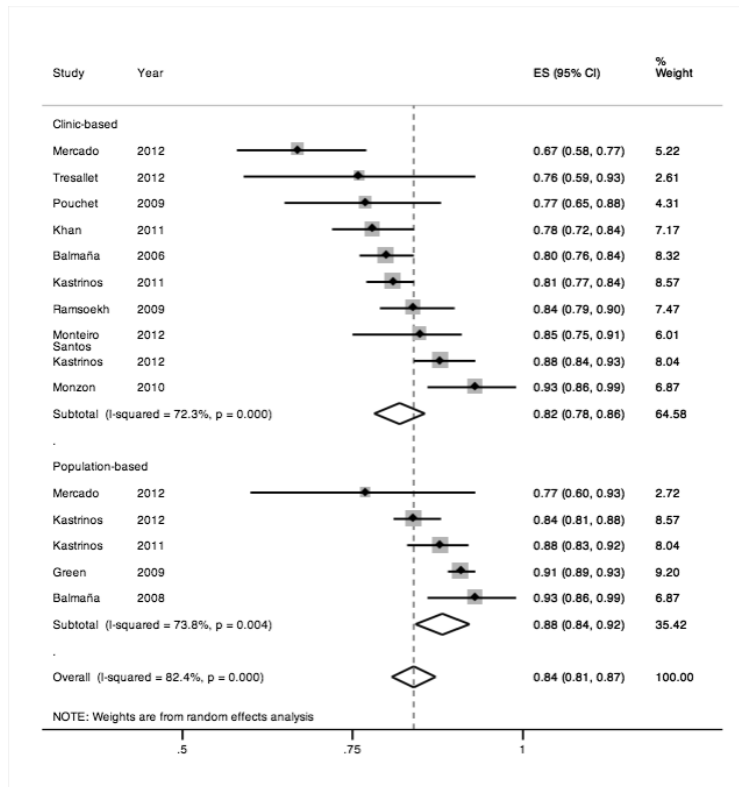
95% CI were estimated using $E/O \exp(\pm 1.96 \cdot \sqrt{1/O})$

Supplementary Table 2 Summary of meta-analyses of the area-under curves from studies that evaluated the three prediction models for mismatch repair gene mutations (PREMM, MMRpro and MMRpredict) within the same study

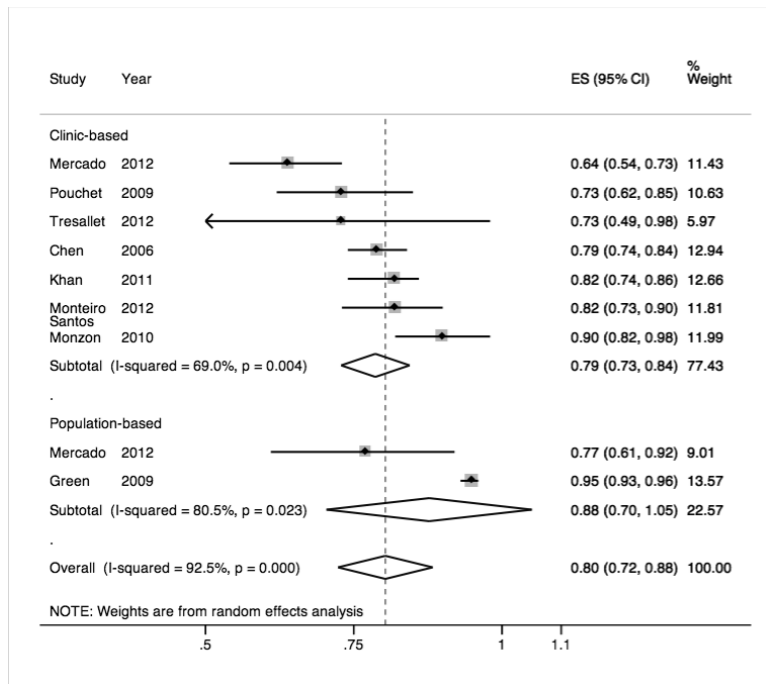
Model	Sample	I-squared (%)	Heterogeneity p-value	Pooled AUC (95%CI)
PREMM	Clinic-based	79.2	<0.001	0.80 (0.72-0.88)
	Population-based	63.3	0.09	0.87 (0.74-0.99)
	Combined	85.4	<0.001	0.82 (0.75-0.88)
MMRpro	Clinic-based	74.1	0.002	0.78 (0.71-0.86)
	Population-based	80.5	0.02	0.88 (0.70-1.05)
	Combined	91.3	<0.001	0.81 (0.72-0.89)
MMRpredict	Clinic-based	78.1	<0.001	0.75 (0.67-0.84)
	Population-based	69.8	0.07	0.89 (0.70-1.08)
	Combined	93.2	<0.001	0.78 (0.68-0.89)

AUC, area-under curve; CI, confidence interval

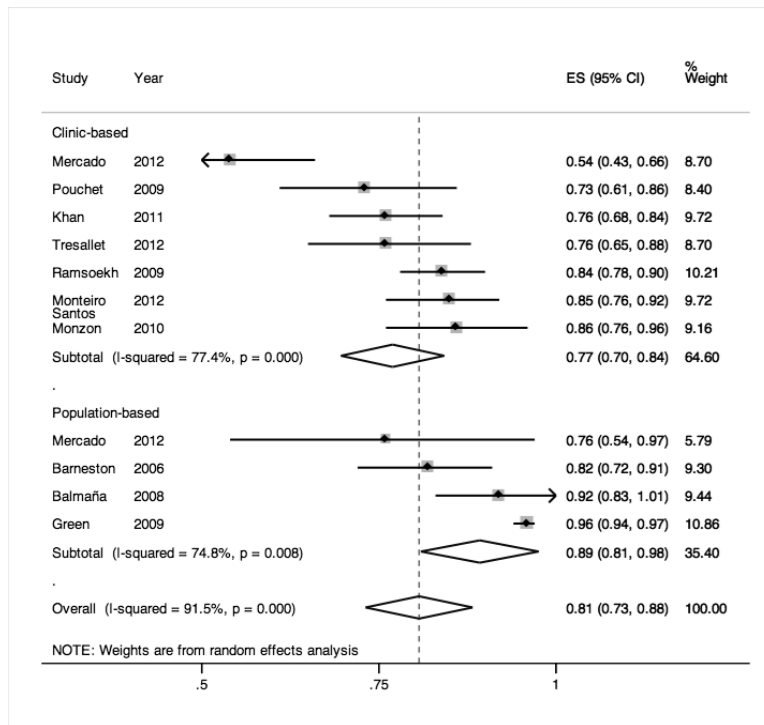
Supplementary Figure 1 Meta-analysis of the area-under curves from studies that evaluated PREMM model of prediction for mismatch repair gene mutations



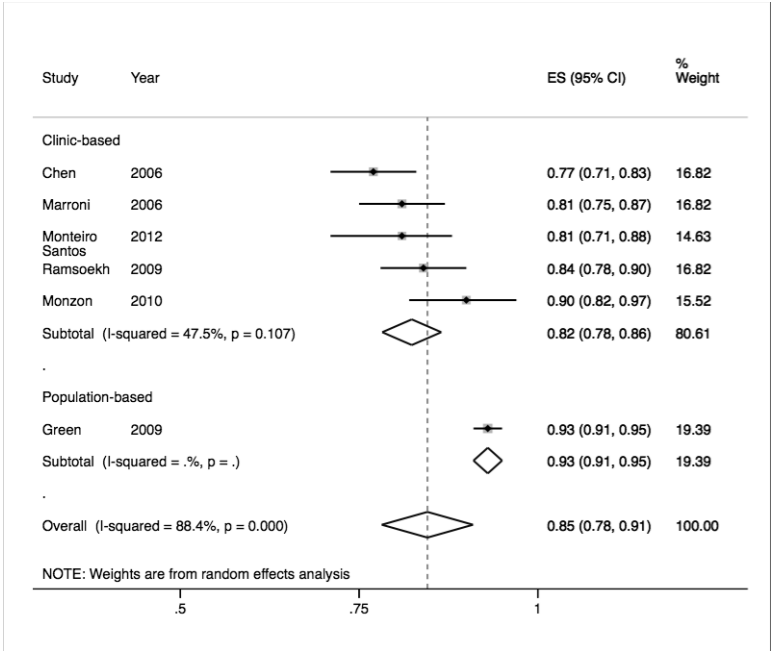
Supplementary Figure 2 Meta-analysis of the area-under curves from studies that evaluated MMRpro model of prediction for mismatch repair gene mutations



Supplementary Figure 3 Meta-analysis of the area-under curves from studies that evaluated MMRpredict model of prediction for mismatch repair gene mutations



Supplementary Figure 4 Meta-analysis of the area-under curves from studies that evaluated Leiden model of prediction for mismatch repair gene mutations



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