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Validation and extension of the PREMM_{1,2} model in a population-based cohort of colorectal cancer patients

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Summary

Background and aims—Early recognition of patients at risk for Lynch syndrome is critical but often difficult. Recently, a predictive algorithm -the PREMM_{1,2} model- has been developed to quantify the risk of carrying a germline mutation in the mismatch repair (MMR) genes, *MLH1* and *MSH2*. However, its performance in an unselected, population-based colorectal cancer population as well as its performance in combination with tumor MMR testing are unknown.

Methods—We included all colorectal cancer cases from the EPICOLON study, a prospective, multicenter, population-based cohort (n=1,222). All patients underwent tumor microsatellite instability analysis and immunostaining for *MLH1* and *MSH2*, and those with MMR deficiency (n=91) underwent tumor *BRAF* V600E mutation analysis and *MLH1/MSH2* germline testing.

Results—The PREMM_{1,2} model with a $\geq 5\%$ cut-off had a sensitivity, specificity and positive predictive value (PPV) of 100%, 68% and 2%, respectively. The use of a higher PREMM_{1,2} cut-off provided a higher specificity and PPV, at expense of a lower sensitivity. The combination of a $\geq 5\%$ cut-off with tumor MMR testing maintained 100% sensitivity with an increased specificity (97%) and PPV (21%). The PPV of a PREMM_{1,2} score $\geq 20\%$ alone (16%) approached the PPV obtained with PREMM_{1,2} score $\geq 5\%$ combined with tumor MMR testing. In addition, a PREMM_{1,2} score of $< 5\%$ was associated with a high likelihood of a *BRAF* V600E mutation.

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Conclusions—The PREMM_{1,2} model is useful to identify *MLH1/MSH2* mutation carriers among unselected colorectal cancer patients. Quantitative assessment of the genetic risk might be useful to decide on subsequent tumor MMR and germline testing.

Introduction

Lynch syndrome, also called hereditary nonpolyposis colorectal cancer, is the most common form of hereditary colorectal cancer (CRC), accounting for 1–5% of all colorectal malignancies^{1–3}. It is characterized by early onset of CRC and other adenocarcinomas, predominantly endometrial cancer. The syndrome is inherited in an autosomal dominant pattern with variable penetrance, and occurs as a consequence of germline mutations in the mismatch repair (MMR) system⁴, mainly in *MLH1* and *MSH2* (>90% of cases)¹, but also in *MSH6*⁵ and *PMS2*⁶. The abnormal function of these genes leads to the accumulation of errors during DNA replication, particularly in repetitive sequences (microsatellites). As a result, tumors in patients with Lynch syndrome characteristically demonstrate microsatellite instability (MSI)⁷, as well as loss of protein expression corresponding to the mutated gene⁸.

The heterogeneity of Lynch syndrome complicates early recognition, which is critical and often not straightforward. The diagnostic criteria continue to evolve as understanding and characterization of this disorder improves. Indeed, identification of Lynch syndrome can be done by tumor MMR screening using MSI testing and/or immunostaining, in combination or not with clinical criteria. At present, the most widely accepted strategy relies on tumor molecular analysis in patients fulfilling the revised Bethesda guidelines⁷. Nevertheless, as in hereditary breast-ovarian cancer syndrome in the past^{9, 10}, Lynch syndrome identification is moving toward more refined algorithms and multivariable models which combine personal and familial data in order to obtain a quantitative estimation of the risk^{11–14}.

The PREMM_{1,2} model¹¹ is a recently developed web-based logistic regression model that predicts the likelihood of germline mutations in the *MLH1* and *MSH2* genes based on personal and family history of individuals. It was developed in a large and diverse cohort of probands undergoing genetic testing on the basis of their clinical history. Whereas the model accurately discriminates gene mutation carriers in this subset of individuals at moderate to high risk for Lynch syndrome¹¹, its usefulness in an unselected CRC population is unknown. Furthermore, efficacy of the PREMM_{1,2} model in combination with tumor MMR testing has not yet been assessed.

Using data from the EPICOLON study^{15, 16}, a prospective, multicenter, population-based cohort collected to establish the incidence and characteristics of hereditary and familial CRC forms in Spain, we assessed the efficacy of the PREMM_{1,2} model, in combination or not with tumor MMR testing, for the identification of *MLH1* and *MSH2* gene mutation carriers among unselected CRC patients.

Patients and methods

Patients

Between November 2000 and October 2001, all newly diagnosed CRC patients in 25 hospitals were included in the EPICOLON study^{15, 16}. Exclusion criteria were familial adenomatous polyposis, personal history of inflammatory bowel disease, and patient or family refusal to participate in the study. The study was approved by the institutional Ethics Committee of each participating hospital, and written informed consent was obtained from all patients.

Demographic, clinical, and tumor-related characteristics of probands, as well as a detailed family history were obtained using a pre-established questionnaire. Pedigrees were traced

backward and laterally as far as possible, or at least up to second degree relatives, in terms of cancer history. Age at cancer diagnosis, type, location, and tumor stage of the neoplasm and current status were recorded for each affected family member^{15, 16}.

Tumor microsatellite instability analysis and immunostaining

Tissue samples from tumor and normal colonic mucosa were obtained from each patient, immediately frozen in liquid nitrogen and stored at -70°C until use. In cases where no frozen tissue was available, formalin-fixed, paraffin-embedded samples were used. Genomic DNA was isolated using the QiaAmp Tissue Kit® (Qiagen, Courtaboeuf, France).

Microsatellite instability testing and immunostaining for MLH1 and MSH2 were performed in all patients regardless of age, personal or family history, and tumor characteristics. In addition, in those patients with a PREMM_{1,2} score $\geq 20\%$, immunostaining for MSH6 and PMS2 was also performed. Paraffin-embedded sections were immunostained with antibodies against mismatch repair proteins (anti-MSH2, Oncogene Research Products, Boston, MA; anti-MLH1, PharMingen, San Diego, CA; anti-MSH6, BD Transduction Laboratories; anti-PMS2, PharMingen, San Diego, CA), as described elsewhere¹⁵. Tumor cells were judged to be negative for protein expression only if they lacked staining in a sample in which normal colonocytes and stroma cells were stained. If no immunostaining of normal tissue could be demonstrated, the results were considered ambiguous.

Microsatellite status was assessed using the 5-marker panel proposed by the National Cancer Institute, as described elsewhere^{15, 17, 18}. Tumors were classified as stable if none of the markers showed instability. Tumors with 2 or more unstable markers were classified as high level MSI (MSI-H) and tumors with 1 unstable marker were classified as low-level MSI (MSI-L).

Germline *MLH1/MSH2* mutation analysis

Patients found to have tumors with MMR deficiency (demonstrated by either MSI-H and/or lack of protein expression) underwent *MSH2/MLH1* germline genetic testing. Moreover, all patients with a PREMM_{1,2} score $\geq 20\%$ with MMR proficient tumors also underwent genetic testing.

Germline mutational analysis was performed by both multiple ligation probe amplification (MLPA) analysis and sequencing, as described elsewhere¹⁵.

Tumor *BRAF* V600E mutation analysis

Tumor *BRAF* V600E mutation analysis was performed in all patients with MSI (high and low) and/or lack of MLH1/MSH2 protein expression by direct sequencing in tumor DNA, as described elsewhere¹⁹.

Application of the PREMM_{1,2} model

The PREMM_{1,2} model is a clinical model to predict the likelihood of finding a *MLH1* or *MSH2* mutation in at-risk individuals¹¹. The original study analyzed *MLH1/MSH2* mutation prevalence in a large cohort of patients undergoing genetic testing at Myriad Genetic Laboratories Inc. (Salt Lake City, UT). A multivariable model was developed using logistic regression including variables related to the proband and relatives. The prediction rule is available as a web-based tool at the Dana-Farber Cancer Institute web site (<http://www.dfci.org/premm>). We calculated the PREMM_{1,2} score for each patient included in the study using the SPSS V11.0 software package (SPSS Inc., Chicago, IL).

Statistical analysis

Sensitivity, specificity and positive predictive value (PPV) of the PREMM_{1,2} model, either alone or in combination with tumor MMR testing, were calculated with respect to the presence of *MLH1/MSH2* germline mutations. These performance characteristics depend on the cut-off used for the predicted risk of mutation and, therefore, we arbitrarily evaluated the following cut-off levels: <5%, ≥5%, ≥10%, ≥20%, and ≥40%. Ninety-five percent binomial confidence intervals were calculated based on the Adjusted Wald method²⁰.

Continuous variables were expressed as mean ± standard deviation and compared by the Student's t test. Categorical variables were compared by the Chi square test, applying the Yates' correction when needed.

All p values were two sided. A p value of less than 0.05 was considered to indicate a statistically significant difference. All calculations were performed by using the 11.0 SPSS software package (SPSS Inc., Chicago, IL).

Results

Characteristics of the patients

During the study period, 1222 patients with pathologically confirmed colorectal adenocarcinoma were diagnosed and included in the EPICOLON project. Demographic, clinical, and tumor-related characteristics of patients included in the study are summarized in Table 1.

One hundred and eleven (9.1%) patients showed tumor MSI, 83 of them (6.8%) were MSI-H and 28 (2.3%) were MSI-L. Likewise, 81 (6.6%) patients had a tumor with loss of protein expression in either *MLH1* (60 cases) or *MSH2* (21 cases). No patients with tumors that were MSI-L had lack of MMR protein expression. On the other hand, expression of both proteins was retained in 10 tumors with MSI-H, whereas loss of *MLH1* or *MSH2* expression was found in 8 patients whose tumor did not show MSI. Overall, 91 (7.4%) patients were found to have a tumor demonstrating MMR deficiency (defined as MSI-H and/or loss of *MLH1* or *MSH2* expression).

BRAF V600E mutation was detected in 20 out of 83 (24.1%) MSI-H tumors, and in 18 out of 60 (30%) tumors exhibiting loss of *MLH1* expression. In addition, only 2 out of 28 (7.1%) MSI-L tumors showed the *BRAF* V600E mutation, whereas it was not observed in any tumor with loss of *MSH2* expression.

Germline genetic testing identified 8 (0.7%) unambiguous mutations in either *MSH2* (5 cases) or *MLH1* (3 cases) genes.

Efficacy of the PREMM_{1,2} model for the identification of *MLH1/MSH2* gene carriers

The distribution according to the PREMM_{1,2} predicted likelihood of carrying a *MLH1/MSH2* germline mutation in the cohort was: <5%, 826 (68%); 5–9%, 266 (22%); 10–19%, 98 (8%); 20–29%, 23 (2%); and >40%, 9 (0.7%).

We first evaluated the PREMM_{1,2} model for its ability to identify *MLH1/MSH2* mutation carriers within the large cohort of CRC patients. Performance characteristics of the PREMM_{1,2} model for the identification of *MLH1/MSH2* gene carriers depended on the cut-off used for the predicted risk of mutation (Table 2). Using a cut-off of ≥5%, the model had a sensitivity of 100%, therefore, no mutation carriers would be missed if molecular evaluation was restricted to individuals with a PREMM_{1,2} score of ≥5%. Using higher cutoffs of 10%, 20% and 40% led to a progressive loss of sensitivity (75%, 62.5% and 25% respectively). As

expected, specificity increased with higher cutoffs and ranged from 68% with a 5% cutoff to 99.4% with a 40% cutoff. Positive predictive values of different cutoffs for the PREMM_{1,2} model are depicted in Figure 1.

Use of the PREMM_{1,2} model in combination with tumor MMR testing

The addition of tumor MMR testing, either by MSI analysis or immunostaining, to the PREMM_{1,2} model enhanced its performance by improving both specificity and PPV (Table 2). A PREMM_{1,2} score of $\geq 5\%$ in combination with abnormal MMR testing was associated with a 100% sensitivity, specificity of 97.4% and a PPV of 20.5%. The maximum PPV (36%) was achieved using a PREMM_{1,2} score of $\geq 20\%$ in combination with an abnormal tumor MMR result. The incremental gain obtained by the addition of MSI/immunohistochemistry testing was less at higher PREMM_{1,2} cut-off values; at a PREMM_{1,2} cut-off of 40%, the addition of MSI/immunohistochemistry testing did not lead to an improvement in specificity.

Characteristics of patients with low PREMM_{1,2} scores

The PREMM_{1,2} score correlated not only with the prevalence of germline mutations but also with the frequency of MMR deficiency (Table 3). Although 52 out of 826 (6.3%) individuals with a PREMM_{1,2} score $< 5\%$ had a MSI-H tumor or showed loss of MLH1 and MSH2 on immunohistochemistry, none of them carried a germline *MLH1/MSH2* mutation and 17 (33%) were associated with *BRAF* V600E mutation in the tumor (Table 3). Interestingly, in patients with abnormal MMR tests, *BRAF* V600E mutation was significantly associated with a PREMM_{1,2} score $< 5\%$ ($p=0.009$). In fact, 17 out of 20 (85%) patients with a MMR deficient tumor associated with *BRAF* V600E mutation had a PREMM_{1,2} score $< 5\%$, whereas none of the 14 patients with a MMR deficient tumor and PREMM_{1,2} score $\geq 20\%$ showed this variant (Table 3). As recent data demonstrate that *BRAF* mutations are rare in Lynch syndrome tumors, these findings are consistent with the conclusion that a low PREMM_{1,2} score indicates a low likelihood that a patient with CRC has Lynch syndrome.

Characteristics of patients with high PREMM_{1,2} scores

When patients with a PREMM_{1,2} score $\geq 20\%$ were stratified according to their MMR status (Table 4), patients with MMR deficient tumors differed from those with MMR proficiency in some clinical characteristics: they are more likely women ($p=0.02$), have a lower prevalence of previous or synchronous adenomas ($p=0.002$), a higher prevalence of endometrial cancer ($p=0.003$), more first-degree relatives with CRC ($p=0.01$), and more second degree relatives with endometrial cancer ($p=0.03$). Therefore, a high PREMM_{1,2} score in combination with MMR proficiency identified a significant group of families, recently characterized in our series, with a less penetrant cancer phenotype¹⁸, in line with a similar group with Amsterdam I criteria recently described as familial CRC type X syndrome²¹.

We further examined potential etiologies of the CRC in the patients based with high PREMM_{1,2} scores based on their MSI status. Five out of 14 (36%) patients with PREMM_{1,2} score $\geq 20\%$ and a MMR deficient tumor carried a *MLH1/MSH2* germline mutation. We further investigated potential etiologies of the high PREMM_{1,2} scores for the 9 individuals who were not found to carry germline *MLH1/MSH2* mutations by performing supplemental analyses of PMS2 and MSH6 immunostaining, and *BRAF* mutation analysis. In these non-mutation carriers, no *BRAF* mutation was found, and normal PMS2 and MSH6 protein expression was observed in all tumors.

In order to better characterize the subset of patients with a PREMM_{1,2} score $\geq 20\%$ and MMR-proficient tumors ($n=18$), MSH6 and PMS2 immunostaining and *MLH1/MSH2* germline gene testing were performed in all of them. With respect to immunostaining, normal MSH6 and PMS2 protein expression was observed in all tumors. Furthermore, *MLH1/MSH2* gene testing

did not show any deleterious mutations. Finally, we performed *MYH* analysis and one patient was found to have a biallelic *MYH* mutation (G382D/Y165C) in a nested study performed in the EPICOLON cohort²². This patient was a 49 years-old male with two synchronous CRCs and 25 synchronous adenomas, and no family history of any neoplasia.

Discussion

Extensive knowledge now available about the Lynch syndrome has encouraged researchers to look for a systematic, quantitative and objective approach to identify these patients^{11–13, 23}. We recently developed the web-based PREMM_{1,2} model¹¹, based on a logistic regression analysis from a large cohort of patients at-risk for hereditary CRC who underwent genetic testing to quantify the relative importance of known clinical parameters in Lynch syndrome and predict the likelihood of carrying a mutation in the *MLH1* and *MSH2* genes. Although the model performed well among individuals at moderate risk for Lynch syndrome, its usefulness and performance in a non-selected, population-based cohort of CRC patients, either alone or in combination with tumor MMR testing, was unknown.

Our study of 1,222 population-based CRC cases demonstrates that the PREMM_{1,2} model constitutes a useful approach to identify *MLH1/MSH2* gene mutation carriers among patients with CRC, either alone or in combination with MMR tumor testing. The quantitative assessment of the genetic risk obtained with the PREMM_{1,2} model may drive subsequent decisions about molecular testing. Moreover, the combination with tumor MMR analysis identified a sizable subgroup of patients with a heterogeneous high CRC risk, potentially involving familial CRC type X syndrome, *MYH*-related cancer and other still unknown inherited disorders.

The first important finding is the demonstration that a PREMM_{1,2} cut-off of $\geq 5\%$ identified all *MLH1* and *MSH2* mutation carriers among unselected CRC patients. The use of a higher PREMM_{1,2} cut-off provided a higher specificity and PPV, at expense of a lower sensitivity. The negative predictive value of a PREMM_{1,2} score $< 5\%$ was 100%, thus reinforcing the consistency of this cut-off point. Therefore, for the clinician in general practice, whose first decision point is to see if a patient with CRC needs further molecular evaluation for Lynch Syndrome, a score of $< 5\%$ indicates that no further referral is likely to be necessary, whereas a score of $\geq 5\%$ should lead to further molecular evaluation. The low specificity of a 5% cut-off for the presence of germline mutations necessitates further refinement of the likelihood of carrying a mutation prior to proceeding to genetic testing. Our data demonstrate that the combination of the PREMM_{1,2} score with tumor MMR testing improved its specificity and PPV. Indeed, using a cut-off of $\geq 5\%$, the addition of an abnormal tumor MMR test result provided a specificity of 98% and a PPV of 21%.

An interesting finding was that the PREMM_{1,2} model, in combination with tumor MMR testing, is able to identify a subset of patients resembling the recently described familial CRC type X syndrome²¹, families who fulfill the Amsterdam I criteria without evidence of MMR deficiency. Individuals in such families have a lower incidence of CRC than those in families with Lynch syndrome, whereas incidence for other cancers may not be increased. The molecular etiology of this disorder remains unknown, with a probable heterogeneous genetic basis. In our study, patients with a PREMM_{1,2} score $\geq 20\%$ with MMR-proficient tumors had similar features to those with familial CRC type X syndrome²¹: lower family history of CRC and other malignancies, and lower incidence of endometrial cancer. Interestingly, one patient with a high PREMM_{1,2} score and no evidence of MMR deficiency carried biallelic *MYH* mutations (Y165C/G382D).

We are aware that our study has some limitations. First, the relatively low number of patients with *MLH1/MSH2* mutations may constitute a potential drawback of the analysis, thus restraining the reliability of performance features. Second, the model does not account for *MSH6* gene mutations, although it is certain that this gene is responsible for a small proportion of Lynch syndrome cases. Finally, genetic testing was mainly performed in those patients whose tumors showed MMR deficiency, even though it is unlikely that gene carriers were undetected when both MSI analysis and immunostaining were performed systematically. In addition, in order to exclude this possibility, patients with a PREMM_{1,2} score $\geq 20\%$ did also undergo genetic testing.

In the last few years, there has been much interest in establishing different strategies to improve the identification of patients with Lynch syndrome. These approaches range from using clinical criteria alone (i.e. the Amsterdam criteria)²⁴ to universal tumor molecular testing (i.e. immunostaining) in any given CRC patient²⁵. The current most widely accepted recommendation, based on combination of the revised Bethesda guidelines and tumor MMR testing⁷, has been found to be an effective and efficient strategy for Lynch syndrome identification¹⁵. However, these clinical criteria have been criticized due to the use of broad and complex variables, which make them difficult to remember for a general health care professional, their low specificity, their inability to establish the likelihood of carrying a mutation in a given patient, and the difficulty of obtaining tumor samples from affected relatives to perform the MMR analyses²³. A potential advantage of the PREMM_{1,2} model with respect to the revised Bethesda guidelines relies on its quantitative nature. In fact, the model demonstrated a reasonable ability to discriminate among risk groups for probability of mutation with respect to the prevalence of mutations observed in the *MLH1/MSH2* genes and the prevalence of MMR deficiency. This latter correlation was especially relevant since a PREMM_{1,2} score of $<5\%$ identified the subset of MMR-deficient tumours associated with the somatic BRAF V600E mutation, a circumstance consistent with what is seen in the sporadic CRC setting^{26–28}. Taking into account these results and the performance characteristics of the predicted model's risk groups, in combination or not with tumor MMR testing, we propose a strategy for *MLH1/MSH2* genetic testing in the clinical practice (Figure 2). According to this algorithm, a PREMM_{1,2} score $<5\%$ could be considered a reliable cut-off to exclude those CRC patients who do not need further risk assessment because of its 100% negative predictive value for detecting germline *MLH1/MSH2* gene mutations. In patients reaching this cut-off, further decisions could also be made on the basis of PREMM_{1,2} score. In patients with a score between 5–19%, tumor MMR testing should be performed in order to achieve a reasonable PPV. Finally, considering the PPV of a PREMM_{1,2} score $\geq 20\%$ alone (16%) and the significant increase of the PPV at that point (Figure 1), it seems reasonable to pursue direct genetic testing in patients reaching such a score, particularly if a tumor sample is not available. It is important to note, however, that in addition to the risk estimate generated from the predictive model, other important factors (i.e. accessibility to genetic services, timelines of genetic information, insurance coverage, and availability of tumor block) may contribute to decide which strategy is the most convenient in a given patient. In that sense, the PREMM_{1,2} model can be used general health care providers to decide whether to refer a patient to a high-risk colorectal cancer clinic to receive appropriate genetic counseling, as well as by geneticists working in such units to decide on the proper molecular strategy. The use of the same algorithm in both clinical settings may contribute to a more rational referral and management approach of patients with suspected Lynch syndrome.

In conclusion, our study demonstrates that the PREMM_{1,2} model is useful to identify *MLH1/MSH2* mutation carriers among unselected CRC patients. The quantitative assessment of the genetic risk might be useful to decide subsequent molecular testing and contribute to identify other high-risk individuals who may benefit from genetic risk assessment.

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Appendix 1

Investigators from the Gastrointestinal Oncology Group of the Spanish Gastroenterological Association who participated in the Epicolon study.

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Figure 1.
Positive predictive value for detecting germline *MLH1/MSH2* gene mutations according to the PREMM_{1,2} score.

Figure 2.

Proposed algorithm for the identification of *MLH1/MSH2* gene carriers among patients with colorectal cancer. CRC, colorectal cancer; MMR, mismatch repair.

Table 1

Clinical and molecular characteristics of patients included in the study

Characteristics	
Age (yrs.) ¹	70 ± 11
Males – no. (%)	731 (59.8)
Site of tumor – no. (%)	
-Proximal to splenic flexure	357 (29.2)
-Distal to splenic flexure	865 (70.8)
Tumor TNM stage – no. (%)	
-I	161 (13.2)
-II	510 (41.7)
-III	337 (27.6)
-IV	214 (17.5)
Degree of differentiation – no. (%)	
-Well	292 (23.9)
-Moderate	835 (68.3)
-Poor	95 (7.8)
Mucinous carcinoma type – no. (%)	142 (11.6)
Synchronous colorectal cancer – no. (%)	71 (5.8)
Microsatellite instability-high – no. (%)	83 (6.8)
Microsatellite instability-low – no. (%)	28 (2.3)
Loss of MLH1/MSH2 protein expression – no. (%)	81 (6.6)
Tumor MMR deficiency ² – no. (%)	91 (7.4)
Germline <i>MLH1</i> mutation – no. (%)	3 (0.25)
Germline <i>MSH2</i> mutation – no. (%)	5 (0.4)

MMR, mismatch repair.

¹Expressed as mean ± standard deviation.²Tumor MMR deficiency demonstrated by either microsatellite instability-high and/or loss of MLH1/MSH2 protein expression.

Table 2
Performance characteristics of the PREMM_{1,2} model for the identification of *MLH1/MSH2* gene mutation carriers

Strategy	Without tumor MMR test results [†]				With MSI-H or abnormal IHC tumor results [†]			
	No. (%)	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (95% CI)	No. (%)	Sensitivity (95% CI)	Specificity (95% CI)	Positive predictive value (95% CI)
PREMM _{1,2} ≥5%	396 (32.0)	100 (70.6–100)	68 (65.4–70.6)	2 (1–4)	39 (3.2)	100 (70.6–100)	97.4 (96.4–98.2)	20.5 (10.5–35.8)
PREMM _{1,2} ≥10%	130 (10.6)	75 (40–93.7)	89.8 (88–91.4)	4.6 (1.9–9.9)	21 (1.7)	75 (40–93.7)	98.8 (97.9–99.3)	28.6 (13.5–50.2)
PREMM _{1,2} ≥20%	32 (2.6)	62.5 (30.4–86.5)	97.7 (96.8–98.5)	15.6 (6.4–32.2)	14 (1.1)	62.5 (30.4–86.5)	99.3 (98.6–99.6)	35.7 (16.2–61.4)
PREMM _{1,2} ≥40%	9 (0.7)	25 (6.3–59.9)	99.4 (98.8–99.7)	22.2 (5.3–55.7)	7 (0.6)	25 (6.3–59.9)	99.6 (99–99.8)	28.6 (7.6–64.8)

MMR, mismatch repair; MSI-H, microsatellite instability-high; IHC, immunohistochemistry; 95% CI, 95% confidence interval.

[†]Tumor MMR testing by either microsatellite instability or *MLH1/MSH2* immunostaining.

Table 3Prevalence of *MLH1/MSH2* germline mutations and mismatch repair deficiency according to the PREMM_{1,2} score

PREMM _{1,2} Score	No.	<i>MLH1/MSH2</i> germline mutation (%) ¹	Tumor MMR deficiency ² (%) ¹	Tumor MMR deficiency ² associated with <i>BRAF</i> V600E mutation (%) ¹
<5%	826	- (-)	52 (6)	17 (2)
5–9%	266	2 (0.8)	18 (7)	2 (0.7)
10–19%	98	1 (1)	7 (7)	1 (1)
20–39%	23	3 (13)	7 (30)	- (-)
≥40%	9	2 (22)	7 (78)	- (-)
Total	1,222	8	91	20

MMR, mismatch repair.

¹ Percentages referred to each PREMM_{1,2} category.² Tumor MMR deficiency demonstrated by either microsatellite instability-high and/or loss of *MLH1/MSH2* expression.

Table 4Personal and familial characteristics of patients with PREMM_{1,2} score $\geq 20\%$ according to the mismatch repair status

	MMR Proficiency(n=18)	MMR Deficiency(n=14)	p value
Personal characteristics			
Age (yrs) ¹	66.0 $\pm$ 15.5	60.7 $\pm$ 19	0.39
Female – no. (%)	4 (22.2)	9 (64.3)	0.02
Previous CRC – no. (%)	6 (33.3)	2 (14.2)	0.34
Synchronous CRC – no. (%)	7 (38.9)	3 (21.4)	0.45
Previous or synchronous adenoma – no. (%)	11 (61.1)	1 (7.1)	0.002
Endometrial cancer – no. (%)	- (-)	6 (42.9)	0.003
Other Lynch-syndrome associated cancers ² – no. (%)	6 (33.3)	7 (50)	0.34
Proximal CRC – no. (%)	7 (38.9)	6 (42.9)	0.82
Familial characteristics			
Age of FDR with CRC (yrs) ¹	45.7 $\pm$ 11.3	40.4 $\pm$ 7.2	0.13
FDR with CRC – no. (%)	6 (33.3)	11 (78.6)	0.01
≥ 2 FDR with CRC – no. (%)	3 (16.7)	7 (50)	0.06
FDR with endometrial cancer – no. (%)	7 (38.9)	3 (21.4)	0.45
≥ 2 FDR with endometrial cancer – no. (%)	2 (11.1)	1 (7.1)	1.0
FDR with other Lynch-syndrome associated cancers ² – no. (%)	2 (11.1)	6 (42.9)	0.09
Age of SDR with CRC (yrs) ¹	45.9 $\pm$ 7.8	46.3 $\pm$ 5.6	0.84
SDR with CRC – no. (%)	1 (5.6)	4 (28.6)	0.14
≥ 2 SDR with CRC – no. (%)	- (-)	2 (14.3)	0.18
SDR with endometrial cancer – no. (%)	- (-)	4 (28.6)	0.03
SDR with other Lynch-syndrome associated cancers ² – no. (%)	1 (5.6)	2 (14.3)	0.57
Amsterdam II criteria – no. (%)	4 (22.2)	10 (71.4)	0.005

MMR, mismatch repair; CRC, colorectal cancer; FDR, first-degree relatives; SDR, second-degree relatives.

¹Expressed as mean + standard deviation.²Stomach, ovaries, urinary tract, small intestine, pancreas, bile ducts, brain or sebaceous glands.