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ORIGINAL REPORT

Lynch Syndrome Caused by Germline *PMS2* Mutations: Delineating the Cancer Risk

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Terms in blue are defined in the glossary, found at the end of this article and online at www.jco.org.

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ABSTRACT

Purpose
The clinical consequences of *PMS2* germline mutations are poorly understood compared with other Lynch-associated mismatch repair gene (MMR) mutations. The aim of this European cohort study was to define the cancer risk faced by *PMS2* mutation carriers.

Methods
Data were collected from 98 *PMS2* families ascertained from family cancer clinics that included a total of 2,548 family members and 377 proven mutation carriers. To adjust for potential ascertainment bias, a modified segregation analysis model was used to calculate colorectal cancer (CRC) and endometrial cancer (EC) risks. Standardized incidence ratios (SIRs) were calculated to estimate risks for other Lynch syndrome–associated cancers.

Results
The cumulative risk (CR) of CRC for male mutation carriers by age 70 years was 19%. The CR among female carriers was 11% for CRC and 12% for EC. The mean age of CRC development was 52 years, and there was a significant difference in mean age of CRC between the probands (mean, 47 years; range, 26 to 68 years) and other family members with a *PMS2* mutation (mean, 58 years; range, 31 to 86 years; $P < .001$). Significant SIRs were observed for cancers of the small bowel, ovaries, breast, and renal pelvis.

Conclusion
CRC and EC risks were found to be markedly lower than those previously reported for the other *MMR*. However, these risks embody the isolated risk of carrying a *PMS2* mutation, and it should be noted that we observed a substantial variation in cancer phenotype within and between families, suggesting the influence of genetic modifiers and lifestyle factors on cancer risks.

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INTRODUCTION

Lynch syndrome (LS) is the most common heritable colorectal carcinoma (CRC) syndrome and is responsible for 2% to 4% of all CRC cases in the Western world.[1] The underlying cause of LS is a pathogenic heterozygous germline mutation in *MLH1*, *MSH2*, *MSH6*, *PMS2*, or *EPCAM*. Previous clinical studies focused primarily on patients with heterozygous mutations in the *MLH1*, *MSH2*, and *MSH6* genes[1-5] and reported high risks for the development of colorectal, endometrial, and other cancers including ovarian, small bowel, pancreatic, gastric, urothelial, breast, and possibly prostate carcinomas.

Although *PMS2* involvement in LS was described around the same time as that for *MSH2* and *MLH1*[6], technical difficulties in analyzing the *PMS2* gene as a result of a large number of pseudogenes has possibly led to underreporting of *PMS2* mutations in patients with LS. Several strategies to overcome this problem, such as the design of long-range amplicons[7,8] and RNA analysis,[9] have led to improvements in *PMS2* mutation detection. As a result of the relatively recent development of improved *PMS2* mutation diagnostic procedures, clinical reports concerning heterozygous *PMS2* mutation carriers published thus far include quite small cohorts.[10-12] These studies reported a lower *PMS2* mutation penetrance for CRC and endometrial cancer (EC)

compared with *MLH1* and *MSH2* mutation carriers and similar or even lower risks as compared with *MSH6* mutation carriers.[1,5,13] Furthermore, parents and other family members of biallelic *PMS2* mutation carriers rarely develop CRC or other LS-related cancers,[14] indicating a reduced penetrance for cancer in these heterozygous family members. One theory regarding the lower penetrance of *PMS2* mutations is that *MLH1*/*MLH3* and/or *MLH1*/*PMS1* heterodimers partially compensate for the loss of *MLH1*/*PMS2*, although it is worth noting that this mechanism has not yet been confirmed by functional studies.[15]

Establishing an accurate cancer risk for mutations in cancer susceptibility genes such as *PMS2* is difficult because families are likely to be ascertained based on the severity of their phenotype and outcomes are thus variable depending on family selection and methods of data analysis (eg, correction for ascertainment bias). In this study, using a modified segregation analysis, we aimed to achieve a reliable estimate of the cancer risk for heterozygous *PMS2* germline mutation carriers by including confirmed carriers together with nontested family members.

METHODS

Data Collection

All probands (index patients) included in the study were referred to a cancer family clinic because of an LS-associated cancer or because of a suspected family history. They all had a confirmed pathogenic germline mutation in the *PMS2* gene. Available pedigree and patient-specific data were collected from 2009 until 2012, in collaboration with the clinical genetic departments of university hospitals in the Netherlands, Norway, Germany, Sweden, Denmark, and Spain and the Leiden-based Netherlands Foundation for the Detection of Hereditary Tumors (Appendix Table A1, online only). The majority of patients were of white northern European origin.

Mutation screening of the probands was performed between 2007 and 2012. *PMS2* mutation analysis was initiated in most patients based on histologic investigations of the tumor suggestive for a *PMS2* germline defect and/or on a family's compliance with the Bethesda criteria.[16] In addition, eight families were recognized via a proband with biallelic *PMS2* mutations. Patients with biallelic *PMS2* mutations have a distinct phenotype, with a typical spectrum of tumors at a young age, so they were excluded from the cancer risk analysis (Data Supplement).[17-19]

Informed consent was obtained according to protocols approved by local ethical review boards (Leiden University Medical Center Ethics Review Board, No. P01.019). Clinical and pathologic data confirming the diagnosis, where available, were obtained from patient records.

Mutation Analysis of PMS2

Mutation detection analysis of the *PMS2* gene was performed in multiple laboratories using a variety of methods all aimed at avoiding interference by pseudogenes. These methods included exon-by-exon DNA sequencing of exons 1 to 11 and simultaneous reverse transcriptase polymerase chain reaction (RNA analysis) of the whole coding region of *PMS2* and/or long-range DNA amplicons that avoid pseudogene amplification.[7,8] Multiplex ligation-dependent probe amplification was used to detect large genomic deletions and duplications. *PMS2* mutations were classified as deleterious based on introduction of a premature stop codon, either directly as a result of a nonsense mutation or as a result of a frameshift mutation, or when a deleterious splice site mutation was identified. Missense mutations were classified as deleterious based on previous studies.[9,20] Mutations are provided in the Data Supplement.

Statistical Analysis

Cancer risk analysis was based on full pedigree information. A previously described protocol was used for the imputation of unknown dates of birth and death from the known dates of their family members. Unknown age at cancer diagnosis was, if possible, imputed from the cohort-, period-, and sex-specific mean age at cancer diagnosis in the general population.[21] Family members were considered to be at risk from birth until the first occurrence of any of the following events: first CRC diagnosis (n = 208); EC diagnosis (n = 39); other cancer diagnosis (n = 218); death; last contact of a family member with the study center or last DNA test of a family member; and 70th birthday. CRC and EC risks were estimated using modified segregation analysis implemented in the pedigree analysis software MENDEL (University of California, Los Angeles, Los Angeles, CA), as previously described.[12,22] Restricting the analysis to confirmed carriers would bias the results because affected family members and those with a strong family history of cancer might be more inclined to pursue mutation testing and deceased individuals would be excluded. The MENDEL program weighs the likelihood contributions of untested individuals according to their probability of being a carrier, which was estimated from their cancer history, age, and position in the pedigree. In the analysis, the penetrance function was modeled in terms of the incidence rates in carriers and noncarriers. The incidence rates were assumed to follow a Cox proportional hazards model in which the noncarriers were assumed to conform to population incidence rates. These population rates were calculated using combined calendar and age-specific incidences from the Netherlands and age-specific rates for the other European countries. All country-specific incidence rates contributed to the mean according to their weight in the total number of families. The relative risk represents the incidence rate in mutation carriers compared with the population incidence rates at age *t*. A single autosomal dominant model and a mutation frequency of 0.001 for *PMS2* were used. The incidences for each disease at age $\lambda(t)$ were assumed to follow a Cox model: $\lambda(t) = \lambda_0(t) \exp[G(t)]$, where $\lambda_0(t)$ is the age-specific disease incidence rate and $\exp[G(t)]$ is the age-specific hazard ratio (HR) or the relative risk in carriers compared with noncarriers.

To estimate the risk of other LS-associated cancers, we calculated the standardized incidence ratio (SIR) in a separate analysis as the ratio of observed cancers in the cohort to the expected cancers derived from the age-, sex-, calendar period–, and site-specific Dutch cancer population incidence rates. We restricted the cohort analysis to known Dutch mutation carriers who were alive and free of cancer in 1960 or born after 1960 (n = 276). Two-sided statistical significance levels for the SIRs were estimated, and 95% CIs were calculated under Poisson distribution of the observed frequencies. Comparative analyses of mean age of cancer development were performed via an independent samples *t* test in IBM SPSS Statistics 20 (SPSS, Chicago, IL).

RESULTS

Our cohort included 98 separate families with 377 proven mutation carriers, of whom 11 were biallelic carriers and 366 were heterozygous carriers. Cohort characteristics are listed in Table 1.

CRC and EC

The cumulative CRC risk (Table 2; Fig 1) calculated using MENDEL was 18.75% (95% CI, 5.60% to 30.06%) for males at age 70 years, with an HR of 6.92 (95% CI, 2.46 to 19.42). The CRC risk at age 70 years for female carriers was 10.56% (95% CI, 2.42% to 18.01%), with an HR of 4.71 (95% CI, 1.51 to 14.72), whereas the cumulative risk at age 70 years for EC (Table 2; Fig 2) was 11.78% (95% CI, 2.61% to 20.09%), with an HR of 8.74 (95% CI, 2.14 to 35.7). The mean age of first CRC development for all patients with a heterozygous *PMS2* mutation was 52 years (range, 26 to 86 years; Table 3). Notably, the age distribution for CRC differed markedly between probands and CRC-affected family members (Appendix Figs A1 and A2, online only); the mean ages of CRC diagnosis in these groups were 47 years (range, 26 to 68 years) and 58 years (range, 31 to 86 years), respectively. There was

Table 1. Cohort Description of the 98 Families

Characteristic	No. of Family Members		
	Total*	Male	Female
Total	2,548	1,284	1,262
Mutation carriers	377	172	205
Homozygotes	11	5	6
Non–mutation carriers	237	108	129
CRC	208	118	90
EC	39	—	39
Other cancer	218	108	110
Lip	1	1	0
Hypopharynx	1	1	0
Lymphoma of pharynx	1	1	0
Esophagus	5	3	2
Stomach	16	8	8
Small intestine, including duodenum	8	5	3
Liver and extrahepatic bile ducts	3	1	2
Pancreas	4	1	3
Other and ill-defined sites within the digestive organs and peritoneum	1	0	1
Nasal cavities, middle ear, and accessory sinuses	1	1	0
Trachea, bronchus, and lung	29	27	2
Bone and articular cartilage	1	0	1
Connective and other soft tissue	1	0	1
Malignant melanoma of the skin	6	3	3
Skin	10	8	2
Female breast	44	—	44
Kaposi's sarcoma	1	0	1
Cervix uteri	7	—	7
Ovary and other uterine adnexa	10	—	10
Other and unspecified female genital organs	1	—	1
Prostate	18	18	—
Testis	3	3	—
Bladder	5	5	0
Kidney and other and unspecified urinary organs	8	4	4
Brain	5	3	2
Thyroid gland	4	3	1
Secondary and unspecified malignant neoplasm of lymph nodes	1	1	0
Secondary malignant neoplasm of respiratory and digestive systems	7	1	6
Other malignant neoplasms of lymphoid and histiocytic tissue	3	0	3
Multiple myeloma and immunoproliferative neoplasms	1	1	0
Leukemia of unspecified cell type	8	6	2
Unknown origin	4	3	1

NOTE. Number of cancers excludes the cancers of the biallelic mutation carriers.
Abbreviations: CRC, colorectal carcinoma; EC, endometrial carcinoma.
*Sex was unknown for two individuals.

Table 2. Age-Specific HRs and Cumulative Cancer Risks for CRC and EC in *PMS2* Mutation Carriers

Cancer and Age	HR	95% CI	CR (%)	95% CI (%)
CRC male				
< 40 years	20.59	3.27 to 129.60	1.27	0.00 to 3.51
40-49 years	17.06	5.15 to 56.50	4.63	0.01 to 9.04
50-59 years	3.66	1.29 to 10.38	7.11	1.76 to 12.17
60-69 years	6.92	2.46 to 19.42	18.75	5.60 to 30.06
CRC female				
< 40 years	8.82	0.72 to 107.55	0.46	0.00 to 1.52
40-49 years	2.63	0.27 to 25.68	0.92	0.00 to 2.42
50-59 years	5.99	1.99 to 18.00	4.74	0.22 to 9.05
60-69 years	4.71	1.51 to 14.72	10.56	2.42 to 18.01
EC				
< 40 years	20.03	0.58 to 688.70	0.19	0.00 to 0.84
40-49 years	7.81	0.81 to 74.80	0.69	0.00 to 2.79
50-59 years	16.18	5.81 to 45.06	7.11	0.52 to 13.26
60-69 years	8.74	2.14 to 35.70	11.78	2.61 to 20.09

Abbreviations: CR, cumulative risk; CRC, colorectal carcinoma; EC, endometrial carcinoma; HR, hazard ratio.

no significant difference in mean age of CRC development between male and female carriers (51 *v* 52 years, respectively; $P = .83$). The mean age at diagnosis of EC was 55 years (range, 35 to 81 years), with no significant difference between probands and affected family members ($P = .76$, Table 3). For both CRC and EC, the mean age at diagnosis of carriers of a pathogenic *PMS2* mutation was higher when compared with carriers of a *MLH1* or *MSH2* mutation, but when compared with *MSH6*, the mean age at diagnosis of CRC was lower and the mean age at diagnosis of EC was similar in our cohort (Appendix Table A2, online only).

Other LS-Associated Cancers

Risks for cancers other than CRC or EC among *PMS2* mutation carriers are listed in Table 4. Significant SIRs were identified for cancers of the small bowel (SIR, 118.9; 95% CI, 38.6 to 277.4), ovaries (SIR, 12.0; 95% CI, 3.3 to 30.7), breast (SIR, 3.8; 95% CI, 1.9 to 6.8), and renal pelvis (SIR, 50.5; 95% CI, 6.1 to 182.4).

DISCUSSION

In an effort to achieve a comprehensive understanding of the cancer risks faced by *PMS2* mutation carriers, we collected and analyzed a

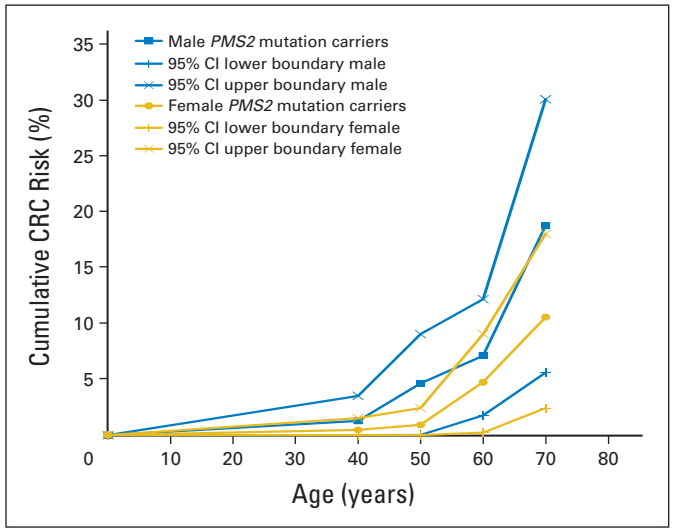

Fig 1. Graphic presentation of cumulative colorectal cancer (CRC) risk with 95% CIs.

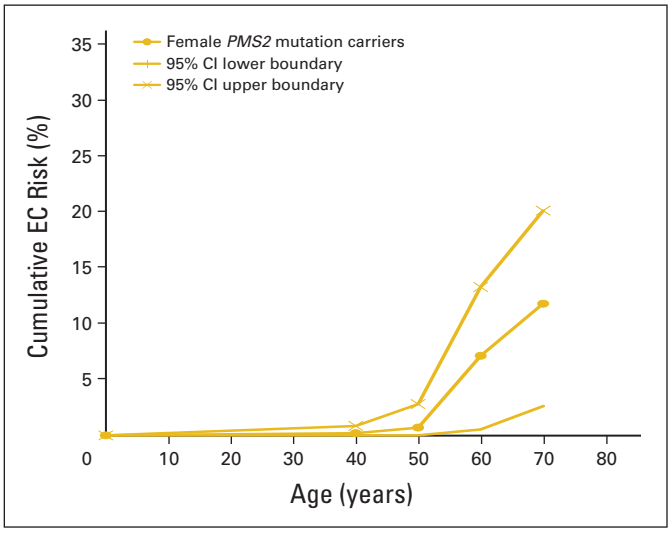

Fig 2. Graphic presentation of cumulative endometrial cancer (EC) risk with 95% CIs.

cohort of 98 *PMS2* mutation–positive families including more than 2,500 family members. Analysis of this large cohort revealed that cumulative CRC risk at age 70 years is almost 19% for males and 11% for females, whereas risk for EC is approximately 12%. Furthermore, we found significant SIRs for cancers of the small bowel, breast, ovaries, and renal pelvis. As reported previously for other mismatch repair genes (MMR), females appear to have a markedly lower CRC risk compared with males, although this observation was not statistically significant in our study. The calculated CRC and EC risks in our study agree with those reported by Senter et al[12] who used the same statistical methodology to calculate cancer risks in a cohort of 55 *PMS2* mutation–positive families. In contrast, cumulative cancer risks at age 70 years reported for *MLH1* and *MSH2* range from 52% to 97% for CRC and 21% to 54% for EC.[1] Far closer to the risks found in our study are the reported risks for carriers of *MSH6* mutations that ranged from 22% to 69% for CRC to 16% to 71% for EC[1]; thus, *MSH6* and *PMS2* mutations seem to represent significantly lower risks to these carriers.

A striking finding in this study is that cancer risk seems to vary widely between members of the same family and does not seem to be solely dependent on an individual's *PMS2* mutation status. This is illustrated by the wide age range at initial CRC diagnosis (26 to 86 years old) and the large difference in mean age (10 years) between probands and mutation-positive family members. The observed heterogeneity of risk between mutation carriers agrees with the findings of Dowty et al,[4] who also described this phenomenon for *MLH1* and *MSH2* mutation carriers. These authors proposed that lifetime CRC risk for both male and female mutation carriers (from birth to age 70 years) follows a U-shaped distribution rather than a normal distribution. This means that most MMR carriers have either a high risk or a low risk of developing CRC, with a relatively low proportion of carriers at moderate risk.[4]

One explanation of this high variance may be the presence of internal (eg, genetic) or external (eg, lifestyle) modifiers. Indeed, certain single nucleotide polymorphisms associated with a slight increase in CRC risk in the general population, as found by genome-wide association studies, are known to significantly influence CRC risk in patients with LS with *MLH1* and *MSH2* mutations.[23,24] Lifestyle factors, such as body mass index and smoking, have also been reported to modify CRC risk and adenoma development in patients with LS.[25,26] This is possibly explained by a difference in the molecular mechanism underlying carcinogenesis in patients with LS that might then result in lifestyle factors having a different effect in these patients compared with patients with sporadic CRC. However, because previous association studies only include limited numbers of *PMS2* mutation carriers, further research on this subject is needed.

Because of the increased identification of *PMS2* mutation carriers (and patients with LS in general) expected to result from the implementation of next-generation sequencing and universal screening programs for patients with CRC and EC, there is now a pressing need to establish *PMS2*-specific risk estimates. This increased detection is illustrated by studies using immunohistochemistry analysis in CRCs from population-based cohorts that showed that isolated PMS2 protein loss in the tumor, which is indicative of a germline *PMS2* mutation, occurs in 0.5% to 1.5% of unselected patients with CRC.[12,15,27] Previously, identification of patients with LS was based on strict Amsterdam and/or Bethesda selection criteria, but recent population-based CRC and EC studies have shown that more than half of patients identified with an LS-like profile do not comply with these criteria and probably would have been missed in the past.[27,28] This problem is even more relevant to *PMS2* families because it is likely that they do not comply with strict selection criteria as a result of low cancer penetrance and a higher mean age at cancer development (> 50 years).[12] Even in our selected cohort, only 19% of the families complied with the Amsterdam II criteria and 78% with the revised Bethesda criteria.[16,29] Almost 22% of the families in our cohort failed to comply with any of the previously mentioned criteria and would have been overlooked based on these criteria alone. The possible current underestimation of *PMS2* mutation–positive families is further suggested and illustrated by next-generation sequencing studies in which *PMS2* mutations were reported as incidental findings in 0.03% to 0.4% of individuals not selected based on CRC.[30-32]

Identifying MMR mutation carriers is important because these individuals should be enrolled in surveillance programs. Currently, LS family members are advised to participate in colonic surveillance at 1- to 2-year intervals beginning at approximately 20 to 25 years of age.[33,34] We now suggest that surveillance in *PMS2* mutation carriers could start at a slightly higher age (eg, 30 years, similar to the previously suggested age for female *MSH6* carriers) because of the later age

Table 3. Age at CRC and EC Diagnosis in *PMS2* Mutation Carriers

Type of Cancer and Population	No. of Mutation Carriers	Age at Diagnosis (years)			*P*
		Median	Mean	Range	
CRC					
Total group	106	51	52	26-86	.83
Male	65	52	51	26-86	
Female	41	51	52	27-78	
Probands	62	48	47	26-68	< .001
Family members	44	57	58	31-86	
EC					
Total group	25	54	55	35-81	
Probands	15	52	56	42-81	.76
Family members	10	58	54	35-68	

Abbreviations: CRC, colorectal carcinoma; EC, endometrial carcinoma.

Table 4. Other Lynch Syndrome–Associated Cancers

Location of Malignancy	Observed No. of Cancers	Expected No. of Cancers	Standardized Incidence Ratio	95% CI	*P*	Age at Diagnosis (years)	
						Mean	Range
Overall cancer*	35	16.9	2.1	1.4 to 2.9	< .001	57	18-80
Small bowel	5	0.042	118.9	38.6 to 277.4	< .001	60	48-79
Breast	11	2.9	3.8	1.9 to 6.8	< .001	55	36-80
Ovary	4	0.33	12.0	3.3 to 30.7	< .001	55	51-59
Prostate	2	1.17	1.7	0.21 to 6.2	.66	56	42-70
Renal pelvis	2	0.040	50.5	6.1 to 182.4	.0015	78	77-79
Brain	1	0.37	2.7	0.069 to 15.2	.62	55	
Leukemia†	1	0.47	2.1	0.054 to 11.9	.75	45	
Stomach	0	0.57	0	0 to 6.5	NA		
Pancreas	0	0.31	0	0 to 12	NA		
Bladder	1	0.50	2.0	0.051 to 11.2	.79	49	

NOTE. The standardized incidence ratio is the observed number of cancers divided by the number of expected cancers in the general population.
Abbreviations: CRC, colorectal carcinoma; EC, endometrial carcinoma; NA, not applicable.
*Excluding CRC and EC.
†Non-Hodgkin lymphoma.

of onset and lower cancer risk than that reported for *MLH1* and *MSH2*.[35] It should be noted, however, that six heterozygous probands (six [1.6%] of 366 mutation carriers) developed CRC at age 30 years or younger. Interestingly, their mutation-positive family members with CRC had a significantly later age of onset (56 years as opposed to 28 years). Moreover, none of the heterozygous family members in our cohort developed CRC before the age of 30 years (Appendix Figures A1 and A2, online only).

The efficacy of surveillance for EC is less well established. However, surveillance is currently still advised and consists of gynecologic examination with transvaginal ultrasound and/or hysteroscopy with aspiration biopsy, starting at the age of 30 to 35, which may lead to the detection of premalignant disease.[33,34,36] Prophylactic surgery (ie, risk-reducing salpingo-oophorectomy) does not seem to be appropriate for female *PMS2* mutation carriers because mortality from EC is relatively low.[37] Indeed, in our cohort, only one of 25 proven *PMS2* mutation carriers with EC died of EC, at age 65. Furthermore, ovarian cancer in patients with LS predominantly presents in early stages of carcinogenesis, and there is evidence that surveillance (transvaginal ultrasound and CA-125) might have a greater efficacy in these patients than in patients with non–LS-related ovarian cancer.[38] Therefore, we only advise use of the surveillance protocol described earlier and not prophylactic surgery.

Additional screening for other LS-associated cancers is currently not advised for patients with LS, except in the case of familial clustering of gastric or urinary tract cancer.[33,39] Our cohort of *PMS2* mutation carriers showed significant SIRs for cancers of the small bowel, ovaries, renal pelvis, and breasts (Table 4). The first three cancers are accepted as part of the LS tumor spectrum, but the association of breast cancer with germline *MMR* mutations is currently still a subject of debate.[1] Because of a relatively low incidence and, in general, a high mean age at diagnosis in our cohort (Table 4), additional surveillance other than for CRC and EC does not seem to be indicated. However, in light of an SIR of 3.8 for breast carcinomas, mammography from age 40 years may be considered, especially in *PMS2* families that show clustering of breast cancer. The relatively small number of patients in the current study and the low frequency of some LS-associated cancers mean that a larger cohort will be required to formulate definitive conclusions and advice.

Besides the implications for surveillance, the risks reported here also have implications for counseling of *PMS2* mutation carriers. Although it is our opinion that gene-specific counseling is justified, it should be clearly explained to patients that these risk estimates were corrected for ascertainment and embody the risk of the *PMS2* mutation itself. These unbiased risks are probably most applicable for patients with a *PMS2* mutation identified via population-based screening programs (and not selected based on their family history of cancer) or as an incidental finding of next-generation sequencing diagnostics now being introduced in many laboratories. However, given that in daily clinical practice, most *PMS2* mutation carriers will still be identified as members of a family severely affected with cancer, it is probable that other genetic or environmental risk factors that contribute to a higher cancer risk are present in these families.

In conclusion, the cumulative CRC and EC risks for *PMS2* mutation carriers are markedly lower than the current risk estimates for LS familiar to clinicians, and a significant proportion of these families are probably missed because of strict selection criteria. In addition, the wide within-family variance suggests that other risk factors are present in most families. While awaiting the advent of personalized risk stratification, we suggest a limited modification of surveillance guidelines for *PMS2* mutation carriers similar to that for *MSH6*, which is to begin colorectal surveillance at age 30 years instead of the current 25 years of age. Furthermore, prophylactic removal of the uterus and ovaries of female *PMS2* mutation carriers does not seem advisable at the present time.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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

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GLOSSARY TERMS

cumulative risk: a measure of risk of an event (usually disease occurrence) during a specified time period.

germline mutation: an inherited variation in the lineage of germ cells. Germline mutations can be passed on to offspring.

immunohistochemistry: the application of antigen-antibody interactions to histochemical techniques. Typically, a tissue section is mounted on a slide and incubated with antibodies (polyclonal or monoclonal) specific to the antigen (primary reaction). The antigen-antibody signal is then amplified using a second antibody conjugated to a complex of peroxidase-antiperoxidase, avidin-biotin-peroxidase, or avidin-biotin alkaline phosphatase. In the presence of substrate and chromogen, the enzyme forms a colored deposit at the sites of antibody-antigen binding. Immunofluorescence is an alternate approach to visualize antigens. In this technique, the primary antigen-antibody signal is amplified using a second antibody conjugated to a fluorochrome. On ultraviolet light absorption, the fluorochrome emits its own light at a longer wavelength (fluorescence), thus allowing localization of antibody-antigen complexes.

Lynch syndrome: hereditary nonpolyposis colorectal cancer (HNPCC). A cancer syndrome characterized by Henry T. Lynch in 1966, this genetic condition has a high risk of colon cancer as well as other cancers including endometrial, ovary, stomach, small intestine, hepatobiliary tract, upper urinary tract, brain, and skin.

mismatch repair genes (*MMR*): genes that recognize and correct errors in DNA replication leading to single base-pair mismatches or insertions/deletions in small repetitive tracts of DNA known as microsatellites.

phenotype: the overall appearance of an organism, or the observable expression of a specific trait, determined by its genotype and environmental factors.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Lynch Syndrome Caused by Germline *PMS2* Mutations: Delineating the Cancer Risk

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Appendix

Table A1. Family Country of Origin

Country	No. of Families
The Netherlands	76
Denmark	6
Spain	6
Sweden	5
Norway	3
Germany	2

Table A2. Mean Age at Cancer Diagnosis of Mismatch Repair Gene Mutation Carriers

	PMS2		*MLH1/MSH2*[4]		*MSH6*[5]	
Cancer	Mean Age (years)	Range (years)	Mean Age (years)	Range (years)	Mean Age (years)	Range (years)
CRC						
Including probands	52	26-86			59	30-90
Excluding probands	58	31-86	47	15-95		
EC						
Including probands	55	35-81			54	32-82
Excluding probands	54	35-68	47	26-75		

Abbreviations: CRC, colorectal carcinoma; EC, endometrial carcinoma.

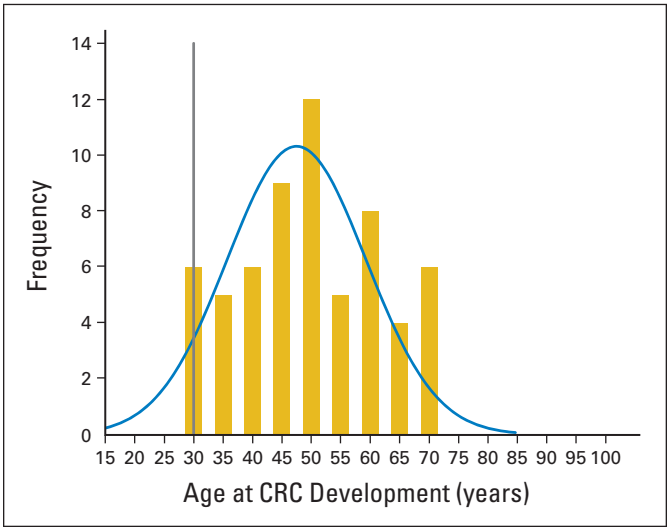

Fig A1. Histogram of mean age of colorectal cancer (CRC) development in probands with a heterozygous *PMS2* mutation. The gray line indicates age 30 years, which is the suggested start of colorectal surveillance.

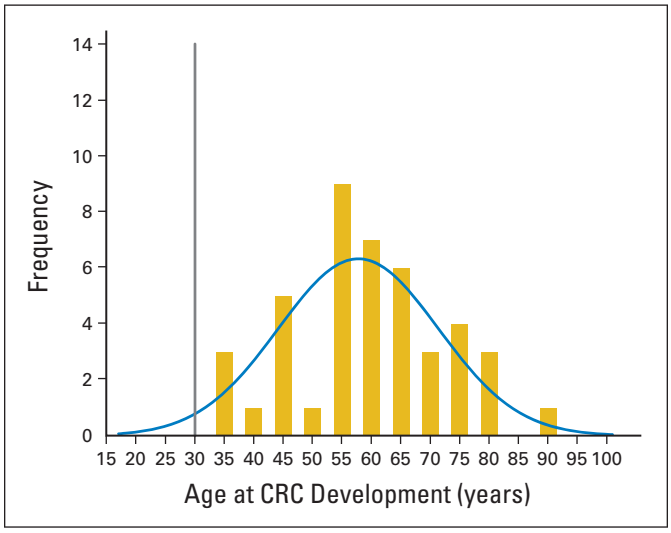

Fig A2. Histogram of mean age of colorectal cancer (CRC) development in family members with a heterozygous *PMS2* mutation. The gray line indicates age 30 years, which is the suggested start of colorectal surveillance.