PMS2-associated Lynch syndrome : the odd one out

Broeke, S.W. ten

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Cancer risks for PMS2-associated Lynch syndrome

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Sanne W ten Broeke, Heleen M. van der Klift, Carli M.J. Tops, Stefan Aretz, Inge Bernstein, Daniel D. Buchanan, Albert de la Chapelle, Gabriel Capella, Mark Clendenning, Christoph Engel, Steven Gallinger, Encarna Gomez Garcia, Jane C. Figueiredo, Robert Haile, Heather L. Hampel, John L. Hopper, Nicoline Hoogerbrugge, Magnus von Knebel Doeberitz, Loic Le Marchand, Tom G.W. Letteboer, Mark A. Jenkins, Annika Lindblom, Noralane M. Lindor, Arjen R. Mensenkamp, Pål Møller, Polly A. Newcomb, Theo A.M. van Os, Rachel Pearlman, Marta Pineda, Nils Rahner, Egbert J.W. Redeker, Maran J.W. Olderode-Berends, Christophe Rosty, Hans K. Schackert, Rodney Scott, Leigha Senter, Liesbeth Spruijt, Verena Steinke-Lange, Manon Suerink, Stephen Thibodeau, Yvonne J. Vos, Anja Wagner, Ingrid Winship, Frederik J. Hes, Hans F.A. Vasen, Juul T. Wijnen, Maartje Nielsen, and Aung Ko Win

ABSTRACT

Purpose

Lynch syndrome due to pathogenic variants in the DNA mismatch repair genes *MLH1*, *MSH2* and *MSH6* is predominantly associated with colorectal and endometrial cancer, although extra-colonic cancers have been described within the Lynch tumor spectrum. However, the age-specific cumulative risk (penetrance) of these cancers is still poorly defined for *PMS2*-associated Lynch syndrome. Using a large dataset from a worldwide collaboration, our aim was to determine accurate penetrance measures of cancers for carriers of heterozygous pathogenic *PMS2* variants.

Patients and methods

A modified segregation analysis was conducted that incorporated both genotyped and non-genotyped relatives, with conditioning for ascertainment to estimates corrected for bias. Hazard ratios (HR) and corresponding 95% confidence intervals (CIs) were estimated for each cancer site for mutation carriers compared with the general population, followed by estimation of penetrance.

Results

In total, 284 families consisting of 4878 first- and second-degree family members were included in the analysis. *PMS2* mutation carriers were at increased risk for colorectal cancer (cumulative risk to age 80 of 13% (95% CI: 7.9-22%) for males and 12% (95% CI: 6.7-21%) for women); and endometrial cancer (13% (95% CI: 7.0-24%)), compared with the general population (6.6%, 4.7% and 2.4%, respectively). There was no clear evidence of an increased risk of ovarian, gastric, hepatobiliary, bladder, renal, brain, breast, prostate or small bowel cancer.

Conclusion

Heterozygous *PMS2* mutation carriers were at small increased risk for colorectal and endometrial cancer but not for any other Lynch syndrome-associated cancer. This finding justifies that *PMS2*-specific screening protocols could be restricted to colonoscopies. The role of risk-reducing hysterectomy and bilateral salpingo-oophorectomy for *PMS2* mutation carriers needs further discussion.

INTRODUCTION

Lynch syndrome is most commonly associated with colorectal cancer and endometrial cancer. However, when first described in 1913, the observation of the co-occurrence of gastric cancer and endometrial cancer led to the initial identification of these families, underlining the apparently diverse phenotype.[1] The genetic background of Lynch syndrome is now known and it is caused by heterozygous germline mutations in one of the four mismatch repair (MMR) genes, *MLH1*, *MSH2*, *MSH6* and *PMS2*, or *EPCAM* deletions. The broad Lynch syndrome-associated tumor spectrum includes not only colorectal cancer and endometrial cancer but also gastric, ovarian, small bowel, brain, urothelial cell, skin, pancreas, prostate and biliary tract cancers.[2, 3] The involvement of germline MMR mutations in the development of breast cancer is still a subject of debate.[4-7] Although the reported cumulative risk (penetrance) to age 70 years for these non-colorectal, non-endometrial cancers in MMR gene mutation carriers is generally below 10%, mutation carriers still have a higher risk relative to the general population.[3] The Lynch syndrome-associated tumor phenotypes and their penetrance could depend on the type of MMR gene mutated or the specific variant.[8, 9]

For heterozygous *PMS2* mutation carriers, accurate estimation of penetrance, especially for extra-colonic cancers, has been hampered both by difficulties in variant analysis related to the existence of multiple pseudogenes, and perhaps more importantly, by problems in identifying *PMS2* mutation carriers due to a markedly lower penetrance.[10-12] Our previous study of penetrance for *PMS2* mutation carriers, using 98 *PMS2* families ascertained through family cancer clinics in several European countries, reported standardized incidence ratios for extra-colonic cancers and found an increased *PMS2*-related risk of cancer of the small bowel, ovaries, renal pelvis and – most notably – of the breast.[11] Although that study presented the largest dataset then available, we were unable to generate reliable estimates of penetrance for these cancers due to their infrequency. In addition, there was an ascertainment bias in this cohort due to the recruitment via family cancer clinics. Another study from Iceland reported significant increases in the risk of colorectal, endometrial and ovarian cancer for two pathogenic *PMS2* founder variants.[13] This study and others have reported relatively high prevalence of *PMS2* variants in the population.[13-15] Thus underlining the need for *PMS2*-specific cancer risks.

In the current study, we have expanded the previous study database to 284 families, including several that were identified through a population-based ascertainment, with

the aim of generating accurate penetrance estimates of colorectal, endometrial and other cancers for *PMS2*-associated Lynch syndrome patients.

METHODS

Data collection

European dataset

Pedigree data on families with a segregating pathogenic variant were originally collected between 2009 and 2012, as previously described.[11] These data were supplemented with *PMS2* families identified between 2012 and 2017. Briefly, data were collected in collaboration with the Netherlands Foundation for the Detection of Hereditary Tumors and with clinical genetic departments in the Netherlands, Norway, Germany, Sweden, Denmark and Spain. Data collection from patient records included demographic data, family pedigrees, age and location of cancer diagnosis, polypectomy, and hysterectomy if applicable. When available, clinical and pathological diagnoses were confirmed using patient records. Data collection and subsequent analysis protocols were approved by the local ethical review board (Leiden University Medical Center Ethics Review Board, protocol ID: P01.019).

Ohio State datasets

For the Ohio State datasets, the first set of patients included both population-based colorectal and endometrial cancer patients from Columbus, Ohio as described elsewhere[10, 16-19] and cancer patients identified at family cancer clinics with absence of PMS2 only on IHC. The second set of patients from Ohio included only population-based colorectal and endometrial cancer patients from 50 hospitals throughout the state of Ohio as described previously.[20] All patients provided informed consent (Ohio State University Biomedical Sciences Institutional Review Board protocol IDs: 1999C0051, 1999C0245 and 2012C0123).

CCFR dataset

The study cohort from the Colon Cancer Family Registry has been described in detail elsewhere[21, 22,] and at www.coloncfr.org. Between 1998 and 2012, the Colon Cancer Family Registry recruited families via population-based probands, recently diagnosed with colorectal cancer, in state or regional population cancer registries in the USA (Washington, California, Arizona, Minnesota, Colorado, New Hampshire, North Carolina, and Hawaii), Australia (Victoria) and Canada (Ontario). In addition, clinic-

based probands were enrolled from multiple-case families referred to family cancer clinics in the USA (Mayo Clinic, Rochester, Minnesota, and Cleveland Clinic, Cleveland, Ohio), Canada (Ontario), Australia (Melbourne, Adelaide, Perth, Brisbane, Sydney and Newcastle) and New Zealand (Auckland). Probands were asked for permission to contact their relatives to seek their enrolment in the Cancer Family Registry (detailed in Newcomb *et al.*[21]). Informed consent was obtained from all study participants, and the study protocol was approved by the institutional research ethics review board at each registry. Information on demographics, personal characteristics, personal and detailed family history of cancer in first- and second-degree relatives, cancer-screening history, history of polyps, polypectomy, and other surgeries was obtained by questionnaires from all probands and participating relatives. Participants were followed approximately every 5 years after baseline to update this information. For the current study, each individual's lifetime cancer history was based on the most recent data (baseline or most recent follow-up). Reported cancer diagnoses and age at diagnosis were confirmed using pathology reports, medical records, cancer registry reports, and death certificates, where possible.

Mutation analysis and clinical variant classification

Probands included in the cohorts were screened for point mutations as well as large genomic rearrangements in the *PMS2* gene (see supplemental methods). Relatives of probands were tested for the specific family mutation. A detailed description of specific variants detected and their classification can be found in Supplementary Table 1 and 2

Statistical analysis

For estimation of the hazard ratios (HRs) and age-specific cumulative risks (penetrance), we used a modified segregation analysis.[23] This analytical method is not subject to population stratification, can rigorously adjust for ascertainment, and uses data on all study participants, whether genotyped or not, thereby maximizing statistical power. Models were fitted by the method of maximum likelihood with the statistical package MENDEL 3.2.[24] Estimates were appropriately adjusted for the ascertainment of families using a combination of retrospective likelihood and ascertainment-corrected joint likelihood. A conditional likelihood was maximized in which each pedigree's data were conditioned on the proband's *PMS2* mutation status, cancer history and ages of cancer diagnoses (for population-based families) or on the proband's *PMS2* mutation status and the cancer history and ages of cancer diagnoses of all family members (for clinic-based families).

For the purposes of analysis, we restricted included subjects to the first- and second-degree relatives of the probands. Observation time started at birth and stopped at age at diagnosis of cancer for affected, and last known age or age at death for unaffected family members. Because age information for each family member was required for the pedigree analysis, missing values were estimated using a defined protocol as follows. If an exact age was unknown but an age range was provided, age was estimated as the midpoint of that range. If age at diagnosis was unknown, it was assumed to be the same as age at death (if the relative was deceased) or the mean age at diagnosis for the specific cancer (if the relative was alive and older than the mean age at diagnosis). For relatives for whom last known age was unknown, ages were censored at the time they were last known to be alive (e.g., at the age at a cancer diagnosis). In the absence of any age information, it was assumed that both parents of the proband were born in the same year, that years of birth differed by 25 years in each generation (e.g., at birth of proband, parents were aged 25 years and grandparents were aged 50 years), and the ages of the siblings were the same. As a sensitivity analysis, we conducted analyses with and without imputing missing age, the results did not differ materially and therefore results from the non-imputed analysis were not shown in detail.

To calculate HRs, we used a likelihood-based approach in which age-specific incidence for *PMS2* mutation carriers was divided by that for non-carriers. Incidence rates for non-carriers were assumed to be the same as age-, sex- and country-specific population incidence rates (Australia, Canada, USA, The Netherlands, Germany) for the period 1998-2002, as obtained from Cancer Incidence in Five Continents.[25] The period of 1998-2002 was selected for analysis because it was the closest available dataset to the mean calendar year of cancer diagnoses in the sample. For each cancer, the age at cancer diagnosis was modeled as a random variable whose hazard was the relevant population incidence multiplied by a cancer-specific HR. For colorectal cancer, HRs for carriers were assumed to be continuous, piece-wise linear functions of age which are constant before age 40 years, linear in the intervals 40-50, 50-60, 60-70 and constant after age 70 years. For all other cancer sites, HRs were assumed to be independent of age. HRs for colorectal cancer, endometrial cancer, and other cancers were estimated simultaneously to allow proper adjustment for colorectal cancer-based ascertainment schemes when estimating the risks of non-colorectal cancers and to increase power (by helping the model identify likely carriers from the placement of Lynch syndrome-associated cancers within each family). HRs were assumed to be independent of country of recruitment.

Age-specific cumulative risks (penetrance) of each cancer site for *PMS2* mutation carriers were calculated separately for males and females, using the formula:

$1 - \exp \int_0^{80} 0\lambda(t)\,dt)$ is the HR multiplied by the US population incidence.[26] Corresponding confidence intervals (CIs) were calculated using a parametric bootstrap. More specifically, 5,000 draws were taken from the multivariate normal distribution that the maximum likelihood estimates would be expected to follow under asymptotic likelihood theory. For each age, corresponding values of the cumulative risk were calculated and the 95% CI for the cumulative risks to that age were taken to be the 2.5th and 97.5th percentile of this sample.

TABLE 1 The study dataset description

	No. of family members		
	Total	Male	Female
Probands (= no. of families)	284	149	136
FDR	1904	953	951
SDR	2974	1487	1487
Confirmed *PMS2* mutation carriers	513	209	304
FDR	339	128	211
SDR	174	81	93
Confirmed *PMS2* non-carriers	404	167	237
FDR	230	100	130
SDR	174	67	107

FDR: first-degree relative. SDR: second-degree relative.

RESULTS

The final analysis included 284 families (211 from the European, 19 from the Ohio State and 54 from the CCFR dataset), with 1904 first- and 2974 second-degree family members, in which 513 were confirmed carriers (Table 1). The numbers and mean ages at diagnosis of each cancer site in first- and second-degree relatives are depicted in Table 2.

Colorectal cancer

PMS2 mutation carriers were at increased risk of developing colorectal cancer, with a HR depending on age and sex of the mutation carrier; 6.51 (95% CI: 2.03-20.9) for males aged <40, 1.70 (95% CI: 0.89-3.24) for males aged >70, 6.48 (95% CI: 2.24-18.8) for females aged <40, and 2.23 (95% CI: 1.21-4.12) for females aged >70). Estimated cumulative risks of colorectal cancer to age 80 for PMS2 mutation carriers were approximately 13% (95% CI: 7.9-22%) for male carriers and 12% (95% CI: 6.7-21%) for female carriers (general population 6.6% and 4.7%, respectively) (Figure 1A).

TABLE 2 The number and mean ages at diagnosis of each cancer site in the first- and second-degree relatives of probands

	FDR (n=1904)		SDR (n=2974)	
Cancer	No.	Mean age at diagnosis, years (SD)	No.	Mean age at diagnosis, years (SD)
Colorectal	116	59.6 (14.7)	112	62.7 (3.0)
Endometrial	33	55.7 (9.04)	21	54.8 (13.7)
Ovarian	9	52.2 (14.8)	5	41.6 (22.8)
Brain	18	42.3 (26.9)	10	56.3 (26.0)
Hepatobiliary	5	56.2 (13.6)	3	60.7 (9.87)
Gastric	14	57.8 (8.72)	11	57.3 (11.0)
Bladder	7	71.7 (14.5)	5	70.0 (14.3)
Breast	47	58.1 (12.0)	50	59.2 (13.5)
Prostate	19	70.7 (12.2)	24	69.8 (14.1)
Renal	7	65 (13.7)	5	61.2 (10.8)
Small bowel	4	45.0 (9.6)	1	38

FDR: first-degree relative. SDR: second-degree relative. SD: standard deviation

Gynecological cancers

PMS2 mutation carriers were also at small increased risk of endometrial cancer, with a HR of 5.73 (95% CI: 2.98-11.0) and estimated cumulative risk to age 80 of approximately 13% (95% CI: 7.0-24%), compared with females from the general population (2.4%) (Figure 1B). There was no clear evidence of increase in the risk of ovarian cancer (HR: 1.52; 95% CI: 0.45-5.05) (Figure 2).

Other cancers

There was no clear increase in risk of gastric, hepatobiliary, bladder, renal, brain, breast or prostate cancer for PMS2 mutation carriers (HR for each cancer shown in Figure 2). There were too few occurrences of small bowel cancer (n=5) to generate a HR.

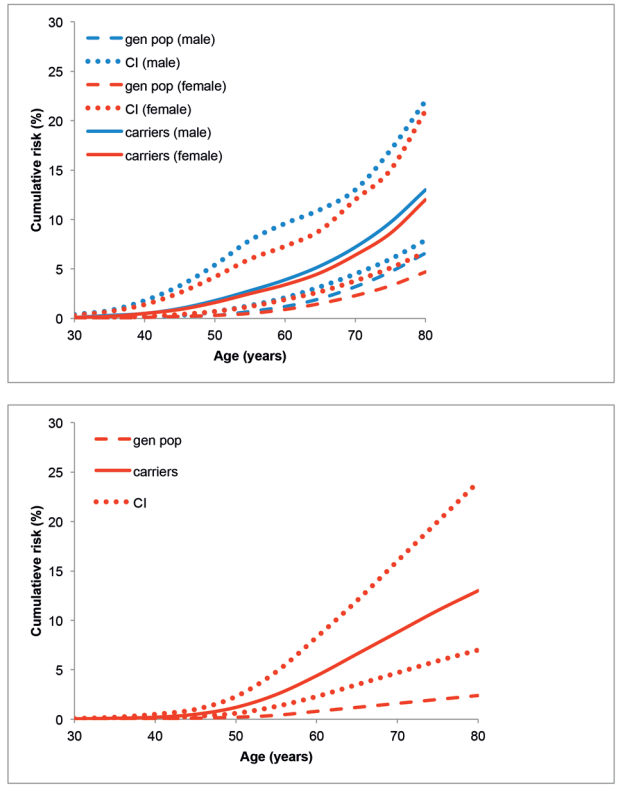

FIGURE 1 Cumulative risks (unbroken lines) and corresponding 95% confidence intervals (dotted lines) of (A) colorectal cancer and (B) endometrial cancer for heterozygous PMS2 mutation carriers, and for the US general population ('gen pop', dashed lines). Blue and red represent males and females, respectively.

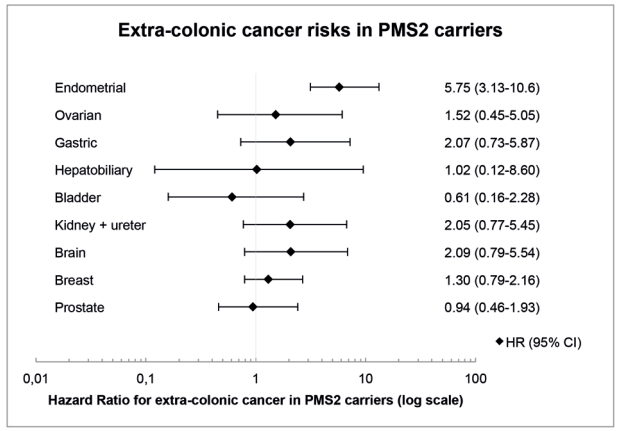

FIGURE 2 Hazard ratios and corresponding 95% confidence intervals of extra-colonic cancers for PMS2 mutation carriers

DISCUSSION

Based on the results from this large, international study of heterozygous *PMS2* mutation carriers, the *PMS2*-associated Lynch syndrome spectrum appears to be restricted to colorectal and endometrial cancer only, underlining the distinct phenotype for *PMS2* mutation carriers. We have also shown that *PMS2* mutation carriers have much lower cancer risks compared with other MMR gene mutation carriers.

The previous two studies of *PMS2* mutation carriers have estimated cumulative risks to age 70 years of 11-20% for colorectal cancer and 12-15% for endometrial cancer.[10, 11] Our current analysis has confirmed that *PMS2* carriers are at small increased risk of colorectal and endometrial cancer. These penetrance estimates are considerably lower than those for other MMR gene mutation carriers, which have been estimated at 35-55% for colorectal cancer and 10-45% for endometrial cancer.[3] A recent report from the Prospective Lynch Syndrome Database (PLSD) described cancer risk and survival for all Lynch syndrome patients, irrespective of the underlying gene variant.[7] This report included 124 *PMS2* mutation carriers, with 524 observation years. The findings support our study data in that endometrial cancer was the sole cancer type observed. Notably, colorectal cancer did not occur in any of the *PMS2* mutation carriers undergoing regular colonoscopic screening. This, together with our penetrance estimates, could justify consideration of less frequent colonoscopy screening for PMS2 mutation carriers. This, together with our low penetrance estimates (Figure 1) could justify modification of the colonoscopy surveillance protocol, for example starting at age 35-40 years, every two-three years, similar to what has been proposed in the NCCN guidelines.[27]

The PLSD database further showed that endometrial cancer survival for all MMR pathogenic variant carriers was excellent, with a 10-year survival of 93% (95% CI: 85-97%). The reported survival for ovarian cancer in Lynch syndrome patients was lower, at 74% (95% CI: 44-90%), but still better than that for sporadic ovarian cancer cases. Current surveillance guidelines advise that risk-reducing hysterectomy and bilateral salpingo-oophorectomy should be considered in women with Lynch syndrome, because transvaginal ultrasound with or without biopsies are ineffective in reducing risk of endometrial cancer and ovarian cancer, and might not have a strong influence on survival.[28] The good survival rates for endometrial cancer, combined with the data presented in the current study showing no evidence of a clinically relevant increase in ovarian cancer risk for *PMS2* mutation carriers, raises questions about the justification of risk-reducing hysterectomy and bilateral salpingo-oophorectomy, which may be too rigorous in carriers of heterozygous pathogenic *PMS2* mutations.

In our previous study of *PMS2*-associated Lynch syndrome patients, we found increased

standardized incidence ratios (SIRs) for cancer of the small bowel, ovary, renal pelvis and of the breast.[11] However, that study was limited by inclusion of confirmed mutation carriers identified through family cancer clinics and a limited number of cancer events. The first factor in particular could have been a potential source of ascertainment bias as a strong family history of cancer and/or early-onset disease increases the likelihood of inclusion and *PMS2* testing. In that report, we did not adjust for this potential ascertainment bias when estimating SIRs for extra-colonic cancers. Traditionally, a strong family history of colorectal and endometrial cancers prompted suspicion of Lynch syndrome and consequently patients were tested for tumor MMR deficiency followed by MMR gene mutation testing. Currently, family histories of *other* cancers are increasingly being ascertained by clinical genetic centers for further evaluation as possible Lynch syndrome. Therefore, it is important to take into account and adjust for ascertainment bias when estimating risks of cancers other than colorectal or endometrial cancer. Furthermore, pathogenic *PMS2* variants are relatively frequently observed using extensive gene panel testing for women with hereditary breast and ovarian cancer.[29-32] Nevertheless, we could not confirm an increased SIR for breast cancer in the present study (HR: 1.30; 95% CI: 0.79-2.16) and the discrepancy with earlier reports can probably be attributed to a high prevalence of *PMS2* (and *MSH6*) mutations in the general population. Conversely, the relative infrequency of *PMS2* variants among Lynch syndrome patients can be explained by the milder phenotype, which makes ascertainment by family cancer clinics less likely.

The current study is the largest to date in estimation of cancer risks for heterozygous *PMS2* mutation carriers. Previous studies have shown that analyses of retrospective data from clinic-based families i.e., ascertained due to family history of cancer, without (statistical) adjustment can lead to overestimation of cancer risks for mutation carriers.[33-35] In the current study, we used a high-level statistical approach to properly adjust for such ascertainment bias. The modified segregation method used data on all family members, regardless of whether they were genotyped, thereby maximizing statistical power while avoiding survival bias.

A potential limitation of the current study was the use of unverified cancer diagnoses that were self- or proband-reported, thus potentially affecting the accuracy of estimates. However, previous studies showed a high probability of agreement between proband-reported cancer status in first-degree relatives and the validated report (for example, 95.4% (95% CI: 92.6-98.3) for breast cancer, 83.3% (95% CI: 72.8-93.8) for ovarian cancer; and 79.3% (95% CI: 70.0-88.6) for prostate cancer).[36] A further possible limitation is that our analysis did not take into account a potential role for genetic or

environmental modifiers of risk. The existence of such modifiers is plausible, as a high degree of variability in penetrance and phenotype has been observed[23], and modifiers of cancer risk such as lifestyle, genetic modifiers and phenotype-genotype correlations have been identified previously.[37-40] Our study estimated cancer risks of all variants combined, however it is plausible that not all *PMS2* variants confer the same risk. A previous study in a selection of the currently analyzed cohort investigated genotype-phenotype correlations and found no difference in risk between the group of variants with retained vs. loss of RNA expression.[40] However, this study did report that those carrying a variant with loss of RNA expression were diagnosed with colorectal cancer on average 9 years younger than those with retained expression. The influence of these modifiers is still not well understood, especially for *PMS2* mutation carriers, although efforts are currently on-going to better define such factors and their potential role in modifying disease risk. Our study results highlight that studies of penetrance modifiers should take the specific MMR gene mutated into account.

In the current study, we analyzed the first dataset large enough to generate the unbiased estimates for the risk of each extra-colonic cancer for *PMS2* mutation carriers. Our results show that *PMS2* carriers are only at small increased risk of colorectal and endometrial cancer. This underlines the importance of gene-specific genetic counseling of Lynch syndrome patients and the development of appropriate clinical guidelines.

REFERENCES

1. Lynch HT, Snyder CL, Shaw TG, et al. Milestones of Lynch syndrome: 1895-2015. Nat Rev Cancer 2015;15:181-94.
2. Umar A, Boland CR, Terdiman JP, et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J.Natl.Cancer Inst. 2004;96:261-268.
3. Barrow E, Hill J, Evans DG. Cancer risk in Lynch Syndrome. Fam.Cancer 2013;12:229-240.
4. Harkness EF, Barrow E, Newton K, et al. Lynch syndrome caused by MLH1 mutations is associated with an increased risk of breast cancer: a cohort study. J Med Genet 2015;52:553-6.
5. Win AK, Lindor NM, Young JP, et al. Risks of primary extracolonic cancers following colorectal cancer in lynch syndrome. J.Natl.Cancer Inst. 2012;104:1363-1372.
6. Scott RJ, McPhillips M, Meldrum CJ, et al. Hereditary nonpolyposis colorectal cancer in 95 families: differences and similarities between mutation-positive and mutation-negative kindreds. Am J Hum Genet 2001;68:118-127.
7. Moller P, Seppala TT, Bernstein I, et al. Cancer risk and survival in path_MMR carriers by gene and gender up to 75 years of age: a report from the Prospective Lynch Syndrome Database. Gut 2017.
8. Lindor NM, Petersen GM, Spurdle AB, et al. Pancreatic cancer and a novel MSH2 germline alteration. Pancreas 2011;40:1138-40.
9. Stuckless S, Parfrey PS, Woods MO, et al. The phenotypic expression of three MSH2 mutations in large Newfoundland families with Lynch syndrome. Fam Cancer 2007;6:1-12.
10. Senter L, Clendenning M, Sotamaa K, et al. The clinical phenotype of Lynch syndrome due to germ-line PMS2 mutations. Gastroenterology 2008;135:419-428.
11. ten Broeke SW, Brohet RM, Tops CM, et al. Lynch syndrome caused by germline PMS2 mutations: delineating the cancer risk. J Clin Oncol 2015;33:319-25.
12. Moller P, Seppala T, Bernstein I, et al. Cancer incidence and survival in Lynch syndrome patients receiving colonoscopic and gynaecological surveillance: first report from the prospective Lynch syndrome database. Gut 2017;66:464-472.
13. Haraldsdottir S, Rafnar T, Frankel WL, et al. Comprehensive population-wide analysis of Lynch syndrome in Iceland reveals founder mutations in MSH6 and PMS2. Nat Commun 2017;8:14755.
14. Espenschied CR, LaDuca H, Li S, et al. Multigene Panel Testing Provides a New Perspective on Lynch Syndrome. J Clin Oncol 2017:Jco2016719260.

15. Win AK, Jenkins MA, Dowty JG, et al. Prevalence and Penetrance of Major Genes and Polygenes for Colorectal Cancer. Cancer Epidemiol Biomarkers Prev 2016.
16. Hampel H, Frankel WL, Martin E, et al. Feasibility of screening for Lynch syndrome among patients with colorectal cancer. J.Clin.Oncol. 2008;26:5783-5788.
17. Hampel H, Frankel WL, Martin E, et al. Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer). N Engl J Med 2005;352:1851-60.
18. Hampel H, Panescu J, Lockman J, et al. Comment on: Screening for Lynch Syndrome (Hereditary Nonpolyposis Colorectal Cancer) among Endometrial Cancer Patients. Cancer Res 2007;67:9603.
19. Hampel H, Stephens JA, Pukkala E, et al. Cancer risk in hereditary nonpolyposis colorectal cancer syndrome: later age of onset. Gastroenterology 2005;129:415-421.
20. Pearlman R, Frankel WL, Swanson B, et al. Prevalence and Spectrum of Germline Cancer Susceptibility Gene Mutations Among Patients With Early-Onset Colorectal Cancer. JAMA Oncol 2017;3:464-471.
21. Newcomb PA, Baron J, Cotterchio M, et al. Colon Cancer Family Registry: an international resource for studies of the genetic epidemiology of colon cancer. Cancer Epidemiol Biomarkers Prev 2007;16:2331-43.
22. Jenkins MA, Win AK, Templeton AS, et al. Cohort Profile: The Colon Cancer Family Registry Cohort (CCFRC). Int J Epidemiol 2018;47:387-388i.
23. Dowty JG, Win AK, Buchanan DD, et al. Cancer risks for MLH1 and MSH2 mutation carriers. Hum Mutat 2013;34:490-7.
24. Lange K, Weeks D, Boehnke M. Programs for pedigree analysis: MENDEL, FISHER, and dGENE. Genet Epidemiol 1988;5:471-472.
25. Ferlay J BF, Steliarova-Foucher E and Forman D. (2014). Cancer Incidence in Five Continents, CI5plus. IARC CancerBase No. 9. Volume 2017, 2014:Lyon: International Agency for Research on Cancer; 2014. Available from: http://ci5.iarc.fr.
26. Win AK, Dowty JG, Cleary SP, et al. Risk of colorectal cancer for carriers of mutations in MUTYH, with and without a family history of cancer. Gastroenterology 2014;146:1208-11.e1-5.
27. Provenzale D, Gupta S, Ahnen DJ, et al. Genetic/Familial High-Risk Assessment: Colorectal Version 1.2016, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Canc Netw 2016;14:1010-30.
28. Renkonen-Sinisalo L, Butzow R, Leminen A, et al. Surveillance for endometrial cancer in hereditary nonpolyposis colorectal cancer syndrome. Int J Cancer 2007;120:821-4.

29. Caminsky NG, Mucaki EJ, Perri AM, et al. Prioritizing Variants in Complete Hereditary Breast and Ovarian Cancer (HBOC) Genes in Patients Lacking known BRCA Mutations. Hum Mutat 2016.
30. Desmond A, Kurian AW, Gabree M, et al. Clinical Actionability of Multigene Panel Testing for Hereditary Breast and Ovarian Cancer Risk Assessment. JAMA Oncol. 2015.
31. Castera L, Krieger S, Rousselin A, et al. Next-generation sequencing for the diagnosis of hereditary breast and ovarian cancer using genomic capture targeting multiple candidate genes. Eur.J.Hum.Genet. 2014.
32. Tung N, Battelli C, Allen B, et al. Frequency of mutations in individuals with breast cancer referred for BRCA1 and BRCA2 testing using next-generation sequencing with a 25-gene panel. Cancer 2015;121:25-33.
33. Antoniou AC, Goldgar DE, Andrieu N, et al. A weighted cohort approach for analysing factors modifying disease risks in carriers of high-risk susceptibility genes. Genet.Epidemiol. 2005;29:1-11.
34. Dowty JG, Win AK, Buchanan DD, et al. Cancer risks for MLH1 and MSH2 mutation carriers. Hum.Mutat. 2013;34:490-497.
35. Vos JR, Oosterwijk JC, Aalfs CM, et al. Bias Explains Most of the Parent-of-Origin Effect on Breast Cancer Risk in BRCA1/2 Mutation Carriers. Cancer Epidemiol Biomarkers Prev 2016;25:1251-8.
36. Ziogas A, Anton-Culver H. Validation of family history data in cancer family registries. Am J Prev Med 2003;24:190-8.
37. Talseth-Palmer BA, Wijnen JT, Brenne IS, et al. Combined analysis of three Lynch syndrome cohorts confirms the modifying effects of 8q23.3 and 11q23.1 in MLH1 mutation carriers. Int.J.Cancer 2013;132:1556-1564.
38. Win AK, Hopper JL, Buchanan DD, et al. Are the common genetic variants associated with colorectal cancer risk for DNA mismatch repair gene mutation carriers? Eur J Cancer 2013;49:1578-1587.
39. Van Duijnhoven FJ, Botma A, Winkels R, et al. Do lifestyle factors influence colorectal cancer risk in Lynch syndrome? Fam.Cancer 2013;12:285-293.
40. Suerink M, van der Klift HM, Ten Broeke SW, et al. The effect of genotypes and parent of origin on cancer risk and age of cancer development in PMS2 mutation carriers. Genet Med 2016;18:405-9.

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SUPPLEMENTAL METHODS

PMS2 mutation analysis

European cohort

This dataset consisted of clinically ascertained families where variant analysis was initiated due to (histological) pre-screening by immunohistochemistry and/or microsatellite instability, usually because a family met Bethesda criteria.[1] *PMS2* mutation screening was performed using Sanger sequencing of PCR or RT-PCR products, and was limited to exons and exon-intron boundaries. Large deletions and duplications were mainly detected by multiplex ligation-dependent probe amplification (MLPA). Comprehensive strategies were applied to avoid unreliable mutation detection caused by interference from pseudogene sequences and frequent gene conversion events.[2]

Ohio State cohort

In the Ohio State cohorts, testing for germline mutations in *MLH1*, *MSH2*, *EPCAM*, *MSH6*, and *PMS2* was performed for all population-based probands who had a colorectal tumor that showed impaired MMR function, as evidenced by tumor microsatellite instability (MSI) or absence of MMR protein expression on immunohistochemical analysis after ruling out MLH1 promoter methylation. In the first cohort, PMS2 testing was performed as published previously.[3] In the second cohort, PMS2 testing was performed by one of two commercial laboratories.[4]

CCFR cohort

Testing for germline mutations in *MLH1*, *MSH2*, *MSH6*, and *PMS2* was performed for all population-based probands who had a colorectal tumor that showed impaired MMR function, as evidenced by tumor microsatellite instability (MSI) or absence of MMR protein expression on immunohistochemical analysis. Testing was also performed for colorectal cancer-affected participants from clinic-based families regardless of tumor MSI or MMR-protein expression status. Sanger sequencing or denaturing high performance liquid chromatography, followed by confirmatory DNA sequencing, was performed to screen for mutations in the *MLH1*, *MSH2*, and *MSH6* genes. Large duplication and deletion mutations were detected by Multiplex Ligation Dependent Probe Amplification (MLPA) according to the manufacturer's instructions (MRC Holland, Amsterdam, The Netherlands).[5-7] *PMS2* mutation testing involved a modified protocol from Senter et al [8], in which exons 1 to 5, 9, and 11 to 15 were amplified in 3 long-range polymerase chain reactions (PCRs), followed by nested exon-specific PCR and sequencing. The remaining exons (7, 8, and 10) were amplified and sequenced directly

from genomic DNA. Large-scale deletions in *PMS2* were detected using the P008-A1 MLPA kit (MRC Holland, Amsterdam, The Netherlands).[9] Relatives of probands with a pathogenic MMR germline mutation[10] who provided a blood sample were tested for the specific mutation identified in the proband.

Variant classification

The InSiGHT Colon Cancer Gene Variant Database (https://insight-database.org/variants/PMS2; last update 20 November 2017) was consulted for presence and clinical classification of the 106 *PMS2* variants reported as disease causing in the families included in this study (supplementary table S1). Fifty-four variants were present with clinical classification in the database including 42 pathogenic (class 5), 10 likely pathogenic (class 4) and 2 variants of uncertain significance (VUS; class 3). Sixteen variants were present but not classified; thirty-six variants were not reported to the Insight database at time of consultation (14 December 2017). Most of these variants could be classified as (likely) pathogenic by applying the variant classification criteria formulated by the InSiGHT Variant Interpretation Committee (VIC).[11] For 9 variants that could not a priori be classified immediately as pathogenic (including two missense variants classified as VUS by the Insight VIC) we provide additional evidence that suggests pathogenicity in supplementary table S2. Variants found in the *PMS2* gene were classified for pathogenicity as reported by the InSiGHT Colon Cancer Gene Variant Database (http://insight-group.org/variants/classifications/) or by applying their classification criteria.[11] The majority of the variants were classified as (likely) pathogenic. Three missense variants (NM_000535.5: c.319C>T p.Arg107Trp, c.2113G>A p.Glu705Lys, and c.2444C>T p.Ser815Leu), not yet classified or classified as a variant of uncertain significance (VUS), were included because additional evidence suggested likely pathogenicity.[2] See Supplementary Table 1 for a description of *PMS2* variants.

REFERENCES

1. Umar A, Boland CR, Terdiman JP, et al: Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J.Natl.Cancer Inst. 96:261-268, 2004
2. van der Klift HM, Mensenkamp AR, Drost M, et al: Comprehensive Mutation Analysis of PMS2 in a Large Cohort of Probands Suspected of Lynch Syndrome or Constitutional Mismatch Repair Deficiency (CMMRD) Syndrome. Hum Mutat, 2016
3. Clendenning M, Hampel H, LaJeunesse J, et al: Long-range PCR facilitates the identification of PMS2-specific mutations. Hum.Mutat. 27:490-495, 2006
4. Pearlman R, Frankel WL, Swanson B, et al: Prevalence and Spectrum of Germline Cancer Susceptibility Gene Mutations Among Patients With Early-Onset Colorectal Cancer. JAMA Oncol 3:464-471, 2017
5. Newcomb PA, Baron J, Cotterchio M, et al: Colon Cancer Family Registry: an international resource for studies of the genetic epidemiology of colon cancer. Cancer Epidemiol Biomarkers Prev 16:2331-2343, 2007
6. Rumilla K, Schowalter KV, Lindor NM, et al: Frequency of deletions of EPCAM (TACSTD1) in MSH2-associated Lynch syndrome cases. J Mol Diagn 13:93-9, 2011
7. Southey MC, Jenkins MA, Mead L, et al: Use of molecular tumor characteristics to prioritize mismatch repair gene testing in early-onset colorectal cancer. J Clin Oncol 23:6524-6532, 2005
8. Senter L, Clendenning M, Sotamaa K, et al: The Clinical Phenotype of Lynch Syndrome Due to Germ-Line PMS2 Mutations. Gastroenterology 135:419-428, 2008
9. Rosty C, Clendenning M, Walsh MD, et al: Germline mutations in PMS2 and MLH1 in individuals with solitary loss of PMS2 expression in colorectal carcinomas from the Colon Cancer Family Registry Cohort. BMJ Open 6:e010293-e010293, 2016
10. Win AK, Lindor NM, Young JP, et al: Risks of primary extracolonic cancers following colorectal cancer in Lynch syndrome. J Natl Cancer Inst 104:1363-1372, 2012
11. Thompson BA, Spurdle AB, Plazzer JP, et al: Application of a 5-tiered scheme for standardized classification of 2,360 unique mismatch repair gene variants in the InSiGHT locus-specific database. Nat Genet 46:107-115, 2014

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SUPPLEMENTARY TABLE 1 PMS2 variants reported as disease-causing in the families included in this study

exon/ intron	PMS2 variant[a]	predicted protein effect	type of variant	InSiGHT class[b]	Nr of families[c]
1	c.1A>G	p.Met1?	start codon	4	2
2	c.137G>T	p.Ser46Ile	missense	4	15
intron 2	c.163+2T>C		canonical splice variant	4	1
intron 2	c.164-2A>G		canonical splice variant	4	1
2	c.24-12_107delinsAAAT	p.Ser8Argfs*5	frameshift	5	3
3	c.219_220dup	p.Gly74Valfs*3	frameshift	5	4
intron 4	c.354-1G>A		canonical splice variant	4	1
5	c.400C>T	p.Arg134*	nonsense	5	2
6	c.697C>T	p.Gln233*	nonsense	5	5
intron 6	c.705+1G>T		canonical splice variant	4	1
7	c.736_741delinsTGTGTGTGAAG	p.Pro246Cysfs*3	frameshift	5	39
7	c.780del	p.Asp261Metfs*46	frameshift	5	1
7	c.802dup	p.Tyr268Leufs*31	frameshift	5	1
intron 7	c.804-60_804-59insJN866832.1		retrotransposal SVA insertion	5	3
8	c.861_864del	p.Arg287Serfs*19	frameshift	5	4
8	c.862_863del	p.Gln288Valfs*10	frameshift	5	1
8	c.903G>T	r.804_903del; p.Tyr268*	exonic splice variant	4	4
9	c.943C>T	p.Arg315*	nonsense	5	4
9	c.949C>T	p.Gln317*	nonsense	5	1
intron 9	c.989-1G>T		canonical splice variant	5	1
intron 9	c.989-2A>G		canonical splice variant	4	3
10	c.1021del	p.Arg341Glyfs*15	frameshift	5	1
10	c.1076dup	p.Leu359Phefs*6	frameshift	5	2
10	c.1079_1080del	p.Ile360Argfs*4	frameshift	5	1
intron 10	c.1144+2T>A	p.Glu330_Glu381del	canonical splice variant	4	1
11	c.1261C>T	p.Arg421*	nonsense	5	1
11	c.1738A>T	p.Lys580*	nonsense	5	1
11	c.1831dup	p.Ile611Asnfs*2	frameshift	5	11
11	c.1840A>T	p.Lys614*	nonsense	5	3
11	c.1882C>T	p.Arg628*	nonsense	5	10
11	c.1927C>T	p.Gln643*	nonsense	5	3
11	c.1939A>T	p.Lys647*	nonsense	5	6
intron 11	c.2007−1G>A		canonical splice variant	4	1
12	c.2113G>A	p.Glu705Lys	missense	3 (see supp tbl S2)	7
intron 12	c.2174+1G>A		canonical splice variant	5	4
13	c.2192_2196del	p.Leu731Cysfs*3	frameshift	5	7
14	c.2404C>T ;	p.Arg802*	nonsense	5	3
14	c.2444C>T	p.Ser815Leu	missense	3 (see supp tbl S2)	1
intron 2	c.163+5G>A		intronic splice variant	present, not classified (see supp tbl S2)	1
3	c.247_250dup	p.Thr84Ilefs*9	frameshift	present, not classified (class 5)	1

SUPPLEMENTARY TABLE 1 PMS2 variants reported as disease-causing in the families included in this study

exon/ intron	PMS2 variant[a]	predicted protein effect	type of variant	InSiGHT class[b]	Nr of families[c]
3	c.247_250dup	p.Thr84Ilefs*9	frameshift	present, not classified (class 5)	1
intron 3	c.251-2A>C		canonical splice variant	present, not classified (class 5)	1
4	c.319C>T	p.Arg107Trp	missense	present, not classified (see supp tbl S2)	1
4	c.325dup	p.Glu109Glyfs*30	frameshift	present, not classified (class 5)	4
7	c.746_753del	p.Asp249Valfs*2	frameshift	present, not classified (class 5)	1
8	c.823C>T	p.Gln275*	nonsense	present, not classified (class 5)	1
8	c.825A>G	r.804_825del, p.Ile269Alafs*31	exonic splice variant	present, not classified (see supp tbl S2)	1
8	c.856_857del	p.Asp286Glnfs*12	frameshift	present, not classified class 5)	1
9	c.904_911del	p.Val302Thrfs*4	frameshift	present, not classified (class 5)	1
11	c.1214C>A	p.Ser405*	nonsense	present, not classified (class 5)	1
12	c.2117del	p.Lys706Serfs*19	frameshift	present, not classified (class 5)	3
12	c.2155C>T	p.Gln719*	nonsense	present, not classified class 5)	2
intron 14	c.2445+1G>T		canonical splice variant	present, not classified (class 4)	5
15	c.2500_2501delinsG	p.Met834Glyfs*17	frameshift	present, not classified (see supp tbl S2)	1
intron 1	c.24-2A>G		canonical splice variant	not present (class 4)	1
intron 2	c.164-1G>C		canonical splice variant	not present (class 4)	1
3	c.215G>A	p.Gly72Glu	missense	not present (see supp tbl S2)	1
intron 4	c.354-2A>G		canonical splice variant	not present (class 4)	2
6	c.613C>T;	p.Gln205*	nonsense	not present (class 5)	1
6	c.658dup	p.Ser220Lysfs*29	frameshift	not present (class 5)	1

SUPPLEMENTARY TABLE 1 PMS2 variants reported as disease-causing in the families included in this study

exon/ intron	PMS2 variant[a]	predicted protein effect	type of variant	InSiGHT class[b]	Nr of families[c]
intron 6	c.705+2T>C		canonical splice variant	not present (class 4)	1
7	c.765C>A	p.Tyr255*	nonsense	not present (class 5)	2
7	c.781del	p.Asp261Metfs*46	frameshift	not present (class 5)	1
8	c.809C>G ;	p.Ser270*	nonsense	not present (class 5)	1
10	c.1067del	p.Lys356Argfs*4	frameshift	not present (class 5)	1
10	c.1107del	p.Lys369Asnfs*2	frameshift	not present (class 5)	2
10	c.1111_1113delinsTTTA	p.Asn371Phefs*11	frameshift	not present (class 5)	1
11	c.1151T>G	p.Leu384*	nonsense	not present (class 5)	1
11	c.1237_1238delinsT	p.Lys413*	frameshift	not present (class 5)	1
11	c.1239dup	p.Asp414Argfs*44	frameshift	not present (class 5)	2
11	c.1281del	p.His428Thrfs*20	frameshift	not present (class 5)	1
11	c.1492_1502del	p.Ser498Glyfs*3	frameshift	not present (class 5)	2
11	c.1687C>T	p.Arg563*	nonsense	not present (class 5)	1
11	c.1874del	p.Leu625*	frameshift	not present (class 5)	1
12	c.2156del	p.Gln719Argfs*6	frameshift	not present (class 5)	1
12	c.2161C>T	p.Gln721*	nonsense	not present (class 5)	1
13	c.2182_2184delinsG	p.Thr728Alafs*7	frameshift	not present (class 5)	1
14	c.2413C>T	p.Gln805*	nonsense	not present (class 5)	1
15	c.2520dup	p.Trp841Leufs*47	frameshift	not present (see supp tbl S2)	1
15	c.2521del	p.Trp841Glyfs*10	frameshift	not present (see supp tbl S2)	1
1	genomic deletion including exon 1		large genomic deletion	5	3
2	genomic deletion including exon 2		large genomic deletion	5	3
6	genomic deletion including exon 6		large genomic deletion	5	1
7	genomic deletion including exon 7		large genomic deletion	5	3
8	genomic deletion including exon 8		large genomic deletion	5	1
9	genomic deletion including exon 9		large genomic deletion	5	2
10	genomic deletion including exon 10		large genomic deletion	5	8
14	genomic deletion including exon 14		large genomic deletion	5	5
1_10	genomic deletion including exons 1-10		large genomic deletion	5	7
1_15	genomic deletion whole gene		large genomic deletion	5	6

SUPPLEMENTARY TABLE 1 PMS2 variants reported as disease-causing in the families included in this study

exon/ intron	PMS2 variant[a]	predicted protein effect	type of variant	InSiGHT class[b]	Nr of families[c]
10	genomic deletion including exon 10		large genomic deletion	5	8
14	genomic deletion including exon 14		large genomic deletion	5	5
1_10	genomic deletion including exons 1-10		large genomic deletion	5	7
1_15	genomic deletion whole gene (exons 1-15)		large genomic deletion	5	6
11_12	genomic deletion including exons 11-12		large genomic deletion	5	2
11_15	genomic deletion including exons 11-15		large genomic deletion	5	5
3_7	genomic deletion including exons 3-7		large genomic deletion	5	4
5_15	genomic deletion including exons 5-15		large genomic deletion	5	3
5_7	genomic deletion including exons 5-7		large genomic deletion	5	3
11_12	genomic duplication including exons 11-12		large genomic in tandem duplication	5	1
1_12	genomic deletion including exons 1-12		large genomic deletion	present, not classified (class 5)	2
2_4	genomic deletion including exons 2-4		large genomic deletion (in frame)	not present (class 4)	1
5_6	genomic deletion including exons 5-6		large genomic deletion (in frame)	not present (class 4)	1
6_8	genomic deletion including exons 6-8		large genomic deletion (in frame)	not present (class 4)	1
11	genomic deletion including exon 11		large genomic deletion	not present (class 5)	3
1_7	genomic deletion including exons 1-7		large genomic deletion	not present (class 5)	1
12_15	genomic deletion including exons 12-15		large genomic deletion	not present (class 5)	1
6_12	genomic deletion including exons 6-12		large genomic deletion	not present (class 5)	1
6_7	genomic deletion including exons 6-7		large genomic deletion	not present (class 5)	1
9_12	genomic deletion including exons 9-12		large genomic deletion	not present (class 5)	1

[a] Variant nomenclature according to HGVS guidelines (http://varnomen.hgvs.org/) with reference to NM_000535.5 for PMS2 accept for the large deletions or duplications. Large deletions and duplications were in some cases detected with the older MLPA kit P008 (MRC Holland) that lacks reliable probes for PMS2 exon 3, 4, 12-15. Therefore, the exact range of exon deletions was not always established. Although for some large deletions the breakpoints have been characterized, we did not include this information.

[b] Clinical variant class as reported on https://insight-database.org/variants/PMS2; last accessed on 14 December 2017; 5 = pathogenic, 4 = likely pathogenic, 3 = variant of uncertain significance. Classification of the variants not present or present but not yet classified in the InSiGHT database is given between brackets, using guidelines provided by https://www.insight-group.org/criteria/. Nonsense and frameshift mutations including large genomic deletions were classified as pathogenic (class 5). Canonical splice variants and large in-frame genomic deletions were classified as likely pathogenic (class 4). Additional evidence that suggests pathogenicity for variants that could not be classified a priori as (likely) pathogenic is provided in supplementary table S2.

[c] Six of the 284 families included index patients that were bi-allelic *PMS2* mutation carriers, of which four were compound heterozygous. For one family the description of the specific *PMS2* variant was not provided.

SUPPLEMENTARY TABLE 2 Additional evidence that suggests pathogenicity for nine PMS2 variants

location	PMS2 variant[a]	type of variant	number of families (this study)	Evidence suggestive for pathogenicity[b]
Intron 2	c.163+5G>A	intronic splice variant	1 (Denmark)	• Predicted decrease of splice site strength of canonical splice donor (-32.8%, Alamut Visual) • Minigene splicing assay shows skip of exon 2 (unpublished data) • Not in control population (ExAC, ESP, 1000G)
Exon 3	c.215G>A p.Gly72Glu	missense	1 (United States)	• Predicted as pathogenic by functional prediction software programs (PolyPhen-2, SIFT, aGVGD, MutationTaster) • Highly conserved on nucleotide and amino acid level. Located in ATP-binding domain • Not in control population (ExAC, ESP, 1000G) • Reported as likely pathogenic (Pearlman et al., 2017)
Exon 4	c.319C>T p.Arg107Trp	missense	1 (Netherlands)	• MMR-deficiency shown by in vitro mmr assay (van der Klift et al., 2016) • Incomplete aberrant splicing (van der Klift et al., 2015) • In trans with pathogenic PMS2 variant in a CMMRD patient (van der Klift et al., 2016)
Exon 8	c.825A>G r.804_825del p.Ile269Alafs*31	exonic splice variant	1 (Netherlands)	• Splice analysis (patient RNA and minigene splicing assay) showed activation of cryptic splice acceptor resulting in a skip of the first 22 basepairs of exon 8 (van der Klift et al., 2015) • In trans with pathogenic PMS2 variant in a CMMRD patient (Johannesma et al., 2011)
Exon 12	c.2113G>A p.Glu705Lys	missense	7 (5x Sweden; 2x United States 2x)	• Predicted as pathogenic by functional prediction software programs (PolyPhen-2, SIFT, aGVGD, MutationTaster) • Highly conserved on nucleotide and amino acid level. Located in metal-binding motif essential for PMS2 endonuclease function • Heterozygous in CMMRD patient (Miyaki et al., 1997) • Abrogated repair function demonstrated in Deschênes et al., 2007 (complementation assay) and in Drost et al., 2013 (in vitro mismatch repair assay) • In a mouse model associated with strong cancer predisposition (van Oers et al., 2010) • Recurrent in Swedish Lynch syndrome population (Lagerstedt-Robinson et al., 2016)
Exon 14	c.2444C>T p.Ser815Leu	missense	1 (Netherlands)	• Predicted as pathogenic by functional prediction software programs (PolyPhen-2, SIFT, aGVGD, MutationTaster) • Highly conserved on nucleotide and amino acid level. Located in MutL dimerisation domain • Not reported in large population cohorts (ExAC, ESP, 1000G) • MMR-deficiency shown by 2 different assays in 2 laboratories (van der Klift et al., 2016; González-Acosta et al., 2017). Diminished protein expression (González-Acosta et al., 2017) • Reported in 3 independent Lynch syndrome patients (van der Klift et al., 2016; González-Acosta et al., 2017) • MLA (performed for 1 family) showed LR of 1.83:1, leading to a posterior probability of pathogenicity of 0.993 (González-Acosta et al., 2017) • Homozygous in CMMRD patient (Suerink et al., 2018)

SUPPLEMENTARY TABLE 2 Additional evidence that suggests pathogenicity for nine PMS2 variants

location	PMS2 variant[a]	type of variant	number of families (this study)	Evidence suggestive for pathogenicity[b]
Exon 14	c.2444C>T p.Ser815Leu	missense	1 (Netherlands)	• Predicted as pathogenic by functional prediction software programs (PolyPhen-2, SIFT, aGVGD, MutationTaster) • Highly conserved on nucleotide and amino acid level. Located in MutL dimerisation domain • Not reported in large population cohorts (ExAC, ESP, 1000G) • MMR-deficiency shown by 2 different assays in 2 laboratories (van der Klift et al., 2016; González-Acosta et al., 2017). Diminished protein expression (González-Acosta et al., 2017) • Reported in 3 independent Lynch syndrome patients (van der Klift et al., 2016; González-Acosta et al., 2017) • MLA (performed for 1 family) showed LR of 1.83:1, leading to a posterior probability of pathogenicity of 0.993 (González-Acosta et al., 2017) • Homozygous in CMMRD patient (Suerink et al., 2018)
Exon 15	c.2500_2501 delinsG p.Met834Glyfs*17	Frameshift in last exon	1 (Canada)	• Frameshift from 29 AA upstream of canonical stop codon. The new reading frame ends in a STOP codon 16 positions downstream. • 2x reported in ClinVar as pathogenic (1 * evidence) • disrupts a highly conserved region in the C-terminal domain of PMS2 possibly disabling heterodimerization with MLH1 (Guerrette et al., 1999; Gueneau et al., 2013)
Exon 15	c.2520dup p.Trp841Leufs*47	Frameshift in last exon	1 (Sweden)	• Frameshift from 22 AA upstream of canonical stop codon. The new reading frame ends in a STOP codon 46 positions downstream. • disrupts a highly conserved region in the C-terminal domain of PMS2 possibly disabling heterodimerization with MLH1 (Guerrette et al., 1999; Gueneau et al., 2013)
Exon 15	c.2521del p.Trp841Glyfs*10	Frameshift in last exon	1 (Germany)	• Frameshift from 22 AA upstream of canonical stop codon. The new reading frame ends in a STOP codon 9 positions downstream. • 1x reported in ClinVar as likely pathogenic (1*evidence) • disrupts a highly conserved region in the C-terminal domain of PMS2 possibly disabling heterodimerization with MLH1 (Guerrette et al., 1999; Gueneau et al., 2013)

[a] Variant nomenclature according to HGVS guidelines (http://varnomen.hgvs.org/) with reference to NM_000535.5 for PMS2.
[b] data on conservation, splice prediction, functional prediction (PolyPhen-2, SIFT, aGVGD, MutationTaster), presence in contr population databases
(ExAC, ESP, 1000G) and in the ClinVar archive were obtained through Alamut Visual v.2.6 last accessed on 23-12-2017
Abbreviations: MMR = mismatch repair; CMMRD = constitutional mismatch repair deficiency;
MLA = multifactorial likelihood analysis; LR = likelihood ratio;
AA = amino acid

References:
Pearlman et al., 2017, JAMA Oncol 3: 464
van der Klift et al., 2015, Mol Genet Genomic Med 3:327–345
van der Klift et al., 2016, Hum Mutat 37:1162–1179