# PMS2-associated Lynch syndrome : the odd one out

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CANCER RISK
ANALYSES

# 2.1

# Lynch syndrome caused by germline *PMS2* mutations; delineating the cancer risk

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# ABSTRACT

## Purpose

The clinical consequences of *PMS2* germline mutations are poorly understood compared to other Lynch-associated mismatch repair gene 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 2548 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 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 and other family members with a *PMS2* mutation: 47 (range 26-68) and 58 years (range 31-78) respectively ($p<0,001$). 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 genes. 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.

# INTRODUCTION



Lynch syndrome (LS) is the most common heritable colorectal carcinoma (CRC) syndrome and is responsible for 2-4 % of all CRC cases in the Western world.[1] The underlying cause for 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 due to a large number of pseudogenes has possibly led to underreporting of *PMS2* mutations in LS patients. 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 consequence 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 colorectal and endometrial cancer (EC) compared to *MLH1* and *MSH2* mutation carriers and similar or even lower risks as compared to *MSH6* mutation carriers.[1,5,13] Furthermore, parents and other family members of biallelic *PMS2* mutation carriers rarely develop colorectal or other Lynch-syndrome 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 on the basis of the severity of their phenotype and outcomes are therefore variable depending on family selection and methods of data analysis, e.g. 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 non-tested family members.

# METHODS

## Data collection

All probands (index cases) included in the study were referred to a cancer family clinic because of a LS associated cancer or because of a suspect 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, Spain and the Leiden-based Netherlands Foundation for the Detection of Hereditary Tumors (table 1). The majority of cases were of white northern European origin.

Mutation screening of the probands was performed between 2007 and 2012. *PMS2* mutation analysis was initiated in most cases on the basis of histological investigations of the tumor suggestive for a *PMS2* germline defect and/or on the basis of 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 very distinct phenotype, with a typical spectrum of tumors at a very young age, so they were excluded from the cancer risk analysis (supplementary table 1).[17-19]

Informed consent was obtained according to protocols approved by local ethical review boards (LUMC Ethics Review Board, No. P01.019). Clinical and pathological 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

TABLE 1 Family country of origin

| | |
|---|---|
| The Netherlands | 76 |
| Denmark | 6 |
| Spain | 6 |
| Sweden | 5 |
| Norway | 3 |
| Germany | 2 |

RT-PCR (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 (MLPA) was used to detect large genomic deletions and duplications. *PMS2* mutations were classified as deleterious on the basis of introduction of a premature stop codon, either directly due to a nonsense mutation or as a result of a frameshift mutation, or when a deleterious splicesite mutation was identified. Missense mutations were classified as deleterious based on previous studies.[9,20] Mutations are depicted in supplementary table 2.



### 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

TABLE 2 Cohort description of the 98 families

| | Total | Male | Female |
|---|---|---|---|
| **Total no. family members** | 2548* | 1284 | 1262 |
| **No. mutation carriers** | 377 | 172 | 205 |
| - homozygotes | 11 | 5 | 6 |
| **No. non-mutation carriers** | 237 | 108 | 129 |
| **No. of CRC** | 208 | 118 | 90 |
| **No. of EC** | 39 | - | 39 |
| **No. other cancer** | 218 | 108 | 110 |
| – Lip | 1 | 1 | 0 |
| – Hypopharynx | 1 | 1 | 0 |
| – Lymphoma of pharynx | 1 | 1 | 0 |
| – Oesophagus | 5 | 3 | 2 |
| – Stomach | 16 | 8 | 8 |
| – Small intestine, including duodenum | 8 | 5 | 3 |
| – Liver and extra-hepatic 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 |

TABLE 2 Cohort description of the 98 families

| | | Total | Male | Female |
|---|---|---|---|---|
| – | 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 |
| – | Leukaemia of unspecified cell type | 8 | 6 | 2 |
| – | Unknown origin | 4 | 3 | 1 |

Note: Number of cancers excluding the cancers of the biallelic mutation carriers.
CRC= colorectal carcinoma; EC= endometrial carcinoma
* Gender was unknown for two individuals.

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, 70$^{th}$ birthday. CRC and EC risks were estimated using modified segregation analysis implemented in the pedigree analysis software MENDEL as previously described.[12, 22] Restricting the analysis to confirmed carriers would bias the results because affected family members and those with a very 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 modelled in terms of the incidence rates in carriers and non-carriers. The incidence rates were assumed to follow a Cox-proportional hazards model in which the non-carriers 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 to 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 to non-carriers.

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% confidence intervals (CI) calculated under Poisson distribution of the observed frequencies.

Comparative analyses of mean age of cancer development were done via an independent samples t-test in IBM SPSS Statistics 20.

# RESULTS

Our cohort included 98 separate families with 377 proven mutation carriers, of whom 11 were biallelic carriers and 366 heterozygous carriers (see table 2 for a description of the cohort).

## CRC and EC

The cumulative CRC risk (table 3, figure 1) calculated using MENDEL was 18.75% (95% CI: 5.60-30.06%) for males at age 70, with a hazard ratio of 6.92 (CI: 2.46-19.42). The CRC risk at age 70 for female carriers was 10.56% (CI: 2.42-18.01%), with a hazard ratio of 4.71 (CI: 1.51-14.72), while the cumulative risk at age 70 for EC (table 3, figure 2) was 11.78% (CI: 2.61-20.09%), with a hazard ratio of 8.74 (CI: 2.14-35.7). The mean age of first CRC development for all cases with a heterozygous *PMS2* mutation was 52 years (range 26-86, table 4). Notably, the age distribution for CRC differed markedly between probands and CRC-affected family members (supplementary figure 1 and 2).

**TABLE 3** Age-specific hazard ratios and cumulative cancer risks for CRC and EC in PMS2 mutation carriers

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

HR = hazard ratio; CR = cumulative risk; CI = confidence interval;
CRC= colorectal carcinoma; EC= endometrial carcinoma

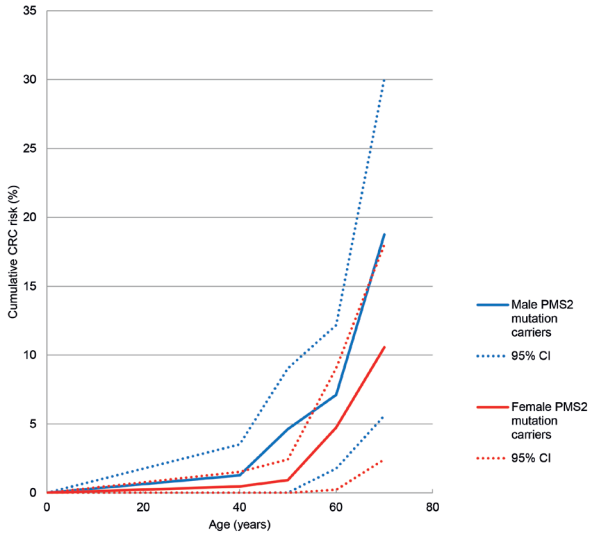



FIGURE 1 Graphic presentation of cumulative cancer risk with 95% confidence intervals (CI) for colorectal carcinoma (CRC).

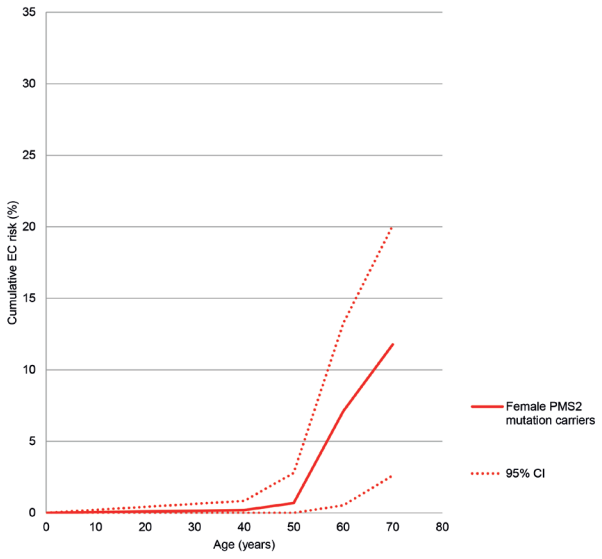



FIGURE 2 Graphic presentation of cumulative cancer risk with 95% confidence intervals (CI) for endometrial cancer (EC).

TABLE 4 Mean age of CRC and EC diagnosis in PMS2 mutation carriers

| | | No. | Median (years) | Mean age (years) | Range | p-value |
|---|---|---|---|---|---|---|
| CRC | Total group | 106 | 51 | 52 | 26-86 | 0.83 |
| | Male | 65 | 52 | 51 | 26-86 | |
| | Female | 40 | 51 | 52 | 27-78 | |
| | Probands | 62 | 48 | 47 | 26-68 | <0.001 |
| | Family members | 44 | 57 | 58 | 31-86 | |
| EC | Total group | 25 | 54 | 55 | 35-81 | |
| | Probands | 15 | 52 | 56 | 42-81 | 0.76 |
| | Family members | 10 | 58 | 54 | 35-68 | |

CRC= colorectal carcinoma; EC= endometrial carcinoma

TABLE 5 Mean ages at cancer diagnosis of MMR mutation carriers

| | PMS2 | *MLH1/MSH2* [#4] | *MSH6* [*5] |
|---|---|---|---|
| CRC | 52 (26-86) including probands<br>58 (31-86) excluding probands | 47 (15-95) | 59 (30-90) |
| EC | 55 (35-81) including probands<br>54 (35-68) excluding probands | 47 (26-75) | 54 (32-82) |

# No probands included
* Including probands
CRC= colorectal carcinoma; EC= endometrial carcinoma MMR: mismatch repair

TABLE 6 Other Lynch syndrome associated cancers

| Location of malignancy | Observed | Expected | Standardized Incidence Ratio (95% CI) | p-value | Mean age of diagnosis (range) |
|---|---|---|---|---|---|
| Overall cancer * | 35 | 16.9 | 2.1 (1.4-2.9) | 0.00016 | 57 (18-80) |
| Small bowel | 5 | 0.042 | 118.9 (38.6-277.4) | $2.12*10^{-6}$ | 60 (48-79) |
| Breast | 11 | 2.9 | 3.8 (1.9-6.8) | 0.0004 | 55 (36-80) |
| Ovary | 4 | 0.33 | 12.0 (3.3-30.7) | 0.0008 | 55 (51-59) |
| Prostate | 2 | 1.17 | 1.7 (0.21-6.2) | 0.66 | 56 (42-70) |
| Renal Pelvis | 2 | 0.040 | 50.5 (6.1-182.4) | 0.0015 | 78 (77-79) |
| Brain | 1 | 0.37 | 2.7 (0.069-15.2) | 0.62 | 55 |
| Leukemia# | 1 | 0.47 | 2.1 (0.054-11.9) | 0.75 | 45 |
| Stomach | 0 | 0.57 | 1.7 (0-6.5) | N/A | |
| Pancreas | 0 | 0.31 | 0 (0-12) | N/A | |
| Bladder | 1 | 0.50 | 2.0 (0.051-11.2) | 0.79 | 49 |

Note: The standardized incidence ratio is the observed number of cancers divided by the number of expected cancers in the general population; Significant SIRs are highlighted in bold; N/A: not applicable; * Excluding CRC and EC; # Non-Hodgkin Lymphoma
CRC= colorectal carcinoma; EC= endometrial carcinoma

The mean ages of CRC diagnosis in these groups were 47 years (range 26-68) and 58 years (range 31-78), respectively. There was no significant difference in mean age of CRC development between male and female carriers (51 years versus 52 years, p=0.83). The mean age at diagnosis of EC was 55 years (range 35-81), with no significant difference between probands and affected family members (p=0.76, table 4). For both CRC and EC, the mean age at diagnosis of carriers of a pathogenic *PMS2* mutation is higher when compared to carriers of a *MLH1* or *MSH2* mutation, but when compared to *MSH6* the mean age of CRC is lower and for EC similar in our cohort (table 5).

### Other LS-associated cancers

Risks for cancers other than CRC or EC among *PMS2* mutation carriers are shown in table 6. Significant SIRs were identified for cancers of the small bowel (118.9 (95% CI: 38.6-277.4)), ovaries (12.0 (CI: 3.3-30.7)), breast (3.8 (CI: 1.9-6.8)) and renal pelvis (50.5 (CI: 6.1-182.4)).

## DISCUSSION

In an effort to achieve a comprehensive understanding of the cancer risks faced by *PMS2* mutation carriers, we collected and analyzed a cohort of 98 *PMS2* mutation-positive families including over 2500 family members. Analysis of this large cohort revealed that cumulative CRC risk at age 70 is almost 19% for males and 11% for females, while risk for EC is around 12%. Furthermore, we found significant SIRs for cancers of the small bowel, breast, ovaries and renal pelvis. As reported previously for other MMR genes, females appear to have a markedly lower CRC risk compared to 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 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 appear 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 appear 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.* 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) 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 (e.g. genetic) or external (e.g. lifestyle) modifiers. Indeed, certain single nucleotide polymorphisms (SNPs) associated with a slight increase in CRC risk in the general population, as found by genome-wide association studies (GWAS), are known to significantly influence CRC risk in LS patients with *MLH1* and *MSH2* mutations.[23,24] Lifestyle factors such as BMI and smoking have also been reported to modify CRC risk and adenoma development in LS patients.[25,26] This is possibly explained by a difference in the molecular mechanism underlying carcinogenesis in LS patients that might then result in lifestyle factors having a different effect in these patients as compared to sporadic CRC patients. However,

as previous association studies only included very limited numbers of *PMS2* mutation carriers, further research on this subject is needed.

Due to the increased identification of *PMS2* mutation carriers (and LS patients in general) expected to result from the implementation of next generation sequencing and universal screening programs for CRC and EC patients, there is now a pressing need to establish *PMS2*-specific risk estimates. This increased detection is illustrated by studies using IHC analysis in CRCs from population-based cohorts that showed that isolated PMS2 protein loss in the tumor - indicative of a germline *PMS2* mutation - occurs in 0.5-1.5% of unselected CRC cases.[12,15,27] Previously, identification of LS patients 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 a 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 as it is likely that they do not comply with strict selection criteria due to low cancer penetrance and a higher mean age of 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 abovementioned criteria and would have been overlooked on the basis of 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 0.03-0.4% of individuals not selected on the basis of 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 from around 20-25 years of age, at 1-2 year intervals.[33,34] We now suggest that surveillance in *PMS2* mutation carriers could start at a slightly higher age, e.g. 30 years, similar to the previously suggested age for female *MSH6* carriers - due to the later age of onset and lower cancer risk than that reported for *MLH1* and *MSH2*.[35] It should be noted however that 6 heterozygous probands (6/367, 1.6% of all mutation carriers) developed CRC below or at the age of 30. 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.

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 30-35, which may lead to the detection of premalignant disease.[33,34,36] Prophylactic surgery,

i.e. risk reducing salpingo-oophorectomy (RRSO), does not seem to be appropriate for female *PMS2* mutation carriers as mortality from EC is relatively low.[37] Indeed, in our cohort only one out of 25 proven *PMS2* mutation carriers with EC died of this cause, at age 65. Furthermore, OC in LS patients 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 cases than in non-Lynch related OC cases.[38] Therefore, we only advise use of the surveillance protocol described above and not prophylactic surgery.

Additional screening for other LS-associated cancers is currently not advised for LS patients, 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 the breasts (table 6). 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] Due to a relatively low incidence and in general a high mean age at diagnosis in our cohort (table 6), additional surveillance other than for CRC and EC does not seem to be indicated. However, in light of a SIR for breast carcinomas of 3.8, mammography from age 40 may be considered, especially in those *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 in order 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 on the basis of 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 colorectal and endometrial cancer 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 due to 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*; begin colorectal surveillance at age 30 instead of the current 25 years of age. Furthermore, prophylactic removal of the uterus and ovaries of female *PMS2* mutation carriers does not appear advisable at the present time.

## REFERENCES

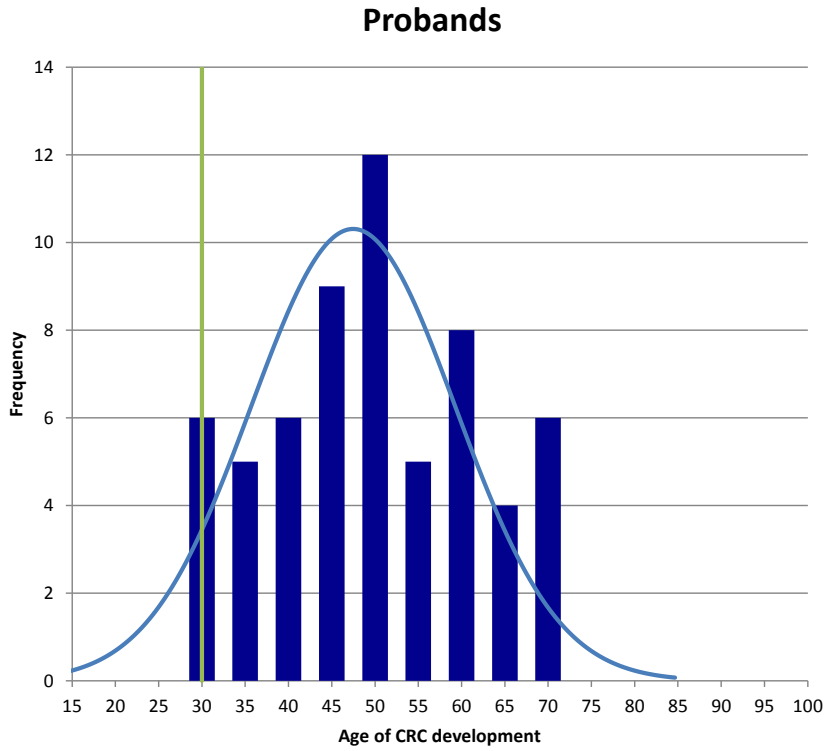

1. Barrow E, Hill J, Evans DG: Cancer risk in Lynch Syndrome. Fam Cancer 12:229-240, 2013
2. Hampel H, Stephens JA, Pukkala E, et al.: Cancer risk in hereditary nonpolyposis colorectal cancer syndrome: later age of onset. Gastroenterology 129:415-421, 2005
3. Stoffel E, Mukherjee B, Raymond VM, et al.: Calculation of risk of colorectal and endometrial cancer among patients with Lynch syndrome. Gastroenterology 137:1621-1627, 2009
4. Dowty JG, Win AK, Buchanan DD, et al.: Cancer risks for MLH1 and MSH2 mutation carriers. Hum Mutat 34:490-497, 2013
5. Baglietto L, Lindor NM, Dowty JG, et al.: Risks of Lynch syndrome cancers for MSH6 mutation carriers. J Natl Cancer Inst 102:193-201, 2010
6. Nicolaides NC, Papadopoulos N, Liu B, et al.: Mutations of two PMS homologues in hereditary nonpolyposis colon cancer. Nature 371:75-80, 1994
7. Clendenning M, Hampel H, LaJeunesse J, et al.: Long-range PCR facilitates the identification of PMS2-specific mutations. Hum Mutat 27:490-495, 2006
8. Vaughn CP, Robles J, Swensen JJ, et al.: Clinical analysis of PMS2: mutation detection and avoidance of pseudogenes. Hum Mutat 31:588-593, 2010
9. van der Klift HM, Tops CM, Bik EC, et al.: Quantification of sequence exchange events between PMS2 and PMS2CL provides a basis for improved mutation scanning of Lynch syndrome patients. Hum Mutat 31:578-587, 2010
10. Worthley DL, Walsh MD, Barker M, et al.: Familial mutations in PMS2 can cause autosomal dominant hereditary nonpolyposis colorectal cancer. Gastroenterology 128:1431-1436, 2005
11. Hendriks YM, de Jong AE, Morreau H, et al.: Diagnostic approach and management of Lynch syndrome (hereditary nonpolyposis colorectal carcinoma): a guide for clinicians. CA Cancer J Clin 56:213-225, 2006
12. 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
13. Bonadona V, Bonaiti B, Olschwang S, et al.: Cancer risks associated with germline mutations in MLH1, MSH2, and MSH6 genes in Lynch syndrome. JAMA 305:2304-2310, 2011
14. Johannesma PC, van der Klift HM, van Grieken NC, et al.: Childhood brain tumours due to germline bi-allelic mismatch repair gene mutations. Clin Genet 80:243-255, 2011

15. Truninger K, Menigatti M, Luz J, et al.: Immunohistochemical analysis reveals high frequency of PMS2 defects in colorectal cancer. Gastroenterology 128:1160-1171, 2005
16. 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
17. Bakry D, Aronson M, Durno C, et al.: Genetic and clinical determinants of constitutional mismatch repair deficiency syndrome: Report from the constitutional mismatch repair deficiency consortium. Eur J Cancer, 2014
18. Herkert JC, Niessen RC, Olderode-Berends MJ, et al.: Paediatric intestinal cancer and polyposis due to bi-allelic PMS2 mutations: case series, review and follow-up guidelines. Eur J Cancer 47:965-982, 2011
19. Wimmer K, Kratz CP, Vasen HF, et al.: Diagnostic criteria for constitutional mismatch repair deficiency syndrome: suggestions of the European consortium 'Care for CMMRD' (C4CMMRD). J Med Genet 51:355-365, 2014
20. Borras E, Pineda M, Cadinanos J, et al.: Refining the role of PMS2 in Lynch syndrome: germline mutational analysis improved by comprehensive assessment of variants. J Med Genet 50:552-563, 2013
21. Brohet RM, Velthuizen ME, Hogervorst FB, et al.: Breast and ovarian cancer risks in a large series of clinically ascertained families with a high proportion of BRCA1 and BRCA2 Dutch founder mutations. J Med Genet 51:98-107, 2014
22. 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 29:1-11, 2005
23. 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 132:1556-1564, 2013
24. Wijnen JT, Brohet RM, van ER, et al.: Chromosome 8q23.3 and 11q23.1 variants modify colorectal cancer risk in Lynch syndrome. Gastroenterology 136:131-137, 2009
25. Van Duijnhoven FJ, Botma A, Winkels R, et al.: Do lifestyle factors influence colorectal cancer risk in Lynch syndrome? Fam Cancer 12:285-293, 2013
26. Pande M, Lynch PM, Hopper JL, et al.: Smoking and colorectal cancer in Lynch syndrome: results from the Colon Cancer Family Registry and the University of Texas M.D. Anderson Cancer Center. Clin Cancer Res 16:1331-1339, 2010
27. van Lier MG, Leenen CH, Wagner A, et al.: Yield of routine molecular analyses in colorectal cancer patients </=70 years to detect underlying Lynch syndrome. J Pathol 226:764-774, 2012

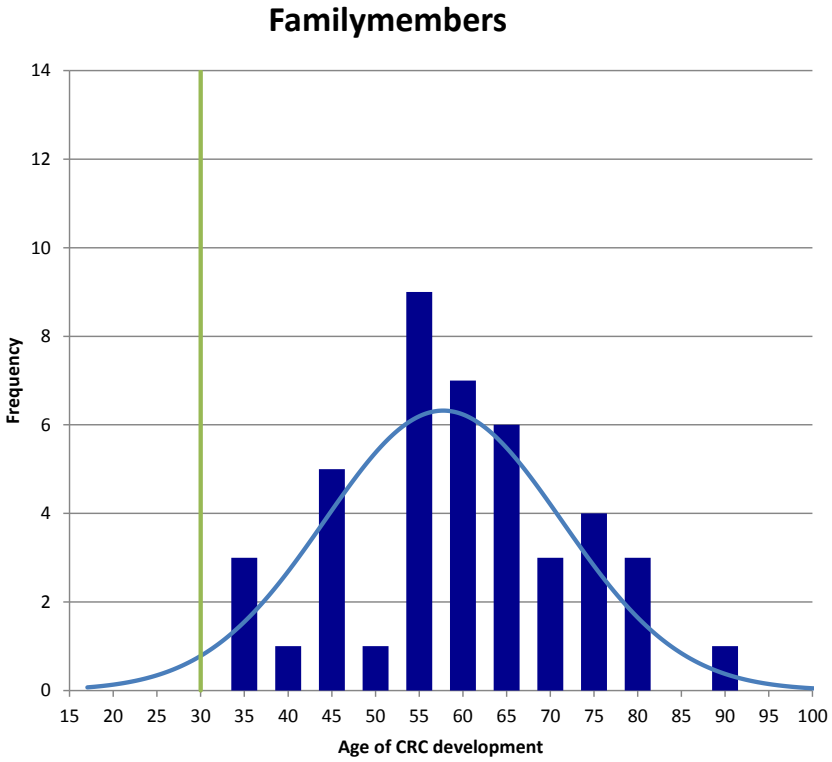
28. Leenen CH, van Lier MG, van Doorn HC, et al.: Prospective evaluation of molecular screening for Lynch syndrome in patients with endometrial cancer </= 70 years. Gynecol Oncol 125:414-420, 2012
29. Vasen HF, Watson P, Mecklin JP, et al.: New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the International Collaborative group on HNPCC. Gastroenterology 116:1453-1456, 1999
30. Dorschner MO, Amendola LM, Turner EH, et al.: Actionable, pathogenic incidental findings in 1,000 participants' exomes. Am J Hum Genet 93:631-640, 2013
31. Boone PM, Soens ZT, Campbell IM, et al.: Incidental copy-number variants identified by routine genome testing in a clinical population. Genet Med 15:45-54, 2013
32. 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
33. Vasen HF, Moslein G, Alonso A, et al.: Guidelines for the clinical management of Lynch syndrome (hereditary non-polyposis cancer). J Med Genet 44:353-362, 2007
34. Vasen HF, Blanco I, Aktan-Collan K, et al.: Revised guidelines for the clinical management of Lynch syndrome (HNPCC): recommendations by a group of European experts. Gut 62:812-823, 2013
35. Hendriks YM, Wagner A, Morreau H, et al.: Cancer risk in hereditary nonpolyposis colorectal cancer due to MSH6 mutations: impact on counseling and surveillance. Gastroenterology 127:17-25, 2004
36. Ketabi Z, Gerdes AM, Mosgaard B, et al.: The results of gynecologic surveillance in families with hereditary nonpolyposis colorectal cancer. Gynecol Oncol 133:526-530, 2014
37. Pylvanainen K, Lehtinen T, Kellokumpu I, et al.: Causes of death of mutation carriers in Finnish Lynch syndrome families. Fam Cancer 11:467-471, 2012
38. Ketabi Z, Bartuma K, Bernstein I, et al.: Ovarian cancer linked to Lynch syndrome typically presents as early-onset, non-serous epithelial tumors. Gynecol Oncol 121:462-465, 2011
39. Bernstein IT, Myrhoj T: Surveillance for urinary tract cancer in Lynch syndrome. Fam Cancer 12:279-284, 2013

2

## SUPPLEMENTAL FIGURES




SUPPLEMENTARY FIGURE 1 Histogram of mean age of CRC development, representing probands with a heterozygous *PMS2* mutation. The green line in figure 4 indicates age 30, which is the suggested start of colorectal surveillance.




SUPPLEMENTARY FIGURE 2 Histogram of mean age of CRC development, representing family members with a heterozygous *PMS2* mutation. The green line in figure 4 indicates age 30, which is the suggested start of colorectal surveillance.

SUPPLEMENTARY TABLE 1 Clinical characteristics of biallelic *PMS2* mutation carriers (N=11)

| Sex | CRC | Age CRC | Other cancer | Age other cancer | Other | Mutation |
|---|---|---|---|---|---|---|
| F | Yes | 19 | Bladder<br>Jejunum | 21<br>Unknown | Multiple adenoma, giant pigmented naevus syndrome, CAL's, hypothyroidism, | c.137G>T AND c.2174+1G>A (compound heterozygous) |
| M | No | | Duodenum<br>Jejunum<br>Acute lymphoid leukaemia (ALL) | 17<br>19<br>21 | Serrated polyp with high grade dysplasia at age 15<br>Small bowel and rectum loaded with polyps at age 16 | c.137G>T AND c.2174+1G>A (compound heterozygous) |
| M | No | | Duodenum | 32 | Polyposis 20 years, complex cor vitium | Deletion exon 3-7 deletion AND deletion exon 1-11(-15) (compound heterozygous) |
| M | No | | Angiosarcoma | 2 | CAL | c.736_741delinsTGTGTGTGAAG AND complete gene deletion (compound heterozygous) |
| F | No | | Brain | 4 | CAL's | c.137G>T AND c.736_741delinsTGTGTGTGAAG (compound heterozygous) |
| M | No | | Brain | 8 | CAL's | c.137G>T AND c.736_741delinsTGTGTGTGAAG (compound heterozygous) |
| F | Yes | 8 | Brain<br>Lymphoma | 9<br>18 | - | c.219_220dup (homozygous) |
| M | No | | -- | - | Rectum adenoma at 24 years | c.325dup AND c.825A>G (splice mutation) (compound heterozygote) |
| F | No | | Brain | 11 | - | c.325dup AND c.825A>G (splice mutation) (compound heterozygote) |
| F | No | | Brain | 2 | 1 adenoma at 10, CAL's and freckling | c.2174+1G>A (homozygous) |
| F | Yes | 43 | Lymphoma, Endometrial carcinoma | 18, 37 and 43<br>39 | Colorectal polyp | c.24-2A>G (splice mutation) (homozygous) |

CAL= Café-au-lait maculae; CRC= colorectal carcinoma

SUPPLEMENTARY TABLE 2 *PMS2* mutations

| Description of PMS2 mutation[1] | Nationality of index patients[2] | Total nr of families | Family description reported before[3] |
|---|---|---|---|
| c.736_741delinsTGTGTGTGAAG | 10x NL; 4x DK; 3x S | 17 | |
| c.1882C>T (p.Arg628X) | 6x NL | 6 | Hendriks, 2006 (family 6) |
| c.697C>T (p.Gln233X) | 4x NL | 4 | |
| Deletion exon 14 | 3x NL | 3 | |
| Deletion exon 11-15 | 3x NL | 3 | Hendriks, 2006 (family 3) |
| c.137G>T (p.Ser46Ile) | 3x NL | 3 | |
| c.2192_2196del | 3x NL | 3 | |
| c.219_220dup | 3x NL | 3 | |
| c.24-12_107delinsAAAT | 2x NL | 2 | |
| c.1831dup | 2x NL | 2 | |
| Deletion exon 3-7 | 2x NL | 2 | |
| Deletion exon 10 | 2x NL | 2 | Hendriks, 2006 (family 2) |
| Whole PMS2 deletion | 2x NL | 2 | Hendriks, 2006 (family 1) |
| c.2113G>A (p.Glu705Lys) | 2x S | 2 | |
| c.823C>T (p.Gln275X) | 2x NL | 2 | |
| unknown | 1x NL | 1 | |
| c.325dup | 1x NL | 1 | |
| c.1111_1113delinsTTTA | 1x DK | 1 | |
| c.2156del | 1x N | 1 | |
| c.861_864del | 1x NL | 1 | Hendriks, 2006 (family 7) |
| c.1079_1080del | 1x NL | 1 | |
| c.2117del | 1x NL | 1 | |
| c.247_250dup | 1x NL | 1 | |
| c.904_911del | 1x NL | 1 | |
| c.658dup | 1x NL | 1 | |
| c.780del | 1x E | 1 | Borras, 2013 (family D) |
| Deletion exon 1 | 1x NL | 1 | |
| Deletion exon 2-15 | 1x NL | 1 | |
| Deletion exon (3-)5-15 | 1x NL | 1 | |
| Deletion exon 1-10 | 1x D | 1 | |
| Deletion exon 6-7 | 1x NL | 1 | |
| Deletion exon 11 | 1x DK | 1 | |
| Deletion exon 6 | 1x E | 1 | Borras, 2013 (family C) |
| Duplication exon 11+12 | 1x N | 1 | |

SUPPLEMENTARY TABLE 2 *PMS2* mutations

| Description of PMS2 mutation[1] | Nationality of index patients[2] | Total nr of families | Family description reported before[3] |
|---|---|---|---|
| c.2444C>T (p.Ser815Leu) | 1x NL | 1 | |
| c.319C>T (p.Arg107Trp and splice effect) | 1x NL | 1 | |
| c.2404C>T (p.Arg802X) | 1x NL | 1 | |
| c.2155C>T (p.Gln719X) | 1x NL | 1 | |
| c.804-60_804-59insJN866832.1 (retrotranspositional insertion of SVA repeat) | 1x NL | 1 | Hendriks, 2006 (family 4); van der Klift, 2012 |
| c.989-1G>T (splice mutation) | 1x N | 1 | Grindedal, 2014 |
| c.989-2A>G (splice mutation) | 1x E | 1 | Borras, 2013 (family G) |
| c.163+2T>C (splice mutation) | 1x NL | 1 | |
| c.903G>T (splice mutation) | 1x NL | 1 | |
| c.2445+1G>T (splice mutation) | 1x NL | 1 | |
| c.1144+2T>A (splice mutation) | 1x NL | 1 | Hendriks, 2006 (family 5) |
| c.354-1G>A (splice mutation) | 1x NL | 1 | |
| c.251-2A>C (splice mutation) | 1x NL | 1 | |
| c.164-2A>G (splice mutation) | 1x E | 1 | Borras, 2013 (family B) |
| c.1A>G (variant in start codon) | 1x E | 1 | Borras, 2013 (family A) |
| c.736_741delinsTGTGTGTGAAG AND complete gene deletion (compound heterozygous) | bi-allelic index (NL) | 1 | Herkert, 2011 |
| c.137G>T AND c.2174+1G>A (compound heterozygous) | bi-allelic index (NL) | 1 | Herkert, 2011 |
| c.137G>T AND c.736_741delinsTGTGTGTGAAG (compound heterozygous) | bi-allelic index (NL) | 1 | Leenen, 2011 |
| c.2174+1G>A (homozygous) | bi-allelic index (NL) | 1 | Herkert, 2011 |
| Deletion exon 3-7 deletion AND deletion exon 1-11(-15) (compound heterozygous) | bi-allelic index (NL) | 1 | Herkert, 2011 |
| c.219_220dup (homozygous) | bi-allelic index (NL) | 1 | |
| c.325dup AND c.825A>G (splice mutation) (compound heterozygote) | bi-allelic index (NL) | 1 | Johannesma, 2011 |
| c.24-2A>G (splice mutation) (homozygous) | Bi-allelic index (E) | 1 | |

[1] Nomenclature according to Human Genetic Variation Society (HGVS) approved guidelines (www.hgvs.org/mutnomen) with reference to NM_000535.5, accept for the large deletions or duplications. Large deletions and duplications were in most 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.
[2]D=Germany, DK=Denmark, E=Spain, N=Norway, NL=Netherlands, S=Sweden
[3]references not in main text: Hendriks, 2006; Leenen, 2011; van der Klift, 2012

Hendriks YM, Jagmohan-Changur S, van der Klift HM, Morreau H, van Puijenbroek M, Tops C, van Os T, Wagner A, Ausems MG, Gomez E, Breuning MH, Brocker-Vriends AH, Vasen HF, Wijnen JT. 2006. Heterozygous mutations in PMS2 cause hereditary nonpolyposis colorectal carcinoma (Lynch syndrome). Gastroenterology 130:312–322.

Leenen CH, Geurts-Giele WR, Dubbink HJ, Reddingius R, van den Ouweland AM, Tops CM, van de Klift HM, Kuipers EJ, van Leerdam ME, Dinjens WN, Wagner A. 2011.
Pitfalls in molecular analysis for mismatch repair deficiency in a family with biallelic pms2 germline mutations.
Clin Genet 80(6):558-65

van der Klift HM, Tops CM, Hes FJ, Devilee P, Wijnen JT. 2012. Insertion of an SVA element, a nonautonomous retrotransposon, in PMS2 intron 7 as a novel cause of Lynch syndrome.
Hum Mutat 33(7):1051-5