Germline variants in the mismatch repair genes: Detection and phenotype

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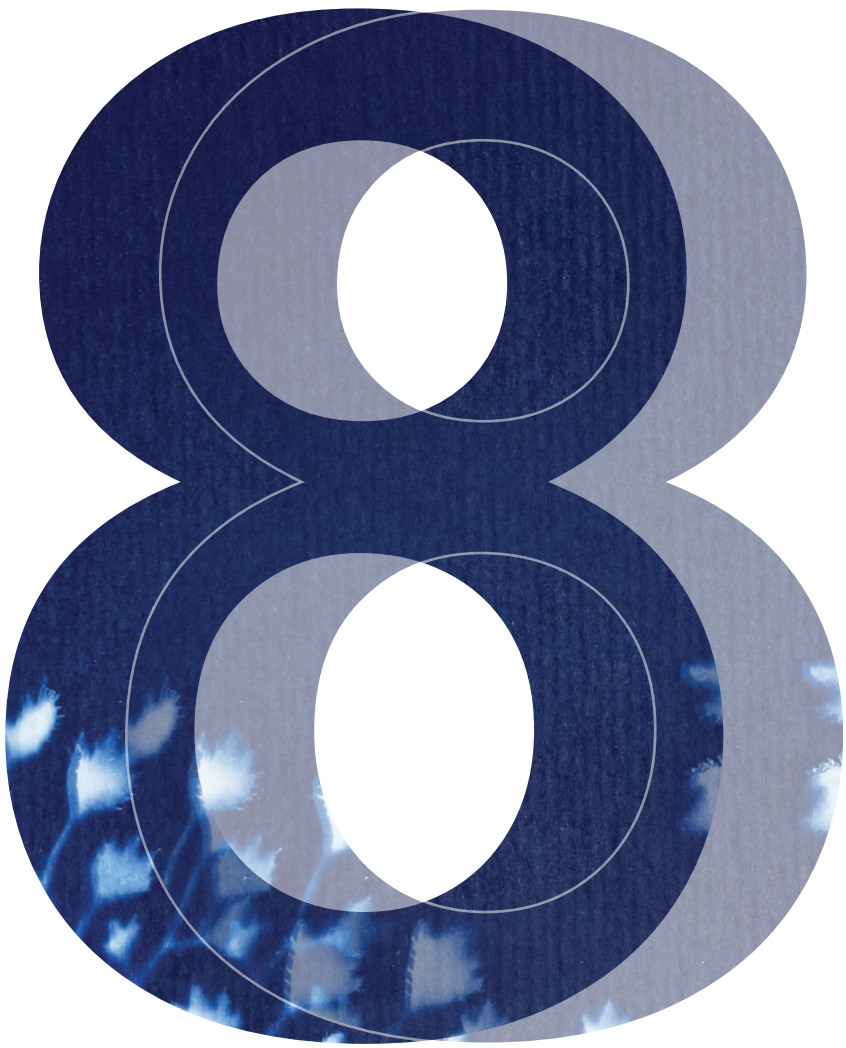

Incidence of (adenomatous) polyps and colorectal cancer in patients with PMS2-associated Lynch syndrome undergoing surveillance: a prospective cohort analysis

Manuscript in preparation

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ABSTRACT

Purpose

Lynch syndrome predisposes carriers of a heterozygous pathogenic germline variant in the *MLH1*, *MSH2*, *MSH6* or *PMS2* genes to the development of mainly colorectal (CRC) and endometrial cancer. Of the four mismatch repair (MMR) genes, *PMS2* variant carriers have the lowest cancer risk, yet surveillance protocols are identical for all Lynch syndrome patients. The aim of this study was to determine the characteristics and incidence of polyps and incident CRC in *PMS2* variant carriers undergoing regular surveillance.

Methods

We collected a cohort of 171 *PMS2* variant carriers and recorded the occurrence and characteristics of incident adenomas and CRC. After receiving consent to request clinical data, we obtained information through PALGA, the Dutch nationwide network and registry of histo- and cytopathology, and by requesting colonoscopy reports at gastroenterology departments. Twenty polyps were available for immunohistochemical staining of the PMS2 protein.

Results

During a total of 675 colonoscopies (1044 observation years, median surveillance interval 2 years), 435 polyps were removed, of which 237 (54.5%) were adenomatous. Forty-one (16.9%) of those adenomas were advanced (i.e. ≥1 cm in diameter, villous component and/or high-grade dysplasia). None of the twenty polyps that were immunohistochemically stained showed loss of PMS2 expression, suggesting late involvement of PMS2 deficiency in the pathway to cancer. One incident CRC was reported.

Conclusion

In this large cohort of *PMS2* variant carriers, only one incident CRC were observed. This tumor was preceded by a colonoscopy with insufficient bowel preparation. Further analyses are required to draw firm conclusions about adenoma risk in *PMS2* carriers compared to the other MMR genes.

INTRODUCTION

Lynch syndrome predisposes carriers of a germline heterozygous pathogenic variant in one of the mismatch repair genes (MMR): *MLH1*, *MSH2*, *MSH6* or *PMS2*, to the development of mainly colorectal and endometrial cancer. Lynch syndrome-associated cancers are characterized by microsatellite instability (MSI) and negative immunohistochemical staining for the involved MMR protein.[1,2] To prevent the development of colorectal cancer, patients with Lynch syndrome are offered surveillance by colonoscopy every 1-2 years, starting at age 25.[3] Despite these regular surveillance colonoscopies, incident colorectal cancers do occur, particularly in *MLH1* and *MSH2* variant carriers.[4-7] Data on polyps and incident colorectal cancer in PMS2-associated Lynch syndrome is sparse, yet highly clinically relevant since recent studies reported a high prevalence of *PMS2* variants in the general population (1:714).[8] *PMS2* variant carriers display a distinct phenotype, with retrospective cohort studies reporting substantially lower cancer risks compared to carriers of *MLH1* and *MSH2* variants,[9-11] which has resulted in discussion of MMR-gene-specific surveillance protocols.[4] This discussion would be greatly assisted by more prospectively collected gene-specific data. Previously, the prospective Lynch syndrome database (PLSD) consortium has confirmed low cancer risks for carriers of pathogenic variants in the *PMS2* gene.[4,12-14] However, these studies did not include exact data on endoscopic detection of adenomas, which is essential for a better understanding of the role of MMR deficiency in Lynch syndrome associated carcinogenesis. Moreover, data on quality of surveillance is usually also lacking. To this aim, we collected prospective colonoscopy data on a large cohort of *PMS2* variant carriers (n=171) and evaluated PMS2 protein expression in twenty polyps.

MATERIALS & METHODS

Data collection

Consent was obtained to request clinical information and pathology samples for 186 Dutch Lynch syndrome patients with a confirmed pathogenic germline *PMS2* variant diagnosed at Dutch family cancer clinics. Obtaining pathology reports was facilitated by PALGA, the nationwide network and registry of histology and cytopathology in the Netherlands.[15] As PALGA encompasses all pathology laboratories in the Netherlands, all pathology reports on each patient can be obtained, even if a patient attended different hospitals for colonoscopies. Corresponding colonoscopy reports were

requested at the respective gastroenterology departments. For fourteen *PMS2* variant carriers both the PALGA search and request for colonoscopy reports did not yield any results, therefore these patients most likely are not undergoing regular surveillance and they were excluded from the analyses. Furthermore, one patient was excluded from the analyses, because of an exceptionally severe phenotype (three synchronous colorectal cancers and 18 adenomas at age 26 and an intellectual disability). This extraordinary phenotype is likely not completely explained by his *PMS2* variant alone. The study was approved by the IRB of the LUMC.

PMS2 variant analysis

Our cohort consisted of clinically ascertained families in which variant analysis was initiated because a family met the Bethesda criteria[16] and/or (histological) pre-screening by immunohistochemistry and/or microsatellite instability was indicative of MMR deficiency. Germline *PMS2* variant screening was performed as previously described.[10,11,17] Comprehensive strategies were applied to avoid unreliable variant detection caused by interference from pseudogene sequences and frequent gene conversion events.[17] All variants found in the included *PMS2* carriers are listed in supplemental tables 1 and 2.

Immunohistochemistry

We retrieved formalin-fixed, paraffin-embedded (FFPE) tissue blocks of 16 adenomas with low-grade dysplasia (one of which was scored as advanced because of a villous component), two sessile serrated lesions and two hyperplastic polyps, and performed immunohistochemical analysis of PMS2 expression. In brief, the FFPE material was sectioned at 4 μm and stained with an antibody to PMS2 (Clone EP51, Agilent, Santa Clara, CA, USA). If the staining results showed absence of nuclear staining in the cells of an adenoma or polyp in the presence of positive control cells (e.g. leukocytes) than this was interpreted as PMS2 deficiency.

Statistical analysis

Descriptive results of colonoscopy findings were computed using Stata (Statacorp version 14). A Kaplan Meier analysis was carried out to estimate time to first adenoma or first (advanced) adenoma. Timepoint zero was the time at first colonoscopy. Advanced adenomas were defined by a size of ≥1 cm in diameter, a villous component of >25%, and/or the presence of high-grade dysplasia.

Results were compared to data from two studies. One study by Engel et al. which reports the occurrence of incident adenomas and advanced adenomas in a large

cohort of *MLH1*-, *MSH2*-, and *MSH6*-associated Lynch syndrome patients.[18] Forsberg et al. report more detailed data on histological subtypes and numbers of (adenomatous) polyps at first colonoscopy in a cohort of MLH1-, MSH2-, and MSH6-associated Lynch syndrome patients and compare this data to control data from an earlier prospective population-based colonoscopy study by the same group.[19]

RESULTS

Between 1987 and 2017, a total of 675 colonoscopies were performed in this cohort of 171 *PMS2* variant carriers, representing 1044 years of follow-up. The median time between follow-up colonoscopies was 2.0 years. All included *PMS2*-associated Lynch syndrome patients had a confirmed germline heterozygous pathogenic variant in the *PMS2* gene (supplemental material) and all have been described in previous studies.[10,11,17,20] A detailed description of the cohort is provided in table 1.

Polyps

In total, 435 polyps were removed from 171 *PMS2* variant carriers, half of which were adenomatous (54.5%). Figure 1 shows the cumulative risk of developing an adenoma after the first colonoscopy. The risk of developing an adenoma is 54.5% (95% CI 41.4 – 68.8%) after 10 years. This is higher than the risks reported for carriers of a mutation in the other genes as reported by Engel et al (44.2% for *MSH2,* 38.4% for *MSH6* and 32.2% for *MLH1*).[18]

Figure 2 shows the cumulative risk of developing an advanced adenoma after first colonoscopy, which was 23.7% (95% CI 12.3 – 43.0%) after 10 years. This risks appears to be higher than for carriers of a pathogenic variant in the other genes as reported by Engel et al.[18] However, because of a wide confidence interval, no reliable comparison can be made.

When comparing the cumulative proportion of individuals with an adenoma at first colonoscopy as a function of age between our *PMS2* cohort (supplemental figure 1) and the cohorts as published by Forsberg et al.[21], the *PMS2* cohort shows a lower adenoma risk than the Forsberg Lynch cohort, but a higher risk than the Forsberg control cohort. The same can be said for the cumulative proportion of advanced adenomas (supplemental figure 2).

The sixteen adenomas with low-grade dysplasia, two sessile serrated lesions and two hyperplastic polyps stained for PMS2 protein expression showed normal staining (table 2).

Table 1. Cohort characteristics

	PMS2 cohort	MLH1 (Engel et al)	MSH2 (Engel et al)	MSH6 (Engel et al)
Patients	171	1407	986	354
Men	69 (40.4%)	47.8%	49%	45.2%
Follow-up (years)				
Total	1044	12798	7961	2550
Mean (s.d.)	6.1 (5.9)			
Median (IQR)	4.2 (1.7-9.0)	8.5 (4.2-13.2)	7.4 (4.4-11.3)	6.5 (4.1-9.4)
Range	0-28.4			
Colonoscopies				
Total	675	8299	6300	1798
Number per patient				
Mean (s.d.)	3.9 (3.0)			
Median (IQR)	3 (1-5)	5 (3-8)	6 (4-8)	4 (3-6)
Range	1-18			
Time interval (years)$				
Mean (s.d.)	2.1 (1.9)			
Median (IQR)	2.0 (1.1-2.2)			
Range	0.02-22.5			
Mean age first colonoscopy (s.d.)	50.6 (12.9)	42.7 (13.5)	44.0 (12.3)	48.7 (13.7)
Mean age first adenoma detected (s.d.)	55.3 (12.5)			
Mean age first advanced adenoma detected (s.d.)	56.8 (13.1)			
Total polyps	435			
Hyperplastic polyps	181 (41.6%)			
Location				
Right-sided	52 (28.7%)			
Left sided	111 (61.3%)			
Not specified	18 (9.9%)			
Sessile serrated polyps/adenomas*	16 (3.7%)			
Location left-sided				
Right-sided	8 (50%)			
Left sided	8 (50%)			
Not specified	0			
Mixed	1 (0.2%)			
Adenomas	237 (54.5%)			
Histology				
Tubular adenoma	154 (65%)			
Tubulovillous adenoma	23 (9.7%)			
Villous adenoma	1 (0.4%)			
Sessile serrated adenoma with dysplasia	12 (5.1%)			
Adenoma n.o.s.	47 (19.8%)			

	PMS2 cohort	MLH1 (Engel et al)	MSH2 (Engel et al)	MSH6 (Engel et al)
Size (mm)				
0-4	134 (56.5%)			
5-10	50 (21.1%)			
10<	21 (8.9%)			
Not specified	32 (13.5%)			
Location				
Right-sided	92 (38.8%)			
Left sided	120 (50.6%)			
Not specified	25 (10.6%)			
Dysplasia				
None	1 (0.4%)			
High grade	6 (2.5%)			
Low grade	222 (93.7%)			
Not specified	8 (3.4%)			
Advanced	41 (16.9%)			

n.o.s.: not otherwise specified, IQR: Interquartile range; s.d.: Standard deviation
Advanced: adenomas ≥1 cm in diameter, villous component, and/or high-grade dysplasia
$ only if >1 colonoscopy was performed
* Sessile serrated adenomas were listed in this category if there was no dysplasia

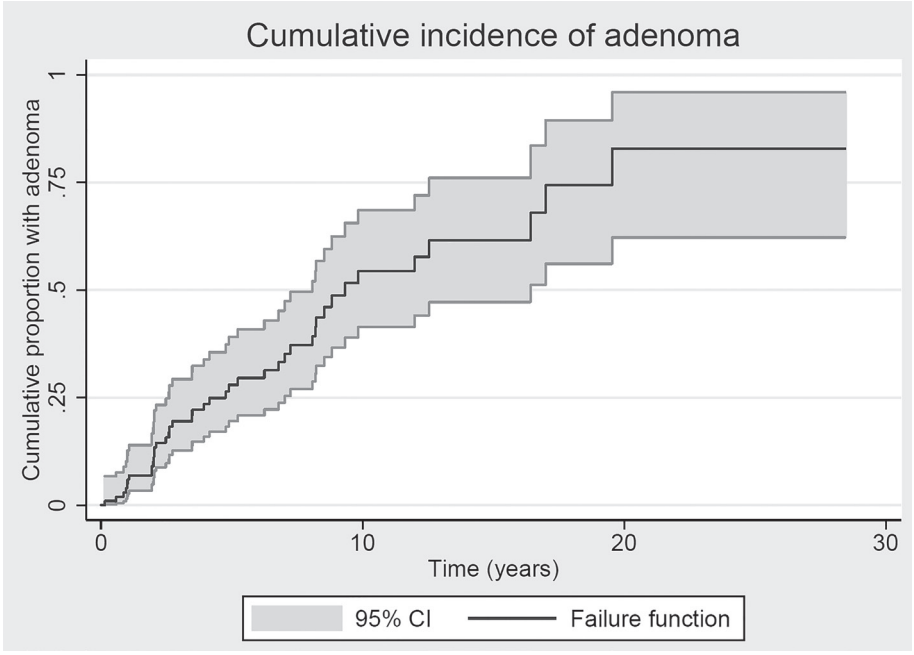

Figure 1 Cumulative proportion of *PMS2* carriers with an adenoma since start of colonoscopy (t=0) with 95% confidence intervals

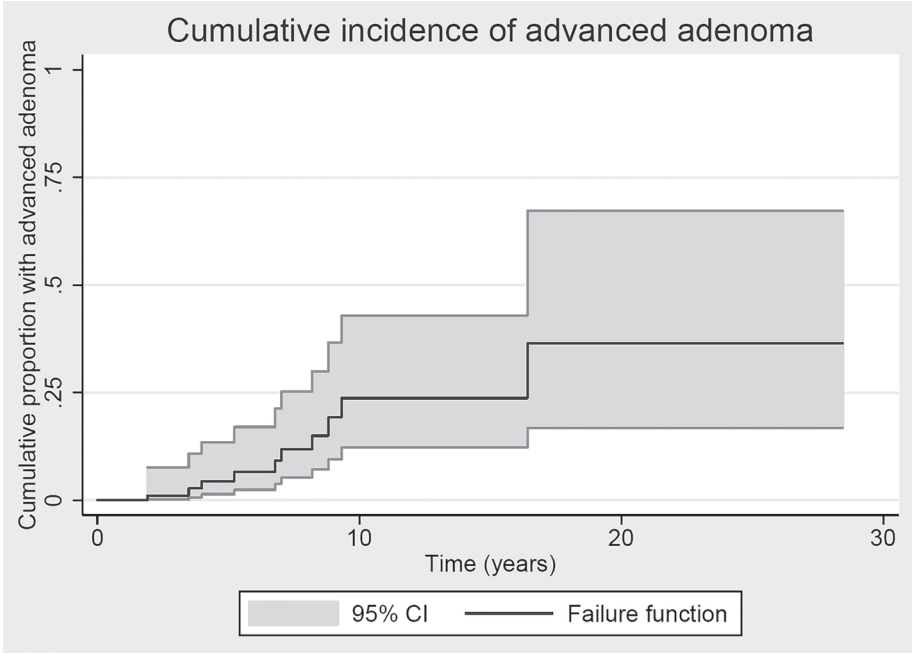

Figure 2 Cumulative incidence of advanced adenomas in *PMS2* carriers with 95% confidence intervals, t=0 is first colonoscopy.

Table 2. Polyps stained for PMS2 protein expression
n.a.: not applicable; n.o.s.: not otherwise specified; CRC: colorectal cancer

Case ID	Gender	CRC	Cumulative number of adenomas	Age of diagnosis first adenoma (years)	Cumulative number of hyperplastic polyps	Cumulative No of sessile serrated lesions	Polyp ID	Site of adenoma	Histology	Grade of Dysplasia	Size (mm)	PMS2 IHC
1	F	No	3	62	1	0	1.1	Right	Tubulovillous adenoma	Low	5	+
							1.2	Left	Tubular adenoma	Low	3	+
2	F	Yes	2	67	23	5	2.1	Pouch	Sessile serrated adenoma	Low	3	+
							2.2	Left	Mixed adenoma	Low	2	+
							2.3	Right	Tubular adenoma	Low	3	+
							2.4	Colon n.o.s.	Hyperplastic polyp	n.a.	8	+
3	F	No	6	57	2	0	3.1	Right	Tubular adenoma	Low	2	+
							3.2	Right	Adenomatous n.o.s.	Low	2,5	+
4	F	No	3	61	0	1	4.1	Right	Tubular adenoma	Low	2	+
							4.2	Left	Tubular adenoma	Low	3	+
							4.3	Left	Sessile serrated polyp	None	10	+
							4.4	Left	Tubular adenoma	Low	2	+
5	M	Yes	3	54	1	0	5.1	Left	Tubular adenoma	Low	2	+
6	F	No	1	45	0	0	6.1	Right	Adenomatous n.o.s.	Low	2	+
7	F	Yes	2	28	1	0	7.1	Left	Adenomatous n.o.s.	Low	3	+
8	M	Yes	15	64	0	0	8.1	Right	Adenomatous n.o.s.	Low	2	+
							8.2	Right	Tubular adenoma	Low	5	+
							8.3	Right	Adenomatous n.o.s.	Low	3	+
9	F	No	1	42	0	0	9.1	Colon n.o.s.	Tubular adenoma	Low	3	+
10	F	Yes	3	76	5	0	10.1	Colon n.o.s.	Hyperplastic polyp	n.a.	5	+

One *PMS2* variant carrier developed an incident colorectal cancer despite undergoing biennial regular colonoscopic surveillance (table 3). The patient presented with colorectal cancer at age 65, was diagnosed with Lynch syndrome, and had a subsequent incident colorectal cancer at age 75. However, this patient had a record of incomplete colonoscopies due to insufficient bowel cleansing, including the colonoscopy preceding the colorectal cancer. The tumor was immunohistochemically stained for MMR protein expression which revealed absent PMS2 staining, as did the initial tumor.

Table 3. *PMS2* carrier with an incident CRC

Sex	Male
Surveillance scopies	10
Years of surveillance	11
Time since last scopy before incident CRC	2 years
Findings at last scopy/clinical evaluation before incident CRC	No adenomas were removed during colonoscopy. There was poor bowel preparation. One adenoma with low grade dysplasia was removed from the stoma of the patient.
Initial CRC	
Age	65
Location	Rectum
IHC	PMS2-
MSI	MSI-H
Incident CRC	
Age	75
Location	Transverse colon
IHC PMS2	Absent
MSI	NA

CRC: colorectal cancer; MSI: Microsatellite instability; IHC: Immunohistochemistry

DISCUSSION

PMS2-associated Lynch syndrome is characterized by relatively low penetrance of colorectal cancer, both in retrospective cohorts of patients who are not under surveillance, as well as in prospective cohorts where patients receive regular colonoscopies.[10,11,22-24] Our study confirms the very low risk for colorectal cancer in *PMS2* variant carriers who undergo regular colonoscopic surveillance. Recent studies have shown that MMR deficient (MMR-d) colorectal cancer in Lynch syndrome patients may develop not only through the traditional MMR proficient (MMR-p) adenoma-to-colorectal cancer progression pathway, but may also arise from the MMR-d crypt pathway.[25-28] Tumors arising via this latter pathway directly proceed from MMR-d crypt to cancer or can first develop into an MMR-d adenoma before becoming malignant.[26,27] The cancers that develop directly from an MMR-d crypt lack a benign precursor lesion and cannot be prevented by colonoscopies. Clinically, these tumors may appear as incident colorectal cancer (i.e. tumors that develop between protocolized follow-up surveillance colonoscopies).[26] Recent work by our group suggests that the MMR-d crypt pathway may be absent in *PMS2* variant carriers.[29] This finding, combined with previous reports that colorectal cancer in non-PMS2 MMR variant carriers develops through the MMR-d crypt pathway, may explain the low penetrance observed in *PMS2* variant carriers, particularly those under surveillance.[10,11,22,23,27,30] This is in line with our current observation of only one incident cancer. This, combined with normal PMS2 staining in all analyzed adenomas, supports the hypothesis that *PMS2* carriers only develop colorectal cancer through de MMR-p adenoma pathway. In this pathway PMS2 deficiency may occur as a relatively late event in (advanced) adenomas, which could then stimulate the malignant transformation. If we assume that this is the only pathway that occurs in these Lynch syndrome patients, it is conceivable that the most important risk factor for colorectal cancer in *PMS2* variant carriers is actually adenoma formation. Indeed, as the *PMS2* variant carriers included in this study were members of families ascertained by high-risk family cancer clinics, our cohort may have been enriched for adenoma risk factors. The observation that the (advanced) adenoma risk at first colonoscopy in our cohort lies between the Forsberg Lynch cohort (which consists of *MLH1*, *MSH2* and *MSH6* carriers) and their control cohort may well be an illustration of this.[21]

When comparing the cumulative 10-year adenoma risk as reported by Engel *et al.*[18], a higher adenoma risk is seen in our cohort compared to the other MMR genes. Engel et al. hypothesize that *MLH1* carriers mainly develop cancer through the MMR-d crypt pathway, *MSH2* carriers through quick progression of an MMR-d adenoma into

a carcinoma and *MSH6* carriers through the MMR-p adenoma-carcinoma pathway. The retained PMS2 expression in the adenomas and previous published data on somatic mutation patterns in *PMS2* associated colorectal cancers,[31] suggest that the predominant pathway to colorectal cancer in *PMS2* carriers is similar to *MSH6* and involves the MMR-p adenoma-carcinoma pathway. However, it is surprising that a high 10-year risk of adenoma development is identified in our cohort. As suggested before, a possible explanation for the relatively high prevalence of adenomas is enrichment for adenoma risk factors in clinically ascertained *PMS2* families. However, interpretation of the comparison of adenoma risks is complicated by the differences in mean age at first colonoscopy between our cohort (50.6 years) compared to the cohort of Engel et al. where it is 42.7 years for *MLH1,* 44.0 years for *MSH2* and *48.7* years in *MSH6*. When factoring in age, a higher risk of adenoma development was noted with increasing age within our own cohort (data not shown), but additional analyses will have to show how much of the difference between the cohorts can be explained by age.

It is striking that different conclusions are drawn when comparing our cohort to two different studies (*i.e.* a relatively low number of adenomas at first colonoscopy compared to the Lynch families as described by Forsberg *et al.*[21] and a relatively high 10-year adenoma risk as compared to the Lynch syndrome patients as reported by Engel *et al.*[18]). Because both studies apply different analyses methods, at this moment it is not possible to find out whether these differences can be attributed to the different approaches in data analysis.

Future studies should investigate the influence of known adenoma risk factors in *PMS2* families, such as obesity and smoking, as this may be important in further decreasing colorectal cancer risk in *PMS2* variant carriers.[32,33] If indeed colorectal cancer development in *PMS2* variant carriers can mostly be prevented by regular surveillance and polypectomies, we would expect a very low cancer risk in this prospective cohort. Nevertheless, we did observe one incident colorectal cancer in our cohort, a finding that on closer inspection of colonoscopy reports appeared to be related to insufficient bowel preparation in this carrier (table 3), highlighting the need for high quality colonoscopy with good bowel preparation to prevent incident colorectal cancer.[34]

Future studies should include a larger number of both tumors and (advanced) adenomas for immunohistochemical staining. Further studies should also elaborate on molecular analysis of, for example, *APC* and *CTNNB1* variants, as specific variants in these genes can help identify the timing of MMR deficiency, as previously shown in the study by Ahadova et al.[27] and Engel et al.[18] This approach might ultimately provide definitive proof of the late involvement of PMS2 deficiency.

In summary, we confirm that *PMS2* variant carriers undergoing regular surveillance colonoscopies are at very low risk for colorectal cancer. This finding supports previous proposals for a less intensive surveillance protocol in these Lynch patients, for example every 2-3 years, starting at age 35-40 years. Comparison of *PMS2* adenoma risk to the adenoma risk in other MMR gene variant carriers is complicated by differences in cohort characteristics and analyses methods between our study and previous publications and requires further investigation.

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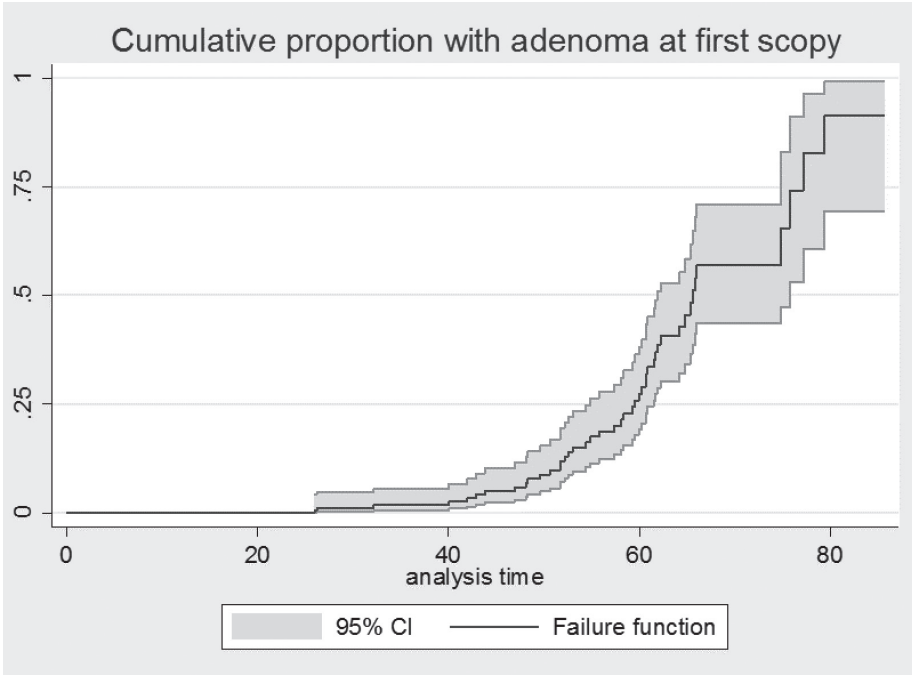
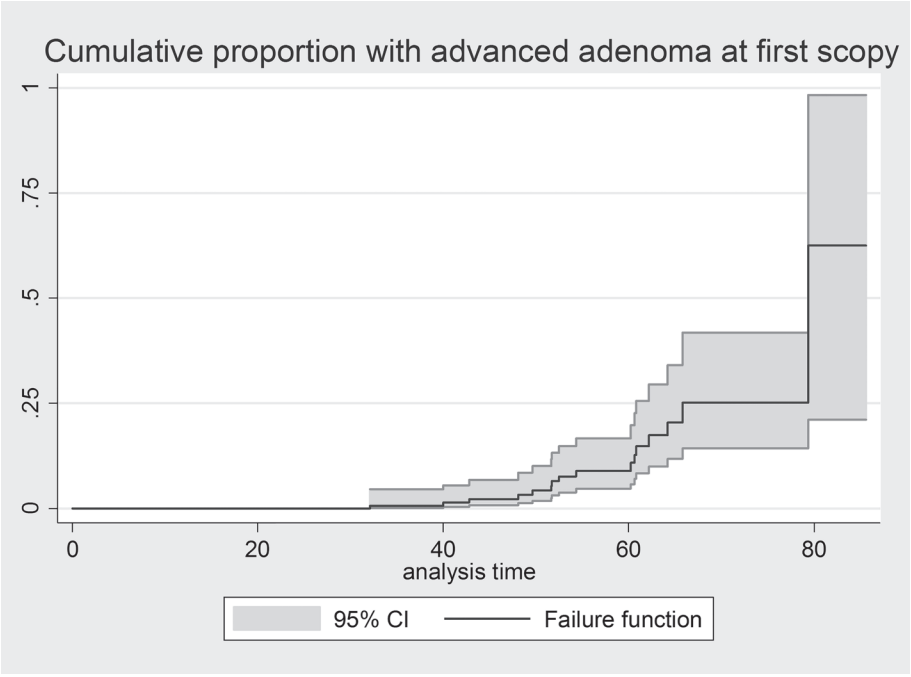
SUPPLEMENTAL INFORMATION



Supplemental Figure 1 Cumulative proportion of *PMS2* carriers with an adenoma at first colonoscopy



Supplemental Figure 2 Cumulative proportion of *PMS2* carriers with an advanced adenoma at first colonoscopy

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]	No of carriers with variant
2	c.137G>T	p.Ser46Ile	missense	4	4
2	c.24-12_107delinsAAAT	p.Ser8Argfs*5	frameshift	5	4
2	c.150delinsAG	p.Ala51Glyfs*3	frameshift	Not present, reported by clinic as pathogenic	1
3	c.219_220dup	p.Gly74Valfs*3	frameshift	5	12
6	c.697C>T	p.Gln233*	nonsense	5	6
7	c.736_741delinsTGTGTGTGAAG	p.Pro246Cysfs*3	frameshift	5	20
intron 7	c.804-60_804-59insJN866832.1		retrotransposal SVA insertion	5	3
8	c.861_864del	p.Arg287Serfs*19	frameshift	5	3
8	c.903G>T	r.804_903del; p.Tyr268*	exonic splice variant	4	2
intron 10	c.1144+2T>A	p.Glu330_ Glu381del	canonical splice variant	4	1
11	c.1831dup	p.Ile611Asnfs*2	frameshift	5	5
11	c.1882C>T	p.Arg628*	nonsense	5	21
13	c.2192_2196del	p.Leu731Cysfs*3	frameshift	5	7
14	c.2404C>T ;	p.Arg802*	nonsense	5	1
14	c.2444C>T	p.Ser815Leu	missense	3 (see supp tbl S2)	1
4	c.325dup	p.Glu109Glyfs*30	frameshift	present, not classified (class 5)	5
8	c.823C>T	p.Gln275*	nonsense	present, not classified (class 5)	4
8	c.856_857del	p.Asp286Glnfs*12	frameshift	present, not classified (class 5)	1
11	c.1214C>A	p.Ser405*	nonsense	present, not classified (class 5)	3
12	c.2117del	p.Lys706Serfs*19	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]	No of carriers with variant
intron 4	c.354-2A>G		canonical splice variant	not present (class 4)	2
11	c.1237_1238delinsT	p.Lys413*	frameshift	not present (class 5)	1
Intron 13	c.2275+1G>A			Not present, ClinVar class 4/5	1
2	genomic deletion including exon 2		large genomic deletion	5	5
10	genomic deletion including exon 10		large genomic deletion	5	1
14	genomic deletion including exon 14		large genomic deletion	5	10
1_15	genomic deletion whole gene (exons 1-15)		large genomic deletion	5	3
11_12	genomic deletion including exons 11-12		large genomic deletion	5	4
11_15	genomic deletion including exons 11-15		large genomic deletion	5	16
3_7	genomic deletion including exons 3-7		large genomic deletion	5	8
5_15	genomic deletion including exons 5-15		large genomic deletion	5	1
5_7	genomic deletion including exons 5-7		large genomic deletion	5	4
1_11	genomic deletion including exons 1-11		large genomic deletion	5	4
2_4	genomic deletion including exons 2-4		large genomic deletion (in frame)	not present (class 4)	4

[a] Variant nomenclature according to HGVS guidelines (http://varnomen.hgvs.org/) with reference to NM_000535.5 for PMS2, except for 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 exons 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.or /criteria/. Nonsense and frameshift mutations, including large genomic deletions, were classified as pathogenic (class 5). Canoni splice variants and large in-frame genomic deletions were classified as likely pathogenic (class 4). Additional evidence that sugg pathogenicity for variants that could not be classified a priori as (likely) pathogenic is provided in supplementary table S2.

Supplementary table 2. Additional evidence that suggests pathogenicity for one PMS2 variants

location	PMS2 variant[a]	type of variant	number of families (this study)	Evidence suggestive for pathogenicity[b]	
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)	2

[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 predictions (PolyPhen-2, SIFT, aGVGD, MutationTaster), presence in control 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

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