PMS2-associated Lynch syndrome : the odd one out

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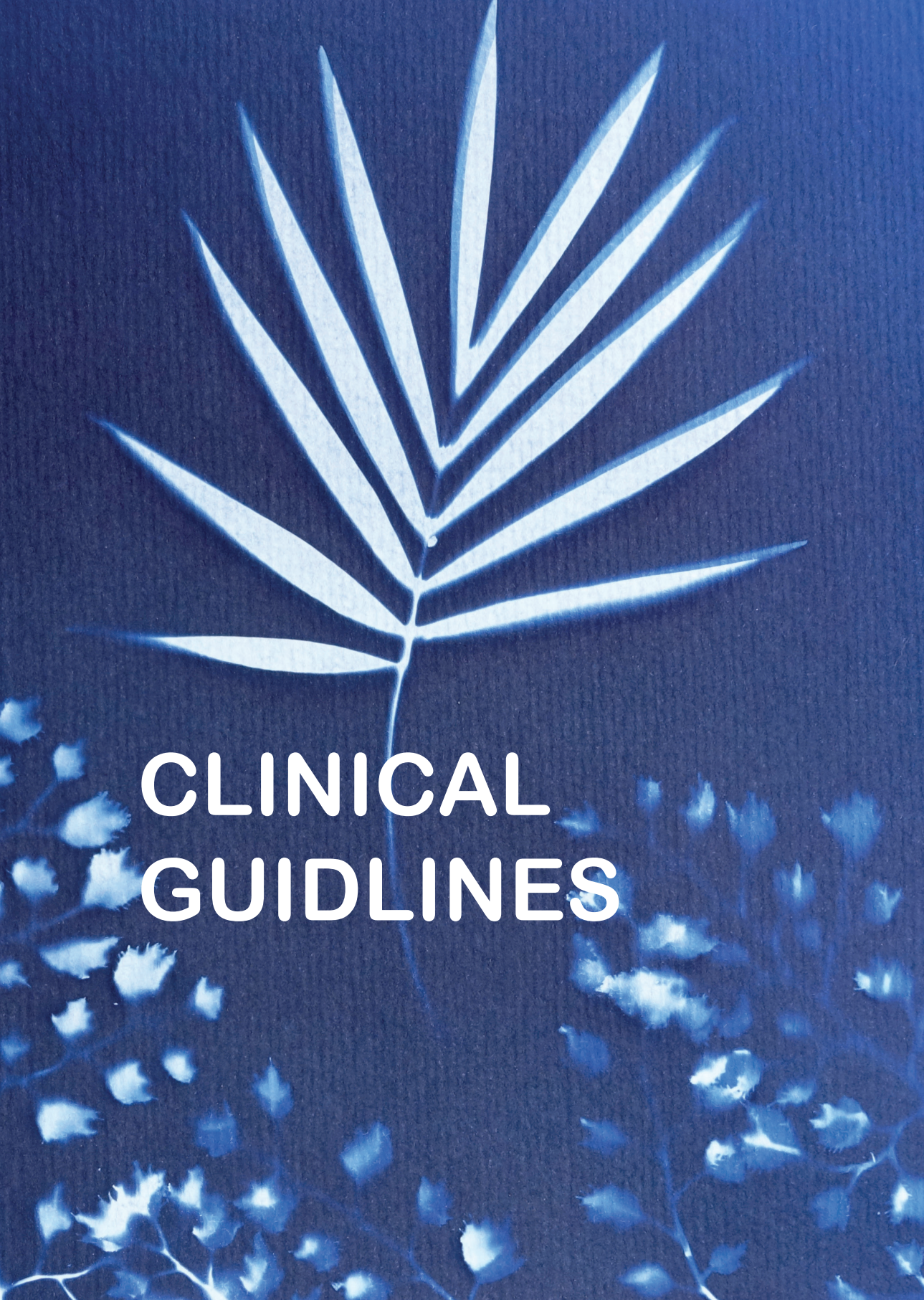
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Incidence of polyps and post-colonoscopy colorectal cancers in patients with PMS2-associated Lynch syndrome: a prospective cohort analysis

Manuscript in preparation

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ABSTRACT

Purpose

Lynch syndrome predisposes carriers of a heterozygous pathogenic germline variant in the *MLH1*, *MSH2* (*EPCAM*), *MSH6* or *PMS2* genes to the development of mainly colorectal and endometrial cancer. Of the four mismatch repair genes, *PMS2* carries 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 post-colonoscopy colorectal cancers (PCCRCs) in *PMS2* carriers undergoing regular surveillance.

Methods

We collected a cohort of 171 *PMS2* carriers and recorded the occurrence and characteristics of PCCRCs and polyps. 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 (1039 observation years), 435 polyps were removed, of which 237 (54.5%) were adenomatous. Forty-one (16.9%) 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. One PCCRC was reported.

Conclusion

This large cohort of *PMS2* carriers showed a low incidence of advanced adenomas and only one PCCRCs. The latter was preceded by difficult and possibly incomplete colonoscopy. Based on these results, widening of the colonoscopy interval can be considered in patients without risk factors for suboptimal colonoscopic surveillance.

INTRODUCTION

Lynch syndrome predisposes carriers of a germline heterozygous pathogenic variant in one of the mismatch repair genes (*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 staining for the involved MMR protein.[1, 2] In order to prevent the development of colorectal cancer in Lynch syndrome patients, patients are offered surveillance by colonoscopy every 1-2 years, starting at the age of 25.[3] Despite these regular surveillance colonoscopies, post-colonoscopy colorectal cancers (PCCRCs) do occur, particularly in *MLH1* and *MSH2* carriers.[4-7] Data on polyp and PCCRC development 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.[8] *PMS2* carriers display a distinct phenotype, with retrospective cohort studies reporting substantially lower cancer risks than carriers of *MLH1* and *MSH2* variants,[9, 10] which has resulted in discussion on the issue of gene-specific surveillance.[4] This discussion would be greatly assisted by more prospectively collected gene-specific data. The prospective Lynch syndrome database (PLSD) consortium already confirmed low cancer risks associated with variants in the *PMS2* gene.[4, 11, 12] However, these studies did not include data on adenoma incidence, while this may be essential for a better understanding of the role of mismatch repair deficiency in Lynch syndrome associated carcinogenesis. To this aim, we collected prospective data on a large cohort of *PMS2* 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.[13] 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 fifteen *PMS2* carriers the PALGA search and request for colonoscopy reports came back with no results,

these patients most likely are not undergoing regular surveillance and they were therefore excluded from the analyses.

PMS2 variant analysis

Our cohort consisted of clinically ascertained families in which variant analysis was initiated due to (histological) pre-screening by immunohistochemistry and/or microsatellite instability, usually because a family met the Bethesda criteria.[14] Germline *PMS2* variant screening was performed as previously described.[10, 15] Comprehensive strategies were applied to avoid unreliable variant detection caused by interference from pseudogene sequences and frequent gene conversion events.[15] 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. Advanced adenomas were defined by a size of ≥1 cm in diameter, a villous component, and/or the presence of high-grade dysplasia.

Results were compared to data from a study by Forsberg et al, in which colonoscopy findings in MLH1-, MSH2-, and MSH6-associated Lynch syndrome patients were compared to control data from an earlier prospective population-based colonoscopy study by the same group.[16]

RESULTS

A description of the cohort is provided in table 1. Between 1987 and 2017 (median 2012), a total of 677 colonoscopies were performed in this cohort of 171 *PMS2* carriers, representing 1039 years of follow-up. 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, 15, 17]

TABLE 1 Description of the cohort

Patients	171
Men	69 (40.4%)
Follow-up (years)	
Total	1039
Mean (s.d.)	9.6 (6.3)
Median (IQR)	8.4 (4.4-14.3)
Range	0-25
Colonoscopies	
Total	675
Number per patient	
Mean (s.d.)	3.9 (3.0)
Median (IQR)	3 (2-5)
Range	1-18
Time interval (years)	
Mean (s.d.)	2.1 (1.9)
Median (IQR)	1.9 (1.1-2.2)
Range	0.02-22.5

IQR: Interquartile range; s.d.: Standard deviation

TABLE 2 Characteristics of polyps

	PMS2 cohort	MLH1/MSH2/MSH6 (Forsberg et al)	Control cohort (Forsberg et al)
Patients	171	138	745
Mean age first colonoscopy (s.d.)	50.6 (12.9)	43,8	51,1
Mean age first adenoma detected (s.d.)	55.3 (12.5)	47,2	59,7
Mean age first advanced adenoma detected (s.d.)	56.8 (13.1)	50,8	62
Total polyps	436	223	474
Hyperplastic polyps	181 (41.6%)	110 (49%)	359 (76%)
Location			
Right-sided	52 (28.7%)		
Left sided	111 (61.3%)		
Not specified	18 (9.9%)		
Sessile serrated polyps/ adenomas*	16 (3.7%)	NA	NA
Location left-sided			
Right-sided	8 (50%)		
Left sided	8 (50%)		
Not specified	0		
Mixed	1 (0.2%)	NA	NA
Adenomas	237 (54.5%)	113 (51%)	115 (24%)
Histology			
Tubular adenoma	154 (65%)	93 (82%)	95 (83%)
Tubulovillous adenoma	23 (9.7%)	14 (12%)	15 (13%)
Villous adenoma	1 (0.4%)		
Sessile serrated adenoma with dysplasia	12 (5.1%)	6 (6%)	5 (4%)
Adenoma n.o.s.	47 (19.8%)		
Size (mm)			
0-4	134 (56.5%)	69 (61%)	76 (66%)
5-10	50 (21.1%)	24 (21%)	31 (27%)
10<	21 (8.9%)	9 (8%)	8 (7%)

TABLE 2 Characteristics of polyps

	PMS2 cohort	MLH1/MSH2/MSH6 (Forsberg et al)	Control cohort (Forsberg et al)
Not specified	32 (13.5%)		
Location			
Right-sided	92 (38.8%)	53 (47%)	39 (34%)
Left sided	120 (50.6%)	56 (49%)	73 (63%)
Not specified	25 (10.6%)	4 (4%)	3 (3%)
Dysplasia			
None	1 (0.4%)		
High grade	6 (2.5%)	13 (12%)	8 (7%)
Low grade	222 (93.7%)	91 (80%)	107 (93%)
Not specified	8 (3.4%)	9 (8%)	
Advanced	41 (16.9%)	27 (24%)	22 (19%)

n.o.s. = not otherwise specified
Advanced: adenomas ≥1 cm in diameter, villous component, and/or high-grade dysplasia
*Sessile serrated adenomas were listed in this category if there was no dysplasia

Polyps

In total, 436 polyps were removed from 171 *PMS2* carriers, the majority of which were adenomatous (54.6%). The most notable difference in *PMS2* carriers compared to Lynch patients carrying other MMR gene variants was the very low frequency of adenomas with high-grade dysplasia (2.5% vs. 12% for the Forsberg Lynch syndrome cohort) and, subsequently a low frequency of advanced adenomas (17.2% vs. 24%). This figure was also slightly lower than that reported in the Forsberg control cohort of average-risk individuals (19%). Mean age at first adenoma detection was 55.3 years (table 2). The proportion of carriers with an adenoma at first colonoscopy is depicted in figure 1 (proportion with advanced adenoma can be found in supplementary figure 1). Figure 2 shows the proportion of *PMS2* carriers free of adenomas as a function of age. The sixteen adenomas with low-grade dysplasia, two sessile serrated lesions and two hyperplastic polyps stained for PMS2 protein expression showed normal staining (table 3).

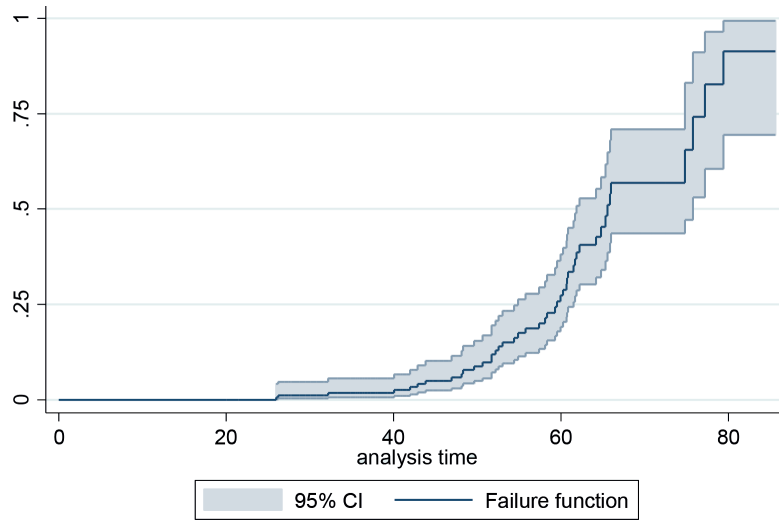


FIGURE 1 Cumulative proportion of PMS2 carriers with an adenoma at first colonoscopy

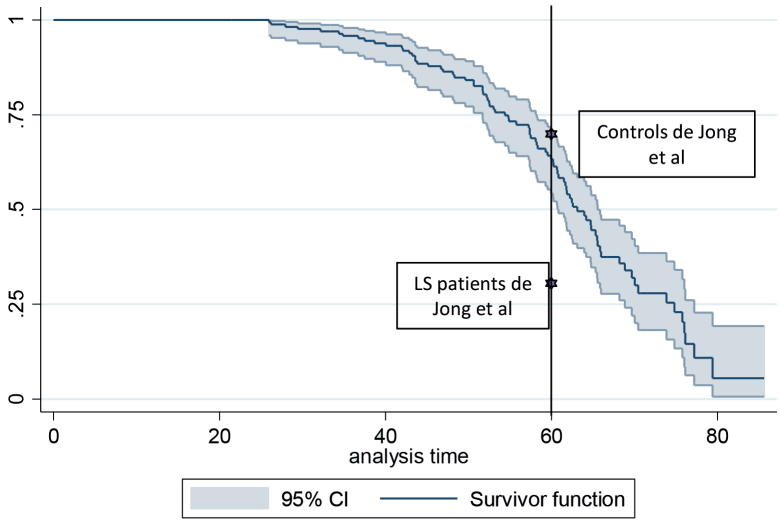


FIGURE 2 Cumulative proportion of PMS2 carriers free from adenomas, compared to the study by de Jong et al [28]

TABLE 3 Polyps stained for PMS2 protein expression

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	+

n.a.: not applicable; n.o.s.: not otherwise specified; CRC: colorectal cancer

TABLE 4 PMS2 carrier with a PCCRC

Sex	Male
Surveillance scopies	10
Years of surveillance	11
Last scopy before interval CRC	2
Initial CRC	
Age	65
Location	Rectum
IHC	PMS2-
MSI	MSI-H
PCCRC	
Age	75
Location	Transverse colon
IHC PMS2	Absent
MSI	NA

CRC: colorectal cancer; PCCRC: Post-colonoscopy colorectal cancer;
MSI: Microsatellite instability;
IHC: Immunohistochemistry

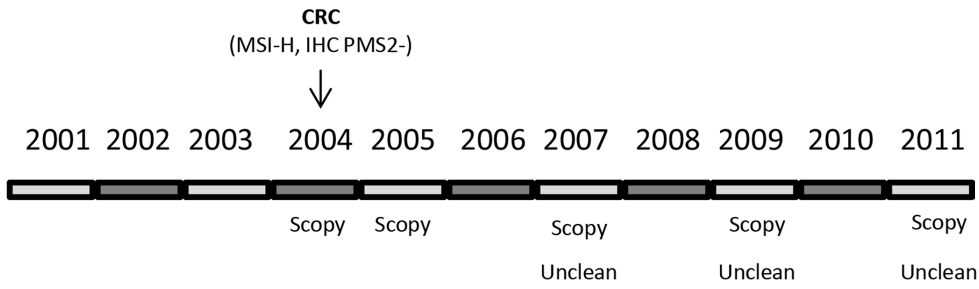
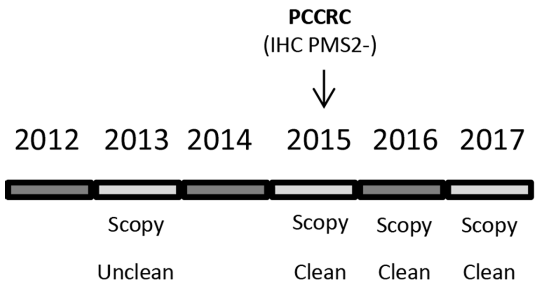


FIGURE 3 Timeline post-colonoscopy colorectal cancer (PCCRC)

Colorectal cancer

One *PMS2* carrier developed a PCCRC despite undergoing biennial regular colonoscopic surveillance (table 4, figure 3). However, this patient had a record of incomplete colonoscopies due to insufficient colon preparation. This patient presented with colorectal cancer at age 65 and had a subsequent PCCRC at age 75. The tumor was immunohistochemically stained for MMR protein expression which revealed absent PMS2 staining, as did the initial tumor.



DISCUSSION

PMS2-associated Lynch syndrome is characterized by relatively low penetrance of colorectal cancer.[10, 18, 19] Our study confirms the very low risk of colorectal cancer development in *PMS2* carriers who undergo regular, complete and good quality colonoscopies and polypectomies. Recent studies have shown that MMR deficient (dMMR) colorectal cancer in Lynch syndrome patients may develop not only through the traditional MMR proficient (pMMR) adenoma-to-colorectal cancer progression pathway, but may also arise from the dMMR crypt pathway.[20-23] Tumors arising via this latter pathway directly proceed from dMMR crypt to cancer or can first develop into an dMMR adenoma before becoming malignant.[21, 22] Clinically, these tumors may appear as PCCRCs (i.e. colorectal cancers that develop between follow-up surveillance colonoscopies and are detected at the next routine colonoscopy).[21] Because the cancers that develop directly from a dMMR crypt lack a benign precursor lesion they cannot be prevented by colonoscopies. Of note, recent work by our group suggests that the dMMR crypt pathway may be absent in *PMS2* carriers.[24] As it has been suggested that dMMR colorectal cancer only rarely arises from pMMR adenomas, this may explain low penetrance in *PMS2* carriers. [10, 18, 19, 25] In other MMR carriers, colorectal cancer is thought to arise mainly from the dMMR crypt pathway, i.e. from adenomas that are dMMR from the beginning of adenoma formation, or even directly from dMMR crypts.[22, 25]

Previous prospective studies in smaller cohorts than the current study have shown that *PMS2* carriers undergoing regular colonoscopies rarely develop colorectal cancer, further supporting the notion that this subset of Lynch syndrome patients may have distinct characteristics.[26, 27] It also underlines the notion that *PMS2* carriers may only develop colorectal cancer through the pMMR adenoma-to-colorectal cancer 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* carriers is actually adenoma formation. Indeed, as the *PMS2* 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. This is illustrated by the similar proportion of *PMS2* carriers where an adenoma is identified at first colonoscopy when compared to the (non-Lynch) familial cancer cohorts described by Forsberg et al. Notably, in their study the proportion of carriers with adenomas at first colonoscopy is much higher for *MLH1* and *MSH2* carriers. The mean age at first adenoma detection in *PMS2* carriers was 55.3 years

(table 2, figure 1), which lies closer to the Forsberg control cohort (59.7 years) than the Forsberg Lynch cohort (47.2 years). Age at first advanced adenoma detection showed a similar pattern (50.8 years for other MMR carriers, 57.6 years for *PMS2* carriers and 62 years for the Forsberg et al. control cohort. Table 2, supplemental figure 1).[16] Another study by de Jong et al found mean age at first adenoma detection in *MLH1* and *MSH2* carries to be even lower, namely 46±9.7 years.[28] It should be noted though that age at adenoma detection is of course related to age at start of colonoscopic surveillance. For our cohort this was comparable to the Forsberg cohorts but higher than the cohorts described by de Jong et al. However, the proportion of carriers that were free from adenomas at age 60 was drastically lower for the PMS2 cohort when compared to the de Jong et al *MLH1*/*MSH2* carrier cohort, but comparable to the MMR variant negative control cohort by the same group (figure 2). This higher adenoma incidence in other MMR carriers may be explained by additional adenoma formation from dMMR crypts.[16] The lack of PMS2 deficient adenomas in this study also provides further evidence for the relatively late involvement of PMS2 deficiency in cancer development, which may be correlated to the later age at first (advanced) adenoma detection (approximately 7-8 years compared to other MMR carriers).[6, 25] Delayed PMS2 deficiency might also be related to the infrequency of advanced adenomas. A recent study suggested that MMR deficiency is often an early and possibly initiating event in tumorigenesis in Lynch patients carrying *MLH1*, *MSH2* or *MSH6* gene variants,[22] and the authors identified an MMR deficiency in 491/640 adenomas (76.7%). This in clear contrast to our data where none of the 16 stained adenomas showed loss of PMS2 expression, suggesting that PMS2 was not involved in the formation of these adenomas.

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* carriers.[29, 30] If indeed colorectal cancer development in *PMS2* carriers can mostly be prevented by regular polypectomies, then we would expect a very low cancer risk in this prospective cohort. Nevertheless, we did observe one case with a PCCRC, a surprising finding that on closer inspection of colonoscopy reports appeared to be related to frequently insufficient bowel preparation in this carrier(figure 3). This could have complicated early detection of adenoma and/or colorectal cancer formation. This PCCRC did exhibit PMS2 abrogation on immunohistochemistry, suggesting that PMS2 deficiency played a role in tumor progression in this patient. Despite this one case, the risk of developing PCCRC in *PMS2* carriers appears to be low and can probably be prevented by regular surveillance and polypectomy, possibly even at extended intervals (e.g. every 2-3 years) provided that the preceding colonoscopy was complete and of good quality.

A limitation of our study is the lack of good control data on adenoma prevalence in the general population. People with Lynch syndrome start colonoscopic surveillance at a very young age, whereas colonoscopies in the general population are generally performed at later ages and only upon clinical indication. This makes a direct comparison challenging. The only study, to our knowledge, to report adenoma occurrence in an unselected, relatively large and age-stratified cohort is that of Forsberg et al., who performed a prospective colonoscopy study that also reported adenoma prevalence in participants aged below 45 years.[35]

A second limitation of our study was that the adenomas stained for PMS2 expressions were all low-grade dysplastic adenomas (table 3). Future studies should include a larger number of both tumors and advanced adenomas. Further studies should also include molecular analysis of, for example, *APC* and *KRAS* variants, as specific variants in these genes can help identify the timing of MMR deficiency, as previously shown in the same study by Ahadova et al.[22] This approach might ultimately provide definitive proof of the late involvement of PMS2 deficiency.

Finally, the reported adenoma frequency in our cohort may have been an overestimate, as a consequence of the previously mentioned possibility of enrichment for adenoma risk factors in high-risk families. This implies that our findings cannot be easily extrapolated to *PMS2* carriers ascertained from the general population. Indeed, we expect families not selected based on a conspicuous phenotype, i.e. at a very young age and/or a positive family history, to become more numerous due to the universal MMR protein screening now being implemented in many countries.[36] In the Netherlands, for example, all colorectal cancers in patients aged below 70 are now screened by MMR immunohistochemistry. More prospectively gathered population-based data is needed and will form a valuable adjunct to data from traditionally selected clinic-based families.

In summary, we can confirm that *PMS2* carriers undergoing regular surveillance colonoscopies show a low risk of developing colorectal cancer and appear to develop less adenomas than other MMR carriers. These findings support previous proposals for an attenuated surveillance protocol in these Lynch patients, for example every 2-3 years, starting at age 35-40 years.

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REFERENCES

1. Shia J. Immunohistochemistry versus microsatellite instability testing for screening colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome. Part I. The utility of immunohistochemistry. J Mol Diagn 2008;10:293-300.
2. Zhang L. Immunohistochemistry versus microsatellite instability testing for screening colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome. Part II. The utility of microsatellite instability testing. J Mol Diagn 2008;10:301-7.
3. 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 2013;62:812-823.
4. 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.
5. Vasen HF, Abdirahman M, Brohet R, et al. One to 2-year surveillance intervals reduce risk of colorectal cancer in families with Lynch syndrome. Gastroenterology 2010;138:2300-6.
6. Edelstein DL, Axilbund J, Baxter M, et al. Rapid development of colorectal neoplasia in patients with Lynch syndrome. Clin Gastroenterol Hepatol 2011;9:340-3.
7. Engel C, Rahner N, Schulmann K, et al. Efficacy of annual colonoscopic surveillance in individuals with hereditary nonpolyposis colorectal cancer. Clin Gastroenterol Hepatol 2010;8:174-82.
8. Win AK, Jenkins MA, Dowty JG, et al. Prevalence and Penetrance of Major Genes and Polygenes for Colorectal Cancer. Cancer Epidemiol Biomarkers Prev 2017;26:404-412.
9. Senter L, Clendenning M, Sotamaa K, et al. The clinical phenotype of Lynch syndrome due to germ-line PMS2 mutations. Gastroenterology 2008;135:419-28.
10. 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.
11. Moller P, Seppala T, Bernstein I, et al. Incidence of and survival after subsequent cancers in carriers of pathogenic MMR variants with previous cancer: a report from the prospective Lynch syndrome database. Gut 2017;66:1657-1664.

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. Casparie M, Tiebosch AT, Burger G, et al. Pathology databanking and biobanking in The Netherlands, a central role for PALGA, the nationwide histopathology and cytopathology data network and archive. Cell Oncol 2007;29:19-24.
14. 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.
15. 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.
16. Forsberg A, Kjellstrom L, Andreasson A, et al. Colonoscopy findings in high-risk individuals compared to an average-risk control population. Scand J Gastroenterol 2015;50:866-74.
17. Ten Broeke SW, Elsayed FA, Pagan L, et al. SNP association study in PMS2-associated Lynch syndrome. Fam Cancer 2017.
18. 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.
19. Goodenberger ML, Thomas BC, Riegert-Johnson D, et al. PMS2 monoallelic mutation carriers: the known unknown. Genet Med 2016;18:13-9.
20. Kloor M, Huth C, Voigt AY, et al. Prevalence of mismatch repair-deficient crypt foci in Lynch syndrome: a pathological study. Lancet Oncol 2012;13:598-606.
21. Ahadova A, von Knebel Doeberitz M, Bläker H, et al. CTNNB1-mutant colorectal carcinomas with immediate invasive growth: a model of interval cancers in Lynch syndrome. Familial Cancer 2016;15:579-586.
22. Ahadova A, Gallon R, Gebert J, et al. Three molecular pathways model colorectal carcinogenesis in Lynch syndrome. Int J Cancer 2018.
23. Staffa L, Echterdiek F, Nelius N, et al. Mismatch repair-deficient crypt foci in Lynch syndrome--molecular alterations and association with clinical parameters. PLoS One 2015;10:e0121980.
24. Ten Broeke SW, van Bavel TC, Jansen AML, et al. Molecular Background of Colorectal Tumors From Patients with Lynch Syndrome Associated With Germline Variants in PMS2. Gastroenterology 2018.

25. Sekine S, Mori T, Ogawa R, et al. Mismatch repair deficiency commonly precedes adenoma formation in Lynch Syndrome-Associated colorectal tumorigenesis. Mod Pathol 2017;30:1144-1151.
26. 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 2015.
27. 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.
28. De Jong AE, Morreau H, Van Puijenbroek M, et al. The role of mismatch repair gene defects in the development of adenomas in patients with HNPCC. Gastroenterology 2004;126:42-8.
29. Winkels RM, Botma A, Van Duijnhoven FJ, et al. Smoking increases the risk for colorectal adenomas in patients with Lynch syndrome. Gastroenterology 2012;142:241-247.
30. Botma A, Nagengast FM, Braem MG, et al. Body mass index increases risk of colorectal adenomas in men with Lynch syndrome: the GEOLynch cohort study. J.Clin.Oncol. 2010;28:4346-4353.
31. Moon SY, Kim BC, Sohn DK, et al. Predictors for difficult cecal insertion in colonoscopy: The impact of obesity indices. World J Gastroenterol 2017;23:2346-2354.
32. Sharara AI, Harb AH, Sarkis FS, et al. Body mass index and quality of bowel preparation: Real life vs. clinical trials. Arab J Gastroenterol 2016;17:11-6.
33. Borg BB, Gupta NK, Zuckerman GR, et al. Impact of obesity on bowel preparation for colonoscopy. Clin Gastroenterol Hepatol 2009;7:670-5.
34. Campbell PT, Jacobs ET, Ulrich CM, et al. Case-control study of overweight, obesity, and colorectal cancer risk, overall and by tumor microsatellite instability status. J.Natl.Cancer Inst. 2010;102:391-400.
35. Forsberg AM, Kjellstrom L, Agreus L, et al. Prevalence of colonic neoplasia and advanced lesions in the normal population: a prospective population-based colonoscopy study. Scand J Gastroenterol 2012;47:184-90.
36. Vindigni SM, Kaz AM. Universal Screening of Colorectal Cancers for Lynch Syndrome: Challenges and Opportunities. Dig Dis Sci 2016;61:969-76.

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
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

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
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
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.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.

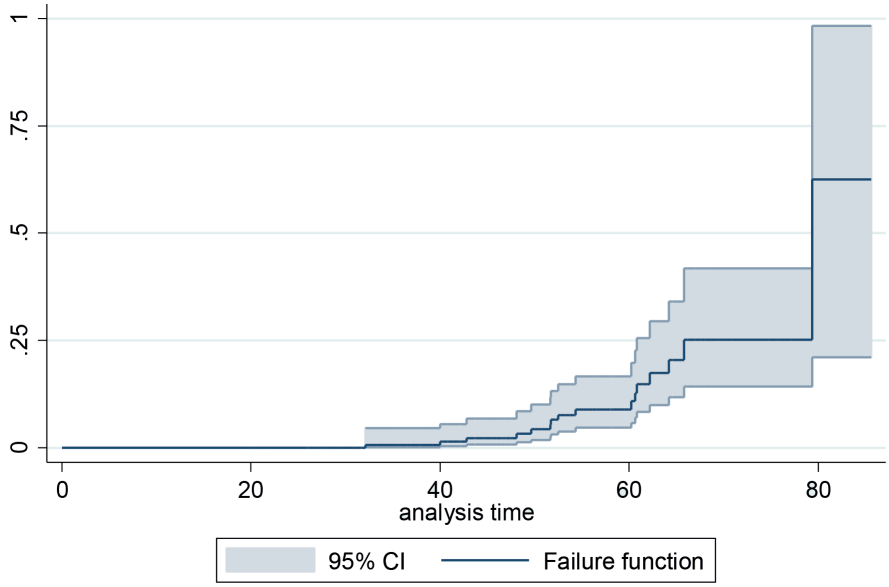
References:

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
Johannesma et al., 2011, Clin Genet 80:243–255
Miyaki et al., 1997
Deschênes et al.,, 2007 Cancer Lett 249(2):148-56
Drost et al., 2013, Hum Mutat 34:1477–1480
van Oers et al., 2010, Proc Natl Acad Sci U S A 107(30):13384-9.
Lagerstedt-Robinson et al., 2016, Oncol Rep 36(5):2823-2835
González-Acosta et al., 2017, Fam Cancer 16(4):501-507
Suerink et al., 2018, Clin Genet 93(1):134-137
Guerrette et al., 1999, J Biol Chem 274(10):6336-41
Gueneau et al., 2013, Nat Struct Mol Biol 20(4):461-8

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



SUPPLEMENTARY FIGURE 1 Cumulative proportion of PMS2 carriers with an advanced adenoma at first colonoscopy