PMS2-associated Lynch syndrome : the odd one out

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Molecular Background of Colorectal Tumors From Patients with Lynch Syndrome Associated With Germline Variants in *PMS2*

Gastroenterology, 2018

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ABSTRACT

Background & Aims

Germline variants in the mismatch repair genes *MLH1*, *MSH2* (*EPCAM*), *MSH6*, or *PMS2* cause Lynch syndrome. Patients with these variants have an increased risk of developing colorectal cancers (CRCs) that differ from sporadic CRCs in genetic and histologic features. It has been a challenge to study CRCs associated with *PMS2* variants (*PMS2*-associated CRCs) because these develop less frequently and in patients of older ages than colorectal tumors with variants in the other mismatch repair genes.

Methods

We analyzed 20 CRCs associated with germline variants in *PMS2*, 22 sporadic CRCs, 18 CRCs with germline variants in *MSH2*, and 24 CRCs from patients with germline variants in *MLH1*. Tumor tissue blocks were collected from Dutch pathology departments in 2017. After extraction of tumor DNA, we used a platform designed to detect approximately 3000 somatic hotspot variants in 55 genes (including *KRAS, APC, CTNNB1*, and *TP53*). Somatic variant frequencies were compared using the Fisher's exact test.

Results

None of the *PMS2*-associated CRCs contained any somatic variants in the catenin beta 1 gene (*CTNNB1*), which encodes β-catenin, whereas 14/24 *MLH1*-associated CRCs (58%) contained variants in *CTNNB1*. Half of PMS2-associated CRCs contained *KRAS* variants, but only 20% of these were in hotspots that encoded G12D or G13D. These hotspot variants occurred more frequently in CRCs associated with variants in *MLH1* (37.5%, *P*=.44) and *MSH2* (and 71.4%, *P*=.035) than with variants in *PMS2*.

Conclusions

In a genetic analysis of 84 colorectal tumors, we found tumors from patients with *PMS2*-associated Lynch syndrome to be distinct from colorectal tumors associated with defects in other mismatch repair genes. This might account for differences in development and less frequent occurrence.

INTRODUCTION

Patients carrying a germline variant in one of the mismatch repair (MMR) genes *MLH1*, *MSH2 (EPCAM)*, *MSH6*, or *PMS2* have Lynch syndrome. These patients have a strongly increased risk of developing colorectal cancer (CRC) and endometrial cancer (EC). In contrast to sporadic tumors, which usually show chromosomal instability, MMR-deficient tumors are characterized by microsatellite instability (MSI) and a high mutational burden. MMR-deficient tumors show different treatment responses and often better patient survival.[1-3] Previous studies also showed notable differences in mutational spectrum between sporadic and Lynch-associated MSI-H tumors, exemplified by a higher degree of activation of the Wnt signaling pathway.[4]

Studies of Lynch-related tumors to date have mainly focused on *MLH1*, *MSH2* and *MSH6*-deficient tumors. Data on tumors associated with *PMS2* variants (*PMS2*-associated) tumors are still relatively sparse, probably due to challenging *PMS2* variant detection.[5] The recognition of *PMS2*-related families can also be challenging, as *PMS2* carriers develop CRCs less frequently and at a later age compared to other MMR variant carriers.[6, 7] One explanation for lower penetrance in *PMS2*-associated Lynch syndrome is that the functional binding partner of PMS2, MLH1, is able to form an alternative heterodimer with MLH3 or PMS1. This alternative heterodimer may then partly rescue MMR function in the absence of PMS2. This redundancy might not only manifest in the clinical phenotype, but might also result in distinct features of these carcinomas. Therefore, our goal was to define the molecular hallmarks of PMS2-associated CRCs compared to other Lynch-associated and sporadic carcinomas.

METHODS

Patients and samples

The cohort consisted of 24 CRCs from Dutch confirmed heterozygous pathogenic *PMS2* variant carriers who had given prior informed consent for use of clinical data and tissue samples. Tissue blocks were retrieved from Dutch pathology centers in 2017. We also collected tissue blocks from *MLH1*- and *MSH2*-associated CRCs from confirmed *MLH1* or *MSH2* carriers that had been previously analyzed for MMR deficiency status at Leiden University Medical Center (n=24 and n=18 respectively). In addition, we retrieved somatic variant data from a cohort of sporadic CRCs which were not suspected of Lynch syndrome (n=22) and were analyzed in the context of routine diagnostics (mainly to assess therapy options) using the same panel as this study, also in the Leiden University Medical Center.

Sample preparation

Tissue samples were extracted from tissue blocks by taking a minimum of three tissue cores of variable length (0.3 mm diameter, or 0.7 mm in case of tissue with a low cell count). Tumor DNA was isolated by use of the Tissue Preparation System with VERSANT Tissue Preparation Reagents (Siemens Healthcare Diagnostics, Tarrytown, NY), as previously described.[8] Briefly, nucleic acid was extracted from paraffin-embedded tissue by use of heat lysis in FFPE buffer and subsequent enzymatic lysis with Proteinase K. The lysate was incubated with silica-coated iron oxide beads, which binds nucleic acid, and subjected to magnetic separation for purification. After being transferred to a new tube, the lysate was again incubated with beads, washed three times and ultimately the nucleic acid was eluted in elution buffer.

Histological and molecular evaluation

Histological hallmarks were scored using the original report, with missing data on specific Lynch-associated hallmarks supplemented by an experienced pathologist when possible. Somatic variant analysis was performed using custom-designed AmpliSeq sequencing panels (Thermo Fisher). Sequencing libraries were prepared according to the manufacturer's recommendations and sequenced on the Ion Torrent Proton Platform (Thermo Fisher). The used cancer hotspot panel is adapted from Ion AmpliSeq™Cancer Hotspot Panel v2 (composition on request) and is designed to detect variations in 207 amplicons covering 21,820 bases of 50 genes with known cancer associations (~3000 COSMIC variants), including well-known somatically mutated genes such as *KRAS*, *APC* and *TP53*. The samples from the PMS2 cohort, were additionally sequenced with a second AmpliSeq panel (custom) covering the coding regions of *MLH1*, *MSH2*, *MSH6*, *PMS2*, *MUTYH* and the exonuclease domain of *POLD1* and *POLE*. In total 19,904 bases were covered spread over 202 amplicons (composition on request). This second panel was also used to detect a second hit (somatic point variants or loss-of-heterozygosity (LOH)) in these tumors). Results for this panel can be found in Table 1 (second hits in *PMS2*) and Supplementary Figure 3 (analysis of *POLE* variants in three *KRAS* mutated CRCs).

The unaligned bam file generated by the Proton sequencer were mapped against the human reference genome (GRCh37/hg19) using the TMAP 5.0.7 software with default parameters (https://github.com/iontorrent/TS). Subsequently variant calling was done using the Ion Torrent specific caller, Torrent Variant Caller (TVC)-5.0.2, using the recommended Variant Caller Parameter for Cancer Hotspot Panel v2. Variant interpretation was done using Genetic Assistant which assigns Functional Prediction, Conservation scores and Disease associated information to each variant

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TABLE 1 Description of the cohort

Cohort description	PMS2 (n=20)	MLH1 controls (n=24)	MSH2 controls (n=18)	Sporadic controls (n=22)
Gender				
Male	10/20 (50%)	13/24 (54%)	11/18 (61%)	
Unknown	0%	0%		100%
Index	18/20 (90%)	16/23 (70%)	11/18 (61%)	N/A
Unknown		1 (4%)		
Age at CRC				
Median (IQR)	48 (43.5-59.5)	46 (39.5-57)	45.5 (38-58)	65 (61-69)
Mean (s.d.)	50.1 (12.3)	47.3 (10.4)	47.8 (12.0)	63 (8.6)
Range	26-70	30-68	29-67	43-73
T-stage				Not available
Advanced: 3/4	12/20 (60%)	14/22 (64%)	10/14 (71%)	
Missing	0	2 (8%)	4	
N-stage				Not available
1/2	6/20 (30%)	9/22 (41%)	2/13 (15%)	
Missing	0	2 (8%)	5 (28%)	
Location				
Proximal location¥	15/20 (75%)	19/20 (95%)	10/16 (63%)	6/20 (30%)
Cecum	8 (40%)	7 (35%)	5	N/A
Ascending	2 (10%)	3 (15%)	2	N/A
Transverse	5 (25%)	4 (20%)	0	N/A
Descending	3 (15%)	0	0	N/A
Rectosigmoid	2 (10%)	0	5	N/A
Missing	0	4 (17%)	2	2 (9%)
Histological hallmarks				Not available
Poor differentiation	6/20 (30%)	15/24 (68%)	4/11 (36%)	
Lymphocyte infiltration (TILs)		Sparse data**	Sparse data**	
Moderate & marked	5/20 (25%)			
Marked	1/20 (5%)			
Crohn's like infiltrate	9/20 (45%)	Sparse data**	Sparse data**	
Mucinous (>50%)	8/20 (40%)	Sparse data**	Sparse data**	
MSI-H	94% (17/18)	18/21 (86%)	13/13 (100%)	Not available
missing	2	4 (17%)	5 (28%)	
Second hit PMS2 identified?*				
Yes	16/20 (80%)	N/A	N/A	N/A
LOH	9/16 (56%)			

Cohort description	PMS2 (n=20)	MLH1 controls (n=24)			MSH2 controls (n=18)			Sporadic controls (n=22)
CTNNB1 variant detected	0/20 (0%)	14/24 (58%)			1/18 (6%)			0%
[4] c.110C>T (p.Ser37Phe)		2 (8%)			0			
[5] c.121A>G (p.Thr41Ala)		4$ (17%)			0			
[5] c.133T>G (p.Ser45Ala)		1 (4%)			0			
[5] c.133T>C (p.Ser45Pro)		2$$ (8%)			0			
[5] c.134C>T (p.Ser45Phe)		5 (21%)			0			
[4] c.133_135delTCT (p.Ser45del)		0			1 (6%)			
Comparison CTNNB1 variant absent/present	N/A	CTNNB1			CTNNB1			N/A
		Absent	Present	p-value	Absent	Present	p-value	
Index		6/10 (60%)	10/13 (77%)	0.337	0/1 (0%)	11/17 (65%)	N/A	
Male		5/10 (50%)	8/14 (57%)	0.527	0/1 (0%)	11/17 (65%)	N/A	
Age at CRC (median, IQR)		46.5 (35-51)	46 (42-58)	0.66	67	45 (38-57)	N/A	
Proximal location		8/9 (89%)	11/11 (100%)	0.45	1/1 (100%)	9/15 (60%)	N/A	
Advanced T stage		6/9 (67%)	8/13 (62%)	0.584	1/1 (100%)	9/13 (69%)	N/A	
Lymphnode metastasis		4/9 (44%)	5/13 (39%)	0.561	0/1 (0%)	2/12 (17%)	N/A	
Poor differentiation		5/9 (56%)	10/13 (77%)	0.276	1/1 (100%)	3/10 (30%)	N/A	
MSI-H		6/8 (75%)	12/12 (100%)	0.147	1/1 (100%)	12/12 (100%)	N/A	

N/A: Not applicable
IQR: Interquartile range
* See supplementary table 1 for a more detailed description of the second hit
¥ *Proximal location: cecum, ascending colon, transverse colon. Includes* tumours described as 'right-sided'
$One of these tumours also harbored a class 3 APC variant: c.4709A>G (p.Asp1470Gly)
$$One of these tumours also harbored class 5 APC mutation: c.4393_4394delAG (p.Ser1465Trpfs*3)
**For the control cohorts MLH1 and MSH2 only sparse data available, see supplementary table 1 for a description of available data for each tumour

(http://softgenetics.com/GeneticistAssistant_2.php). Variants were called using an in-house developed pipeline and analyzed using the Geneticist Assistant NGS Interpretative Workbench (version 1.1.8, SoftGenetics, State College, PA). All variants were manually curated and visualized in the integrative genomics viewer (IGV).[9, 10] Variants with low coverage (<100 reads) and/or with an allele frequency of <10% were not considered. Although the latter approach is conservative, it was adopted due to the substantial background noise due to FFPE treatment of the tissue samples, as evinced by a high transition/transversion (TsTv) ratio. Four samples of *PMS2*-associated CRCs, showed a high number of variants and a high TsTv ratio and were excluded from the final analysis. The ratio of these samples were considered to be too high for meaningful interpretation, regardless of the known association MMR deficient cancer and C>T transitions (mutational signature 6).[11]
Proportions were compared using the Fisher's exact test in Stata version 14 (Statacorp). Reported p-values are two-tailed.

RESULTS

Cohort description

A description of the cohort is given in Table 1. The analyzed cohorts were comparable in terms of index status, gender and age at CRC diagnosis. A full description of all available histological and molecular characteristics (including germline MMR and somatic variants) for each analyzed CRC can be found in Supplementary Table 1 and Supplementary Figure 1.

Most frequently mutated genes

The most frequently mutated genes were *TP53*, *KRAS*, *FBXW7*, *CTNNB1*, *APC* and *PIK3CA* (Table 2). The relative overrepresentation of C>T transitions observed matched mutation 'signature 6', previously associated with MMR deficiency (Supplementary Figure 2).[11] PMS2-associated CRCs harbored proportionally more variants in *FBXW7* (p=0.043) and *PIK3CA* (p=0.229) compared to sporadic CRCs, and relatively fewer functional variants in *TP53* (p=0.007) and *APC* (p=0.531).

CTNNB1

CTNNB1 variants were identified in 14/24 (58%) *MLH1*-, 1/18 (6%) *MSH2*-, and in none of *PMS2*-associated CRCs. In the *MLH1* tumors, all *CTNNB1* variants were missense while the *CTNNB1* variant in the *MSH2* tumor was a deletion of three base pairs. All

TABLE 2 Proportion of samples with a variant in the most frequently mutated genes

					p-value (Two-sided Fisher's exact test)			
	Sporadic (n=22)	MLH1 (n=24)	PMS2 (n=20)	MSH2 (n=18)	Overall	Sporadic vs. PMS2	MLH1 vs. PMS2	MSH2 vs. PMS2
TP53	15 (68%)	6 (25%)	5 (25%)	5 (28%)	0.007	0.007	1.00	1.00
KRAS	18 (42%)*	7 (29%)	10 (50%)	7 (39%)	0.564	0.594	0.218	0.532
FBXW7	0 (0%)	4 (17%)	4 (20%)	3 (17%)	0.126	0.043	1.00	1.00
CTNNB1	0 (0%)	14 (58%)	0 (0%)	1 (6%)	<0.001	N/A	<0.001	0.474
APC	9 (41%)	3 (13%)	6 (30%)	6 (33%)	0.158	0.531	0.261	1.00
PIK3CA	2 (9%)	8 (33%)	5 (25%)	7 (39%)	0.124	0.229	0.742	0.489

Note:
Class 3-5 variants were included in the analysis.
*Sporadic tumors underwent routine diagnostic pre-screening for several common hotspot variants (e.g. *KRAS* c.35G>T, *BRAF* c.1799T>A) and when found were excluded from full panel analysis. To prevent skewing of the results for variants in KRAS, sporadic tumors with one of these common variants not entered in the full panel analysis have also been included in the comparison. In total, 18/43 carried a *KRAS* variant.

variants but one targeted a Serine at amino acid location 37 or 45, the remaining variant involved a Threonine located at amino acid location 41 (see Table 1 for a full description of the identified variants). No statistically significant differences in index status, gender, age, tumor location, stadium, lymph node metastasis or MSI were identified when *CTNNB1*-mutated CRCs were compared to non-mutated CRCs (see Table 1). *MLH1* tumors with a *CTNNB1* variant appeared to be more likely poorly differentiated (56% vs. 77%), however this finding was not statistically significant (p=0.276). All *CTNNB1*-mutated tumors were microsatellite instable.

KRAS

KRAS variants were more frequently observed in the *PMS2* cohort (11/20 (55%)) compared to the *MLH1* cohort (7/24 (29%), p=0.218) and – to a lesser extent – to *MSH2*-associated CRC (7/18 (39%), p=0.532). Two hotspot variants in *KRAS* are known to occur frequently in CRC in general, but even more frequently in Lynch syndrome CRCs,

namely G12D (c.35G>A) and G13D (c.38G>A).[12] Notably, these hotspots accounted for only a minority of identified *KRAS* variants in PMS2-associated CRCs (20%). The contribution of these variants appears to be lower when compared to *MLH1* tumors (37.5%, p=0.44) and even more so when compared to *MSH2* tumors (71.4%, p=0.035). Sporadic CRCs harbored hotspot and non-hotspot *KRAS* variants to an equal extent (Figure 1).

FBXW7

PMS2-associated CRCs harbored *FBXW7* variants in 20% (4/20), which was similar to what we found in the *MLH1* (4/24, 17%) and *MSH2* (3/18, 17%) cohorts but markedly higher than in sporadic CRCs (0/22, p=0.043). It is worth mentioning that the most common hotspot mutation c.1993C>T (p.R465C) was the only identified variant in the PMS2 cohort, while this was only the case for 1 in 4 *FBXW7* variants in *MLH1*- and 1 in 3 *MSH2*- associated CRCs.

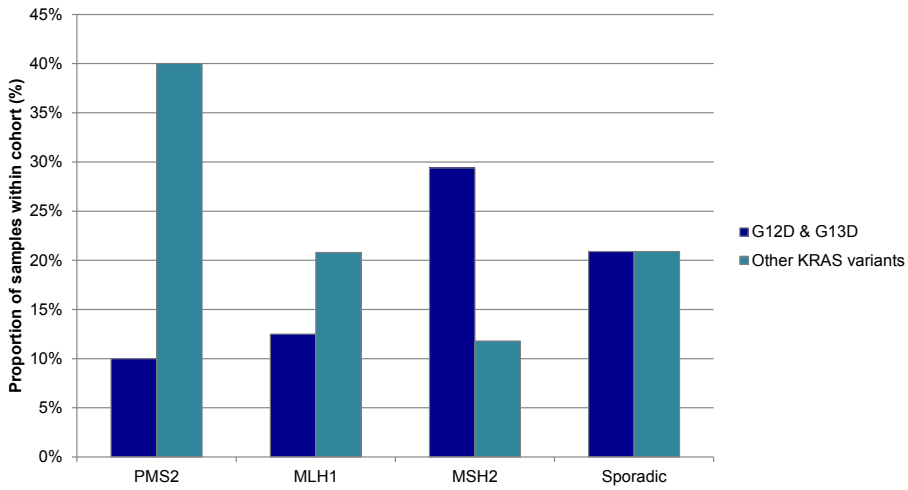

FIGURE 1 Proportion of samples with common hotspot variants G12D and G13D, or other KRAS variants.
Note: p-values represent comparison between PMS2-associated colorectal tumors and MLH1- or MSH2-associated tumors, respectively.

DISCUSSION

This is the first study to investigate molecular hallmarks of *PMS2*-associated CRCs. Overall, the variant spectrum of *MLH1*-, *MSH2*- and *PMS2*-associated CRCs look very similar. The molecular profile of *PMS2*-associated CRC is characterized by *KRAS* and *FBXW7* variants. However, the most notable difference ($p<0.001$) was in the relative frequencies of the catenin beta 1 gene (*CTNNB1*, which encodes β-catenin) variants (all classic exon 3 variants - Table 1), with *CTNNB1* variants entirely absent in all *PMS2* tumors but (very) frequent in *MLH1*-associated CRC (58%, 14/24). β-catenin is involved in the Wnt signaling pathway which plays a key role in colorectal carcinogenesis, exemplified by the high frequency of *APC* variants in CRCs.[13] Briefly summarized, Wnt/β-catenin signaling inhibits the degradation of β-catenin in the cytoplasm, which leads to accumulation of the protein. β-catenin then translocates to the nucleus where it trans-activates target genes involved in cell maintenance, differentiation and proliferation. To prevent over-accumulation of β-catenin, a destruction complex that includes *APC* degrades β-catenin by phosphorylation before it can translocate to the nucleus. A tumor cell can exploit Wnt signaling by disabling both *APC* alleles or through an activating variant in the *CTNNB1* gene. Activating variants in exon 3 of *CTNNB1* are relatively frequent in *MLH1*-associated tumors[4, 14], and the combined previously reported prevalence of 65% for *APC* and *CTNNB1* variants in Lynch-associated CRC[14] is very similar to our *MLH1* cohort (71%), further underlining the remarkable absence of these variants in *PMS2* tumors.

Interestingly, it had been reported that *CTNNB1* variants seem to occur at a later stage of adenoma-to-CRC progression in Lynch syndrome patients, with 18.2% of CRCs harboring *CTNNB1* variants compared to only 1.2% of adenomas.[4] This observation suggests that these variants are not initiating, in contrast to *APC* variants for example, and may therefore play a role in enhancing tumor cell survival, a conclusion also supported by another observation from the same report that *CTNNB1* variants are more prevalent at higher tumor stages (i.e. the majority were found in Dukes' tumor stages C and D).[4] It was also suggested that *CTNNB1* variants may not be a direct consequence of general MMR deficiency per se, because no *CTNNB1* variants were found in a sporadic MSI-H CRC cohort.[14]

A recent paper, by Ahadova et al., suggested that *CTNNB1* variants characterize a distinct type of CRC, with an aggressive and invasive growth pattern, that develops from MMR-deficient (dMMR) crypts instead of through the more commonly accepted adenoma-to-CRC pathway.[15] These tumors are suggested to present as interval CRCs, i.e. tumors that cannot be prevented by colonoscopies due to absence of a benign

precursor stage, i.e. adenomas. In the adenoma-to-CRC pathway, the second hit in a germline-mutated MMR gene occurs at a later stage of tumor progression and results in a significantly decreased time to CRC development. Thus, the lack of *CTNNB1* variants in *PMS2*-associated CRCs suggests that the dMMR crypt model might not apply to *PMS2* variant carriers (see Figure 2). This is supported by our detection of relatively frequent deleterious variants in the *APC* gene which are typical for adenoma development and may represent the adenoma-to-CRC progression pathway, although this finding was not statistically significant. Moreover, a recent paper by the same group showed that *KRAS* variants likely occur frequently only after loss of the wildtype MMR allele, which is in line with other work showing that *KRAS* variants are involved in a later stage of tumorigenesis.[12, 16] While the two known hotspot variants in the *KRAS*

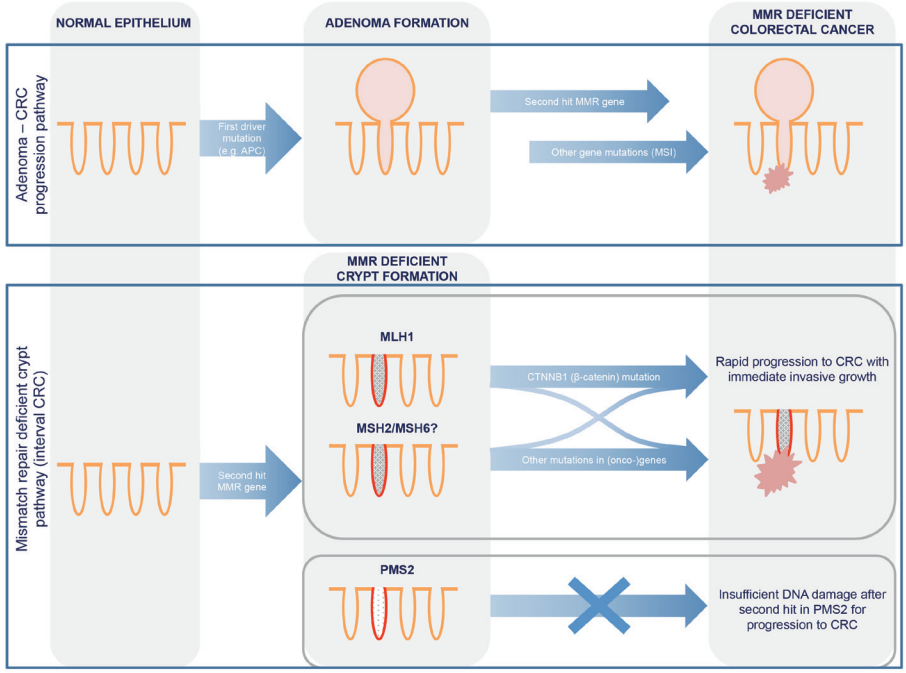

FIGURE 2 Proposed gene-specific development of CRC in Lynch syndrome patients
CRC: colorectal carcinoma. MMR: mismatch repair
Note: Figure inspired by Figure 4 from the paper by Ahadova et al[15]. At this moment it is unsure whether MMR deficient (dMMR) crypts exist in PMS2 carriers. Further studies should elucidate this.

gene, G12D and G13D, occur frequently in CRC overall, they are even more prevalent in MSI-H CRC.[12] The prevalence of these two variants was relatively sparse in the *PMS2* cohort (Figure 1) as compared to the *MLH1* and *MSH2* cohort, which may suggest a later chronological timing of PMS2 deficiency in tumor progression, i.e. the *KRAS* variants in PMS2 deficient tumors may occur before loss of the *PMS2* wildtype allele. Indeed, a recent paper by Alpert et al. may further corroborate the late occurrence of PMS2 deficiency in tumorigenesis as they reported that CRCs showing isolated loss of PMS2 display significantly less histological features associated with MSI, including immune-related hallmarks.[17] Larger studies are needed to more accurately determine timing of PMS2 deficiency in adenoma-to-CRC progression.

The results of our study may (partly) explain clinical observations in PMS2 carriers such as lower penetrance and the absence of surveillance interval CRCs in *PMS2* carriers, observed in prospective cohort studies.[6, 7, 18] Indeed, in contrast to *PMS2* carriers, both *MLH1* and *MSH2* carriers develop such interval CRCs between surveillance colonoscopies, with cumulative risks reported to be up to 46% and 43% to age 75 years, respectively.[18] The high risk of interval CRCs for *MSH2* carriers is interesting in light of the markedly lower prevalence of *CTNNB1* variants observed in our cohort (1/16, 6%) and a previous report (2/27, 7%) when compared to *MLH1*-associated CRCs, which may be explained by their different role in the MMR machinery.[15] In mammalian cells MMR proteins function as heterodimers in two main complexes existing of MutS homologues MSH2 with either MSH6 or MSH3, or MutL homologues MLH1 binding to PMS2, PMS3, or MLH3.[19] *PMS2* and *MLH1* carriers may therefore follow similar tumorigenesis as they function within the same heterodimer, making the difference in *CTNNB1* variant prevalence all the more striking. A possible reason for a difference in carcinogenesis of *MLH1* and *PMS2* carriers may be the ability of the MLH1 protein to form a heterodimer with MLH3 or PMS1 in the absence of PMS2, which means that in the absence of PMS2 some MMR function might still remain.[20] Our finding of low *CTNNB1* variant prevalence in *MSH2*-associated CRC despite observed high interval CRC risk suggests that CRC development from dMMR crypts in *MSH2* carriers not only utilizes *CTNNB1* variants but might require or utilize variants in other (onco-)genes as well (Figure 2). However, this is only speculation at this point and further research is needed to shed more light on the precise tumorigenesis in *PMS2*- and other Lynch syndrome-associated CRCs, as our study is limited by the number of analyzed tumors. A further limitation was the use of a hotspot panel which means that we may have missed Lynch specific somatic variants which could play an essential role in progression from either adenoma or dMMR crypt to CRC. This is especially relevant for the latter pathway as we did not identify specific genes that were mutated more frequently in the

MSH2 cohort. A possible candidate gene that has been proposed in CRC development where MMR deficiency occurs at very early stages is *RNF43*.[21] Subsequent studies should therefore focus on more extensive (onco-)panels or preferentially whole exome sequencing. This may also allow for more thorough analysis of MMR deficiency timing by looking into Lynch syndrome specific variants in for example *KRAS* and *APC*, similar to what has been done in a recent study by Ahadova et al and the preliminary results from our *KRAS* analysis.[12] Lastly, future studies should have a prospective design in which interval carcinomas with aggressive, non-polypous growth patterns could be sequenced and compared to CRCs identified in index carriers. However, based on our data, the existence of such tumors in *PMS2*-associated Lynch syndrome is doubtful.

In conclusion, this study supports the hypothesis that *PMS2* carriers represent a distinct entity among Lynch syndrome patients. If confirmed, the possible absence of CRC formation through the dMMR crypt pathway in *PMS2* carriers could have major implications for surveillance guidelines and might justify a longer colonoscopic surveillance interval in PMS2-associated Lynch patients, for example every 2-3 years. The current biennial surveillance interval of 1-2 years was selected primarily on the basis of data from *MLH1* and *MSH2* patient cohorts and therefore might be too rigorous for PMS2 carriers.[22, 23]

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SUPPLEMENTARY FIGURE 1 Schematic overview of variants in most commonly mutated genes for each tumor sample
N/A: Not available. * Patient ID from supplementary table 1.

PMS2

Patient ID*	4	13	6	14	1	11	9	16	5	7	17	8	12	20	18	15	2	3	10	19
TP53																				
KRAS																				
FBXW7																				
CTNNB1																				
APC																				
PIK3CA																				
Tumorstage	3	2	4	2	3	4	4	2	3	3	4	2	2	3	1	3	2	2	1	4

MLH1

Patient ID*	9	19	23	18	3	15	1	21	5	12	22	4	13	20	7	11	10	6	24	2	16	14	8	17
TP53																								
KRAS																								
FBXW7																								
CTNNB1																								
APC																								
PIK3CA																								
Tumorstage	4	2	N/A	3	1	1	3	4	3	3	3	3	4	2	2	2	3	2	N/A	4	1	3	4	3

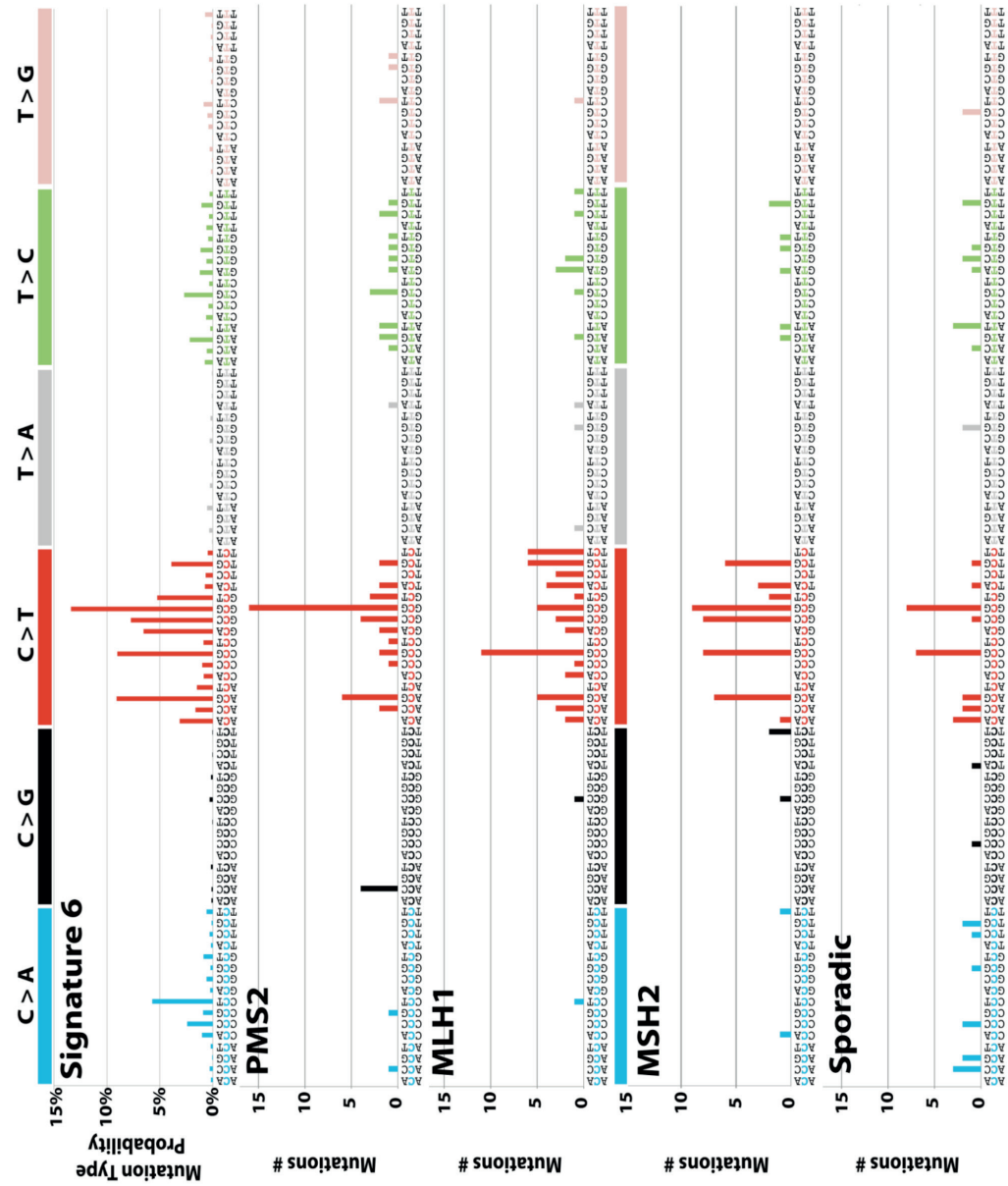
MSH2

Patient ID*	1	11	14	6	3	2	9	10	7	8	5	12	13	17	15	16	18	4
TP53																		
KRAS																		
FBXW7																		
CTNNB1																		
APC																		
PIK3CA																		
Tumorstage	3	2	N/A	3	2	3	3	N/A	3	3	N/A	N/A	3	2	4	3	Intra mucosal	3

Sporadic

TP53																						
KRAS																						
FBXW7																						
CTNNB1																						
APC																						
PIK3CA																						
Tumorstage	N/A	N/A	1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	3	N/A

SUPPLEMENTARY FIGURE 2 Mutational signatures
Note: The PMS2, MLH1, MSH2-associated colorectal cancer cohorts show overrepresentation of C>T transitions, similar to what is found in signature 6 (1), which has been associated with mismatch repair deficient tumors. The PMS2 cohort appears to have relatively few transitions targeting CCG trinucleotides. However, the panel used in this study is not well suited for mutational signature analysis, therefore results should be interpreted carefully.
Reference : (1) Alexandrov, L.B. et al. Nature 500, 415-21 (2013).



SUPPLEMENTARY FIGURE 3 Proportion of samples with specific KRAS mutation
Note: PMS2 associated CRCs frequently display KRAS mutations. Which is important to know for therapy options, e.g. KRAS mutated tumors have reduced sensitivity to anti-EGFR treatments (such as cetuximab).(1) It is worth noting that in 3/20 CRCs the specific KRAS mutation was c.436G>A (p.A146T), which only accounts for 0.6-1.2% of KRAS mutations in sporadic CRCs.(2,3) Strikingly, this mutation was found in a mother and son, as well as in a non-related PMS2 carrier, with different germline PMS2 mutations (c.2192_2196delTAACT (p.Leu731CysfsX3) and c.804-60_804-59ins2kb respectively). This specific KRAS mutation in codon 146 has previously been associated with the POLE associated phenotype. (4) Therefore we hypothesized that these PMS2 carriers might also carry a (germline) variant in POLE. Pathogenic mutations in this gene reside in the exonuclease domain, which was covered by DNA repair panel. However, there was no overlapping POLE variant in the mother and son. Moreover, only the mother had a somatic nonsynonymous POLE variant (44% reads, NM_006231:exon7:c.659A>G, p.D220G), which lies somewhat before the exonuclease domain and has not been previously described. Consequently underlying POLE mutations do not appear to explain the association of PMS2 CRCs with this specific KRAS variant. Although it should be noted that we also identified this KRAS mutation in one of the MLH1 associated CRCs. Hence this could also represent an association of this variant with MMR deficient CRCs in general, as opposed to only PMS2.
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SUPPLEMENTARY TABLE 1 is available online: S.W. ten Broeke et al, Gastroenterology 2018, doi: 10.1053/j.gastro.2018.05.020