Germline variants in the mismatch repair genes: Detection and phenotype

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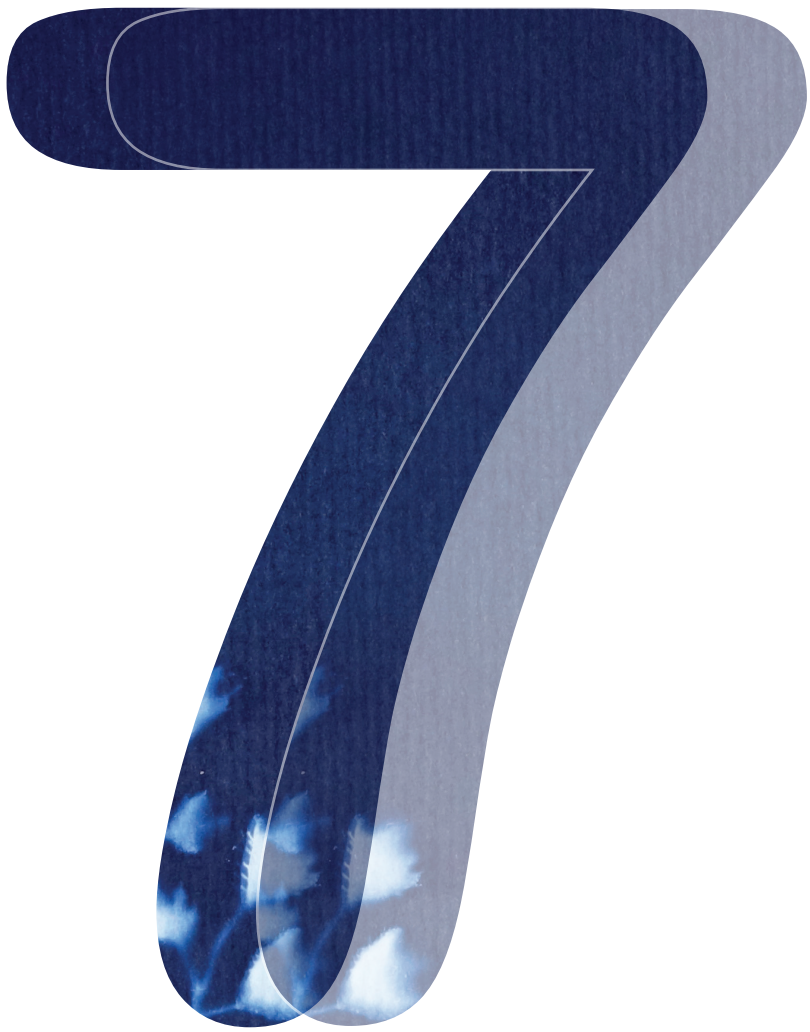

The effect of genotype and parent-of-origin on cancer risk and age of cancer development in PMS2 mutation carriers

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ABSTRACT

Purpose

Lynch syndrome (LS), a heritable disorder with an increased risk of primarily colorectal cancer (CRC) and endometrial cancer (EC), can be caused by mutations in the *PMS2* gene. We wished to establish whether genotype and/or parent-of-origin effects (POE) explain (part of) the reported variability in severity of the phenotype.

Methods

European *PMS2* mutation carriers (n=381) were grouped and compared based on RNA expression and whether the mutation was inherited paternally or maternally.

Results

Mutation carriers with loss of RNA expression (group 1) had a significantly lower age at CRC diagnosis (51.1 years versus 60.0 years, p=0.035) and a lower age at EC diagnosis (55.8 years versus 61.0 years, p=0.2, non-significant) compared to group 2 (retention of RNA expression). Furthermore, group 1 showed slightly higher, but non-significant, hazard ratios (HRs) for both CRC (HR: 1.31, p=0.38) and EC (HR: 1.22, p=0.72). No evidence for a significant parent-of-origin effect was found for either CRC or EC.

Conclusions

PMS2 mutation carriers with retention of RNA expression developed CRC nine years later than those with loss of RNA expression. If confirmed, this finding would justify a delay in surveillance for these cases. Cancer risk was not influenced by a parent-of-origin effect.

INTRODUCTION

A germline mutation in one of the mismatch repair (MMR) genes causes Lynch Syndrome (LS), an autosomal dominant disorder characterized by the clustering of colorectal (CRC) and endometrial cancer (EC) within affected families. Also higher risks have been reported for other cancers such as ovarian and urothelial cell cancer. However, thus far only one study confirmed these risks in *PMS2* mutation carriers.[1]

The MMR proteins normally act together to repair mismatches that occur during cell replication. MSH2 and MSH6 form a heterodimer that recognizes base–base mismatches and insertion/deletion mispairs, whereas MLH1 and PMS2 form a heterodimer that supports initiated repair.[2] A mutation can result in complete loss of protein or a protein with impaired function. Cancer risks associated with *PMS2* are lower than those reported for *MLH1* and *MSH2*.[1,3]

Phenotypes resulting from germ-line MMR gene mutations vary both among and within families.[4] Interfamilial variance might be partly attributable to known genotype–phenotype correlations of MMR genes,[5-7] whereas intrafamilial variance could be due to the influence of parental transmission on penetrance of the disease, a so-called parent-of-origin effect (POE). Although a POE has previously been reported in LS, studies have shown conflicting results.[8,9]

The aims of our study were to investigate genotype–phenotype relationships in *PMS2* mutation carriers and to explore a possible parent-of-origin effect in *PMS2*. Significant results would have implications for the surveillance and management of patients and their families.

MATERIALS AND METHODS

Patients

The study cohort included 381 pre-symptomatic and symptomatic mutation carriers (from 130 families) with a heterozygous *PMS2* mutation, and consisted of 120 apparently unrelated index patients and 261 relatives. Index cases with missing clinical data (n=1) or constitutional mismatch repair deficiency (CMMR-D) (n=9)[10] were excluded from analysis (due to a much younger age-of-onset and a different tumor spectrum compared to heterozygous mutation carriers, CMMR-D is considered a separate syndrome). When available, clinical data from participating clinical genetics departments (the Netherlands, Spain, Norway, Denmark, Sweden and Germany) were used to confirm the diagnosis. DNA analysis of patients and family members was conducted between 2007 and 2013, and in the majority of cases the analysis was

indicated due to compliance with the Bethesda criteria[11] or MSI-testing-indicated-by-a-Pathologist (MIPA) criteria[12]. When applicable, informed consent was obtained according to local approved protocols (LUMC Ethics Review Board, P01.019). Information on inheritance was available for 183 *PMS2* mutation carriers.

PMS2 mutations

The *PMS2* mutations included in this study were detected using a range of mutation detection strategies as applied by the diagnostic laboratories connected to the above-mentioned clinical genetic departments. All laboratories aimed at avoiding interference by pseudogenes by applying different methods, see table S1 for more details. Data on RNA splicing and transcript expression were available for around half of all mutations [13-17] The mutations were therefore classified into three genotype groups:

1. Mutations with an observed reduction in mRNA expression or entirely absent expression in RT-PCR screenings assays
2. Mutations without an effect on mRNA expression
3. Mutations for which no or inconclusive data on RNA analysis were available

PMS2 mutations were described according to Human Genetic Variation Society approved guidelines (http://www.hgvs.org/mutnomen/); see table 1 and table S1 for more details. Group 1 included a missense,c.903G>T (p.Tyr268) and a silent mutation,c.825A>G (p.Ile269Alafs*31) with a known effect on splicing, large genomic out-of-frame deletions, and deletions that involve the start and/or the end of the gene. Group 2 consisted of two missense mutations, 137G>T (p.Ser46Ile) and c.2113G>A (p.Glu705Lys), that were shown to be mismatch-repair deficient in a cell-free functional test.[18] Although no functional assay was available for a third missense mutation, c.2444C>T (p.Ser815Leu), analysis with three in-silico prediction programs classified this variant as deleterious (SIFT; score 0.00), probably damaging (Polyphen-2; score 1.00) and likely to interfere with function: (aGVGD; class C65).

Group 3 consisted of all mutations for which protein expression from the mutated allele was uncertain, including large genomic in-frame deletions, splice variants causing in-frame exon skipping (e.g. exon 10 deletion or skip), splice variants inducing multiple aberrant transcripts of which some are in-frame, and nonsense or frame-shift mutations that escape NMD due to their location in the gene.

Statistics

The Chi-square test and the Cox regression analyses were carried out separately for CRC and EC, using IBM SPSS Statistics 20. A p-value of <0.05 was considered to be statistically significant. Because the majority of group 3 mutations probably result in loss of RNA expression, mutation carriers in groups 1 and 3 were combined and then compared with group 2 in a sensitivity analysis.

For the CRC risk analyses, using the Cox regression method, mutation carriers were considered to be informative from birth until complete or partial colectomy, start of surveillance and/or first polyp detection, last contact or death. In the case of the EC risk analyses, mutation carriers were considered to be informative until hysterectomy, last contact or death. The development of CRC or EC was taken as the end point. Mutation carriers could reasonably be considered informative from birth because a very young age of cancer development would prompt genetic testing, resulting in no young index case being missed. These analyses resulted in hazard ratios comparing the two groups.

Generalized estimating equations (GEE) were used to rule out the possibility that Cox regression results were influenced by the coincidental clustering of family characteristics other than the mutation itself.

RESULTS

In 381 mutation carriers from 130 families, a total of 53 different *PMS2* germline mutations were found, with 248 mutation carriers carrying a mutation that results in loss of RNA expression. The most common mutations were c.736_741delCCCCTinsTC TCTCTGAAG, present in 61 mutation carriers, and c.1882C>T, present in 47 mutation carriers. Together, these two mutations accounted for 28.3% of all mutation carriers (table S1).

Genotype groups

Of the 282 mutation carriers in RNA groups 1 and 2, ninety-six developed CRC. a significant difference (p=0.035) was noted in mean ages of CRC development (51.1 versus 60.0 years) (see table 1). Comparing groups 1 and 2 produced no evidence for a significantly elevated risk of CRC development (HR1.31, p=0.38). Of the 155 women included in the analyses, 27 developed EC, with a slightly lower, but non-significant, mean age of EC development in group 1 compared to group 2 (55.8 years versus 61.0, p=0.2).

Evidence that males have a higher risk of developing an MMR-related CRC prompted us to include gender as a co-variate.[8,11] As expected, males had a higher risk (HR 1.72) of developing CRC than females (p=0.012). No statistically significant differences in EC development were found for the 140 women in the various genotype groups (HR 1.22, p=0.72).

Sensitivity analyses

For this analysis, mutation carriers in group 1 were combined with mutation carriers in group 3. This new and larger group of mutation carriers was then compared to mutation carriers in group 2. The HR for CRC development (HR 1.30, p=0.39) was

Table 1. Description of cohort.

		RNA-group		
		Group 1: Loss of RNA expression N = 248 (% or range)	Group 2: Retained RNA expression N=34 (% or range)	p-value*
Sex	male (%)	97 (39.1)	18 (52.9)	0.20
	Female (%)	142 (57.3)	16 (47.0)	
	Unknown (%)	9 (3.6)	0	
Cancer[#]	CRC (%)	84 (33.9)	12 (29.4)	0.87
	EC (% of females)	23 (16.2)	4 (25.0)	0.40
	other cancer (%)	32 (12.9)	3 (8.8)	0.50
	no cancer (%)	121 (48.7)	18 (52.9)	
Mean age of Cancer	age CRC (age range)	51.1 (25 – 86)	60.0 (43 – 79)	0.035
	age EC (age range)	55.8 (46 – 68)	61.0 (54 – 68)	0.2
Parent-of-Origin	father (%)	54 (21.8)	8 (23.5)	0.96
	mother (%)	64 (25.8)	9 (26.5)	
	unknown (%)	130 (52.4)	17 (50.0)	

CRC=colorectal cancer, EC=endometrial cancer, *Variables were tested for assumed equal distribution using a Pearson-Chi square test.[#] Some individuals had more than one form of cancer.

similar to the HR for just groups 1 and 2. The HR for EC was slightly lower (HR 1.07, p=0.91). Additional analysis using GEE showed no significant differences for the different genotype groups after clustering the data, although male gender remained a significant risk factor for the development of CRC.

Parent-of-origin

The parent of origin was known for 183 of the 381 mutation carriers. Of these, 39 developed CRC and 9 females developed EC. Inheritance of a mutation via either the paternal or maternal line did not significantly influence the mean age of CRC (46.9 versus 45.6 years, p= 0.68) or EC onset (49.2 versus 55.5 years, p=0.23). Cox regression analysis, in both the group as a whole and following separate analysis of males and females, also produced no evidence for a POE. The colorectal cancer HR associated with paternal inheritance of mutations was comparable to that for maternal inheritance (0.80, p=0.51). For endometrial cancer, the hazard ratio was 1.73 (p=0.46, table 2).

Table 2. Hazard ratios for genotype effect (genotype group 1 vs. group 2) and POE paternally vs. maternally inherited mutations)

			HR	Confidence Interval	P-value
Genotype	CRC	All carriers	1.31	0.71 – 2.42	0.38
		Index cases	1.58	0.67 – 3.71	0.30
		c.2444C>T excluded	1.39	0.74 – 2.61	0.31
	EC	All carriers	1.22	0.42 – 3.56	0.72
		Index cases	0.91	0.21 – 4.05	0.91
		c.2444C>T excluded	1.32	0.39 – 4.47	0.65
POE	CRC	All patients	0.80	0.41 – 1.57	0.51
		Males	0.94	0.39 – 2.24	0.89
		Females	0.68	0.24 – 1.97	0.48
	EC		1.73	0.41 – 7.22	0.46

HR = hazard ratio, CRC = colorectal cancer, EC = endometrial cancer,
POE = parent-of-origin effect.

DISCUSSION

Mutation carriers with a *PMS2* mutation that results in the loss of RNA expression develop CRC, on average, nine years earlier than carriers of mutations that do not effect RNA expression. An explanation for this finding could be that, in the latter group, protein with some residual function is still produced. Indeed, while some (functional) protein expression (~25%)[19] and a (limited) repair function was found for the c.2113G>A mutation, functional studies demonstrated severely impaired repair efficiency,[18,19] perhaps suggesting that a severely impaired protein is still superior to no protein at all.

Further support for the notion that mutations leading to retention of mRNA expression result in a milder phenotype comes from the underreporting of this type of mutation. Both in the present study and that of Senter *et al.*, the majority of *PMS2* mutations found in patients result in loss of RNA expression.[3] On a population basis, there is no obvious reason why fewer group 2 mutations would occur compared to group 1 mutations, suggesting that individuals with group 2 mutations may have less severe phenotypes and/or no family history and are therefore less likely to be referred to a clinical geneticist. This idea has been suggested previously by Beck *et al.*[20], who found a relative overabundance of missense *MLH1* and *MSH2* mutations in 10 families which failed to meet the Amsterdam I criteria, compared to families that meet the criteria.

There are a number of shortcomings of our study. For one, the inclusion of affected family members of index patients might have resulted in bias due to the phenotypes of relatives being more similar than phenotypes of unrelated index cases. We attempted to overcome this problem using GEE analysis. We also repeated the analyses for index patients alone, which resulted in similar HR and p-values (Table 2). Another shortcoming was the relatively small number of patients in group 2 (retained RNA expression), reducing statistical power. This may explain non-significant results and indicates the need for analyses in larger patient groups. Also, in this group, one mutation, the c.2444C>T, found in one family, is classified as VUS/Class 3 in the InSight database (link: http://insight-group.org/variants/database/), and larger cohorts are still needed to prove its pathogenicity. When excluding the family (4 relatives) form the analyses similar HR and p-values were found (Table 2). Lastly, two mutations in the cohort are overrepresented in our cohort (namely the c.736_741delinsTGTGTGTGAAG and the c.1882C>T mutation) might dominate the results. When comparing patients with these mutations and patients with other mutations no significant differences in mean age of CRC or CRC risk (HR) were found though.

We were unable to confirm earlier reports that found a significant POE.[8,9] However, a trend was observed towards a lower HR for CRC in females with a paternally-inherited mutation. This is broadly in line with the results of van Vliet *et al*. for the males in their research population, although their results showed a much higher, and significant, HR of 3.2 (p=0.03) for males when comparing maternally-inherited mutations to paternally-inherited mutations.[9]

A possible explanation for the differences in POE findings could be the fact that van Vliet *et al*. used another statistical approach - a modified segregation analyses.[9] We did not use this broad approach because, to the best of our knowledge, no bias or confounders were present in our cohort that would make a modified segregation analysis necessary. The possible exception would be a POE-dependent selection bias; for example, if mutation carriers with a maternally-inherited mutation were more severely affected, more carriers of a maternally-inherited mutation with CRC would be expected in our database. Using a chi square test, we therefore analyzed whether there was a bias in maternal inheritance for mutation carriers with CRC compared to those without CRC. This was not the case (p-value = 0.12).[8,9]

With the ever-wider adoption of whole genome DNA analysis, more families with *PMS2* mutations will be identified in the near future, including some with no apparent history suggestive of Lynch syndrome. Because many of these families may have milder phenotypes, studies such as ours provide useful advice on surveillance programs for these mutation carriers. Should our results be confirmed in larger studies, the significant age differences in CRC development reported here provide some justification for starting surveillance at a later age for mutation carriers who show retention of *PMS2* RNA expression.

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SUPPLEMENTARY INFORMATION

Table S1

Mutation[a,b]	Predicted protein change	RNA group[d]	references RNA analysis[e]	Number of mutation carriers (families)	Frequency (%)
c.736_741delinsTGTGTGTGAAG	p.Pro246Cysfs*3	1	1	61 (25)	15.9
c.1882C>T	p.Arg628*	1	1	47(14)	12.3
deletion exon 11 - 15 (c.1145-1350_ *20545del)	p.?	(1)	na	23(4)	4.7
c.2192_2196del	p.Leu731Cysfs*3	1	1	18(6)	4.4
c.697C>T	p.Gln233*	1	1	13(5)	3.7
c.1831dup	p.Ile611Asnfs*2	(1)	na	10(3)	2.6
deletion exon 1 – 11[c]	p.?	(1)	na	9(1)	2.3
c.823C>T	p.Gln275*	1	2	8(2)	2.1
deletion of the whole gene	p.0	(1)	na	7(3)	1.8
c.1112_1113delinsTTTA	p.Asn371Ilefs*2	(1)	na	5(1)	1.3
c.325dup	p.Glu109Glyfs*30	1	2	5(3)	1.3
c.1079_1080del	p.Ile360Argfs*4	(1)	na	4(1)	1
c.2117delA	p.Lys706SerfsX19	1	2	4(1)	1
c.861_864del	p.Arg287Serfs*19	1	1	4(1)	1
c.903G>T (skips exon 8)	p.Tyr268*	1	3	3(1)	1
c.1145-?_c.2006-?del (deletion exon 11)[c]	p.?	(1)	na	3(1)	0.8
c.2155C>T	p.Gln719*	1	2	3(2)	0.8
c.804-60_804-59insJN866832.1	p.?	1	4	3(2)	0.8
c.1214C>A	p.Ser405*	(1)	na	2(1)	0.5
c.2156delA	p.Gln719Argfs*6	(1)	na	2(1)	0.5
c.354-1G>A	p.?	(1)	na	2(1)	0.5
c.251-2A>C	p.?	(1)	na	2(2)	0.5
c.856_857del	p.Asp286Glnfs*12	(1)	na	1(1)	0.3
c.1261C>T	p.Arg421*	(1)	na	1(1)	0.3
c.211_214delAATG	p.Asn71Aspfs*4	(1)	na	1(1)	0.3
c.658dup	p.Ser220Lysfs*29	(1)	na	1(1)	0.3
c.904_911delGTCTGCAG	p.Val302Thrfs*4	(1)	na	1(1)	0.3
c.989-?_2275+?del (deletion exon 10-13)[c]	p.?	(1)	na	1(1)	0.3
deletion exon 5 - 15[c]	p.?	(1)	na	1(1)	0.3
deletion exon 9 -11[c]	p.?	(1)	na	1(1)	0.3
c.247_250dupTTAA	p.Thr84Ilefs*9	1	2	1(1)	0.3
c.825A>G (first 22 nucleotides exon 8 spliced out)	p.Ile269Alafs*31	1	5	1(1)	0.3
c.137G>T	p.Ser46Ile	2	1	19(8)	5
c.2113G>A	p.Glu705Lys	(2)	na	11(2)	2.9
c.2444C>T	p.Ser815Leu	2	1	4(1)	1
deletion exon 5 – 7[c]	p.?	3	na	18(5)	4.7
deletion exon 14	p.?	3 (no NMD observed)	1	11(3)	2.9
c.219_220dup	p.Gly74Valfs*3	3 (partial NMD observed)	1	10(3)	2.6

Mutation[a,b]	Predicted protein change	RNA group[d]	references RNA analysis[e]	Number of mutation carriers (families)	Frequency (%)
c.24-12_107delinsAAAT	p.Ser8Argfs*5	3 (no NMD observed)	1	9(2)	2.3
c.989-1G>T	p.?	3 (no NMD observed)	6	9(1)	2.3
c.989-2A>G	p.Glu330_Glu381del	3 (no NMD observed)	7	8(1)	2.1
deletion exon 2[c]	p.?	3	na	7(4)	1.8
c.319C>T	p.Arg107Trp	3 (change in ratio alternative transcripts)	2	7(1)	1.8
c.2404C>T	p.Arg802*	3	na	4(2)	1
c.1144+2T>A	p.Glu330_Glu381del	3 (no NMD observed)	1	4(1)	1
deletion exon 10	p.?	3	na	3(2)	0.8
c.2174+1G>A	p.?	3 (multiple transcripts)	2	3(1)	0.8
c.1A>G	p.?	3	na	1(1)	0.3
c.989-296_1144+706del (deletion exon 10)	p.Glu330_Glu381del	3	na	1(1)	0.3
deletion exon 6 - 7	p.?	3 (multiple transcripts)	2	1(1)	0.3
c.163+2T>C	p.Ser8Argfs*5	3 (no NMD observed)	1	1(1)	0.3
deletion exon 3 - 7	p.?	3 (no NMD observed)	1	1(1)	0.3
c.2445+1G>T	p.?	3 (no NMD observed)	2	1(1)	0.3
Total				381 (134[f])	100

[a] Except large genomic deletions, mutations were described according to the Human Genetic Variation Society approved guidelines (http://www.hgvs.org/mutnomen/) with reference to PMS2 GenBank reference sequence NM_000535.5. The large genomic rearrangements, nonsense, frame-shift, and canonical splice site mutations in this study are considered pathogenic or likely pathogenic (class 5 or 4).[1]

[b] To avoid interference of pseudogene sequences using long range PCR, either with cDNA or genomic DNA as template was used for detection of point mutations and small insertions and deletions.[2-5] Mutations were found using different techniques, depending on the involved diagnostic laboratory.

[c] The large deletions were mostly detected using the multiplex ligation-dependent probe amplification (MLPA) kit P008-A1 (MRC-Holland, Amsterdam, the Netherlands). This MLPA kit version lacks (reliable) probes for PMS2 exon 3, 4, 12, 13, 14 and 15. Because the exact extent of these deletions is often not characterized, they are included with an informal description.

[d] 1=no mRNA expression from mutated allele, 2=normal mRNA expression; 3=RNA expression unknown, or mRNA present but with exon(s) skipped

[e] references 1=van der Klift et al 2010[4]; 2=van der Klift, unpublished observations; 3= microattribution Mensenkamp & Ligtenberg in LOVDdb ; 4=van der Klift, 2012[6]; 5=Johannesma et al.2011[7]; 6=Sjursen et al 2009[8]; 7=Borras et al 2013[9]; na=not available

[f] the total number of families in is 134 because four families carry two different segregating mutations.

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