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Recurrent and founder mutations in the *PMS2* gene

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Abstract

Germline mutations in *PMS2* are associated with Lynch syndrome (LS), the most common known cause of hereditary colorectal cancer. Mutation detection in *PMS2* has been difficult due to the presence of several pseudogenes, but a custom-designed long-range PCR strategy now allows adequate mutation detection. Many mutations are unique. However some mutations are observed repeatedly, across individuals not known to be related, due to the mutation being either recurrent, arising multiple times *de novo* at hot spots for mutations, or of founder origin, having occurred once in an ancestor. Previously, we observed 36 distinct mutations in a sample of 61 independently ascertained Caucasian probands of mixed European background with *PMS2* mutations. Eleven of these mutations were detected in more than one individual not known to be related and of these, six were detected more than twice. These six mutations accounted for 31 (51%) ostensibly unrelated probands. Here we performed genotyping and haplotype analysis in four mutations observed in multiple probands and found two (c.137G>T and exon 10 deletion) to be founder mutations, one (c.903G>T) a probable founder, and one (c.1A>G) where founder mutation status could not be evaluated. We discuss possible explanations for the frequent occurrence of founder mutations in *PMS2*.

Keywords

colon cancer; founder mutation; genetic predisposition; *PMS2*

INTRODUCTION

Mutations in at least four mismatch repair genes cause Lynch syndrome (LS), a condition that predisposes to colorectal and endometrial cancer and to a lesser degree to a number of other cancers (1). Even though the proportion of all colorectal cancer caused by LS is a

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modest ~3% (but greater if diagnosis is at young age) there is a need for improved strategies to diagnose LS because clinical surveillance and prophylactic surgery can greatly reduce cancer morbidity and mortality (2–4). Different strategies have been devised to detect as many LS mutation carriers as possible as cost-effectively as possible and in many institutions, it is standard practice to perform immunohistochemistry (IHC) for the mismatch repair proteins as a first step in screening for Lynch syndrome (5–8).

While standard mutation detection methods apply well to *MLH1, MSH2,* and *MSH6*, testing for *PMS2* gene mutations has been problematic due to the presence of numerous pseudogenes. The use of carefully-designed long-range PCR to avoid amplifying the pseudogenes has virtually solved the problem (9–12) so that presently, all four mismatch repair genes can be readily studied for mutations. Deletions in these mismatch repair genes are relatively common; therefore multiplex ligation-dependent probe amplification is also commonly used. Among the four mismatch repair genes, mutations in two (*MLH1* and *MSH2*) cause high lifetime risks (penetrance) and together account for some 60–80% of all LS. Mutations in the other two genes (*MSH6* and *PMS2*) have lower penetrance and each accounts for some 10–20% of all LS (13–18).

This communication deals with mutations in the *PMS2* gene that were observed multiple times. Mutations that are observed in ostensibly unrelated individuals can be either *recurrent* (repeated spontaneous *de novo* occurrence; also known as “hot spot” mutations) or of *founder* type (inherited from a shared ancestor).

Material and Methods

Patients

This study is an extension of a previous study in which 99 probands with colon and/or endometrial cancer who demonstrated isolated absence of tumor staining for PMS2 by IHC were analyzed for *PMS2* mutations (16). In total, 61 of the 99 probands (61%) had deleterious mutations (55 monoallelic; 6 biallelic). Of these, 36 were distinct mutations; 25 occurred in just one proband each, 5 occurred in 2 ostensibly unrelated probands and one (c. 736_741del6ins11), a previously described ancient founder mutation (10), occurred in 12 ostensibly unrelated probands. The present paper describes four of the remaining five mutations which respectively occurred in seven, three, three and three ostensibly unrelated probands each (16). For this analysis, we have included an additional seven, previously unreported probands with these four *PMS2* mutations (total 21 probands with four different mutations). For the fifth mutation, namely the complete gene deletion that occurred in 3 probands in Senter et al. (16) a DNA sample from only one patient was available; therefore this mutation was not studied.

Samples from 21 subjects were studied. All research was conducted under approval of the Institutional Review Board (IRB) at The Ohio State University. Fourteen of these subjects were described previously (16) and five of these previously described subjects were accrued anonymously through research collaborations with the Australian Registry of the National Cancer Institute-funded Colon Cancer Family Registry (19). Anonymized samples from an additional 7 subjects were provided from the ARUP Laboratories (Salt Lake City, UT) (11). All subjects studied here had LS-associated tumors displaying absence of PMS2 protein with retention of MLH1, MSH2 and MSH6 protein. To determine the population frequency of the mutation-associated haplotypes we genotyped 80 control individuals. These samples were randomly drawn from Caucasians belonging to a collection of samples obtained from residents of central Ohio for the purpose of serving as controls for genetic studies.

Exon 10 deletion mutant breakpoint analysis

To determine breakpoints for patients with exon 10 deletions, patient DNA was first amplified by long-range PCR using TaKaRa LA Taq and primers specific for *PMS2*, spanning exon 8 to exon 11. Long-range amplicons were diluted 1:10 and used as template for nested PCR using primers spanning the breakpoint region. Amplicons were then sequenced using BigDye Terminator chemistry on the Applied Biosystems 3730 and compared to NC_000007.13, complement positions 6012870..6048737.

Genotyping

To characterize the haplotypes present in cases and controls we utilized 5 out of 6 microsatellite markers and 7 out of 9 SNPs previously reported (10) that span the *PMS2* locus. The available subjects and 80 controls were typed for these *PMS2* markers.

In order to prevent the amplification of pseudogenes, DNA samples were amplified using a previously described long-range PCR procedure (9, 11). Amplicons spanning exons 1–5 (long-range amplicon LR1) and 7–9 (LR2) were generated using the previously published primers. For the region encompassing exons 11–15, rather than generating two long-range products spanning exons 10–12 and 12–15, we used the forward primer located in exon 10 and the reverse primer located 3’ of exon 15. This generates an 18,341 bp product (LR3) (11). With this design modification, all of the long-range products have at least one primer anchored in an exon not present in any of the pseudogenes.

Using each of the long-range primer sets, 100 ng of DNA were amplified in 25 μl reactions containing 0.2 μM each primer, 1.25 U TaKaRa LA Taq (TaKaRa Bio Inc., Otsu, Shiga, Japan), 1 × LA PCR Buffer II, and 400 μM each dNTP. Cycling consisted of an initial denaturation at 94°C for 1 minute and 30 cycles of 10 seconds at 98°C and 10 or 18 minutes (for LR2 or LR3 respectively) at 68°C. Final elongation entailed 10 minutes at 72°C. The amplification result of long-range PCR was confirmed by gel electrophoresis and diluted (1 in 20) prior to marker-specific amplification.

Microsatellite markers were typed either by direct labeling of a PCR primer or by utilizing a labeled M13 primer in conjunction with an M13-tailed, amplicon-specific primer in a three primer PCR. Each 15 μl PCR reaction contained 7.5 μl AmpliTaq Gold master mix (PE Applied Biosystems, Foster City, CA), 100 ng genomic DNA, 10 pmol untailed primer, 5 pmol M13-tailed primer, and 10 pmol FAM-labeled M13 primer. Reactions were cycled using the following profile: 96°C for 10 min, 36 cycles of 96°C for 30 sec, 58°C for 30 sec, 72°C for 30 sec, and final extension at 72°C for 5 min. The PCR product was sized using an ABI 3730 DNA Analyzer.

For the genotyping of SNPs we used the same PCR conditions as above in the presence of 10 pmol forward and reverse primer with the appropriate long-range PCR product or genomic DNA used as template. The PCR product containing the SNP was subjected to the SNaPshot reaction (PE Applied Biosystems, Foster City, CA). The sequences of the primers used for microsatellite genotyping have been published (10) while sequences for the primers used in the SNaPshot reaction can be obtained upon request.

Haplotype construction

Genotyping data were used to construct haplotypes using the PHASE 2.0 program (20) according to the manual. Haplotypes associated with the mutation are shown.

RESULTS

Genotyping of control samples

We genotyped the controls and used the data to calculate the frequency of the alleles (see Fig. 1). The controls did not have any of the 4 mutations we discuss in this study. Using PHASE we constructed haplotypes in the controls. The haplotypes associated with each mutation were searched for in the controls and the number reported.

c.137G>T

We had access to samples from a total of 10 mutation-positive probands with c.137G>T, 6 from the original series (16) and an additional 4 from the ARUP collection. The results (Fig. 1) show that a disease-associated haplotype comprising some 375kb was shared by all subjects. The haplotype stretches from microsatellite D7S481 upstream of exon 1 to SNP rs1468996 downstream of exon 15. This 375kb shared haplotype was observed in one of 80 control individuals from the central Ohio area. All subjects were Caucasian and while ancestral information was not available for all probands, 3 probands reported ancestry in the United Kingdom and another reported Australian ancestry.

Exon 10 deletion

The exon 10 deletion was found in three unrelated probands from Australia (16). For this study we had access to an additional 2 probands and a mutation-carrying sister of the third proband, all from the ARUP collection. Breakpoints were confirmed (c. 989-296_144+706del) and were identical to breakpoints previously reported in individuals with exon 10 deletions (21, 22). A shared haplotype extending from rs7788441 in intron 7 to microsatellite marker Clen37 downstream of exon 15 was observed in six probands (Fig. 1). The same haplotype occurred in 2 of 80 controls. These data are consistent with a relatively short shared ancestral haplotype. All subjects were Caucasian with unknown ancestral origin.

c.903G>T

The c.903G>T mutation leads to the skipping of exon 8 (16). We studied all 3 probands and detected a shared haplotype spanning from microsatellite marker Clen35 upstream of exon 1 to SNP rs1468996 some 280kb downstream of *PMS2*. This haplotype was seen in 0 of the 80 controls. All subjects were Caucasian and two of three probands reported ancestry from Austria, Hungary, and Germany.

c.1A>G

Of the 3 probands originally detected carrying this mutation, 2 were available for study. A shared haplotype was seen from microsatellite marker Clen35 upstream of exon 1 to D7S2201 some 390kb downstream of *PMS2*. This haplotype was seen in 7/80 controls. Ancestral information is known for only one of the two subjects, being mixed Irish, French, and Native American.

DISCUSSION

The existence of numerous pseudogenes has made it more difficult to search for mutations in *PMS2* than in the other three MMR genes. As a consequence, data on the proportion of all Lynch syndrome that is caused by *PMS2* mutations are scarce. Moreover, the documented low penetrance of *PMS2* mutations relative to the penetrance of the MMR genes *MLH1* and *MSH2* (see below) means that *PMS2* mutations will be underdiagnosed in the clinical setting where mutation analyses typically are applied to individuals displaying the “high

risk" features of strong family history of early onset Lynch syndrome cancers. For these reasons the proportion of LS caused by mutations in *PMS2* can best be estimated by studies in which unselected cases of Lynch syndrome-associated cancers are screened. The data summarized in a large review (7) show *MLH1* and *MSH2* involvement in 32% and 39% respectively while *PMS2* and *MSH6* were reported to be present in 15% and 14% respectively of all Lynch syndrome cases. An additional population-based study of 500 CRC cases (23) disclosed 18 LS probands with a similar distribution of mutations. Thus, as an overall conclusion *MSH2* and *MLH1* account for ~70% of diagnosed LS while *MSH6* and *PMS2* together account for the remaining ~30% (7). From a practical point of view these numbers suggest that to adequately assess the presence of LS all 4 genes must be considered.

This study focuses on those mutations in *PMS2* that occurred repeatedly in a series of 99 probands whose tumors did not stain for PMS2 protein by IHC (16). The subjects emanated from numerous institutions mainly in Northern Europe, North America and Australia, being mostly Caucasians of European origin. It is important to bear in mind that we cannot therefore make inference about other ethnicities or nationalities. Moreover, because the initial series of 99 ostensibly unrelated probands contained at least 24 population-based probands while at least 19 probands were from high risk clinics (exact numbers are not available), there may be a bias in favor of higher rather than lower penetrance mutations if such exist. Nevertheless, with these limitations our series of subjects is by far the largest of its kind and therefore allows at least some tentative conclusions of population relevance.

We show that repeated mutations in *PMS2* are common and whenever feasible to assess, are likely to be of founder nature. Among the 61 probands, 31 carried a mutation seen in at least three probands and one mutation was observed in seven probands. In addition, as shown in Table 1 several of these mutations have been seen and published in patients who were not part of the initial series described in Senter et al. (16). Thus it appears that approximately half of all *PMS2* mutations occur repeatedly in the Caucasian population.

The most common mutation described in Senter et al. (16), c.736_741del6ins11, has been studied in detail previously (10). The second most common mutation described in Senter et al. (16), c.137G>T (Ser46Ile) has the characteristics of a deleterious missense change (24,25). Our data allow us to conclude that this mutation is inherited from a single shared ancestor. The haplotype is short suggesting that the mutation occurred many generations ago, but with the limited number of affected individuals available for study we are not able to estimate the age of the mutation with any degree of precision. Additionally the exon 10 deletion (the second mutation examined in our study) very likely is of a founder type, due to the presence of shared breakpoints and a shared haplotype and the same shared haplotype occurred only in a very small number (2/80) of controls.

The third mutation investigated in this study, namely c.903G>T, that leads to the skipping of exon 8 (16) showed somewhat longer shared haplotype. For this shared haplotype we were unable to determine the upstream start. Since this haplotype was never detected in controls and although we have studied only 3 probands we conclude that this is a probable founder mutation.

The last mutation analyzed (c.1A>G) disrupts the first translation initiation codon leading to 5' truncation of the putative protein. Only 2 samples were available from subjects with this mutation and they shared an even longer haplotype which was present in 7/80 controls. In this case too we were unable to determine the upstream start of the shared haplotype. Thus, while shared ancestry is a distinct possibility, this mutation could also be the one that recurs

frequently *de novo*. We note here that *de novo* mutations are rare in the mismatch repair genes (26).

Founder mutations are not unique to *PMS2*. Founder mutations are well known in *MLH1* (27–31), and *MSH2* (32–34). At least one recurrent "hot spot" mutation is widespread worldwide. This is the intronic *MSH2* c.942+3A>T splice site mutation that apparently arises frequently de novo as a result of meiotic misalignment at a stretch of 26 adenines in the 5' region of intron 5 (35). Are founder or recurrent mutations less common in *MLH1*, *MSH2* and *MSH6* than in *PMS2*? The large multicenter study on *MSH6* by Baglietto et al. (18) identified 74 distinct mutations in a total of 113 probands. Among the 74 mutations, 22 were observed in more than one proband (range = 2 to 6 probands). In total, 29/113 families displayed mutations seen more than twice, as compared to the 31/61 noted by us for *PMS2*. Thus it is possible that the two MMR genes with the lowest penetrance (*PMS2* and *MSH6*) also share the property of having frequent recurrent or founder mutations, but they may be more abundant in *PMS2* than *MSH6*. It is documented that the penetrance of cancer is lower in *PMS2* (lifetime risk of CRC ~20%) than in *MLH1* and *MSH2* (lifetime risk of CRC ~ 40–60%) (Senter et al. (16) and references cited within). Unfortunately, data establishing the proportion of repeated mutations in *MLH1* and *MSH2* are not readily available. We are not aware of publications in which the occurrence of mutations has been determined in large numbers of probands from panmixing (as opposed to isolated; geographically distinct) populations. Nevertheless, population-based studies reviewed in Palomaki et al. (7) list the MMR mutations found in altogether only 82 probands with Lynch syndrome. These data are too few to conclude anything with certainty about the proportion of founder mutations in the two most prevalent MMR genes compared to *PMS2* and *MSH6*. We suggest that further, much larger population-based studies are desirable to shed light on this question.

Founder mutations are believed to become enriched by at least 2 alternative mechanisms. First, if a rare mutation is introduced into an isolated population that subsequently expands without significant influx of genes, it can become enriched simply by genetic drift. (More often however, it can decrease or become extinct from genetic drift.) This mechanism is believed to account for those numerous examples of frequent founder mutations seen in Icelanders, Finns, Ashkenazi Jews, French Canadians, and other typical isolated founder populations. This mechanism does not readily apply to our findings in *PMS2* which are derived from large panmixing Caucasian populations. Another well known cause by which a particular mutation can become enriched occurs when its effect carries an advantage (positive selection). This mechanism is well known e.g. from the hemoglobin gene where heterozygosity for the most common sickle cell anemia mutation confers protection against malaria (36). We are unaware of any evidence about positive selection of mutations in *PMS2*.

In summary, founder mutations appear to be common in *PMS2*. As more *PMS2* mutations are identified through population-based screening of colon and/or endometrial cancers using IHC followed by appropriate germline genetic testing, more *PMS2* mutation carriers are likely to be identified and could provide much more detailed estimates of the prevalence of these mutations. It is possible that if certain mutations are identified in a significant number of patients, standard methodology of *PMS2* mutation detection could be altered by testing for common mutations before sequencing the entire gene.

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c.137G>T

Sample ID	D7S2478		Clen 37		D7S2201		rs1468996		rs2228007		rs12702460		rs7788441		rs7793254		rs12702463		rs6949598		Mut	D7S481		Clen35	
A17*	156	160	180	182	255	259	**G**	A	A	**A**	**T**	T	**G**	A	A	**A**	**C**	C	**G**	G	Yes	**298**	304	277	279
A13*	154	154	180	182	251	255	**G**	G	G	**A**	**T**	T	**G**	A	G	**A**	**C**	G	**G**	A	Yes	**298**	304	261	277
A18*	154	160	182	182	255	257	**G**	G	A	**A**	**T**	T	**G**	A	A	**A**	**C**	C	**G**	G	Yes	**298**	312	277	279
A15*	154	154	172	184	259	259	**G**	G	A	**A**	**T**	T	**G**	A	A	**A**	**C**	C	**G**	G	Yes	**298**	308	263	273
50087-01	154	160	180	182	255	259	**G**	G	A	**A**	**T**	T	**G**	A	G	**A**	**C**	G	**G**	A	Yes	**298**	310	261	275
U01-1574	154	160	180	182	255	259	**G**	A	A	**A**	**T**	T	**G**	A	A	**A**	**C**	C	**G**	G	Yes	**298**	298	261	283
4460-01	154	160	180	192	255	259	**G**	A	A	**A**	**T**	T	**G**	A	A	**A**	**C**	C	**G**	G	Yes	**298**	314	253	275
23922-01	154	160	180	184	255	259	**G**	G	A	**A**	**T**	T	**G**	A	A	**A**	**C**	C	**G**	G	Yes	**298**	298	277	285
A00370	140	154	180	182	255	259	**G**	A	A	**A**	**T**	T	**G**	A	G	**A**	**C**	C	**G**	G	Yes	**298**	298	263	277
A06361	154	160	178	182	255	259	**G**	G	A	**A**	**T**	T	**G**	A	A	**A**	**C**	C	**G**	G	Yes	**298**	310	267	275
							~ 375 kb																		
Control frequencies**							**62%**		**97%**		**90%**		**79%**		**76%**		**89%**		**93%**			**25%**			

Frequency of disease haplotype in controls: 1/80

exon 10 deletion

Sample ID	D7S2478		Clen 37		D7S2201		rs1468996		rs2228007		Mut	rs12702460		rs7788441		rs7793254		rs12702463		rs6949598		D7S481		Clen35	
A19*	154	162	180	**180**	251	**259**	**G**	A	**A**	A	Yes	**T**	T	**G**	G	G	G	C	C	G	A	298	314	277	281
A5*	154	154	180	**180**	259	**259**	**G**	G	**A**	A	Yes	**T**	T	**G**	G	G	G	C	C	G	A	298	314	275	277
sis. Prob. A22*	154	160	180	**180**	255	**259**	**G**	G	**A**	A	Yes	**T**	T	**G**	G	A	A	C	C	G	A	298	314	261	277
A06119	140	162	180	**180**	259	**259**	**G**	A	**A**	A	Yes	**T**	T	**G**	G	G	A	C	G	G	A	306	314	253	277
A05689	154	162	180	**180**	255	**259**	**G**	A	**A**	A	Yes	**T**	T	**G**	G	G	G	C	C	G	G	294	314	267	277
A02656	154	162	177	**180**	251	**259**	**G**	G	**A**	A	Yes	**T**	T	**G**	G	G	A	C	G	G	A	312	314	271	277
			~ 446 kb																						
Control frequencies**			**52%**		**53%**		**62%**		**97%**			**90%**		**79%**												

Frequency of disease haplotype in controls: 2/80

c.903G>T

Sample ID	D7S2478		Clen 37		D7S2201		rs1468996		rs2228007		rs12702460		Mut	rs7788441		rs7793254		rs12702463		rs6949598		D7S481		Clen35	
38422-01	154	154	182	182	259	259	G	**A**	**A**	A	**T**	T	Yes	**G**	G	**A**	A	**C**	C	**G**	G	298	**310**	259	**273**
40102-01	154	154	180	182	251	255	G	**A**	**A**	A	**T**	T	Yes	**G**	G	**A**	A	**C**	C	**G**	G	**310**	312	**273**	277
73781-01	154	154	180	180	251	251	G	**A**	**A**	A	**T**	T	Yes	**G**	A	**A**	A	**C**	C	**G**	G	**310**	312	**273**	285
Control frequencies**							**38%**		**97%**		**90%**			**79%**		**76%**		**89%**		**93%**		**6.9%**		**3.7%**	

Frequency of disease haplotype in controls: 0/80

c.1A>G

Sample ID	D7S2478		Clen 37		D7S2201		rs1468996		rs2228007		rs12702460		rs7788441		rs7793254		rs12702463		rs6949598		Mut	D7S481		Clen35	
42002-01	154	154	180	188	259	**259**	**G**	G	**A**	A	**T**	T	A	**A**	A	**A**	**C**	C	**G**	G	Yes	298	**304**	**261**	279
36727-01	154	154	190	190	255	**259**	**G**	A	**A**	A	**T**	T	G	**A**	G	**A**	**C**	C	**G**	G	Yes	292	**304**	**261**	263
Control frequencies**					**53%**		**62%**		**97%**		**90%**		**19%**		**76%**		**89%**		**93%**			**15%**		**20%**	

Frequency of disease haplotype in controls: 7/80

Fig. 1.

Genotype data spanning the PMS2 locus in probands carrying mutations c.137G>T, exon 10 deletion, c.903G>T or c.1A>G. The alleles associated with the mutation are bolded and the shared haplotypes are represented by the empty bars.

*the samples from the ARUP Laboratories; **frequencies in controls of the bolded alleles.

Table 1

PMS2 mutations found in two or more of 61 probands studied by Senter et al. (16) and number of probands with the same mutations reported in the literature.

Mutation	# Probands		
	Senter et al. (16)	Literature	Total
c.736_741del6ins11 (P246CfsX3) *	12	11,37	16
c.137G>T (S46I)	7	11,37−40	15
Deletion exon 10 (c.989– 296_1144+706del)	3	11,21,22	7
c.903G>T (skips exon 8)	3	-	3
c.1A>G (5' truncation)	3	-	3
Complete gene deletion	3	22,37	5
c.1840A>T (K614X)	2	-	2
c.1831_1832insA (1611NfsX2)	2	11	3
c.2113G>A (E705K)	2	-	2
c.949C>T (Q317X) **	2	-	2
Deletion of exons 5, 6, 7	2	-	2

* (Clendenning et al. (10))

** This mutation occurred monoallelic in one proband and homozygous biallelic in one proband. In the latter case the parents were first cousins and each was heterozygous for the mutation.