Frequency of 3′ Deletions in *PMS2*

Cecily P. Vaughn[1], Jeffrey J. Swensen[1,2], Christine L. Baker[1], and Wade S. Samowitz[1,2]
[1]ARUP Institute for Clinical and Experimental Pathology
and the [2]Department of Pathology, University of Utah Health Sciences Center, Salt Lake City, Utah

ARUP LABORATORIES
An enterprise of the University of Utah and its Department of Pathology

The University of Utah
Department of Pathology

ABSTRACT (updated)

Introduction: Lynch syndrome is characterized by mutations in one of four mismatch repair genes, *MLH1*, *MSH2*, *MSH6*, or *PMS2*. While the detection of mutations in *PMS2* is greatly complicated by numerous pseudogenes, long-range PCR can be employed to specifically amplify the gene and detect sequence changes. For detection of large deletions, multiplex ligation-dependent probe amplification (MLPA) has been employed for exons 1–11. We have recently described an MLPA-based method that avoids *PMS2CL*, a pseudogene with extensive homology to the 3′ end of *PMS2*, and now permits detection of deletions for this region of the gene (exons 12–15) as well. However, the frequency of 3′ deletions of *PMS2* has not yet been determined. **Methods:** Seventy-one samples for which immunohistochemical staining suggested a *PMS2* mutation, but for which no mutation was identified using previously available methods, were evaluated for 3′ deletions in *PMS2* using our new method. This method utilizes MLPA probes for *PMS2* and *PMS2CL* with the selection of appropriate reference samples and sequencing the gene and pseudogene in this region. **Results:** Evaluation of this cohort of samples identified seven samples with deletions in the 3′ region of the gene, including three previously reported samples with deletions in intron 12 – exon 15, exons 13–15, and exons 14–15. Of the additional four samples with deletions, one sample harbored an exon 12–15 deletion, one sample harbored an exon 13 deletion and two samples harbored exon 14 deletions. Overall, 7/71 (9.9%) samples in which *PMS2* mutations had not been identified by existing methodologies harbored deletions in the 3′ region of *PMS2*. **Conclusion:** These results indicate that ~10% of samples suspected of harboring a *PMS2* mutation based on immunohistochemical staining, for which mutations have not yet been identified, may benefit from testing using the new methodology.

INTRODUCTION

Lynch syndrome is caused by mutations in the mismatch repair genes *MLH1*, *MSH2*, *MSH6*, and *PMS2*, which are inherited in an autosomal dominant manner. Immunohistochemical (IHC) staining of tumors can guide genetic testing in patients. Specifically, a loss of PMS2 protein staining without a corresponding loss of MLH1 staining suggests a mutation in *PMS2*. Clinical analysis of the suspected gene to identify mutations utilizes sequencing of exonic regions and deletion/duplication testing.

Until recently, deletion/duplication analysis of *PMS2* was hindered by the presence of the pseudogene *PMS2CL*, which has homology to *PMS2* exons 9 and 11–15. Additionally, *PMS2* and *PMS2CL* have been shown to undergo extensive gene conversion involving exons 12–15, further complicating efforts to analyze deletions in this region of the gene.

We have recently described a new method whereby deletions in *PMS2* exons 12–15 may be detected. This method utilizes a new version of the MRC-Holland PMS2 MLPA kit, paired with sequencing analysis of the gene and pseudogene to verify that detected deletions are indeed located in the gene and not the pseudogene.

Prior to the description of this methodology, clinical testing utilizing MLPA for deletion/duplication analysis did not include analysis of exons 12–15. Thus, samples previously thought to be negative for pathogenic mutations may in fact harbor a deletion in this region of the gene. We sought to establish the percentage of the samples with associated tumors exhibiting isolated loss of PMS2 expression that would benefit by re-analysis utilizing this new method.

MATERIALS AND METHODS

Patient Samples

Patient samples were selected from specimens submitted to ARUP Laboratories for *PMS2* whole gene analysis. All samples had previously been subjected to *PMS2* mutational analysis consisting of long-range PCR followed by exon-specific amplification and Sanger sequence analysis. Clinical testing also included MLPA analysis using the P008-A1 PMS2 MLPA kit (MRC-Holland) to detect deletions in exons 1, 2 and 5–11. The immunohistochemical profile of corresponding tumors for each patient sample was provided by the submitting clinician. All samples whose tumors exhibited isolated loss of PMS2 staining by IHC were included in this study; 129 samples met this criteria. Of these, a germline mutation was not detected using available clinical testing methodologies in 75 samples. DNA was available for 71 of these samples and was subjected to the testing described below. All samples were used with University of Utah IRB approval.

Deletion Analysis for *PMS2* Exons 12–15

Samples were analyzed using the MRC-Holland P008-B1 MLPA kit. This kit includes probes for exons 12–15 that bind both *PMS2* and *PMS2CL*, i.e. four alleles. It also contains probes for both sequences of paralogous sequence variants (PSVs) in intron 12 and exons 13–15 (see Figure 1).

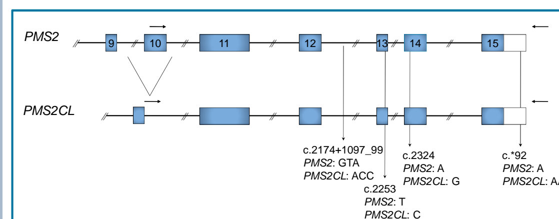

Figure 1. Representation of homologous regions of *PMS2* and *PMS2CL* encompassing exons 9 and 11-15. The locations of paralogous sequence variants (PSVs) under MLPA probes are noted for exons 12-15. Nucleotides corresponding to the reference sequence for *PMS2* and *PMS2CL* are indicated for each PSV. Arrows indicate the location of primers used for long-range amplification. The forward primer for *PMS2* (is located in exon 10, a region of the gene deleted from *PMS2CL*. The forward primer for *PMS2CL* is located over unique sequence spanning the 2.7-kb deletion in *PMS2CL*. A common reverse primer was used for both reactions.

All MLPA runs included four reference samples selected to harbor two copies of each PSV. Since MLPA results are expressed as a ratio of the probe signal in the patient sample versus the corresponding probe signal in the reference samples, selection of specific reference samples allows proper interpretation of ratios calculated for the patient samples. Analysis of MLPA results was performed using SoftGenetics GeneMarker software, version 1.85.

Additionally, sequencing of *PMS2* and the pseudogene *PMS2CL* was performed to verify the location of deletions in patient samples. Briefly, long-range PCR, using a forward primer specific to *PMS2* or *PMS2CL* and a common reverse primer, was employed to amplify the 3′ region of the gene and pseudogene, encompassing exons 11–15 (see Figure 1). Exon-specific amplification and sequencing was performed using the long-range amplicons as templates. Amplicons were sequenced and analyzed in Mutation Surveyor (SoftGenetics).

Determination of whether detected deletions occurred in the gene or pseudogene was achieved by comparing MLPA ratios results for each PSV probe with sequencing results of corresponding exons in the gene and pseudogene.

Breakpoint Analysis

Breakpoints for the three intragenic deletions were determined using primers located outside the breakpoint region of each deletion. Amplified products were analyzed by gel electrophoresis and novel smaller bands (corresponding to the allele harboring the deletion) were cut from the gel and purified using the Promega Wizard SV Gel and PCR Clean-up System. Purified amplicons were subjected to sequencing on the ABI 3730 and analyzed in Mutation Surveyor with comparison to NC_000007.13, 6012870..6048737 complement. Nucleotide numbering is with respect to reference sequence NM_000535.5. Breakpoints for deletions extending beyond the 3′ end of *PMS2* were not determined.

RESULTS

Frequency of 3′ Deletions in Samples Previously Negative for Pathogenic Mutations

Evaluation of the cohort of samples (those with immunohistochemical staining suggestive of a *PMS2* mutation, but for which a *PMS2* mutation had not yet been detected) identified 7 samples harboring 3′ deletions. These included three previously reported samples with deletions in exons 13–15 (2 samples) and exons 14–15 and samples with deletions of exons 12–15, exon 13, and exon 14 (2 samples). Identified deletions are shown in Table 1. Overall, 7/71 (9.9%) samples in which immunohistochemical staining suggested a *PMS2* mutation, but for which no mutation was previously clinically identified, harbored deletions in the 3′ region of *PMS2*.

Table 1. Identified Deletions in *PMS2* Exons 12–15

Sample	Deleted exons	Breakpoints
10	13 - 15	undetermined
15	13	c.2175-226_2276-91
35	14	c.2176-173_2445+1536
40	13 - 15	undetermined
45	14	c.2176-138_2445+1571
54	14 - 15	undetermined
70	12 - 15	undetermined

Description of Breakpoints

Breakpoints for the three samples with intragenic deletions (samples 15, 35 and 45) are listed in Table 1. In all three cases, both the 5′ and 3′ boundaries of the deletions occur in *Alu* sequences, as shown in Figure 2. These breakpoints are consistent with *Alu*-mediated non-allelic homologous recombination. Deletions for the remaining four samples are also illustrated in Figure 2.

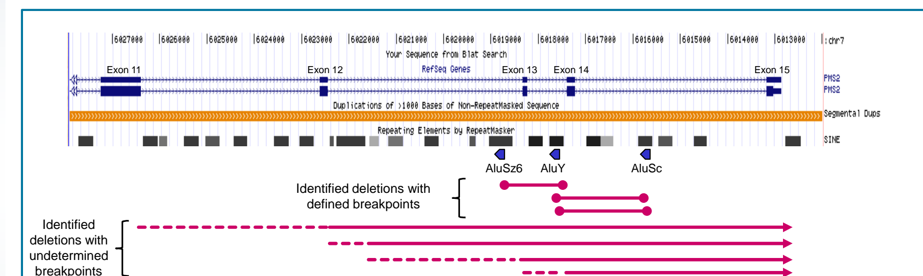

Figure 2. Localization of identified *PMS2* deletion breakpoints. The region including *PMS2* exons 11–15 is shown. These exons are within a segmental duplication found in the pseudogene *PMS2CL*. The SINE (primarily *Alu)* repeats for this region are also shown, with arrowheads denoting the direction of the repetitive element for those *Alu* sequences involved in characterized breakpoints in three of the samples. For the remaining four samples, dotted lines indicate breakpoint regions, as identified by MLPA probes; the 3′ ends of these deletions extend beyond the *PMS2* gene.

Overall Frequency of Pathogenic Mutations and Deletions

Analysis of all samples whose tumors exhibited an absence of PMS2 staining by IHC without a corresponding loss of MLH1 staining, including samples previously described, show that 61/129 (47%) harbored deleterious mutations. Four samples included in the total were not available for re-testing to determine the presence of 3′ deletions. Of the 61 patients with detected mutations, 4 harbored two mutations each, bringing the total number of detected mutations to 65. Twenty-eight (43%) of the detected deletions were due to large deletions involving one or more exons, and of these, 10, including the 7 deletions identified in this study, are due to deletions that include one or more exons known to undergo gene conversion with the pseudogene *PMS2CL*.

CONCLUSIONS

- Approximately 10% of samples suspected of harboring a *PMS2* mutation based on immunohistochemical staining, for which a mutation has not yet been identified, may benefit from testing for 3′ deletions using the new methodology.
- The three intragenic deletions identified in this study are consistent with *Alu*-mediated non-allelic homologous recombination.
- Overall, deletions in *PMS2* comprise 43% of detected mutations in this gene, with 36% of these deletions involving one or more exons known to undergo gene conversion with the pseudogene *PMS2CL*.

REFERENCES

Clendenning M, et al. Long-range PCR facilitates the identification of PMS2-specific mutations. *Hum Mutat* 2006;27:490-5.
Hayward BE, et al. Extensive gene conversion at the PMS2 DNA mismatch repair locus. *Hum Mutat* 2007;28:424-30.
Van der Klift HM, et al. Quantification of sequence exchange events between PMS2 and PMS2CL provides a basis for improved mutation scanning of Lynch syndrome patients. *Hum Mutat* 2010;31:578-87.
Vaughn CP, et al. Clinical analysis of PMS2: mutation detection and avoidance of pseudogenes. *Hum Mutat* 2010;31:588-93.
Vaughn CP, et al. Avoidance of pseudogene interference in the detection of 3′ deletions in *PMS2*. *Hum Mutat* 2011;32:1063-71.