Frequency of 3’ Deletions in PMS2

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MATERIALS AND METHODS

Patient Samples
Patient samples were selected from specimens submitted to ARUP Laboratories for PMS2 genetic analysis. All samples had previously been subjected to PMS2 mutation analysis consisting of long-range PCR followed by exon-specific amplification and Sanger sequencing 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 tissues from 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 with University of Utah IRB approval.

Detection Analysis for PMS2 Exons 12-15
Samples were analyzed using the MRC-Holland P008-B1 MLPA kit. This kit includes PMS2 probes for exons 12–15 that bind both PMS2 and PMS2CL, i.e. four alleles. It also includes 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.

RESULTS

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

Table 1: Identified Deletions in PMS2 Exons 12–15

<table>
<thead>
<tr>
<th>Sample</th>
<th>Deletions</th>
<th>Breakpoints</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>13-15</td>
<td>undetermined</td>
</tr>
<tr>
<td>35</td>
<td>14-15</td>
<td>c.2175-173_2445+1536</td>
</tr>
<tr>
<td>40</td>
<td>13-15</td>
<td>undetermined</td>
</tr>
<tr>
<td>44</td>
<td>13-15</td>
<td>c.2173_130_2445+1571</td>
</tr>
<tr>
<td>54</td>
<td>12-15</td>
<td>undetermined</td>
</tr>
<tr>
<td>70</td>
<td>12-15</td>
<td>undetermined</td>
</tr>
</tbody>
</table>

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.

INTRODUCTION

Lynch syndrome is caused by mutations in the mismatch repair gene 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 ligational-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 in 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 described methodologies 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, 77 (49.2%) samples in which PMS2 mutations had not been identified by existing methodologies harbored deletions in the 3’ region of PMS2. Conclusions: 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.

REFERENCES