Representation of PMS2 mutations in patients seeking genetic testing for Lynch syndrome

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BACKGROUND

Lynch syndrome (also known as Hereditary Nonpolyposis Colon Cancer), is an autosomal dominant genetic syndrome that is responsible for 2-4% of colorectal cancers, as well as other cancers, within the United States.1,2

Lynch Syndrome-Associated Cancers

- Colon Cancer
- Endometrial Cancer
- Ovarian Cancer
- Stomach Cancer
- Small Intestinal Cancer
- Hepatobiliary Tract Cancer
- Urinary/Renal Pelvis Cancer
- Skin Cancer (Sebaceous Tumor)
- Brain Cancer

Disease-associated mutations have been identified in multiple DNA mismatch repair genes. Previous studies indicate that ~70-80% of causative Lynch syndrome mutations occur in the MSH2 and MSH6 genes. MSH6 mutations and EPCAM mutations represent ~15% and ~1.3% of identified mutations, respectively, and ~3-15% of Lynch syndrome-associated mutations occur in the PMS2 gene.3,4

Clinical diagnostic testing, which detects mutations within MSH2, MSH6, and EPCAM, is usually performed using standard sequencing and large rearrangement methodologies. Although PMS2 mutations have been shown to be responsible for a significant proportion (3-15%) of DNA mutations resulting in Lynch syndrome, clinical testing for PMS2 mutations has not become clinically available until recently. Diagnostic analysis is complicated by the presence of multiple highly homologous pseudogenes, which span exons 1-5, 9, and 11-15 of the PMS2 gene.3,4

RESULTS

METHODS

After informed consent was obtained by a healthcare provider, peripheral blood or buccal mouthwash samples were collected by the healthcare provider from patients with a personal and/or family history suggestive of Lynch syndrome or hereditary colon cancer. Genomic DNA was extracted from each sample for subsequent genetic analysis. Comprehensive analysis, which includes concurrent sequencing and large rearrangement analyses for DNA mutations in the MLH1, MSH2, MSH6, PMS2, and EPCAM genes, was performed.

For sequencing analysis, genomic DNA was directly amplified utilizing the Polymerase Chain Reaction (PCR) to generate exon-specific amplicons for MLH1, MSH2, MSH6, EPCAM, and portions of the PMS2 gene. Due to the presence of pseudogenes, long range PCR with a nested PCR approach was utilized for the remaining portions of PMS2, allowing for the detection of sequencing mutations along the entire PMS2 coding region. PCR products were sequenced in the forward and reverse orientations using fluorescent dye-labeled sequencing primers, and resulting chromatograms were compared to a known wild type sequence.

MLH1 and MSH2 large rearrangement analysis (CART) was performed using a quantitative multiplex PCR assay, which detects copy number variations indicative of a deletion or insertion. PMS2, EPCAM, and MSH6 large rearrangement analyses were performed using Multiplex Ligation-dependent Probe Amplification (MLPA) assays. In addition to concurrent genetic analysis of the MLH1, MSH2, MSH6, PMS2, and EPCAM genes, some patients opted for a more targeted gene approach, allowing for identification of additional sequencing and large rearrangement mutations in the PMS2 gene.

Sequencing and large rearrangement analyses of the MLH1, MSH2, MSH6, and EPCAM genes were performed on patient DNA. Approximately 14% of disease-associated mutations identified were found in the PMS2 gene.

RESULTS

Frequency of PMS2 Mutations

Molecular genetic testing of a patient cohort for Lynch syndrome was performed in our clinical diagnostic laboratory. Testing included concurrent sequencing and large rearrangement analyses of the PMS2, MLH1, MSH2, MSH6, and EPCAM genes. 327 disease-associated mutations were identified within this patient population. 44/327 (14%) of the identified mutations were within the PMS2 gene (Figure 2). This observed prevalence of PMS2 mutations is at the high end of previously reported estimates of PMS2 mutation frequency.5,6

In addition to patients receiving comprehensive testing for mutations in PMS2, MLH1, MSH2, MSH6, and EPCAM, some patients received more targeted genetic analysis for mutations in PMS2 alone or PMS2 in combination with one or more genes. These analyses have identified 37 unique sequencing and 14 unique large rearrangement PMS2 mutations (Figure 3). This corresponds to a PMS2 mutation profile of ~73% sequencing versus ~27% large rearrangement mutations.

Analysis of PMS2 Pseudogene Regions

Analysis of the PMS2 gene is complicated by the presence of multiple pseudogenes which span exons 1-5, 9, and 11-15. For sequencing analysis, long range PCR with subsequent nested PCR amplification and sequencing can be utilized to generate coding gene-specific DNA sequences. This allows for sequencing analysis of the entire PMS2 coding region.

Due to the presence of multiple single nucleotide polymorphisms in or near exons 1-5 and 9, it is usually possible to distinguish the PMS2 coding gene from confounding pseudogenes, allowing for MLPA large rearrangement analysis to be more readily performed for exons 1-10. However, the pseudogene spanning exons 11-15 bears strong homology to the coding gene, and gene conversions events are common. Thus, the interpretation of MLPA test results within this region may require additional confirmatory analyses such as long range PCR and sequencing of both coding gene and pseudogene regions of interest. Understanding these complexities, we have identified multiple large rearrangements within PMS2 coding regions known to have interfering pseudogenes (Figure 4).

CONCLUSIONS

- PMS2 gene mutations are responsible for ~14% of Lynch syndrome cases for which causative mutations were identified.
- A PMS2 mutation profile of ~73% sequencing versus ~27% large rearrangement mutations was identified.
- PMS2 pseudogenes increase the complexity of genetic analysis, but sequencing mutations and large rearrangements are readily detected.
- The identification and interpretation of PMS2 large rearrangements within exons 11-15 is complicated by the presence of a highly homologous pseudogene and gene conversion. However, additional analyses can clarify these results.
- Comprehensive diagnostic genetic testing for Lynch syndrome should include sequencing and large rearrangement analyses of the PMS2 gene.

REFERENCES


Figure 1.

Extract DNA from Blood/Buccal Mouthwash Samples

Sequencing and Large Rearrangement Analyses

PMS2 Analyses

MLH1-MSH2, MSH6, & EPCAM Analyses

Percentage of Lynch Syndrome Mutations By Gene

MSH2
MLH1
MSH6
PMS2
EPCAM
EPCAM/MSH2

-0.5
0.5
1.5
2.5
3.5
Percentage of Mutations Identified

PMS2 Large Rearrangements Identified

<table>
<thead>
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<th>Interpretation Complicated by Pseudogenes</th>
<th>Interpretation Not Complicated by Pseudogenes</th>
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<tr>
<td>del exons 1-11</td>
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<tr>
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<tr>
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<tr>
<td></td>
<td>del exon 10</td>
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The PMS2 gene is composed of 15 exons. Multiple pseudogenes spanning exons 1-5, 9, and 11-15 have been identified elsewhere in the genome. However, the interpretation of large rearrangement analysis results is significantly impacted for exons 11-15. We have identified 14 unique large rearrangements within this gene that are associated with increased cancer risk.