Lynch syndrome patients harbor a mutation in one of the mismatch repair genes, MLH1, MSH2, MSH6, or PMS2. While screening for mutations in MLH1, MSH2, and MSH6 is fairly straightforward, the detection of mutations in PMS2 is greatly complicated by the presence of numerous pseudogenes, which represent an obstacle to obtaining reliable data. To address this issue, the immunohistochemical (IHC) profile most closely associated with a germline PMS2 mutation. We used a modification of a previously described long-range PCR method to evaluate the PMS2 gene in 145 patient samples submitted for clinical testing. Our modification avoids potential interference from the pseudogene, PMS2L2. An intragenic probe was designed spanning exons 11–15, the forward primer anchored in exon 10, an exon which is not shared by PMS2L2. Long-range PCR was followed by nested PCR using exon-specific primers and Sanger sequencing. Large deletions were identified by MLPA. IHC results were provided by submitting clinicians. We identified deleterious PMS2 mutations in 22 of 59 patients whose tumors showed isolated loss of PMS2 by IHC. In 27 patients whose tumors showed absence of either another protein or combination of proteins, including 25 samples with concurrent MLH1/PMS2 loss, no deleterious mutations were identified. Three additional patients with deleterious PMS2 mutations were identified from 53 samples without corresponding IHC data. Identified mutations are listed in Table 1. In total, 27 mutations were detected. Of these, 10 (27%) were large deletions encompassing one or more exons. Twelve of the deleterious mutations identified in our subset of patients, to the best of our knowledge, have not previously been reported, including c.325dupG, c.394C>T, c.1392dupA, c.1687T>C, c.1981G>T, c.2007dupT, and c.2174+1G>A. These three mutations have previously been identified as founder or potential founder mutations. Three of the patients (Patients 21, 22, and 23) harbored biallelic mutations, and an additional two patients had suspected biallelic mutations. All five of these patients presented with tumors at a very young age as shown in Table 1.

### METHODS

#### Patient Samples

Specimens consisted of whole blood from 145 untreated patient samples submitted to ARUP Laboratories for PMS2 whole gene analysis over a 13-month period. DNA was extracted from each blood sample using the QIAamp DNA Blood Maxi Kit (Qiagen). Whole blood samples submitted for family-specific testing were obtained by direct consent from family members, analyzed to aid in the confirmation of biallelic mutations. Immunohistochemical profiles of tumors and patient histories were provided by the submitting clinicians. Samples were used with University of Utah IRB approval.

#### Long-range PCR

DNA samples were amplified using primers spanning exons 1-5 and 7-9. For the region encompassing exons 11–15, which is often amplified as a single primer, a primer pair was designed allowing for amplification of exons 11–15. The forward primer contained in exon 10, the reverse primer located in exon 15. This generated a 18.341-kb product. 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. Samples were amplified using the TaKaRa LA Taq polymerase and reagents. Amplification of long-range PCR products was confirmed by gel electrophoresis prior to proceeding to exon-specific amplification.

#### Exon Specific PCR and Sequencing Analysis

For exons 6 and 10, areas without pseudogene overlap, 100 ng of genomic DNA was used as the template. For all remaining exons, the appropriate long-range PCR product was diluted 1:10, and 2 µL of the dilution was used as the template. PCR was performed using the ABI AmpliTaq Gold PCR Master Mix. PCR products were then sequenced using M13 primers and an ABI 377 sequencer. The ABI 3770, Sequences were compared to the NCBI reference sequence NT_007381. Only knockout sequence changes (those resulting in a stop codon or frameshift), splice site mutations, and a single missense mutation (c.1370G>T) previously determined to be pathogenic were considered deleterious.

### CONCLUSIONS

- **Successful detection of mutations in PMS2 relies on the ability to distinguish between gene and pseudogene sequences. This is particularly crucial for the 3' region of the gene, which is susceptible to gene conversion. To avoid pseudogene amplification, long-range PCR primers anchored in regions unique to the gene can be used.**

- **We detected pathogenic mutations in 22 of 59 (37%) individuals with isolated loss of PMS2 protein expression. In total, 27 mutations were detected. Of these, 10 (27%) were large deletions.**

- **Five individuals with confirmed or suspected biallelic mutations presented with phenotypes suggestive of biallelic mismatch repair gene mutations, including cancers at a very young age and the NF1 phenotype.**

- **Pathogenic mutations were seen only in individuals whose tumors showed isolated loss of PMS2, the IHC profile typically associated with germline PMS2 mutations.** The fact that deleterious mutations were not associated with other IHC profiles suggests that the long-range PCR method employed did not generate false positives due to inadvertent amplification of pseudogene sequences.

### REFERENCES

