Identification of an Alu insertion in MSH2 by Next-Generation Sequencing in a Family with Lynch Syndrome: An 8-year Diagnostic Odyssey

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Background
- Lynch syndrome (LS) is a well-known cause of hereditary colon cancer.
- Pathogenic variants and likely pathogenic variants in one of the mismatch repair (MMR) genes (MLH1, MSH2, MSH6 and PMS2 along with deletions of EPCAM) are known to cause LS.
- Alu insertions are the most abundant retrotransposon in the human genome and insertions of Alu elements have been shown to cause disease by either disrupting a coding region or a splice signal.
- Retroelement insertions have been observed in cancer predisposition genes and were recently reported to be more common (1/325; 0.3%) than previously estimated (1/600; 0.16%).
- There have been previous reports of Alu insertions in MMR genes in families with LS.2,3

Methods
- The mobile element (ME) detection software Mobster4 and the commercial laboratory’s in-house developed software was used to detect unaligned and soft-clipped reads from the BAM file.
- The variant was confirmed by Sanger sequencing.
- The mother’s WGS BAM file data were again reviewed and reads covering this insertion were not identifiable (Figure 2).
- Mobster was implemented to run on the maternal WGS.
- Split reads were detected on WGS in the same variant location.
- Standard WGS BWA alignment did not map the reads which contained more than 50% Alu reads and trimmed the reads with less than 50% Alu reads.
- This splice site was not detected by standard variant calling.
- Reads are assessed for small variants and structural variation (Figure 3).
- The MSH2/Alu insertion was undetected by conventional NGS variant calling methods.

Case Report
- 16-year-old female diagnosed with stage 4 colon cancer (Figure 1).
  - Colon tumor specimen showed abnormal microsatellite instability (MSI) and loss of protein expression of MSH-2 and MSH-6 by immunohistochemistry (IHC).
  - Clinical history was unremarkable leading up to her diagnosis and she passed away 10 months later.
  - Patient’s mother had a history of multiple colon polyps starting in her mid-20s.
  - Results from a LS screen performed on a colon tubular adenoma with focal high-grade dysplasia revealed abnormal MSI and the same absent protein expression.
  - She also had a history of a sebaceous adenoma and a squamous cell carcinoma of the scalp.
- Maternal family history:
  - Fulfilled Amsterdam Criteria II.
- Paternal family history:
  - Significant for multiple generations of breast cancer.
  - Multiple polyps starting mid-20s.

Results
- Multiple genes analyzed, no causative variants identified.
- Clinical sequencing and deletion/duplication analysis of APC, MLH1, MSH2, MSH6, and PMS2.
- Research testing of EPCAM (TACSTD1) though Dr. Lichtenberg’s laboratory in Nijmegen.
- DNA was isolated and banked.
- Patient’s mother underwent whole genome sequencing (WGS).
  - No causative variants identified to explain family history of cancer.
  - A pathogenic variant was found in the FBN1 gene, leading to a diagnosis of Marfan syndrome in the mother.
- Testing performed on banked DNA utilizing a commercial laboratory’s custom cancer panel on a Next-Generation sequencing (NGS) platform.
  - A total of 81 cancer susceptibility genes analyzed.
  - A likely pathogenic variant was observed at c.1442_1443insAlu in the MSH2 gene.
  - Patient’s parents underwent confirmatory genetic testing via GSPMC.
  - Mother: positive for the likely pathogenic variant in the MSH2/Alu insertion.
  - Father: negative/nornal genetic findings.

Conclusion
- There is a subset of patients with a phenotype strongly suggestive of LS and no identifiable germline pathogenic variant.
- This case demonstrates the importance of critically assessing the testing methodologies previously performed in this patient cohort.
  - Specifically taking into account if previous testing was capable of identifying retroelement insertions.
- This case demonstrates the value of reanalyzing short-read sequencing data for structural variants and retroelement events for cases that have not been previously diagnosed.

References