RNA Analysis and Long Read Sequencing Identify Causative *APC* Complex Rearrangement in Unsolved FAP Case

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Introduction: Missing heritability remains a challenge for individuals with a clinical diagnosis of a hereditary cancer syndrome for whom germline genetic testing has thus far been uninformative. Newer technologies, including RNA analysis and long read sequencing (LRS), can be informative in germline-negative cases with strong suspicion of a hereditary cancer syndrome. LRS can identify hard-to-detect variants including, among others, complex structural variants (SV). Here we describe a family with a strong history of Familial Adenomatous Polyposis (FAP) with prior uninformative genetic testing that was solved after RNA and LRS.

Methods: The patient sample was submitted for multi-gene panel testing (MGPT) which included paired short-read (SR) capture DNA and RNA sequencing. Analysis of the RNA data identified allele specific expression (ASE) of a single *APC* allele, and this case was prioritized as a candidate for LRS. HiFi long-read genome sequencing was conducted on the PacBio Revio.

Results: A 12-year-old female with a personal history of hepatoblastoma diagnosed at 15 months, tubular adenomas of the stomach, rectum and entire colon, and a family history of colonic polyposis and early-onset colorectal cancer had uninformative *APC* SR sequencing and deletion/duplication analysis. ASE analysis showed that only one allele of *APC* was expressed, suggesting the presence of an unidentified variant causing the loss of this part of the transcript on one allele. Long read genome sequencing identified a translocation event between chromosomes 5 and 17 with a breakpoint deep within intron 8 of *APC*. This led to a fusion transcript with the *STXBP4* gene. This complex rearrangement was classified as pathogenic and the underlying cause of FAP in this patient.

Conclusions: Conventional DNA-only MGPT via SR sequencing may be unable to detect several variant types including deep intronic variants and complex rearrangements. This example highlights how newer technologies, including RNA sequencing and LRS, can be utilized to identify causative variants in hereditary cancer predisposition genes in patients who previously received negative clinical genetic testing. Further studies utilizing RNA analysis and LRS to elucidate the genetic etiology of hereditary cancer predisposition syndromes are warranted in cases with highly suspicious clinical presentations.