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

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BACKGROUND

Long read sequencing (LRS) is emerging as a key technology to elucidate complex variants that are missed and/or not fully characterized by conventional short read (SR) sequencing approaches[1]. Among these, structural variants and mobile elements are particularly challenging to resolve. We sought to apply LRS to address missing heritability in a family with a strong history of Familial Adenomatous Polyposis (FAP) with prior uninformative genetic testing.

METHODS

Multigene panel testing (MGPT), which included paired SR DNA and RNA sequencing, was completed as part of the proband's clinical testing. Clinical history was ascertained from test requisition forms and clinic notes. Allele specific expression (ASE) analysis identified the loss of an *APC* SNP in exon 10. HiFi long-read whole genome sequencing (LR-WGS) was conducted on the PacBio Revio. Familial testing including gel electrophoresis and Sanger sequencing of the known breakpoints was completed.

RESULTS

- 12 y/o Female with personal history of hepatoblastoma, diagnosed at 15 months, tubular adenomas of stomach, rectum and entire colon.
- Family history of colonic polyposis and early-onset colon cancer.
- MGPT was negative.

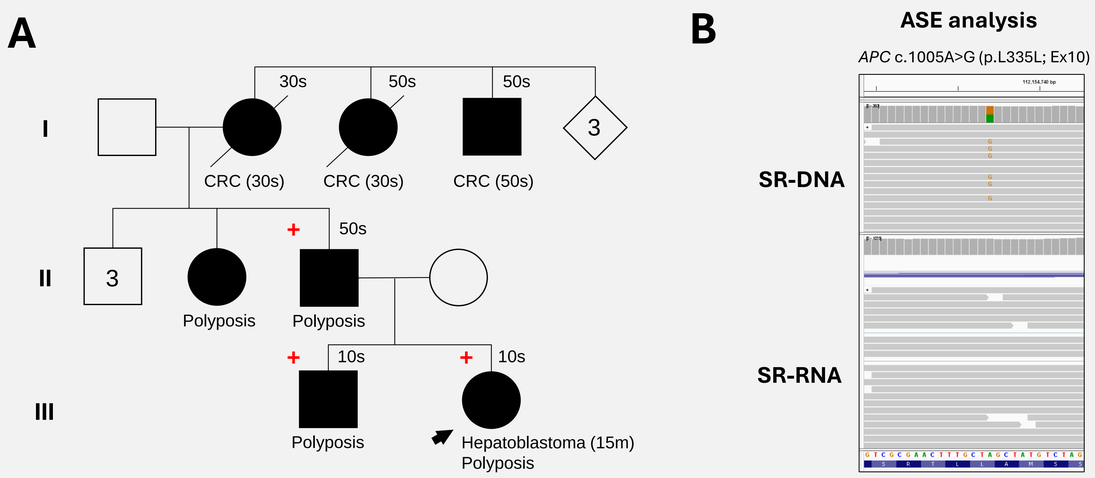


Figure 1: **A)** Pedigree-Affected status (polyposis or CRC) is indicated by filled shape. Age at diagnosis is noted in parenthesis and current age or age at death is noted in top right corner. CRC: Colorectal Cancer. **B)** ASE analysis from the proband shows loss of expression of one *APC* allele.

- ASE analysis identified monoallelic expression of the wild-type allele for heterozygous SNP *APC* c.1005A>G (p.L335L) in exon 10, indicating the presence of an unidentified variant affecting one *APC* allele.
- HiFi LR-WGS revealed the presence of a balanced translocation between chromosomes 5 and 17.

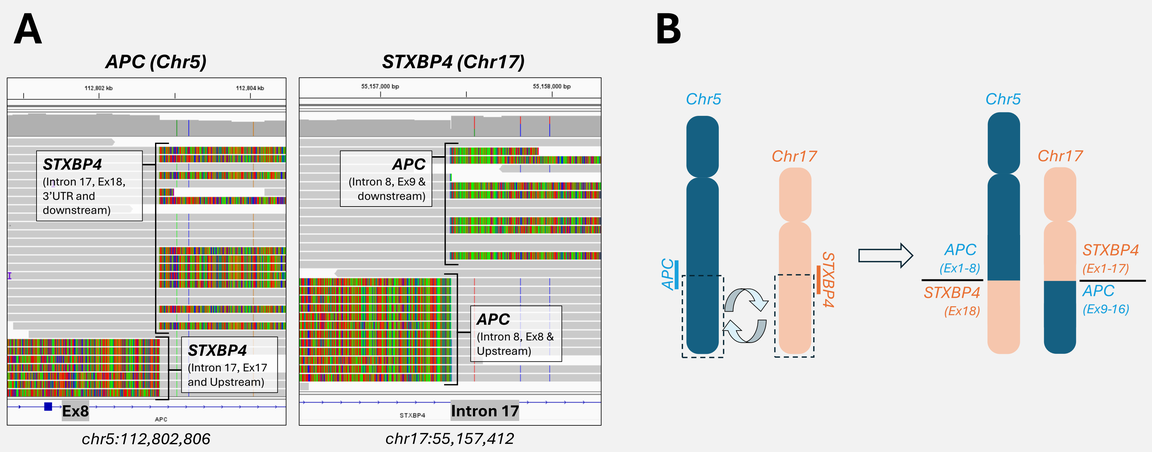
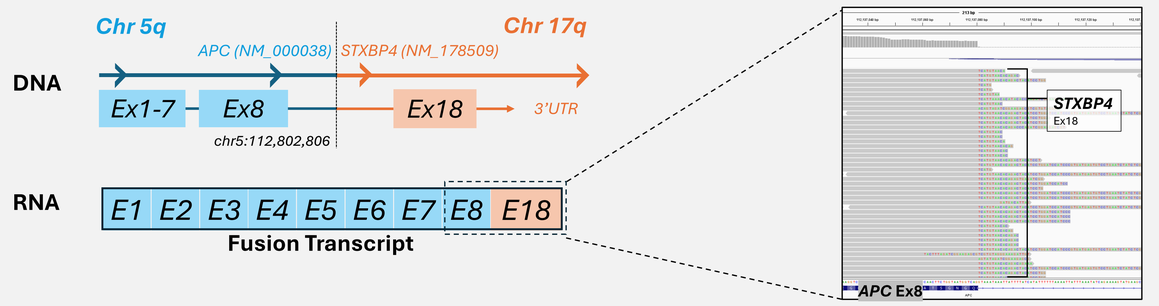
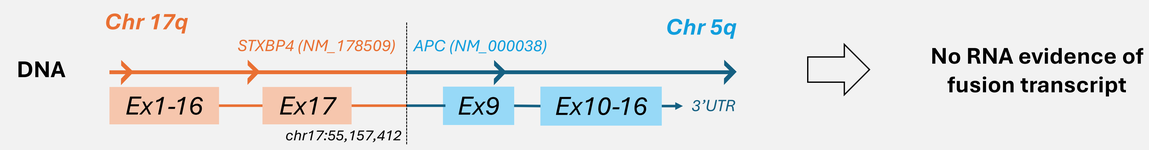


Figure 2: **A)** HiFi LR-WGS identified split reads in Chr5 and Chr17 supporting a translocation event. Breakpoint coordinates in hg38. **B)** Schematic representation of the translocation, which breakpoints disrupt *APC* in Chr5 and *STXBP4* in Chr17.

- The translocation breakpoints disrupt *APC* coding sequence by displacing the last 8 exons, which are critical for function, resulting in pathogenicity[2,3].
- Review of RNA data uncovered evidence of a fusion transcript *APC-STXBP4*, that is not expected to retain *APC* function.



- Conversely, no evidence of a fusion transcript *STXBP4-APC* was observed, likely due to low expression levels and/or lack of probe coverage for *STXBP4*.



- Cosegregation analysis was performed showing that the proband's brother and father, both diagnosed with polyposis, are also carriers of the translocation.

TAKE HOME POINTS

- Conventional DNA-only MGPT via SR sequencing may be unable to detect several variant types, including complex rearrangements.
- RNA sequencing and ASE analysis can inform the value of investigating missing heritability cases with LRS, which can drastically shorten the diagnostic odyssey for families.
- Further studies of the utility of RNA analysis and LRS to elucidate the genetic etiology of hereditary cancer predisposition syndromes in clinically suspicious unsolved cases are warranted.

REFERENCES

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