A Novel Pathogenic Variant in *APC* Intron 14 Contributing to FAP

Maria C. Elias, PhD, HCLD[1]; Paola Nix, PhD, HCLD[1]; Jack W. Landon, BS[1]; Susana San Roman Rivera, MS, CGC[2]; Marcie Parker, MS, LGC[3]; Catherine Ward-Melver, MD[3]; Debora Mancini-DiNardo, PhD, FACMG[1]; Bradford Coffee, PhD, FACMG[1]; Nanda Singh, PhD, FACMG[1]

1. Myriad Genetics, Inc., 2. Myriad Genetics Laboratories, Inc., 3.Akron Children's Hospital

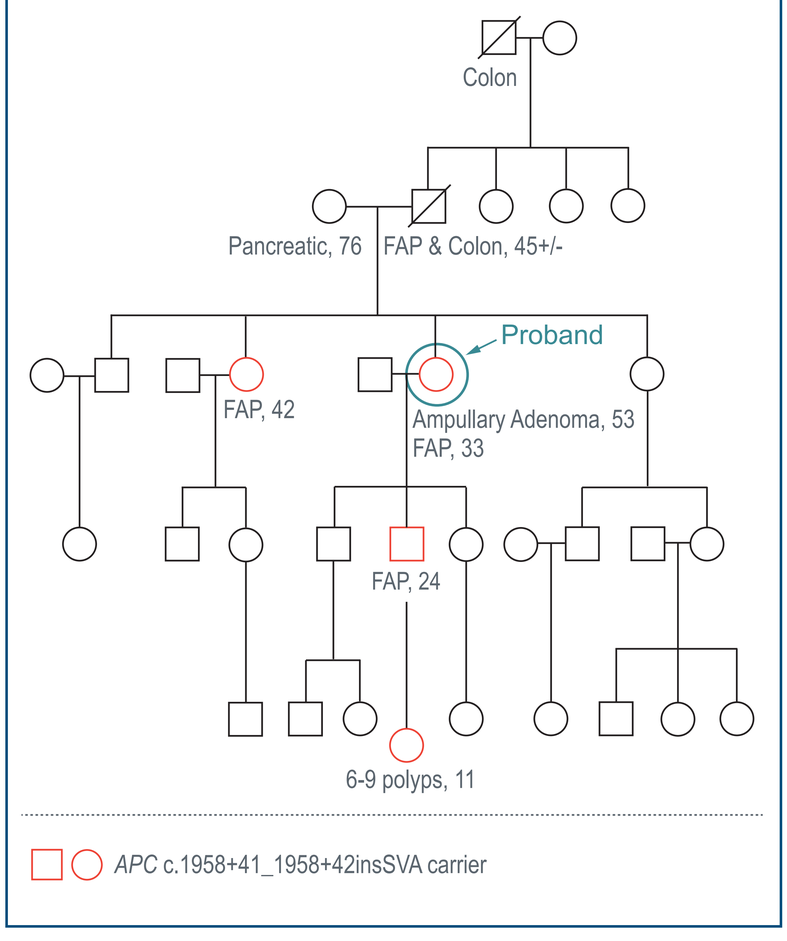
BACKGROUND

- Monoallelic pathogenic variants (PVs) in the adenomatous polyposis coli (*APC*) gene cause Familial Adenomatous Polyposis (FAP).
- This condition is characterized by 100s-1000s of colorectal adenomatous polyps and/or cancer.
- PVs in *APC* are typically frameshifts, small indels, canonical splice site changes, and single to multi-exonic deletions and duplications that result in truncation of the APC protein.
- Here we describe an FAP family with a novel PV in *APC* intron 14.

METHODS

- Genetic testing of a multi-generational FAP family (Figure 1) was performed using genomic DNA on various testing platforms including Sanger sequencing, Southern blot, Array CGH, and Next Generation Sequencing (NGS) for sequencing and large rearrangement analysis.
- A blood sample for RNA analysis was obtained from one family member according to our laboratory's IRB-approved research protocol.

Figure 1. Multi-Generational FAP Family Pedigree

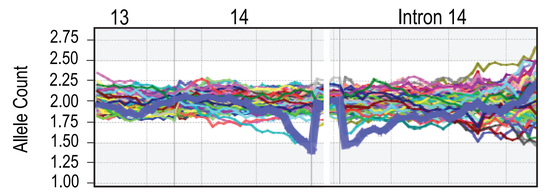


RESULTS

- Initial testing results using Sanger Sequencing and Southern Blot analysis in 2005, followed by NGS analysis in 2018 (Figure 2), identified a rearrangement in *APC* intron 14, but the size and nature of the rearrangement could not be characterized.
  - NGS dosage analysis demonstrated decreased dosage at the exon 14/intron 14 border.
  - A representative NGS raw read demonstrates foreign sequence beginning at c.1958+42.

Figure 2. Next Generation Sequencing (NGS) Data

A. NGS Dosage Analysis



B. NGS Representative Raw Read

(EXON 14) (Intron 14)

GCTACAAATGAGGACCACAGgtatatatagagttttatattacttttaa

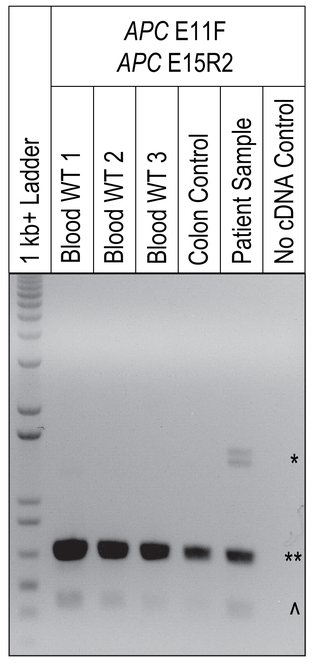
agtacagaattcGGCGACAGAGCGAGACTCCATCTCAAAAAAAA

(INSERTED SEQUENCE)

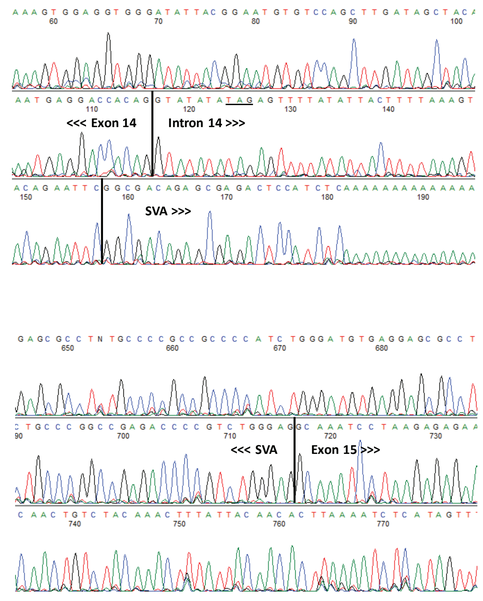
- RNA analysis by Reverse Transcriptase-PCR (RT-PCR) identified an aberrant transcript in the patient sample only and not in normal controls (Figure 3A).
- Subsequent sequencing analysis demonstrated a transcript that included retention of intron 14 and insertion of a SINE/VNTR/ALU (SVA) element (Figure 3B).

Figure 3. RNA Analysis Identifies c.1958+41_1958+42insSVA

A. RT-PCR gel

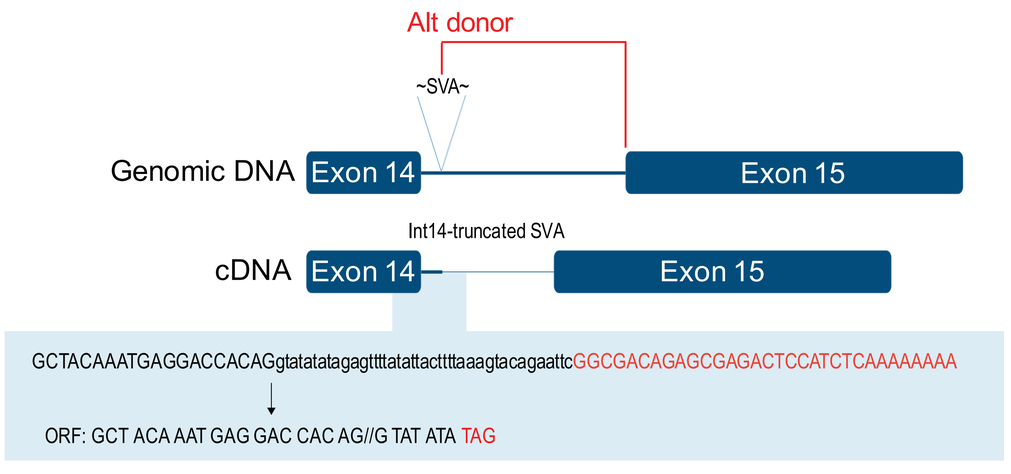


B. Sequence analysis of 1.3 Kb mutant band



*mutant splice product (1.3 Kb), **wild type splice product (0.65 Kb), ^Δexon 14 splice product (0.45 Kb)

Figure 4. c.1958+41_1958+42insSVA Mechanism of Action



- The proposed mechanism of pathogenesis suggests that the SVA element introduces an alternate splice donor that is utilized in place of the native exon 14 splice donor, resulting in an mRNA retaining portions of intron 14 and the SVA element (Figure 4).
- The remaining intron 14 sequence introduces a stop codon 8 bases after the last base of exon 14, resulting in premature truncation of the APC protein.

CONCLUSIONS

- Pathogenic intronic variants, beyond canonical splice site changes, are generally difficult to identify and characterize.
- We used a variety of methods to identify a novel SVA insertion in *APC* intron 14 that introduces a new splice donor site causing aberrant RNA splicing and premature truncation of the APC protein after exon 14.
- The results of this study have clarified the causative PV in a classic FAP family.

Presented at CGA-ICC on November 3, 2019.