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Expanding the Limits of DNA-Based Testing – Simultaneous RNA Analysis Identifies Pathogenic Variants in *APC* that May Otherwise Not Be Identified

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BACKGROUND

- The underlying genetic cause of familial adenomatous polyposis (FAP) remains unidentified in some patients with a clinical diagnosis of the condition.
- While there have been previous reports of deep-intronic, pathogenic variants in *APC*, clinical genetic tests focus only on exons and limited parts of the intron, potentially limiting the identification of disease-causing variants.[1]
- Here we describe the use of concurrent DNA and RNA genetic testing (DGT/RGT) to identify disease-causing intronic variants in *APC* in patients with a phenotype suggestive of FAP.

METHODS

- Patients were clinician-referred for DNA and RNA hereditary cancer panel testing between March 2019 and June 2020.
- Molecular results were reviewed to identify patients with clinically-actionable intronic variants in *APC.*

TAKE-HOME POINTS

- Without RNA evidence, detection and interpretation of deep intronic DNA variants is limited and molecular diagnoses may be missed
- Simultaneous DGT/RGT improves genetic diagnosis of high-risk families with a clinical FAP phenotype
- The addition of RGT can identify clinically actionable results, enabling familial cascade testing of at-risk relatives and informing cancer risk counseling and risk management strategies
- Two deep intronic variants (c.933+829A>G and c.423-3958C>T) represent 4.1% of pathogenic/likely pathogenic variants in *APC* reported during the study period

REFERENCES

1. Spier, I *et al*. Deep intronic APC mutations explain a substantial proportion of patients with familial or early-onset adenomatous polyposis. Hum Mutat 33, 1045-50 (2012).

Novel Intronic Variant: *APC* c.933+829A>G

- Proband presented with a personal history of 100+ colorectal polyps
- Paternal history suggestive of a hereditary basis for FAP
- RGT identified presence of an alternate, out-of-frame transcript
- Sanger sequencing confirmed presence of *APC* c.933+829A>G

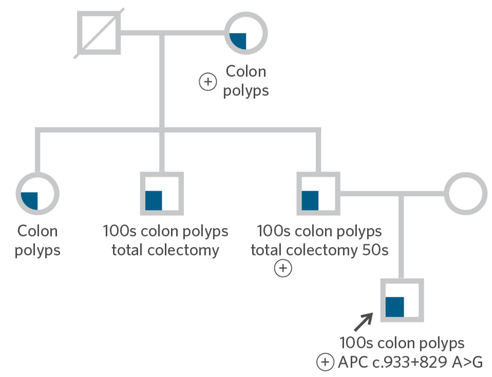

Figure 1: Family pedigree.
(+) indicates an individual is a confirmed carrier of *APC* c.933+829A>G

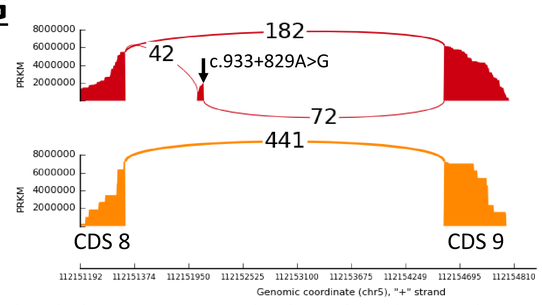

Figure 2: Sashimi plot demonstrating inclusion of a cryptic exon in RNA derived from the proband (red) with *APC* c.933+829A>G but not in RNA from the WT control (orange).

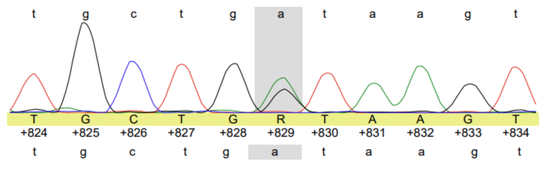

Figure 3: Sanger sequencing confirmation of c.933+829A>G

Novel Intronic Variant: *APC* c.423-3958C>T

- One proband had a personal history of colorectal cancer (age 50s), >20 adenomatous polyps, fundic gland polyps and a family history of polyposis in multiple first-degree relatives. Also identified in a second, unrelated proband with history of polyposis, family history unknown.
- RGT identified presence of an alternate, out-of-frame transcript in both probands.
- Sanger sequencing confirmed presence of *APC* c.423-3958C>T.

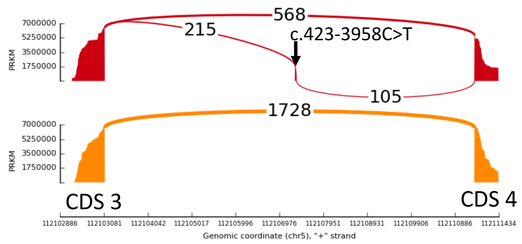

Figure 4: Sashimi plot demonstrating inclusion of a cryptic exon in RNA derived from the proband (red) with *APC* c.423-3958C>T but not in RNA from the WT control (orange).

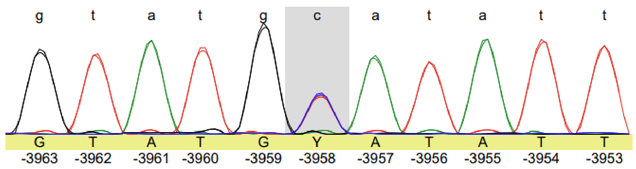

Figure 5: Sanger sequencing confirmation of c.423-3958C>T

Novel Intronic Variant: Unidentified complex variant in Intron 14 of *APC*

- Proband had a personal history of >50 adenomatous and hyperplastic colon polyps.
- DGT identified a heterozygous loss in *APC* intron 14.
- RGT identified an increase in out-of-frame skipping of exon 14.
- Due to the complex nature of this variant additional data is needed to determine the causal nature of this variant.

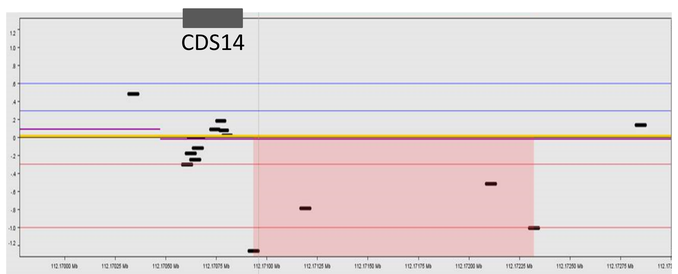

Figure 6: Array data is consistent with a heterozygous loss of non-coding sequence in intron 14 just downstream, but not including the consensus splice donor.

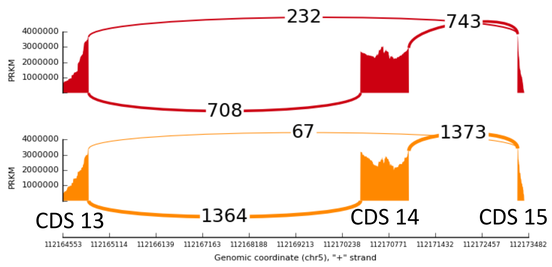

Figure 7: Sashimi plot demonstrating a significant increase in the level of CDS14 exon skipping in RNA derived from the proband (24.2%, red) compared with RNA from the WT control (4.7%, orange).