•. 1998;11(3):197-201.

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No nonsense: When a premature termination is not what it appears

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BACKGROUND

- Pathogenic mutations in *APC* cause Familial Adenomatous Polyposis (FAP), a highly penetrant disorder characterized by thousands of polyps, among other pathologies.
- Patients with pathogenic mutations at the 5' and 3' ends as well as in an alternatively spliced part of *APC* exon 9 may present with attenuated disease characterized by 10s to 100s of polyps due to dosage effect [1, 2, 3].
- The functional impact of the in-frame transcript that results from alternative splicing of exon 9 is unknown but may partially rescue loss of function explaining attenuated disease.

CLINICAL HISTORIES

Family 1: Proband >50 yo with gyn cancer dx 50s. Family History: Breast, Colon, Cervical.
Family 2: Proband >50 yo, unaffected. Family History: Breast, Cervical
Family 3: Proband >50 yo, with Breast dx 60s. Family History: Breast, Uterine, Genital Organ, Mesothelioma. Proband C-scope results not provided but on 10-year C-scope follow up schedule.
Family 4: Proband >50 yo, unaffected. Family History: Pancreatic, Breast, Colon. Proband C-scope results not provided but on 10-year C-scope follow up schedule.

TABLE 1: RNA RESULTS SUMMARY

Baseline	r.	p.	PSI Experiment A	PSI Experiment B	PSI Experiment C
Natural Partial Exon 9 Skipping ("9a")	r.934_1236del	p.V312_Q412del	Pro 1: 24.0% Cont: 18.2%	Pro 1: 33.6% Cont: 19.0%	Pro 1: 32.5% Pro 2: 31.0% Cont 1: 21.2% Cont 2: 19.7%
Natural Total Exon 9 Skipping	r.934_1312del	p.V312Cfs*16	Pro 1: 5.6% Cont: 5.0%	Pro 1: 11.9% Cont: 6.4%	Pro 1: 12.4% Pro 2: 12.9% Cont 1: 8.5% Cont 2: 9.5%
Aberrant Partial Exon 9 Skipping	r.934_1074del	p.V312_Q358del	Pro 1: 17.5% Cont: 0.2% (ns)	Pro 1: 15.6% Cont: 0.3% (ns)	Pro 1: 14.4% Pro 2: 13.6% Cont 1: 0.1% Cont 2: 0.3% (ns)

ns=not shown (reads were filtered at 50X to remove low-level noise

FIGURE 1: *APC* TRANSCRIPTS AND GENOTYPE-PHENOTYPE CORRELATION[1]

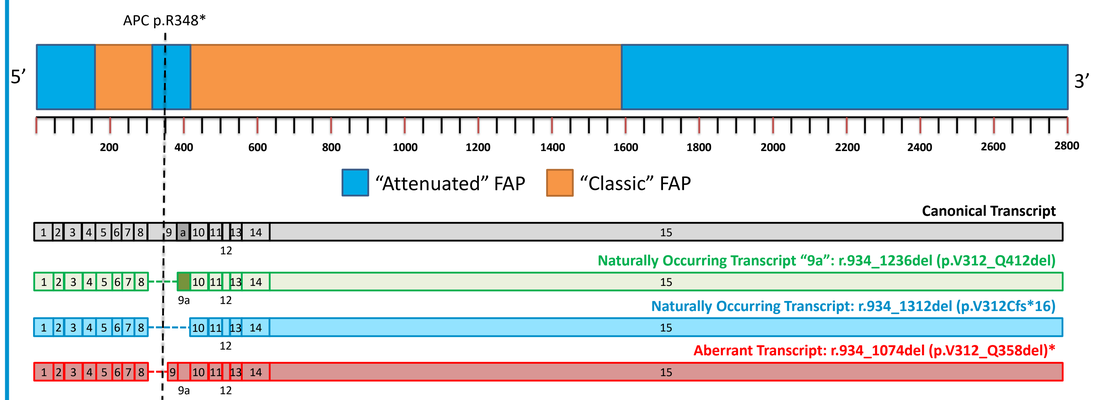

Figure 1. TOP: A to-scale depiction of the amino acid regions where pathogenic mutations are associated with attenuated FAP (blue) and classic FAP (orange). BOTTOM: The exon structure of the canonical transcript (black), the naturally occurring isoform known as "9a" (green), the naturally occurring transcript lacking coding exon 9 (blue), and the aberrant transcript identified in carriers of APC p.R348* (red with asterisk). The location of APC p.R348* is shown as a black hashed line indicating its inclusion in the canonical transcript but its exclusion in all other transcripts.

FIGURE 2: RNA RESULTS

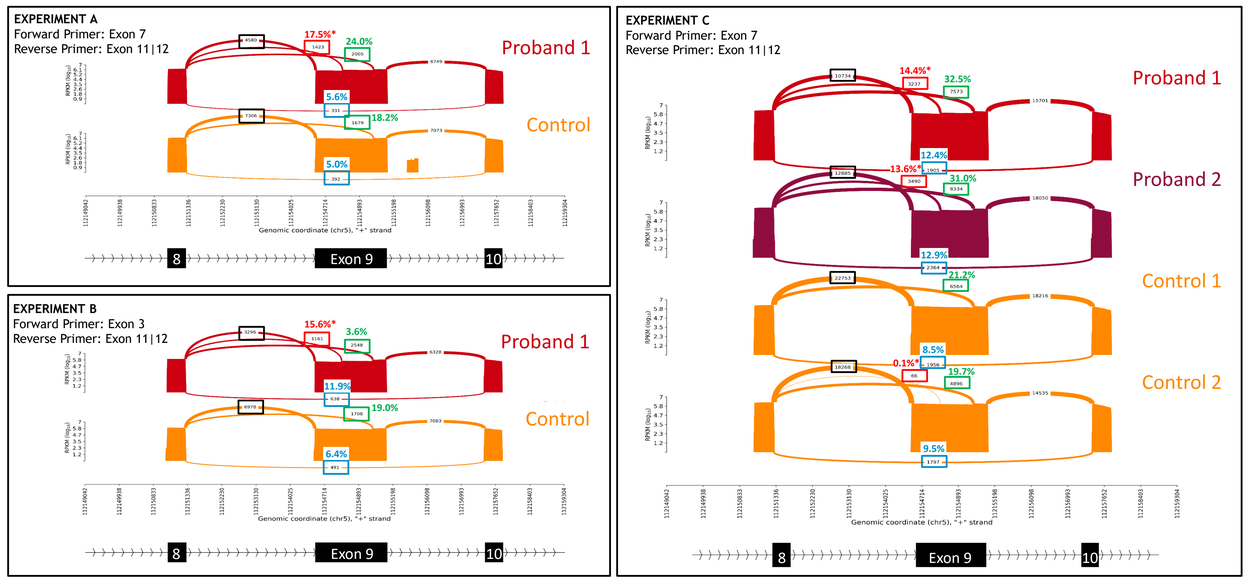

Figure 2. RNA from Patient 1 and Patient 2 was ascertained multiple times with multiple RTPCRSeq methodologies and displayed here as Sashimi plots. Patient RNA is depicted in shades of red and RNA from control blood samples are depicted in orange. Experiment A and C were conducted with a forward primer designed in coding exon 7 and reverse primer spanning the exon-exon junction of coding exons 11 and 12. Experiment B ascertained the possibility of longer aberrant transcripts by utilizing a forward primer in Exon 3 and the same reverse primer as in Experiments A and C. All alternatively spliced- and aberrant transcripts impacting this region are displayed and the percent splicing index (PSI) and number of reads supporting each transcript are depicted. The identity of all transcripts are color-coded to correspond to Figure A and Table 1: Aberrant transcript (red with asterisk); Naturally occurring "9a" (green); Naturally occurring exon 9 skipping (blue); Canonical transcript (black). The exon structure of the canonical transcript is displayed in black at the bottom of each Sashimi plot. The relative quantification of each alternate and aberrant transcript are provided in Table 1 and with text in the Sashimi plot. Reads were filtered at 50X to remove low-level noise.

METHODS

- A retrospective review of the clinical features of four unrelated probands carrying *APC* c.1042C>T (p.R348*) was undertaken
- Where available, corresponding RNA from each proband was analyzed using multiple RT-PCRSeq designs.

RESULTS

- Carriers of *APC* c.1042C>T (p.R348*) are each over 50 years old and do not report a personal or family history consistent with FAP
- RNA analysis identified *APC* r.934_1074del141 (p.V312_Q358del), an in-frame aberrant transcript that splices-out the nonsense alteration.

TAKE-HOME POINTS

- *APC* c.1042C>T encodes a stop codon in coding exon 9.
- Four probands carrying *APC* c.1042C>T (p.R348*) are not overtly affected with FAP/AFAP.
- *In silico* and RNA analyses show that this alteration leads to a pool of NMD-escaping transcripts that splices-out the nonsense mutation and may rescue function.
- *APC* c.1042C>T (p.R348*) is considered a variant of uncertain significance by this group.
- Splice prediction and RNA analysis should be undertaken for all variant types, including *a priori* loss-of-function variants.

REFERENCES

1. Leoz ML, *et al.* The genetic basis of familial adenomatous polyposis and its implications for clinical practice and risk management. *Appl Clin Genet*. 2015 Apr 16;8:95-107.
2. Van der Luijt RB, *et al.* APC mutation in the alternatively spliced region of exon 9 associated with late onset familial adenomatous polyposis. *Hum Genet* 1995 Dec;96(6):705-10
3. Curia, MC, *et al.* Transcript dosage effect in familial adenomatous polyposis: model offered by two kindreds with exon 9 APC gene mutations. *Hum Mutat*. 1998;11(3):197-201.

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