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

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Background: *APC* exon 9 is subject to naturally occurring alternative splicing leading to an in-frame (IF) transcript. Patients with loss-of-function alterations in the spliced region frequently have attenuated Familial Adenomatous Polyposis (FAP). Three patients in our diagnostic laboratory cohort carrying *APC* c.1042C>T (p.R348*) in exon 9, did not report a personal or family history of polyps or colon cancer. This variant has *in silico* splice predictions suggesting the creation of a weak, novel acceptor site. Patient RNA was analyzed for use of this alternate site.

Methods: Retrospective review of clinical data and patient RNA was performed on three carriers of this variant who underwent pan-cancer multigene panel testing.

Results: Probands were >50y without a personal or family history of AFAP. One reported a 10-year colonoscopy schedule while the others did not provide colonoscopy information. Both CaptureSeq and RT-PCRSeq analyses consistently revealed the use of a novel cryptic acceptor site leading to incomplete expression of *APC* r.934_1074del141 (p.V312_Q358del). This transcript is predicted to lead to the IF loss of 47 amino acids of unknown function including codon 348 where the nonsense alteration occurs.

Conclusions: The pool of possibly functional aberrant and naturally expressed IF transcripts exclude the nonsense alteration and may explain the lack of overt AFAP these probands. Based on splicing and clinical data, this variant is classified as a variant of uncertain significance at this laboratory. These cases highlight the importance of considering splicing in the interpretation of every variant type including nonsense and frameshift variants by minimally evaluating *in silico* splice predictions and ideally by analyzing patient RNA. This can mitigate incorrect *a priori* classification of variants as pathogenic when they might otherwise have a splicing rescue effect.

We hereby confirm that the relevant patient data submitted in this case series is exempt from IRB review.

Keywords: APC, FAP, AFAP, Polyposis, RNA, alternative splicing