A Deep Dive into *APC* Introns: Paired DNA/RNA Testing Identifies Novel FAP and AFAP Alleles in the Deep Intronic Regions of *APC*

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Background:

Pathogenic alleles in *APC* cause the autosomal dominant syndromes of familial adenomatous polyposis (FAP) and attenuated familial adenomatous polyposis (AFAP). FAP and AFAP are characterized by the development of dozens to thousands of adenomatous colon polyps and significantly increased lifetime risks of developing colon cancer. Multigene panel and deletion/duplication testing has been successful in identifying causal mutations in an estimated 85-90% of FAP families and nearly 50% of AFAP families, however, there remains many families with clinical FAP/AFAP diagnoses in which a causal variant is unidentified. Therefore, paired DNA/RNA sequencing can be an important tool to help identify previously undiscovered *APC* mutations.

Methods:

Paired DNA/RNA sequencing via multigene panel testing at a diagnostic laboratory was used to screen patients for deep intronic variants in *APC* that result in aberrant RNA splicing. Aberrant RNA transcripts were identified by RNA sequencing and variants were subsequently identified by Sanger sequencing. RT-PCR was performed on patient samples for additional quantification. These alterations were classified according to the ACMG/AMP variant classification guidelines.

Results:

Using paired DNA/RNA sequencing, we identified three novel, deep intronic APC variants, c.730-494C>G, c.933+829G>A, and an as-of-yet unidentified complex intron 14 variant identified by a high expression of r.1744_1958del215. These variants were identified in patients with clinical characteristics consistent with FAP/AFAP and shown to segregate in these families. RNA RT-PCR confirmed the splice impact resulting from these variants, and the combination of evidence lead to clinical classifications of likely pathogenic or pathogenic.

Conclusions:

Paired DNA/RNA testing is able to identify deep intronic variants that have been previously uncharacterized using DNA testing alone and therefore RNA testing is an important tool to characterize the complete spectrum of causal mutations in *APC*.

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

Keywords: *APC*, RNA testing, Clinical Classification, FAP, AFAP