Truncating variants in 5' exons of *APC* are associated with highly attenuated phenotypes and emphasize the need for genotype-phenotype correlation.

Jamie D. Weyandt, PhD; Colin C. Young, PhD; June Mikkelson, MS, CGC; Marcy E. Richardson, PhD

Background: Loss of function (LoF) alterations at the N-terminus of *APC* are associated with Attenuated Familial Adenomatous Polyposis (AFAP); however, we have identified multiple truncating variants in *APC* coding exons 1 and 2 (CDS1 and 2) in individuals whose polyp burden is below the threshold typically described for AFAP.

Clinical Presentation: Retrospective clinical data was curated for individuals in which multigene panel testing for cancer identified alterations predicted to result in premature termination codons (PTCs) in CDS1 and 2 of *APC.* Although AFAP is associated with N- and C- terminal LoF alterations, we observed an unusually attenuated phenotype (0-10 polyps) in multiple patients with these alterations, including p.M1?, p.R24*, p.Q25Rfs*5, p.E46Sfs*4, p.K49*, p.L68Yfs*2, and p.E74*.

Discussion: Several possibilities could explain this observation, including escape of nonsense mediated decay (NMD), use of an alternate start codon, and/or alternative splicing. To investigate these possibilities, we identified the location of the PTC for each alteration with respect to the N-terminus and in alternative transcripts. While some of these alterations could escape NMD due to their N-terminal location, others that are more distal to the N-terminus are expected to be NMD-prone. Exon skipping due to alternative splicing is also unlikely as all known transcripts for *APC* (NCBI) contain CDS2. CDS1 and 2 encode the oligomerization domain which is required for homodimerization; therefore, it is possible that retention of this domain is critical for pathogenicity.

Conclusions: Highly attenuated phenotypes observed in individuals with PTCs in CDS1 and 2 of *APC* emphasize a need for robust clinical variant interpretations that are not based solely on assumptions of predicted impact. A thorough understanding of variant effect and genotype-phenotype correlation is critical for reporting accurate classifications and interpretive information to providers.

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

Keywords: APC, AFAP, N-terminus, LoF, attenuated, alterations