Don't MISS the mark: Rare missense variants in *APC* associated with polyposis phenotypes.

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Background and Aim: Most pathogenic variants in *APC* are truncating or expected to cause aberrant splicing resulting in premature truncation; however, missense variants are rarely considered deleterious. The ClinGen expert panel applies "BP1" evidence toward benign classification for missense variants in *APC*, excluding only one 15-amino acid repeat within the β-catenin binding domain from eligibility (codons 1021-1035, where multiple likely pathogenic true missense variants have been identified). We investigated whether there are any other regions in *APC* where missense variants are associated with phenotype and, thus, should also be considered for non-application of BP1.

Methods: We retrospectively reviewed clinical data for missense variants in *APC* classified as Pathogenic/Likely Pathogenic (P/LP) based on ACMG/AMP criteria in individuals undergoing multigene panel testing for hereditary cancer at a clinical diagnostic laboratory.

Results: We classified 23 missense variants in *APC* as P/LP in association with classic or attenuated familial adenomatous polyposis (FAP/AFAP). Thirteen of these were predicted and/or observed to impact splicing and were excluded from further analysis. Five were within the β-catenin binding domain repeat already excluded for BP1 application. Four were within a SAMP motif involved in actin binding (codons 1475-1584). One was in a different β-catenin binding domain repeat.

Conclusions: Rare missense variants in *APC* are associated with FAP/AFAP. While some lead to aberrant splicing, others may directly impair protein function, as nearly half of the missense variants classified as P/LP have no predicted or observed splicing impact. In addition to the known β-catenin binding domain repeat, multiple variants within the SAMP motif from codons 1475-1584 are also associated with disease and this region should be considered for exclusion from BP1 eligibility. These findings emphasize that, although rare, missense variation can result in loss-of-function in *APC*, and that at least two regions in the protein may be hotspots for phenotype-associated alterations.