Coverage matters: High rate of promoter 1B deletions in a large *APC* testing cohort

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Germline analysis of the *APC* gene is a long-standing first-tier test for patients with multiple colorectal adenomas. Improvements in testing methodologies (i.e. gross deletion/duplication (del/dup) analysis), increased gene coverage (i.e. promoter 1B), and the emergence of next-generation sequencing (NGS) multi-gene panel tests (MGPTs) have optimized detection rates of *APC*-associated polyposis conditions in recent years. Here we present results from >5 years of high volume clinical *APC* testing. Data from *APC* single gene tests (SGTs) and MGPTs containing *APC* ordered 10/01/2011-12/31/2016 were retrospectively reviewed. In all cases, *APC* analyses included sequencing and gross del/dup coverage of all coding regions and gross del/dup coverage of promoters 1A and 1B. Cases with an alteration classified as "pathogenic" or "likely pathogenic" (P/LP) were considered positive. The common p.I1307K moderate-risk mutation was excluded from the analysis. The final dataset contained 2,501 SGTs and 62,868 MGPTs. A total of 355 unique P/LP alterations were identified among 716 positive cases. *APC* positive rate varied drastically by test ordered: 17.8% (446/2,501) for SGTs and 0.4% (270/62,868) for MGPTs. Nonsense and small insertions/deletions were the most commonly identified mutation types (75.1%, 538/716), followed by gross deletions (12.3%, 88/716), splice junction (9.9%, 71/716), missense (2.5%, 18/716), and gross duplications (0.1%, 1/716). Promoter 1B deletions accounted for nearly 5% of positive cases (4.6%, 33/716) and represented the most common *APC* deletion (37.5%, 33/88). No gross deletions isolated to promoter 1A were identified, suggesting these alterations may not be responsible for *APC*-associated polyposis. Notably, 5 positive probands had reported previous negative *APC* testing. In 4/5 of these cases, a gross deletion had escaped detection on previous testing due to sequence analysis only (2 cases) or lack of promoter 1B gross deletion coverage (2 cases). In the final case, a point mutation was not detected previously by Sanger sequencing at an external laboratory, but was detected on re-analysis by NGS. In summary, promoter 1B gross deletions are common *APC* mutations. For patients suspected of having an *APC*-associated polyposis condition who previously tested negative, our data supports re-testing for promoter 1B deletions if not covered in the initial testing. Phenotype analysis is in progress to better understand possible genotype-phenotype correlations.