

Title: Into the Abyss: Mapping the Landscape of Deep Intronic Pathogenicity

Authors: Kelly Fulk, Kristin Kohler, Heather Zimmermann

Background: Pathogenic variants in hereditary cancer genes are typically identified within coding regions or near canonical splice sites; however, deep intronic variants (DIVs) can also disrupt splicing and contribute to disease risk. These DIVs are not routinely interrogated in clinical genetic testing and remain poorly characterized with respect to prevalence, genomic architecture, and functional impact. As expanded sequencing and RNA analysis increasingly enable detection of intronic variation, improved understanding of DIVs is needed to inform hereditary cancer variant interpretation. Accordingly, this study sought to define the spliceogenic landscape of clinically actionable DIVs across hereditary cancer genes.

Methods: Pathogenic (P) and likely pathogenic (LP) DIVs with demonstrated splice impact were identified from paired DNA/RNA hereditary cancer testing performed at a clinical laboratory (2019-2026). DIVs were defined as ≥ 20 bp from the nearest exon boundary. DIV enrichment was assessed by gene, distance to exon boundary, and intron length. Observed-to-expected ratios were calculated using expected counts derived from average DIV burden per intronic base pair and evaluated using two-sided Poisson testing with Benjamini-Hochberg false discovery rate correction. SpliceAI scores were evaluated to assess prediction performance.

Results: A total of 105 unique P/LP DIVs were analyzed across 18 hereditary cancer genes. Most variants (77.1%;) were initially classified using internal paired DNA/RNA evidence, with RNA support for 94.3% overall; 5.7% relied exclusively on external functional evidence. Cryptic exon inclusion was the predominant splice consequence (62.9%), followed by partial intron inclusion (26.7%). *ATM* and *NF1* demonstrated significant enrichment of DIV burden. DIVs showed nonrandom positional enrichment relative to exon boundaries, with the strongest enrichment observed within the -20 to -50 bp interval upstream of exons. SpliceAI performed well overall, with 85.7% of RNA-confirmed P/LP DIVs exceeding a ≥ 0.2 threshold and 63.8% exceeding ≥ 0.5 .

Conclusions: Collectively, these findings define a nonrandom spliceogenic landscape of P/LP DIVs across hereditary cancer genes. Most clinically actionable DIVs were identified through paired DNA/RNA testing, underscoring the critical role of RNA analysis in detecting pathogenic splice-altering variants that could be missed by DNA-only hereditary cancer testing approaches.