

Title: “The *ATM* Splice-ome: Charting the multifaceted splicing landscape of *ATM*”

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Background: Pathogenic variants in the DNA damage response gene *ATM* are associated with hereditary cancer susceptibility and ataxia-telangiectasia. While many pathogenic variants directly result in protein truncation, disruption of splicing represents an additional and underrecognized mechanism of pathogenicity. Splice-altering variants may arise not only at canonical splice sites but also from exonic substitutions that alter splicing regulatory elements or intronic variants that activate pseudoexonization. Because exonic splice variants can be misinterpreted as missense changes and deep intronic variants may escape routine annotation, the landscape of *ATM* variants that disrupt splicing remains incompletely characterized in clinical diagnostic cohorts.

Methods: Likely pathogenic (LP) and pathogenic (P) single nucleotide variants with a demonstrated splice impact were analyzed. Variants identified during routine clinical diagnostic testing included canonical splice variants, first/last exonic nucleotide variants, exonic missense and synonymous variants, and noncanonical intronic variants. SpliceAI predicted events with scores >0.2 across a 500bp flanking region around each variant were analyzed.

Results: Among 253 eligible *ATM* splice-altering variants, canonical splice alterations were the most frequent (56.52%), followed by first/last exonic nucleotide variants (10.28%), exonic missense variants (9.88%), and deep intronic variants (7.51%). Splice-altering variants were distributed across the gene but showed exon-level enrichment in CDS 20 and 52, each having 10 events. Exonic missense splice variants occurred throughout *ATM* but were non-uniformly distributed with CDS 52 showing the highest burden (5 events). Deep intronic variants were also observed across *ATM* but were not detected beyond CDS 43.

SpliceAI showed strong performance for noncanonical splicing variants. Among exonic missense splice variants 96% had SpliceAI scores >0.2 and 73% exceeded 0.5. Deep intronic variants showed even stronger prediction, with 100% scoring >0.2 and 75% exceeding 0.5.

Conclusions: Over 30% of splice-altering LP/P *ATM* variants are noncanonical and likely missed without RNA analysis but accurately predicted by SpliceAI. These findings highlight that deep intronic and exonic missense variants can carry a substantial risk of pathogenicity through splice-altering mechanisms and that SpliceAI is a dependable tool for identification.