Unlocking the Code: When SpliceAI Falls Short in Variant Assessment

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BACKGROUND

- RNA splicing involves intricate biological mechanisms and predicting how a variant will impact this process is particularly challenging.
- Splice prediction algorithms, like SpliceAI, are helpful but not definitive for predicting splicing impacts.
- Incorporating RNA data is essential for accurate variant interpretation.

Aims: Describe 5 cases with clinically relevant variants with observed splicing impacts that were not predicted by SpliceAI based on the commonly accepted benign threshold of 0.1.[1,2]

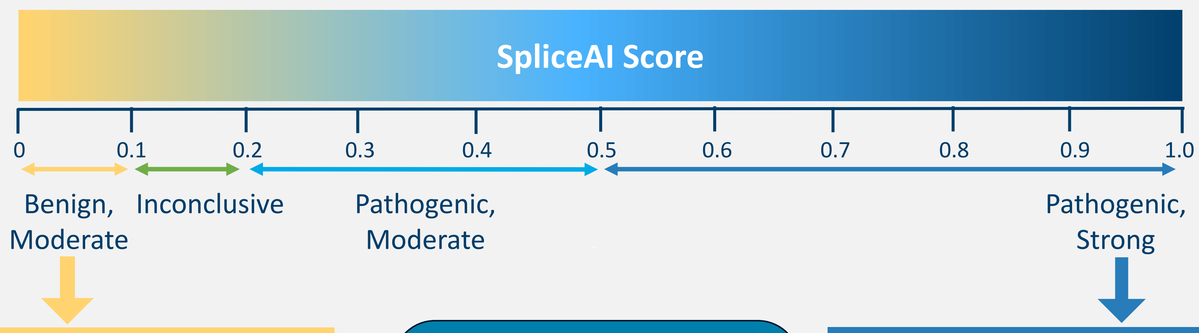
TAKE HOME POINTS

- Variants with low SpliceAI scores can still result in clinically relevant splicing alterations.
- Reliance solely on *in silico* predictions can lead to variant misclassification.
- RNA analysis provides detailed insights into splicing alterations and improves interpretation accuracy.

METHODS & RESULTS

- 5 variants with splice predictions below the typical benign threshold were identified in cases with clinical features consistent with variant pathogenicity **[Figure 1]**
- RNA studies detected substantial aberrant splicing in all 5 cases **[representative cases in Figure 2]**
- Incorporation of RNA evidence leads to clinically significant upgrades (VUS to P/LP) **[Table 1]**

FIGURE 1: SPLICEAI THRESHOLDS[1,2]



Variant of Interest Scores	
APC c.1744-22C>G	0.07
CDH1 c.1613A>T	0.01
JAG1 c.886+3A>G	0
NF1 c.6084G>A	0.08
PMS2 c.1144G>C	0.02

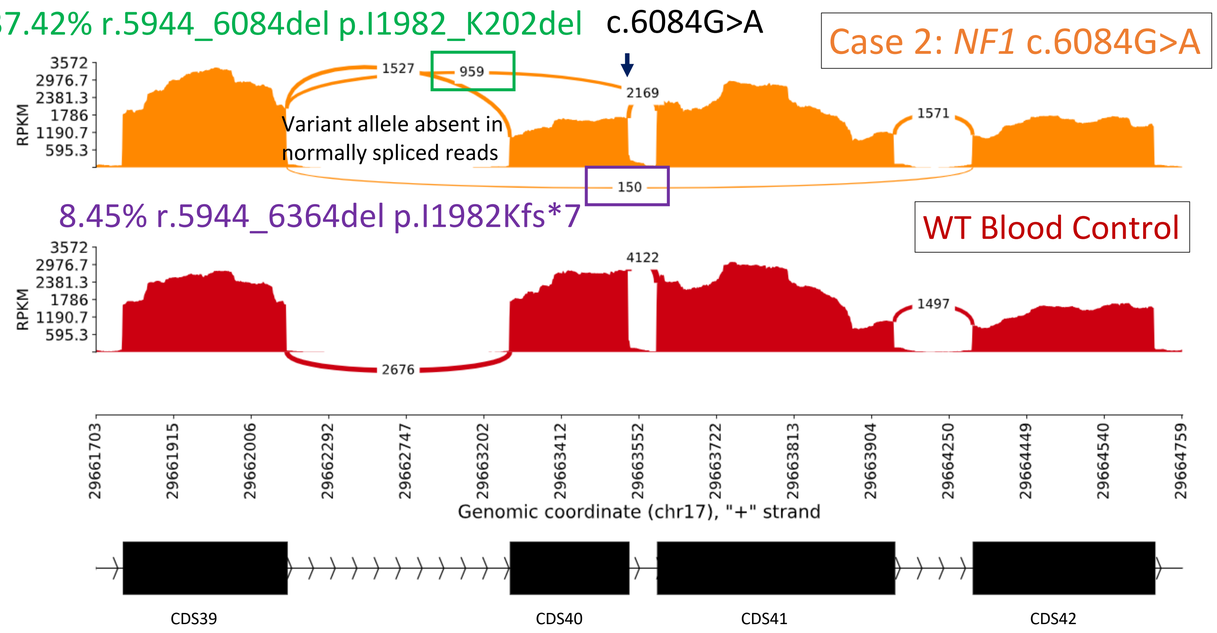
Despite SpliceAI scores below the benign threshold, variants of interest had significant splice impacts.

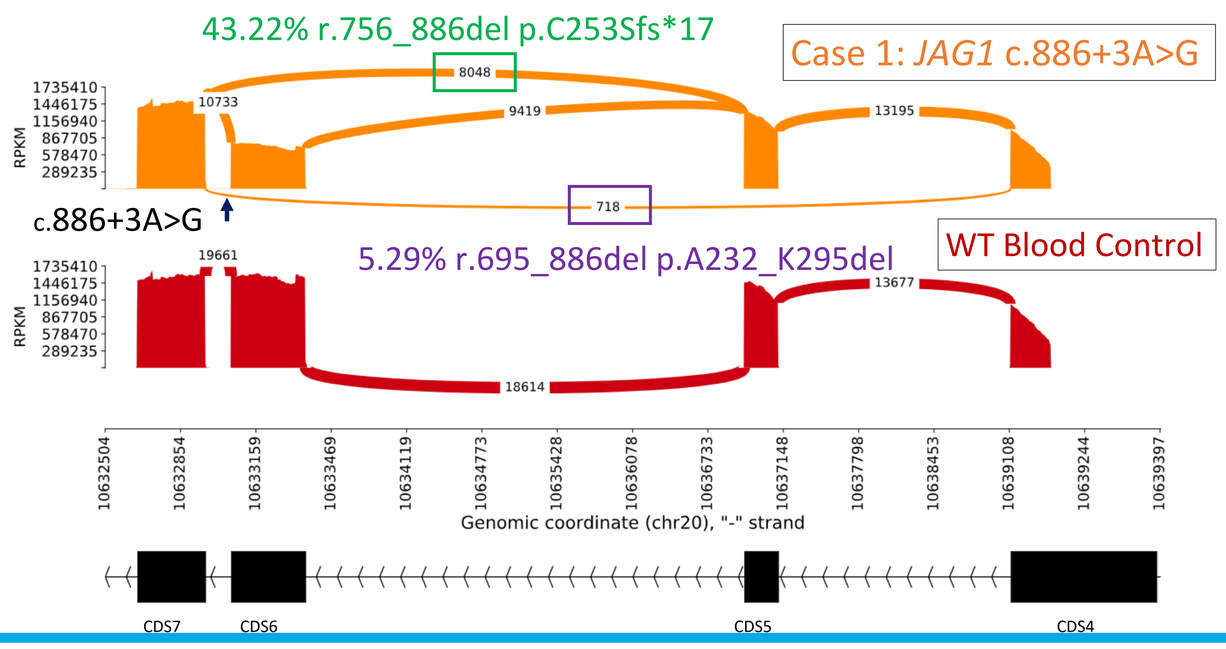
Canonical Splice Scores	
APC c.1744-2A>G	0.97
CDH1 c.1711+1G>C	1.0
JAG1 c.886+2T>G	1.0
NF1 c.6084+1G>A	0.99
PMS2 c.1144+1G>C	1.0

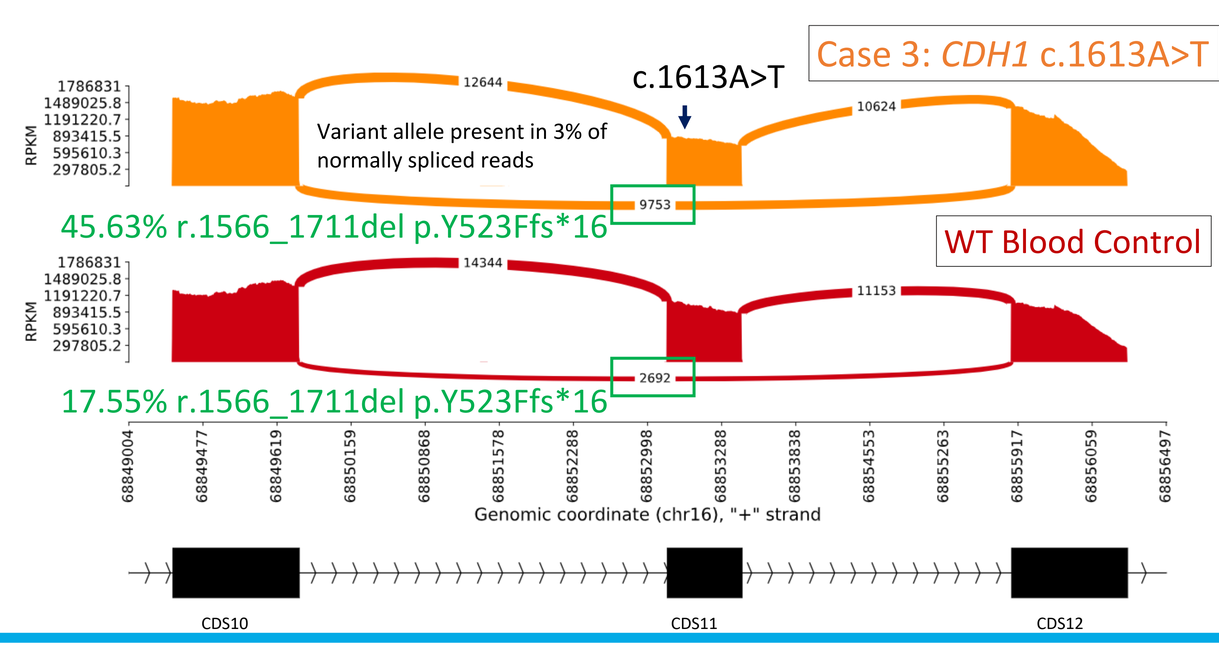
TABLE 1: IMPACT OF RNA EVIDENCE

Case ID	Variant (Location)	SpliceAI[2] DL = donor loss AL = acceptor loss	PSI (Percent spliced in) (ESF = full exon skip)	– RNA	+ RNA
1	*JAG1* c.886+3A>G (Intronic)	DL = 0	43.22% ESF4 5.29% ESF4-5	VUS	LP[3]
2	*NF1* c.6084G>A p.K2028K (Last nucleotide)	DL = 0.08	37.42% ESF40 8.45% ESF40-41 0% A allele	VUS	P
3	*CDH1* c.1613A>T p.D538V (Mid-exonic)	AL= 0.01	45.56% ESF13 3% T allele	VUS	LP
4	*APC* c.1744-22C>G (Intronic)	AL = 0.07	64.62% ESF	VUS	P
5	*PMS2* c.1144G>C p.G382R (Last nucleotide)	DL = 0.02	45.64% ESF 2% C allele	VUS[4]	P

FIGURE 2: RNA STUDIES RESULTS







REFERENCES 1. PMID 36865205; 2. PMID 30661751; 3. PMID 12442286; 4. PMID 26437257