

# RNA-seq–informed germline variant curation in pediatric acute myeloid leukemia



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## Background & significance

### Germline predisposition is clinically relevant in pediatric AML

- In the pediatric AML cohort analyzed by Harmon et al., ≥5.5% of patients had P/LP variants in familial myeloid malignancy genes.
- An additional 3.3% had P/LP variants in lymphoid malignancy or solid tumor predisposition genes.
- Across myeloid malignancies, germline prevalence exceeds the >5% threshold cited to support routine genetic testing.
- Classification can influence surveillance, treatment selection, transplant donor choice, and testing of relatives.

### Why careful classification matters

#### Shwachman–Diamond syndrome

- Biallelic SBDS variants
- Bone marrow failure + MDS/AML risk
- Poor outcomes after AML; early recognition may guide surveillance and transplant planning

#### CHEK2 risk alleles

- Identified in pediatric AML cohort
- p.I157T enriched in hematopoietic malignancy
- Likely lower penetrance; requires careful interpretation

**Central question: can RNA-seq evidence clarify which candidate germline variants truly disrupt splicing and warrant classification changes?**

## Methods

### Cohort and variant selection

AAML 1031  
(1,087 patients)

Whole genome sequencing  
from bone marrow at diagnosis/  
relapse and remission

Identify likely germline variants  
(variant allele frequency > 0.3 at both  
diagnosis/relapse and remission)

Interrogate 555  
candidate genes

Annotate pathogenicity according to  
ACMG/AMP guidelines

## Methods continued

### Interpretation path

#### DNA-level prediction

What might happen?  
Variant location, SpliceAI,  
predicted protein effect

#### RNA-level observation

What actually happened?  
Exon skipping, intron retention,  
cryptic splice-site use

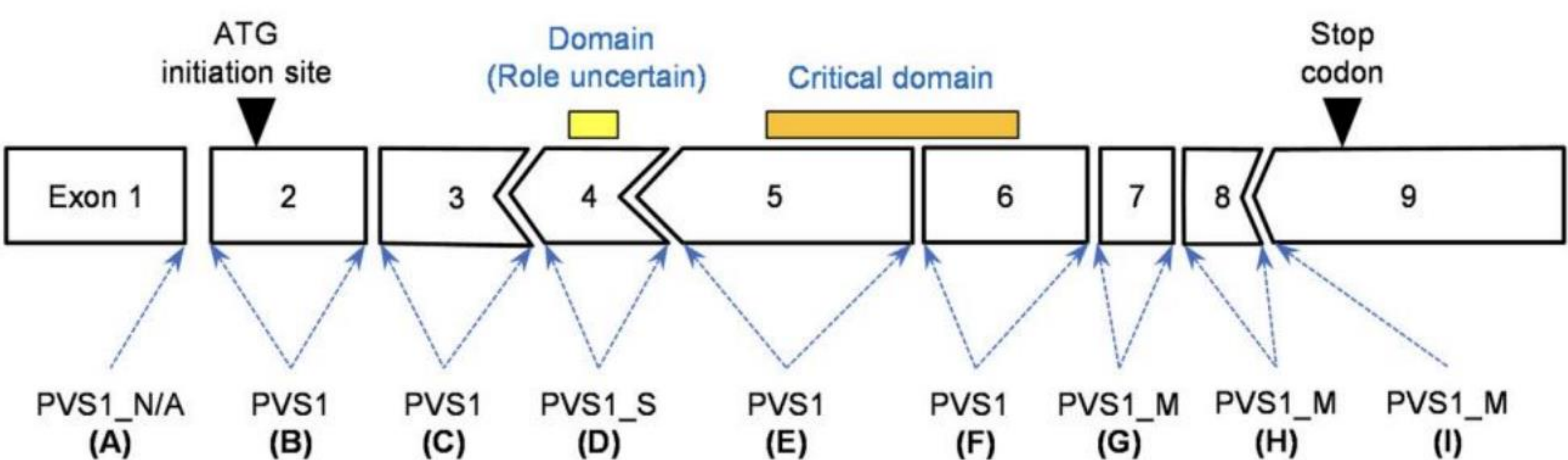
### Clinical interpretation

What does it mean?  
PVS1(RNA) strength and  
updated ACMG classification

### RNA-seq review

- Visualized matched RNA-seq in IGV
- Compared variant-containing samples with controls
- Classified observed effects:
  - exon skipping,
  - intron retention
  - cryptic splice-site use
  - no measurable effect
- BRCA2 and NF1 served as positive controls

### Gene-specific PVS1 assessment



- PVS1(RNA) strength depends on where the RNA event occurs and what it does to the transcript/protein.
- High-impact events include start codon loss, frameshift with predicted NMD, or loss of a critical protein region.
- Lower-impact/noncoding events or genes without an established LoF mechanism are not automatically upgraded.

## Results

87

variants  
assessed

39

RNA-seq  
informed PVS1  
ratings

22

gene-specific  
reassessments

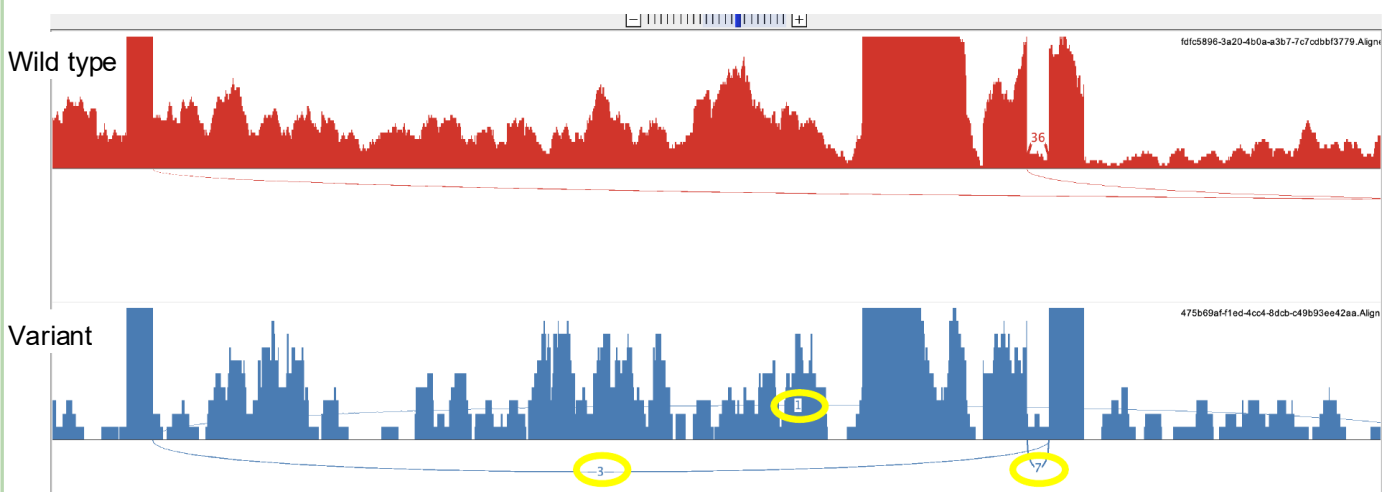
4

classification  
labels revised

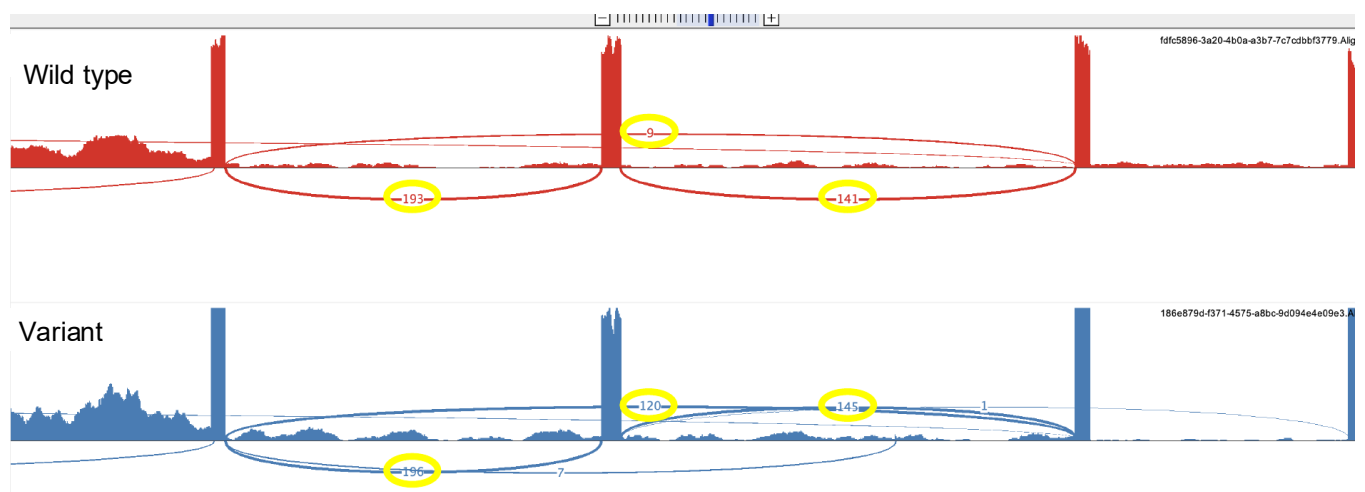
PVS1(RNA) strengths:  
16 very strong • 10 strong • 13 moderate  
Gene-specific outcomes among 22 reassessed variants:  
13 full-strength PVS1 • 7 PVS1\_M • 2 PVS1 N/A

### Revised classification RNA findings

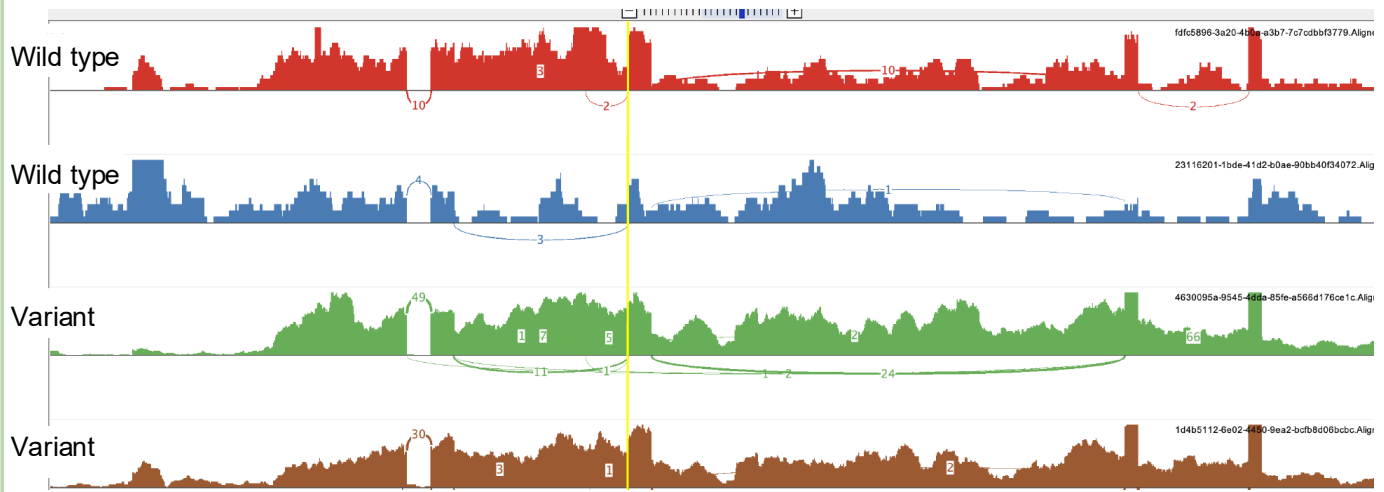
#### IFNAR1 — exon skipping; predicted NMD



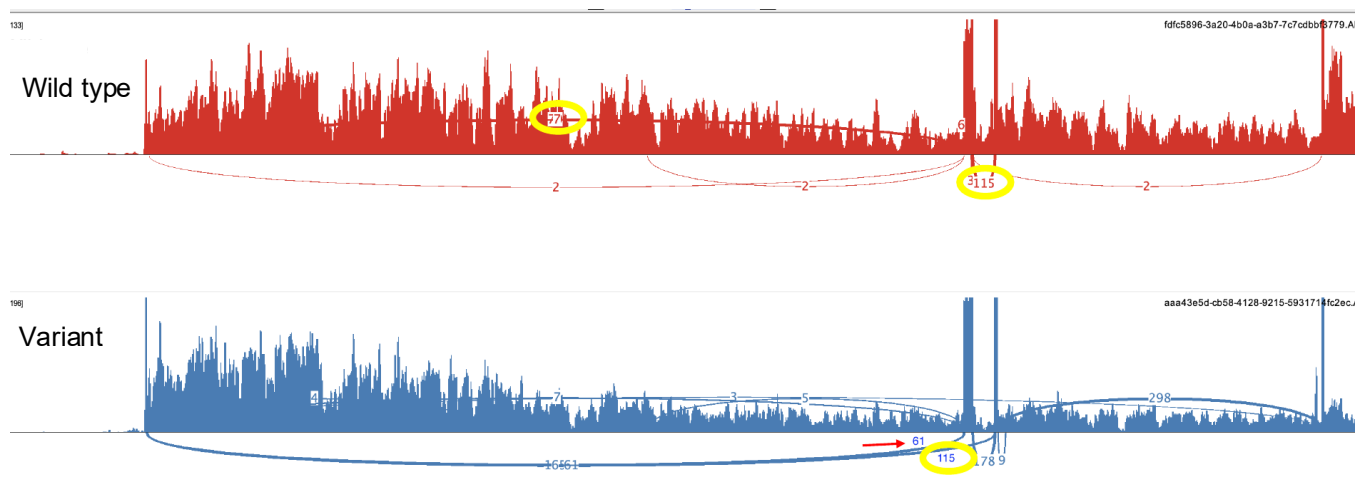
#### RNASEH2B — exon skipping; predicted NMD



#### CARD9 — intron retention; predicted NMD



#### SYK — exon skipping; LoF caution



### Biologic profile of revised genes

- IFNAR1 — type I interferon receptor; biallelic LoF causes severe viral susceptibility.
- RNASEH2B — RNase H2 subunit; biallelic LoF causes Aicardi–Goutières syndrome.
- CARD9 — innate immune adaptor for fungal recognition/NF-κB signaling; biallelic LoF causes invasive fungal susceptibility.
- SYK — immune-receptor tyrosine kinase; known dominant disease is primarily gain-of-function, so LoF interpretation requires caution.

Observed RNA abnormalities are usually only upgraded when the transcript consequence matched the gene's established disease mechanism.

## Conclusions & Next Steps

- RNA-seq provided direct evidence of variant-associated splicing.
- Controls prevented overcalling physiologic/background alternative splicing.
- Gene-specific PVS1 assessment translated RNA abnormalities into ACMG evidence.
- 4/22 reassessed variants were revised to likely pathogenic or pathogenic.
- Next step: evaluate association with treatment toxicity and outcomes.

**RNA observation plus gene-specific biology improves germline variant classification in pediatric AML. Proper classification can influence surveillance, treatment selection, transplant donor choice, and testing of relatives.**