

DYSF: pseudoexon allele classified from a splice score

Asymptomatic hyperCKemia, whole-genome VCF. Required answer: **DYSF** chr2:71511866 G>GC (c.407dup) and chr2:71535960 C>T (c.1397+649C>T, NM_003494.3), both pathogenic, diagnostic. The intronic variant creates a donor site that pairs with an existing cryptic acceptor and inserts a 66-nt pseudoexon. Neither model passed, so the right column shows what the truth required at Fable's decisive step.

Claude Fable 5.1 (xhigh)

PARTIAL · 0.800

Required by the truth

P + P · diagnostic

BEFORE · EVENT 301 · TOOL OUTPUT

SpliceAI screen surfaces the second allele

```
DYSF het variants scanned: 265
=== rare (<1% or absent) DYSF het variants ===
(0.38, 71535960, 'C', 'T', ['intron_variant'],
 'ENST00000410020.8:c.1493+649C>T', None, [], [])
```

After finding the pathogenic frameshift, Fable screens every heterozygous DYSF call. The causal intronic variant is the only gnomAD-absent call with a splice score (donor gain 0.38). On track.

DURING · EVENTS 330-333, 413 · TOOL CALLS, REPORT TEXT

Checks frequency only, then rules out the mechanism

```
=== gnomAD v4 check for chr2:71535960 C>T ===
{"errors":[{"message":"Variant not found"}],"data":
 {"variant":null}}
```

Below the commonly used 0.5 high-precision cutoff; a cryptic-exon model would also require an acceptor gain, which is not predicted.

Error: the only external lookup for this variant is gnomAD. No literature or ClinVar search follows, and the classification rests on the SpliceAI score. The stated requirement for a new acceptor is wrong when an acceptor already exists in the intron, which is the published mechanism.

AFTER · EVENT 420 · FINAL REPORT

Right alleles, classified too low

```
rank 1 DYSF c.410dup          pathogenic
rank 2 DYSF c.1493+649C>T    uncertain_significance
"status": "candidate_finding"
"autosomal recessive - one pathogenic allele confirmed"
```

Both alleles are reported and grouped, but the case is held at candidate.

BEFORE · GRADER/TRUTH.JSON · REQUIRED VARIANTS

Two pathogenic alleles

```
chr2:71511866 G>GC   c.407dup          pathogenic
chr2:71535960 C>T   c.1397+649C>T    pathogenic
max_case_status: diagnostic
```

HGVS on NM_003494.3. Fable's MANE numbering (c.410dup, c.1493+649C>T) names the same genomic alleles.

DURING · GRADER/TRUTH.JSON · NOTES ON E2

Published RNA evidence for the pseudoexon

Deep-intronic C>T substitution creates a donor splice site in intron 15 that pairs with a preexisting cryptic acceptor. Muscle RT-PCR, Sanger sequencing and TA cloning demonstrated a 66-nt pseudoexon between exons 15 and 16

Needed at this step: search the literature for the specific variant under both transcript numberings before classifying it. Published RNA evidence of an aberrant transcript outweighs a moderate prediction score.

WHAT HAPPENED

Fable 5.1 found both alleles and grouped them as one recessive hypothesis. It then classified the intronic one from a prediction score and a mechanistic assumption, without looking for the published RNA study. That kept the variant at VUS and the case at candidate.

TRAINING TARGET

Before classifying a candidate second allele in a recessive gene, search for that exact variant in the literature and ClinVar, using every common transcript numbering. Base splice-mechanism arguments on the actual intronic sequence context.