RS1 PVS1 Decision Tree

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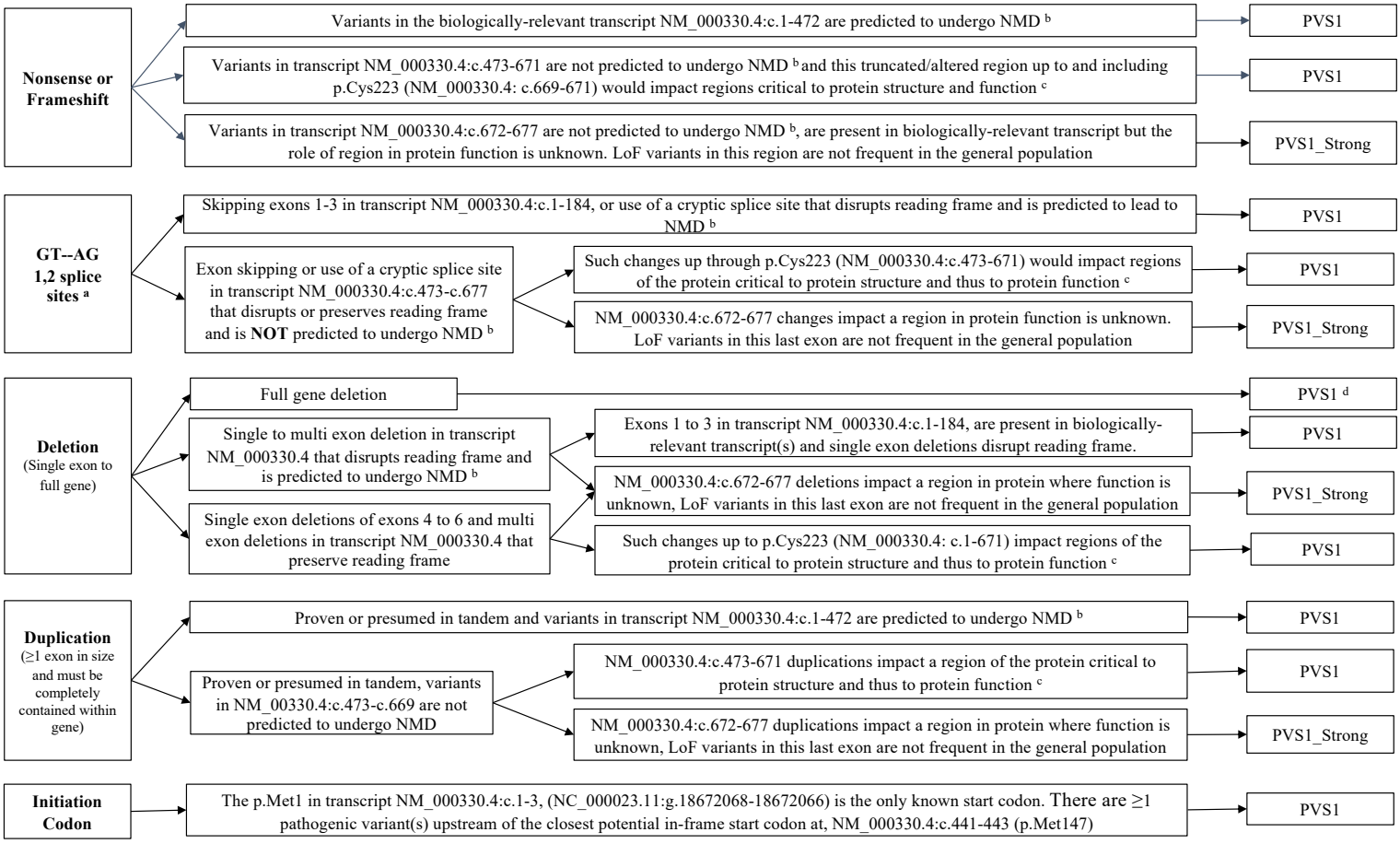
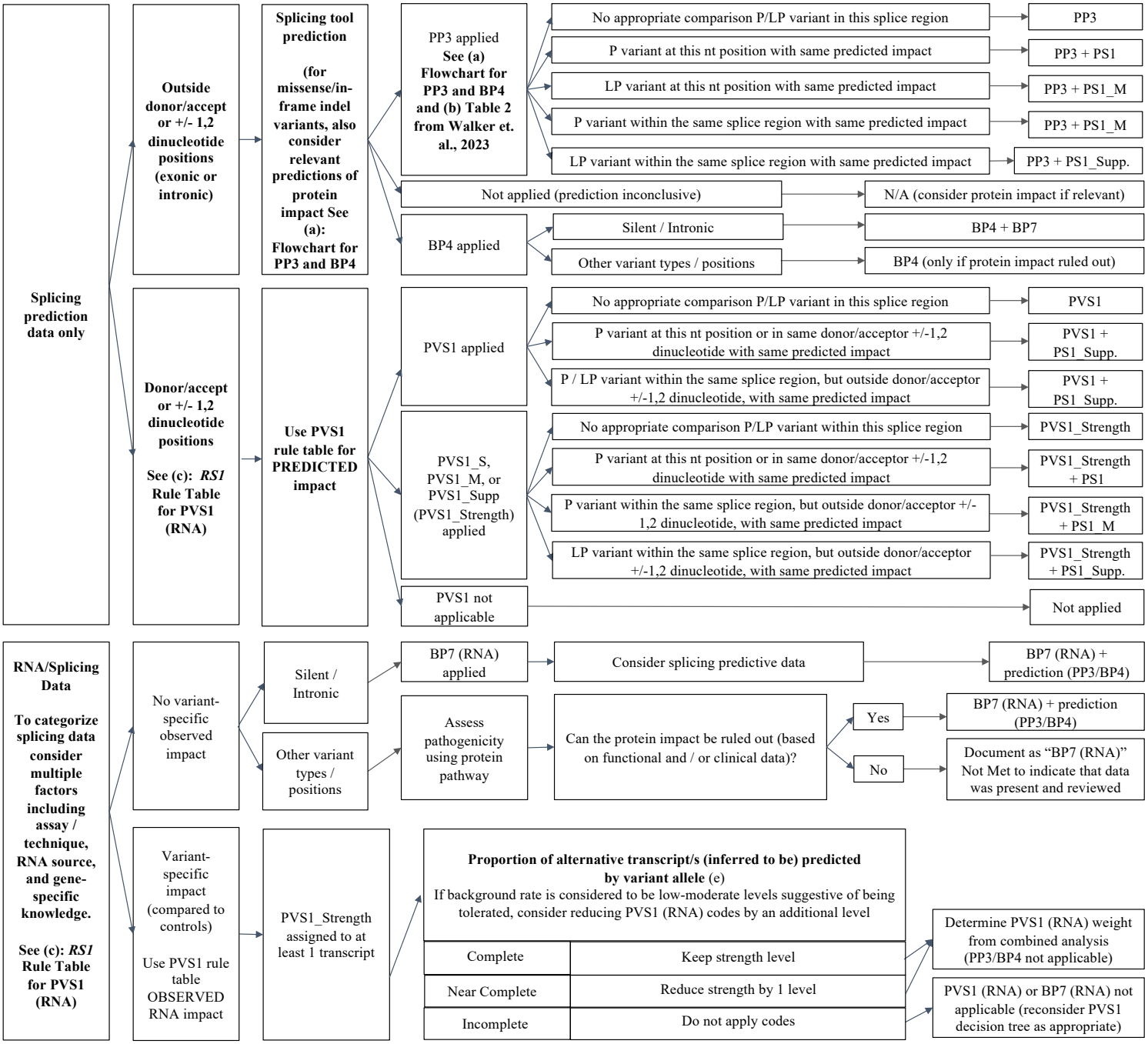
PVS1 Decision Tree for *RS1*

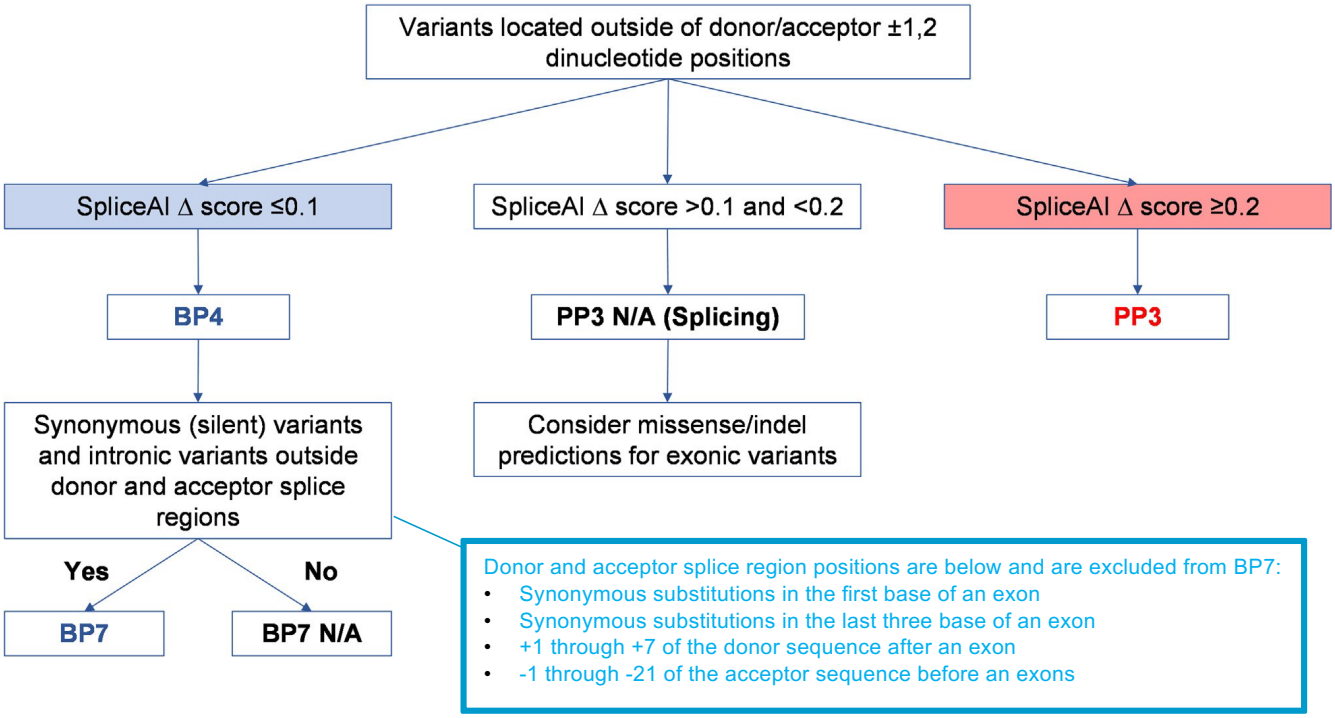


Legend for I:
RS1 PVS1 Decision Tree. This is taken from Figure 1 in SVI recommendations for PVS1 interpretation (Tayoun et al., 2018). See also *RS1* PVS1 (RNA) Decision Tree below (taken from Walker et al 2023). (a) This criterion should not be applied in combination with *in silico* splicing predictions (PP3). Additionally, splice site variants must have no detectable nearby (+/- 20nts) strong consensus splice sequence that may constitute in-frame splicing. (b) NMD prediction based on the premature termination codon not occurring in the 3' most exon or in the 3' most 50bp of the penultimate exon (p.Ser2-p.Asp158 in transcript NM_000330.4:c.4-472, NC_000023.11:g. 18,672,065-18,644,575). (c) Relevant domain indicated by experimental evidence proving a critical role of the domain and/or presence of non-truncating pathogenic variants in the region NM_000330.4:c.1-671, include the discoidin domain, and specific residues involved in forming disulfide bridges and salt bridges that are important for protein structure. (d) For a full gene deletion of a known haploinsufficient gene, a pathogenic classification is warranted (in the absence of conflicting data) even though application of PVS1 alone would not reach a pathogenic classification using the combining rules in Richards et al., 2015). For a full gene deletion of *RS1* in a male, a pathogenic classification is warranted. NMD, nonsense-mediated decay; LoF, loss of function.

RS1-specific PVS1 (RNA) Decision Tree for Splicing



(a) SpliceAI Flowchart (based on Walker et al., 2023, Figure 4)



(b) Table 2 from Walker et al., 2023

Table 2. PS1 code weights for variants with same predicted splicing event as a known (likely) pathogenic variant

Variant under assessment (VUA)	Baseline computational/ predictive code applicable to VUA	Position of comparison variant relative to VUA	PS1 code applicable to VUA	
			with P comparison variant	with LP comparison variant
Located outside splice donor/ acceptor ±1,2 dinucleotide positions	PP3	same nucleotide	PS1	PS1_Moderate
	PP3	within same splice donor/acceptor motif (including at ±1,2 positions)	PS1_Moderate	PS1_Supporting
Located at splice donor/acceptor ±1,2 dinucleotide positions	PVS1	within same splice donor/ acceptor ±1,2 dinucleotide	PS1_Supporting	N/A
	PVS1	within same splice donor/acceptor region, but outside ±1,2 dinucleotide[a]	PS1_Supporting	PS1_Supporting
	PVS1_Strong, PVS1_Moderate, or PVS1_Supporting	within same splice donor/ acceptor ±1,2 dinucleotide	PS1	N/A
	PVS1_Strong, PVS1_Moderate, or PVS1_Supporting	within same splice donor/acceptor motif, but outside ±1,2 dinucleotide[a]	PS1_Moderate	PS1_Supporting

Prerequisite for all: the predicted event of the VUA must precisely match the predicted event of the comparison (likely) pathogenic variant (e.g., both predicted to lead to exon skipping, or both to lead to enhanced use of a cryptic splice motif, AND the strength of the prediction for the VUA must be of similar or higher strength than the strength of the prediction for the comparison [likely] pathogenic variant). For an exonic variant, predicted or proven functional effect of missense substitution(s) encoded by the VUA and (likely) pathogenic variant should also be considered before application of this code. Dinucleotide positions refer to donor and acceptor dinucleotides in reference transcript(s) used for curation. Designated donor and acceptor motif ranges should be based on position weight matrices for intron category (see methods). For GT-AG introns these are defined as follows: the donor motif, last 3 bases of the exon and 6 nucleotides of intronic sequence adjacent to the exon; acceptor motif, first base of the exon and 20 nucleotides upstream from the exon boundary. Consider other motif ranges for non-GT-AG introns.

[a]If relevant, splicing assay data for a pathogenic variant outside a ±1,2 dinucleotide position may be used to update a PVS1 decision tree and hence the applicable PVS1 code for a ±1,2 dinucleotide variant.

(c) *RS1* PVS1 (RNA) rule table (for +- 1,2 changes and RNA splicing assays) for in the transcript NM_000330.4.

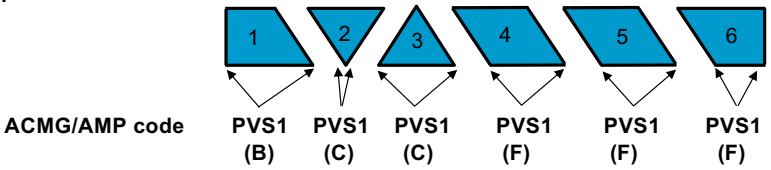
Based on generic gene schematic proposed by Walker et al., 2023 with the following modifications:

1. Pathogenic missense variants have been identified in exons 1-6 which are all considered to be "critical to protein function", requirement for being more that 10% of total protein length does not apply.
2. ATG initiation site is located in exon 1 so 5' UTR recommendation (A) does not apply.
3. No potential "rescue isoforms" are known.
4. Use these tables to assign appropriate PVS1 code and rationale:

NM_000330.4	Transcript start 3' acceptor position	Transcript end 5' donor position	HG38 coordinates (reverse strand)		Exon skipping leads to preserved reading frame or frameshift (FS) with nonsense mediated decay (NMD)		PVS1 code for +/- 1,2 dinucleotide change and rationale from Walker et al. below	Critical exon?	Domain
			Genome start	Genome ends	FS	NMD?			
Exon 1	c.-40	c.52	18,672,108	18,672,017	FS	NMD	PVS1 (B)	Yes	Leader sequence
Exon 2	c.53	c.78	18,657,665	18,657,640	FS	NMD	PVS1 (C)	Yes	Leader sequence
Exon 3	c.79	c.184	18,656,758	18,656,653	FS	NMD	PVS1 (C)	Yes	RS1 domain
Exon 4	c.185	c.326	18,647,332	18,647,191	In-frame	NMD	PVS1 (F)	Yes	Discoidin domain
Exon 5	c.327	c.522	18,644,625	18,644,430	In-frame	No NMD	PVS1 (F)	Yes	Discoidin domain
Exon 6	c.523	c.*2316	18,642,156	18,639,688	In-frame	No NMD	PVS1 (F)	Yes	Discoidin domain

NM_000330.4	Transcript start 3' acceptor position	Transcript end 5' donor position	Notes
Start codon	c.1-3	p.1	
Nearest in-frame start codon	c.441-443	p.147	This alternate start codon is in exon 5.
Next nearest in-frame start codon	c.639-641	p.231	This alternate start codon is in exon 6.
Stop codon	c.675-677	p.225	
NMD predictd cutoff NM_000330.4	c.472		
Stop codon after stop-loss	c.*127		Adds 43 amino acids

(d) *RS1* exon map: The retinal-specific transcript NM_000330.4 is shown. Splice sites for exons 1-6 are indicated. The overhang shown at the top is a two-nt overhang, overhang on bottom is a one-nt overhang. Parallel lines represent in-frame junctions.



(e) Generic PVS1 scoring taken from Figure 2 from Walker et al., 2023

(A) 5' UTR region - No splicing alteration predicted or use of a cryptic splice motif does not affect the coding sequence.
(B) Exon skipping or use of a cryptic splice motif eliminates the initiation codon and there are no alternative start codons.
(C) Exon skipping or use of a cryptic splice motif disrupts reading frame and is predicted to undergo NMD
(D) Exon skipping or use of a cryptic splice motif preserves reading frame, and removes a region (>10% of the protein) which has not been established as critical to protein function.
(E) Exon skipping or use of a cryptic splice motif disrupts reading frame and is predicted to undergo NMD
(F) Exon skipping or use of a cryptic splice motif preserves reading frame, and removes a region which has been established as critical to protein function
(G) Exon skipping or use of a cryptic splice motif preserves reading frame, and removes a region (<10% of the protein) which has not been established as critical to protein function.
(H) Exon skipping or use of a cryptic splice motif disrupts reading frame and is not predicted to undergo NMD, and removes a region (<10% of the protein) which has not been established as critical to protein function.
(I) Exon skipping or use of a cryptic splice motif disrupts reading frame and is not predicted to undergo NMD, and removes a region (<10% of the protein) which has not been established as critical to protein function.

Figure 2. Schematic demonstrating assignment of gene-specific codes to splice donor/acceptor ±1,2 dinucleotide variants based on a modified version of the original ClinGen SVI PVS1 framework
Original framework refers to recommendations as published.[2] It is important to note that each PVS1 assigned weight may be reduced if there is evidence of potential rescue mechanisms. For example, skipping of either exon 4 or 7 may lead to a protein that retains partial function. Annotating gene-specific lists of naturally occurring splicing events can provide greater evidence of potential "rescue" isoforms. Also see Box S1.