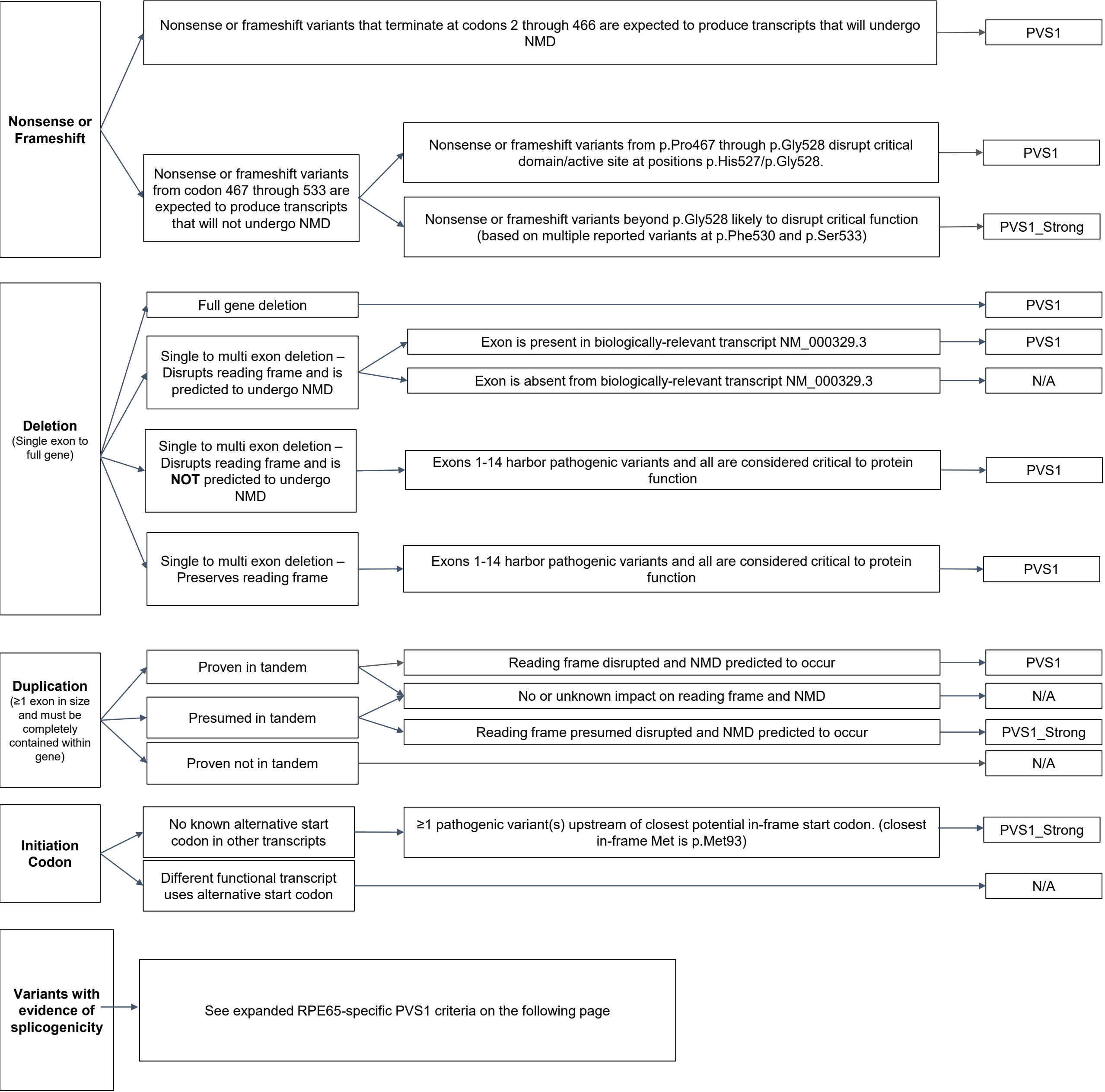
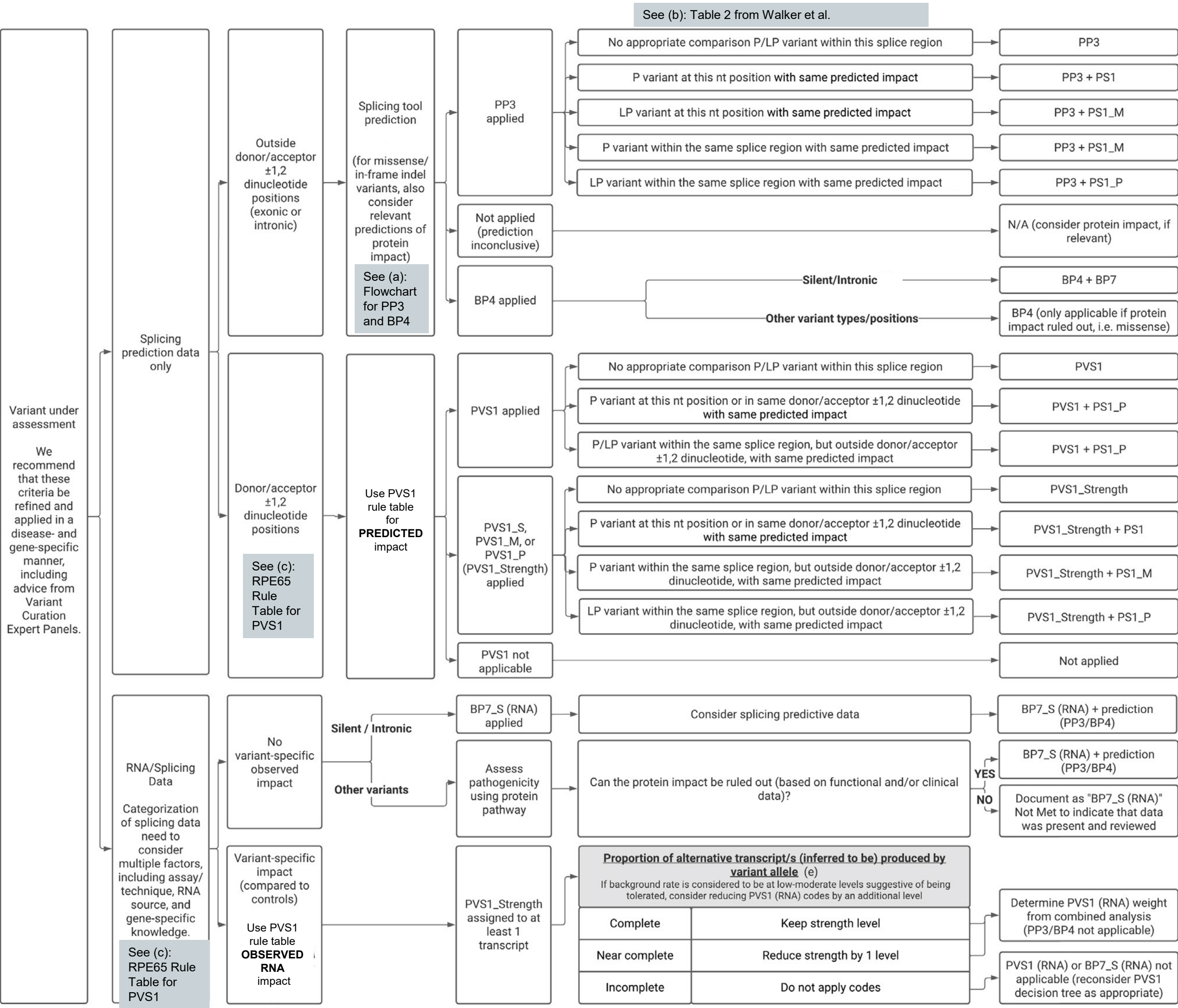


RPE65-specific PVS1 criteria – based on ClinGen SVI Working Group publication of Tayoun 2018 (PMID:30192042) and the PVS1 decision tree of ClinGen SVI Splicing Subgroup (Walker 2023, PMID: 37352859)



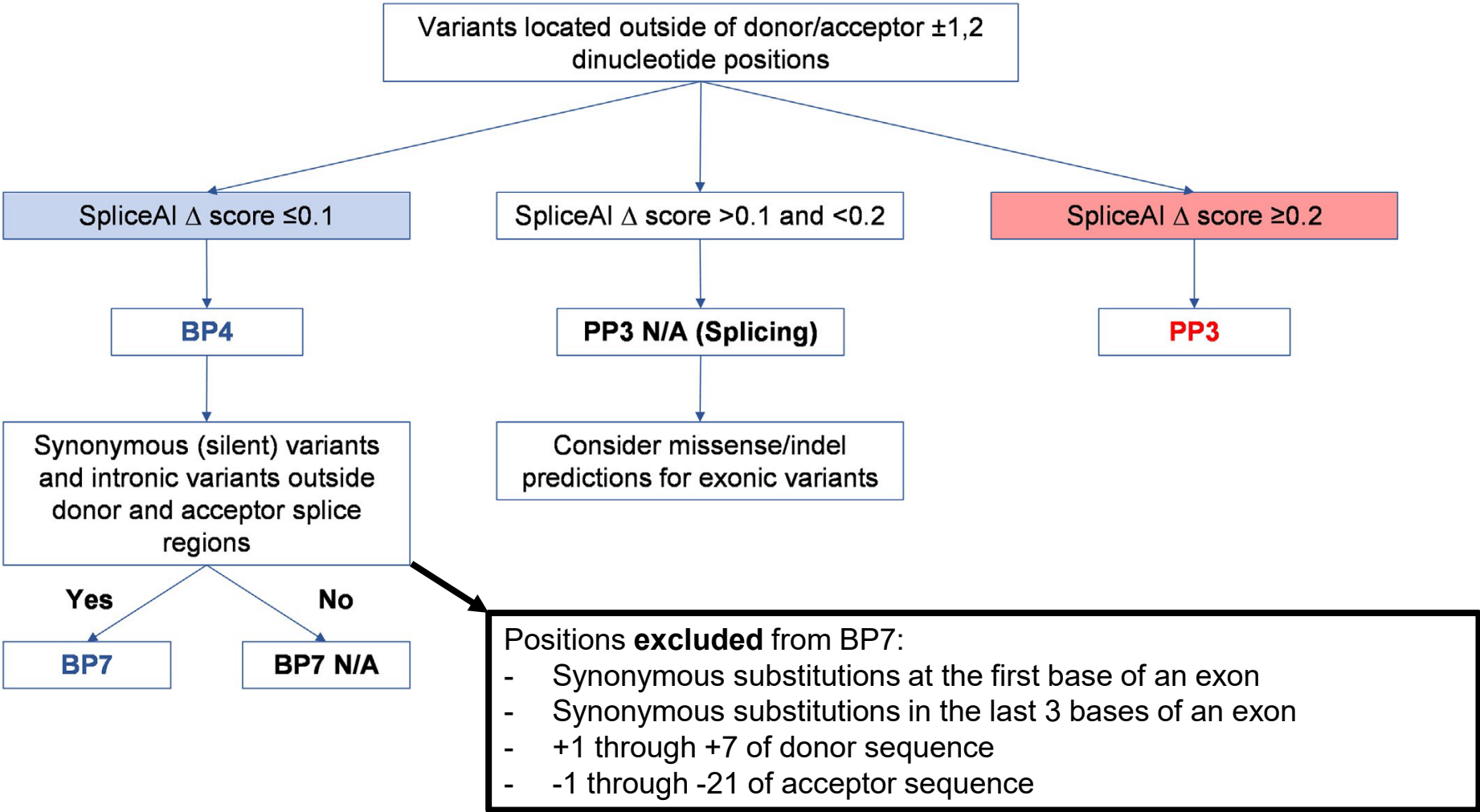
RPE65-specific PVS1 decision tree for splicing



Gene-specific modifications for RPE65 (additional details on following pages):

- (a) SpliceAI Flowchart (based on Walker et al., 2023) for PP3 and BP4 – calibrated cutoff scores for SpliceAI splicing prediction. If BP4 applies, consider BP7 based on location of variant.
- (b) Table 2 from Walker et al., 2023: If PP3 applies, consider PS1 code weights for variants with the same predicted splicing event as a known (likely) pathogenic variant.
- (c) RPE65 rule table for PVS1 – based on Walker et al. but RPE65-specific.
- Use when variant under assessment affects the donor/acceptor +/- 1,2 dinucleotide positions. As noted in Walker et al., PS1 code can be added where applicable.
 - Use to evaluate RNA splicing data - **observed** results of the splicing assay are classified in the same manner as predicted results. As detailed in flowchart above, use PVS1(RNA) if there is evidence of impact on splicing or BP7_Strong(RNA) if evidence suggests no impact.

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



(b) Table 2 from Walker 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.

^aIf 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.

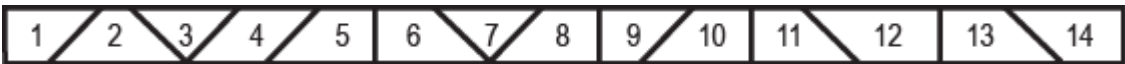
(c) RPE65 PVS1 rule table (for +- 1,2 changes and RNA splicing assays):

Based on generic gene schematic (shown below) proposed by Walker et al with the following modifications:

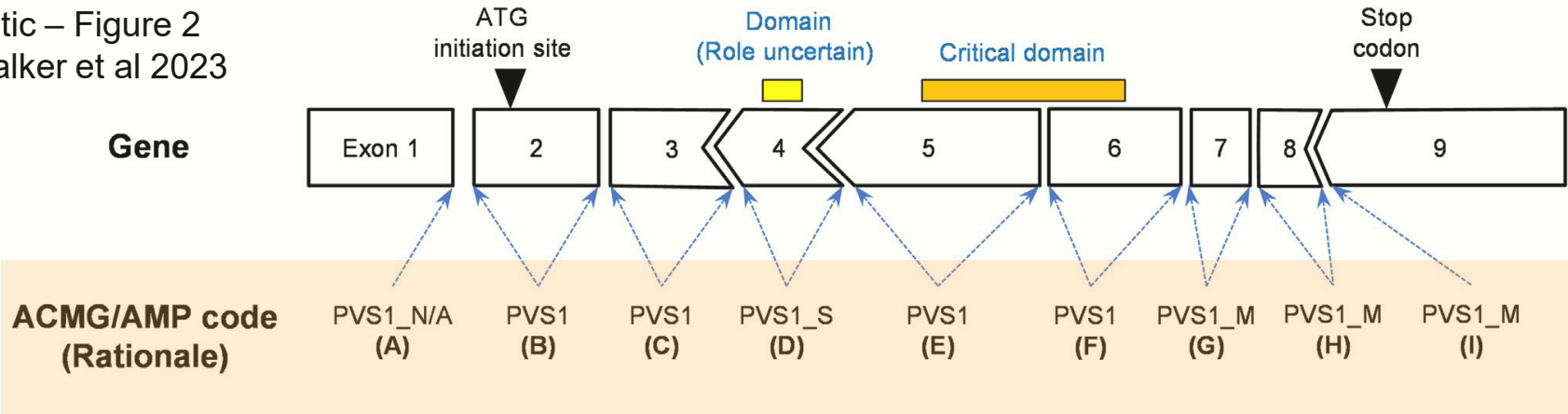
- 1. Pathogenic variants have been identified in every exon so all exons are considered to be “critical to protein function”, requirement for being more than 10% of total protein length does not apply.
- 2. ATG initiation site is located in exon 1 so 5’ UTR recommendation (A) does not apply
- 3. No potential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

	3' acceptor position	5' donor position	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	exon known to be critical to protein function
exon 1		c.11	fs/nmd	PVS1 (B)	yes
exon 2	c.12	c.94	fs/nmd	PVS1 (C)	yes
exon 3	c.95	c.245	fs/nmd	PVS1 (C)	yes
exon 4	c.246	c.353	in frame/no nmd	PVS1 (F)	yes
exon 5	c.354	c.495	fs/nmd	PVS1 (C)	yes
exon 6	c.496	c.643	fs/nmd	PVS1 (C)	yes
exon 7	c.644	c.725	fs/nmd	PVS1 (C)	yes
exon 8	c.726	c.858	fs/nmd	PVS1 (C)	yes
exon 9	c.859	c.998	fs/nmd	PVS1 (C)	yes
exon 10	c.999	c.1128	fs/nmd	PVS1 (C)	yes
exon 11	c.1129	c.1243	fs/nmd	PVS1 (C)	yes
exon 12	c.1244	c.1338	fs/nmd	PVS1 (C)	yes
exon 13	c.1339	c.1450	fs but no nmd because new stop is still in the final exon	PVS1 - no equivalent in generic model - transcript is not expected to undergo nmd but is also considered critical to protein function, therefore PVS1 at standard level.	yes
exon 14	c.1451		N/A	PVS1 - no equivalent in generic model - transcript is not expected to undergo nmd but is also considered critical to protein function, therefore PVS1 at standard level.	yes

RPE65 exon map: overhang on top is a two-nt overhang, overhang on bottom is a one-nt overhang. Parallel lines represent in-frame junctions (eg. del of exons 8+9 is in frame, del of 7+8 is out of frame)



Generic PVS1 schematic – 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.