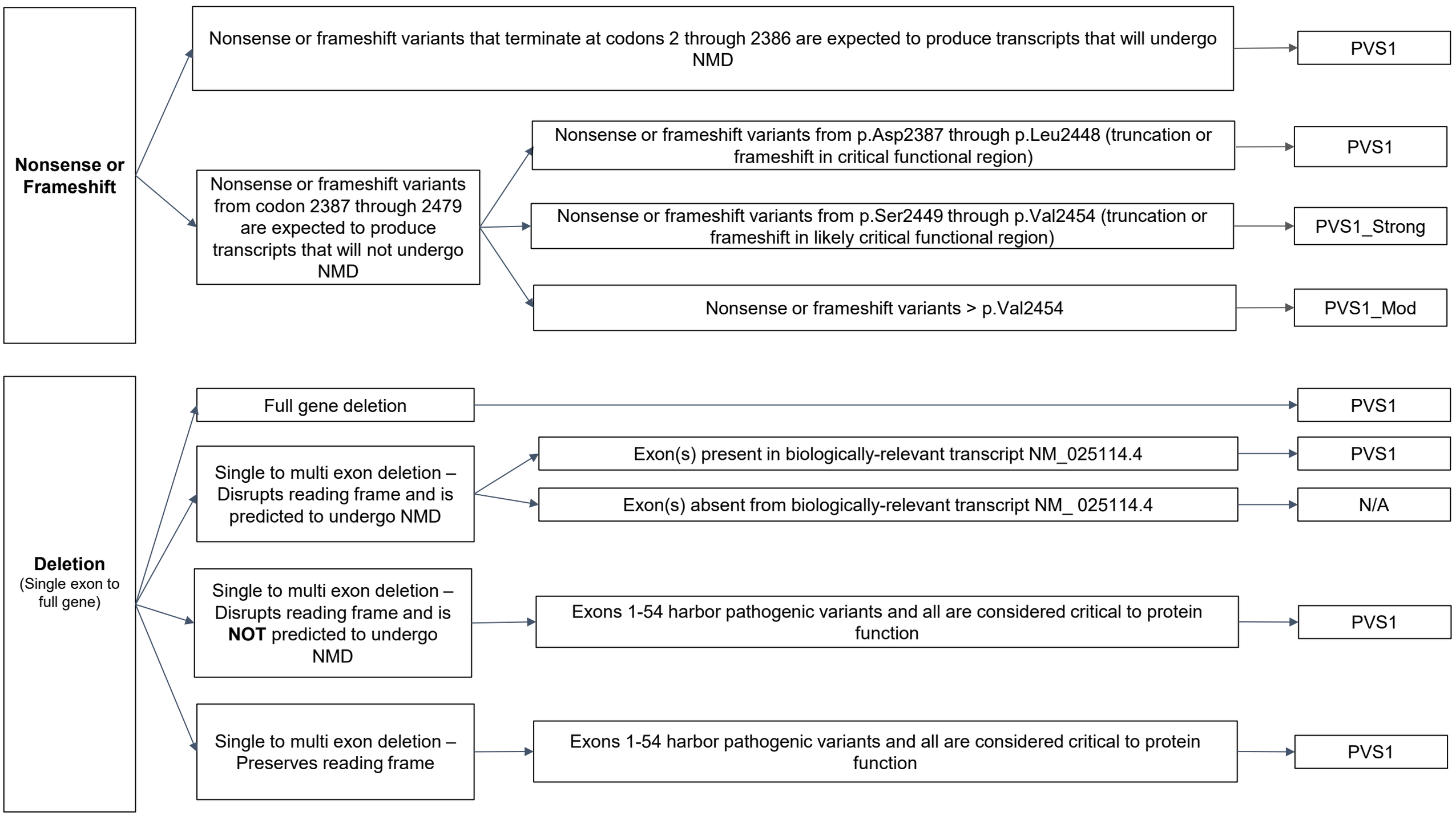
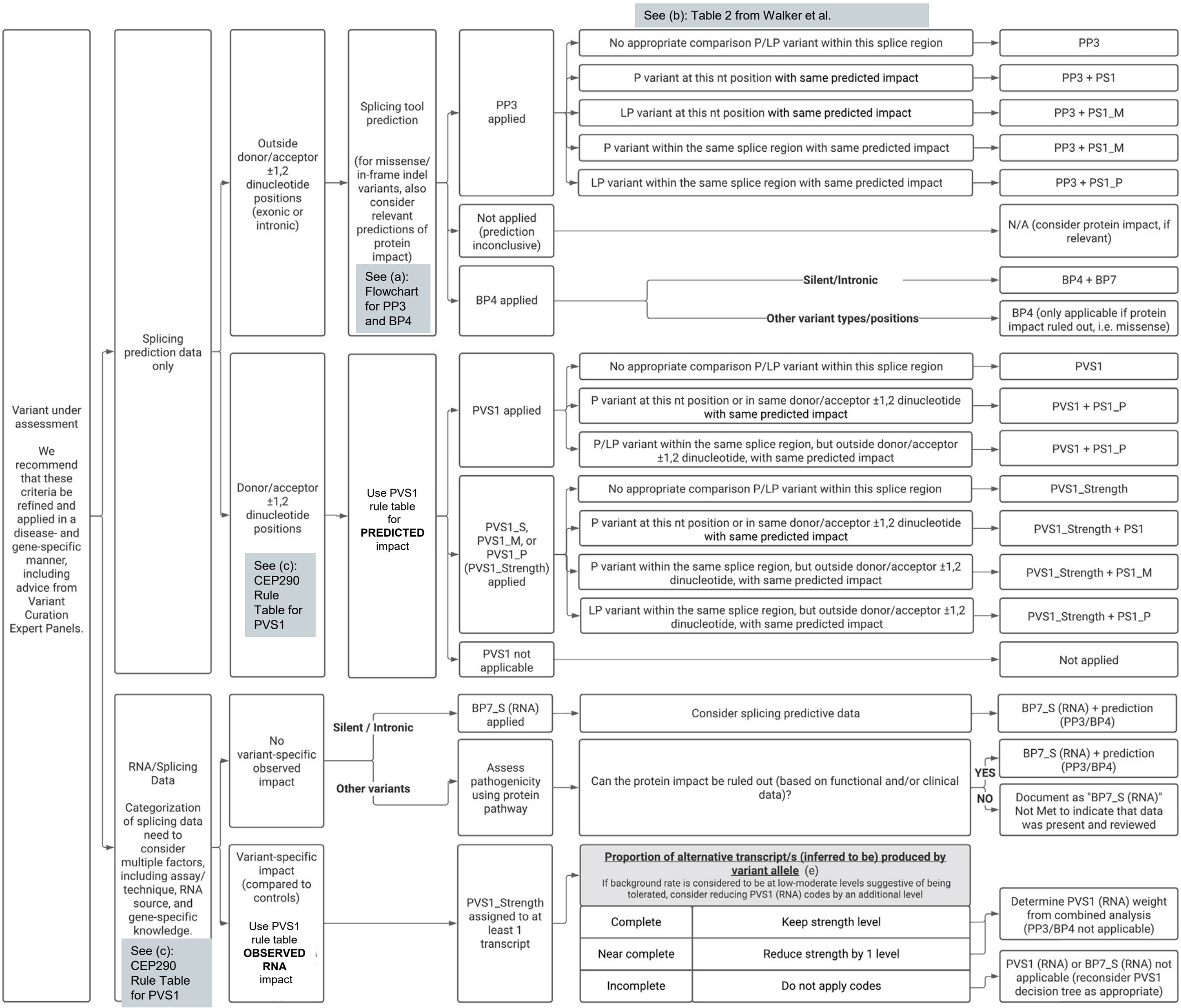
CEP290-specific PVS1 criteria – based on ClinGen SVI Working Group publication of Tayoun 2018 (PMID:30192042) and the PVS1 decision tree of ClinGen SVI Splicing Subgroup (Walker 2023, PMID: 37352859)



CEP290-specific PVS1 decision tree for splicing

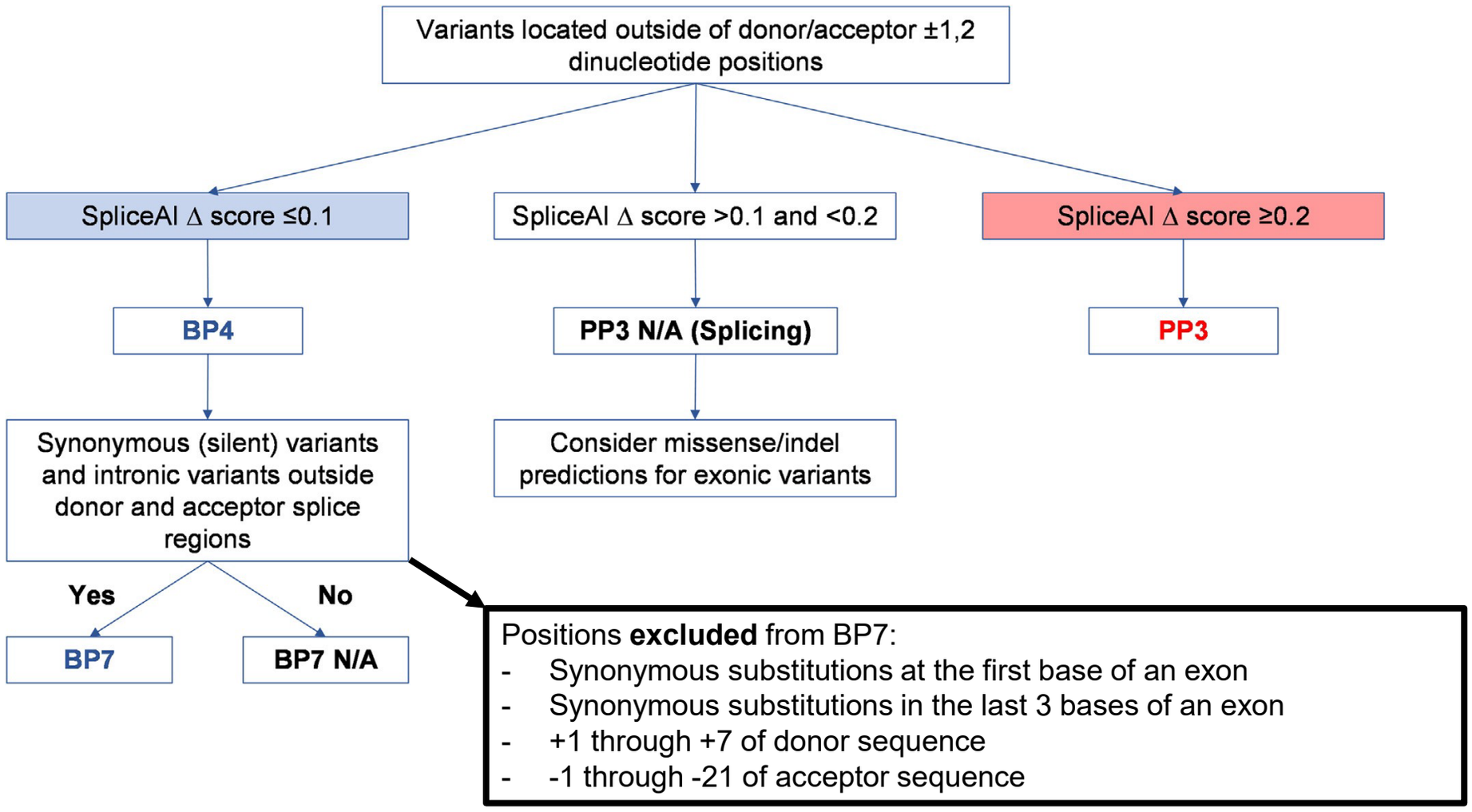


Gene-specific modifications for CEP290 (additional details on following pages):

(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) CEP290 rule table for PVS1 – based on Walker et al. but CEP290-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.

[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) *CEP290* PVS1 rule table (for +- 1,2 changes and RNA splicing assays):

Based on proposal by Walker et al 2023 with the following modifications:

1. All exons are considered to be “critical to protein function” based on either frameshifts (see below), pathogenic variants having been identified flanking them (exons 2-8 and 11-53), or being required for CEP290 self-association (exons 9-10, see below).
2. Requirement for being more than 10% of total protein length does not apply.
3. ATG initiation site is located in exon 2
4. No potential “rescue isoforms” are known

Use this table to assign appropriate PVS1 code:

exon	c.start	c.end	length (bp)	aa start	aa end	protein domains	PVS1 strength	exon skipping leads to preserved reading frame or frameshift (fs) with nmd?
1	-344	-28	317				NA	
2	-27	102	129	1	34	self-association	PVS1	In frame/no nmd
3	103	180	78	35	60	CCI, self-association	PVS1	In frame/no nmd
4	181	250	70	61	84	CCI,self-association	PVS1	fs/nmd
5	251	297	47	84	99	CCI, self-association	PVS1	fs/nmd
6	298	441	144	100	147	CCI, self-association	PVS1	In frame/no nmd
7	442	495	54	148	165	CCI, self-association	PVS1	In frame/no nmd
8	496	516	21	166	172	CCI, self-association	PVS1	In frame/no nmd
9	517	669	153	173	223	CCI, self-association	PVS1	In frame/no nmd
10	670	852	183	224	284	CCI, TM I, self-association	PVS1	In frame/no nmd
11	853	942	90	285	314	CCI, self-association	PVS1	In frame/no nmd
12	943	1065	123	315	355	CCI, self-association	PVS1	In frame/no nmd
13	1066	1189	124	356	397	CCI, TM II, self-association	PVS1	fs/nmd
14	1190	1359	170	397	453	CCI, self-association	PVS1	fs/nmd
15	1360	1522	163	454	508	CCI, TM III, self-association	PVS1	fs/nmd
16	1523	1623	101	508	541	CCI, self-association	PVS1	fs/nmd
17	1624	1711	88	542	571	CCI, self-association	PVS1	fs/nmd
18	1712	1824	113	571	608	CC II, self-association	PVS1	fs/nmd
19	1825	1909	85	609	637	CC II, self-association	PVS1	fs/nmd
20	1910	2052	143	637	684	CC II, self-association	PVS1	fs/nmd
21	2053	2217	165	685	739	CC III, self-association, NPHP5 binding	PVS1	In frame/no nmd
22	2218	2367	150	740	789	CC III, CC IV, NPHP5 binding	PVS1	In frame/no nmd
23	2368	2483	116	790	828	CC IV, NPHP5 binding	PVS1	fs/nmd
24	2484	2586	103	828	862	CC IV, NPHP5 binding	PVS1	fs/nmd
25	2587	2817	231	863	939	CC IV, NPHP5 binding	PVS1	In frame/no nmd
26	2818	2991	174	940	997	CC V	PVS1	In frame/no nmd
27	2992	3103	112	998	1035	CC V	PVS1	fs/nmd
28	3104	3309	206	1035	1103	CC VI	PVS1	fs/nmd
29	3310	3461	152	1104	1154	CC VI, CC VII	PVS1	fs/nmd
30	3462	3573	112	1154	1191	CC VII	PVS1	fs/nmd
31	3574	4029	456	1192	1343	CC VIII, CC IX, KID I	PVS1	In frame/no nmd
32	4030	4194	165	1344	1398	CC IX	PVS1	In frame/no nmd
33	4195	4302	108	1399	1434	CC IX	PVS1	In frame/no nmd
34	4303	4437	135	1435	1479	CC X	PVS1	In frame/no nmd
35	4438	4704	267	1480	1568	CC X, CC XI	PVS1	In frame/no nmd
36	4705	4812	108	1569	1604	CC XI	PVS1	In frame/no nmd
37	4813	5012	200	1605	1671	CC XII	PVS1	fs/nmd
38	5013	5226	214	1671	1742	CC XII, MM binding	PVS1	fs/nmd
39	5227	5364	138	1743	1788	CC XII, MM binding	PVS1	In frame/no nmd
40	5365	5586	222	1789	1862	CC XII, MM binding	PVS1	In frame/no nmd
41	5587	5709	123	1863	1903	CC XII, KID II, MM binding, MM binding	PVS1	In frame/no nmd
42	5710	5855	146	1904	1952	CC XII, KID III, BP_NLS, MM binding	PVS1	fs/nmd
43	5856	6011	156	1952	2004	CC XII, MM binding	PVS1	In frame/no nmd
44	6012	6135	124	2004	2045	CC XII	PVS1	fs/nmd
45	6136	6270	135	2046	2090	CC XIII	PVS1	In frame/no nmd
46	6271	6357	87	2091	2119	CC XIII, P-loop	PVS1	In frame/no nmd
47	6358	6522	165	2120	2174	CC XIII	PVS1	In frame/no nmd
48	6523	6645	123	2175	2215	CC XIII, KID IV	PVS1	In frame/no nmd
49	6646	6818	173	2216	2273	CC XIII	PVS1	fs/nmd
50	6819	6960	142	2273	2320	CC XIII	PVS1	fs/nmd
51	6961	7034	74	2321	2345	CC XIII	PVS1	fs/nmd
52	7035	7129	95	2345	2377	CC XIII	PVS1	fs/nmd
53	7130	7209	80	2377	2403	CC XIII, KID V	PVS1	fs/nmd
54	7210	*171	402	2404	2479	CC XIII, KID VI	PVS1	NA
		2479 amino acids				CC, coiled-coil domain; TM, tropomyosin homology domain; KID, RepA/Rep+ protein KID; NLS_BP, bipartite nuclear localization signal; P-loop, ATP/GTP-binding site motif A (P-loop); MM, microtubule/membrane binding.		

CEP290 exon map: overhang on top indicates a two-nt overhang, overhang on bottom is a one-nt overhang. Parallel lines represent in-frame junctions (eg. del of exons 6+7 is in frame, del of 12+13 is out of frame). Locations of known protein homology are shown in table.