AIPL1-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)

Nonsense or Frameshift

- Nonsense or frameshift variants that terminate at codons 2 through 244 are expected to produce transcripts that will undergo NMD → PVS1
- Nonsense or frameshift variants from codon 245 through 384 are expected to produce transcripts that will not undergo NMD
  - Nonsense variants from p.Glu245 through p.Ser328 (truncation in critical functional region) → PVS1
  - Nonsense variants from p.Glu329 through p.Ser346 (significant truncation of the proline-rich domain(PRD)) → PVS1_Strong
  - Nonsense variants from p.Ser347 through p.His384 (~10% truncation of the proline-rich domain (PRD) → PVS1_Mod
  - Frameshift variants from p.Glu245 – p.Glu337 (likely to disrupt critical function) → PVS1
  - Frameshift variants from p.Pro338 through p.His384 (likely to disrupt critical function) → PVS1_Strong

Deletion (Single exon to full gene)

- Full gene deletion → PVS1
- Single to multi exon deletion – Disrupts reading frame and is predicted to undergo NMD
  - Exon(s) present in biologically-relevant transcript NM_014336.5 → PVS1
  - Exon(s) absent from biologically-relevant transcript NM_ 014336.5 → N/A
- Single to multi exon deletion – Disrupts reading frame and is **NOT** predicted to undergo NMD → Exons 2-6 harbor pathogenic variants and all are considered critical to protein function → PVS1
- Single to multi exon deletion – Preserves reading frame → Exons 2-6 harbor pathogenic variants and all are considered critical to protein function → PVS1

Duplication (≥1 exon in size and must be completely contained within gene)

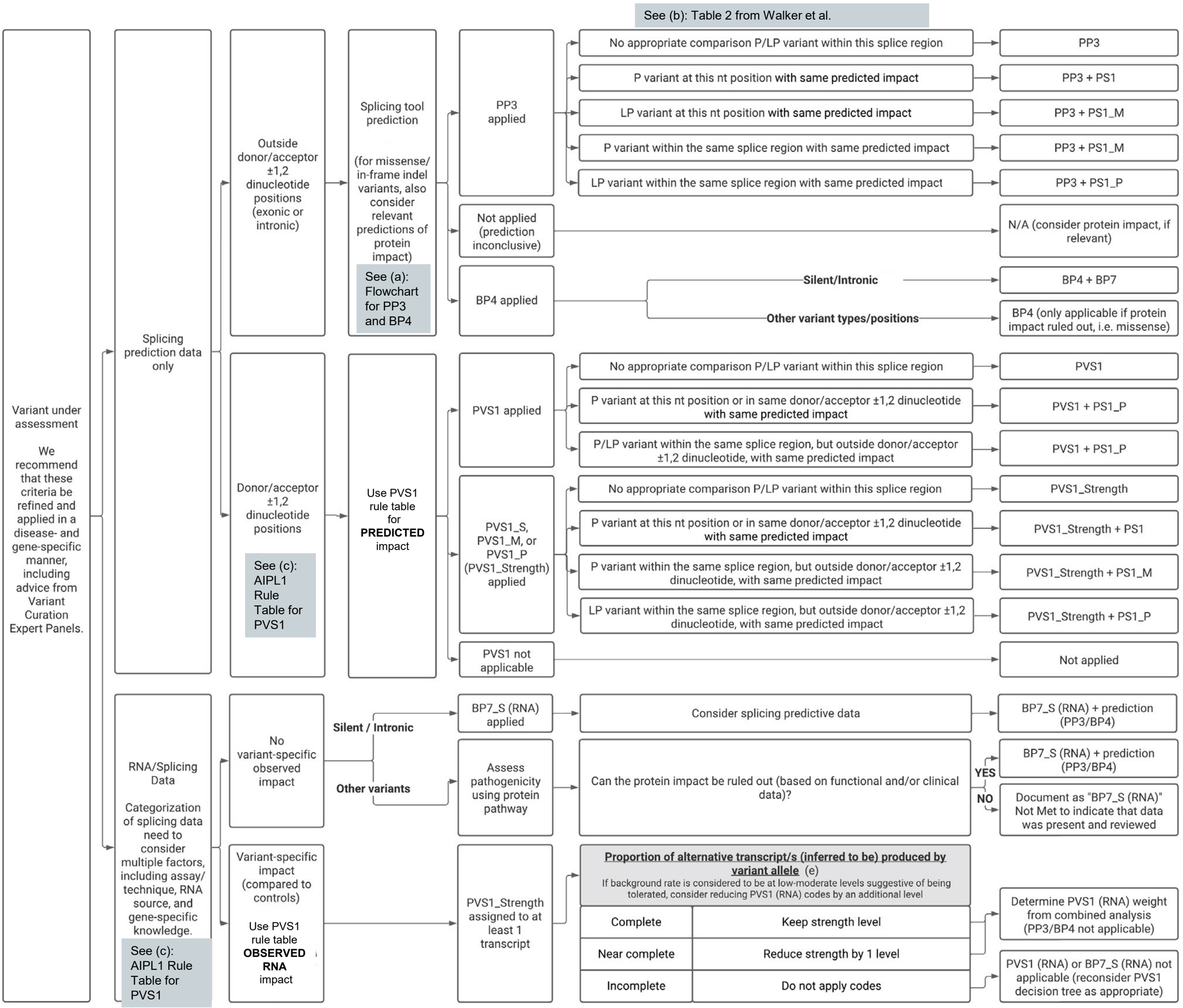
- Proven in tandem
  - Reading frame disrupted and NMD predicted to occur → PVS1
  - No or unknown impact on reading frame and NMD → N/A
  - Reading frame presumed disrupted and NMD predicted to occur → PVS1_Strong
- Presumed in tandem
  - Reading frame disrupted and NMD predicted to occur → PVS1
  - No or unknown impact on reading frame and NMD → N/A
  - Reading frame presumed disrupted and NMD predicted to occur → PVS1_Strong
- Proven not in tandem → N/A

Initiation Codon

- No known alternative start codon in other transcripts → ≥1 pathogenic variant(s) upstream of closest potential in-frame start codon. (closest in-frame Met is p.Met40) → PVS1
- Different functional transcript uses alternative start codon → N/A

Variants with evidence of splicogenicity → See expanded AIPL1-specific PVS1 criteria on the following page

AIPL1-specific PVS1 decision tree for splicing

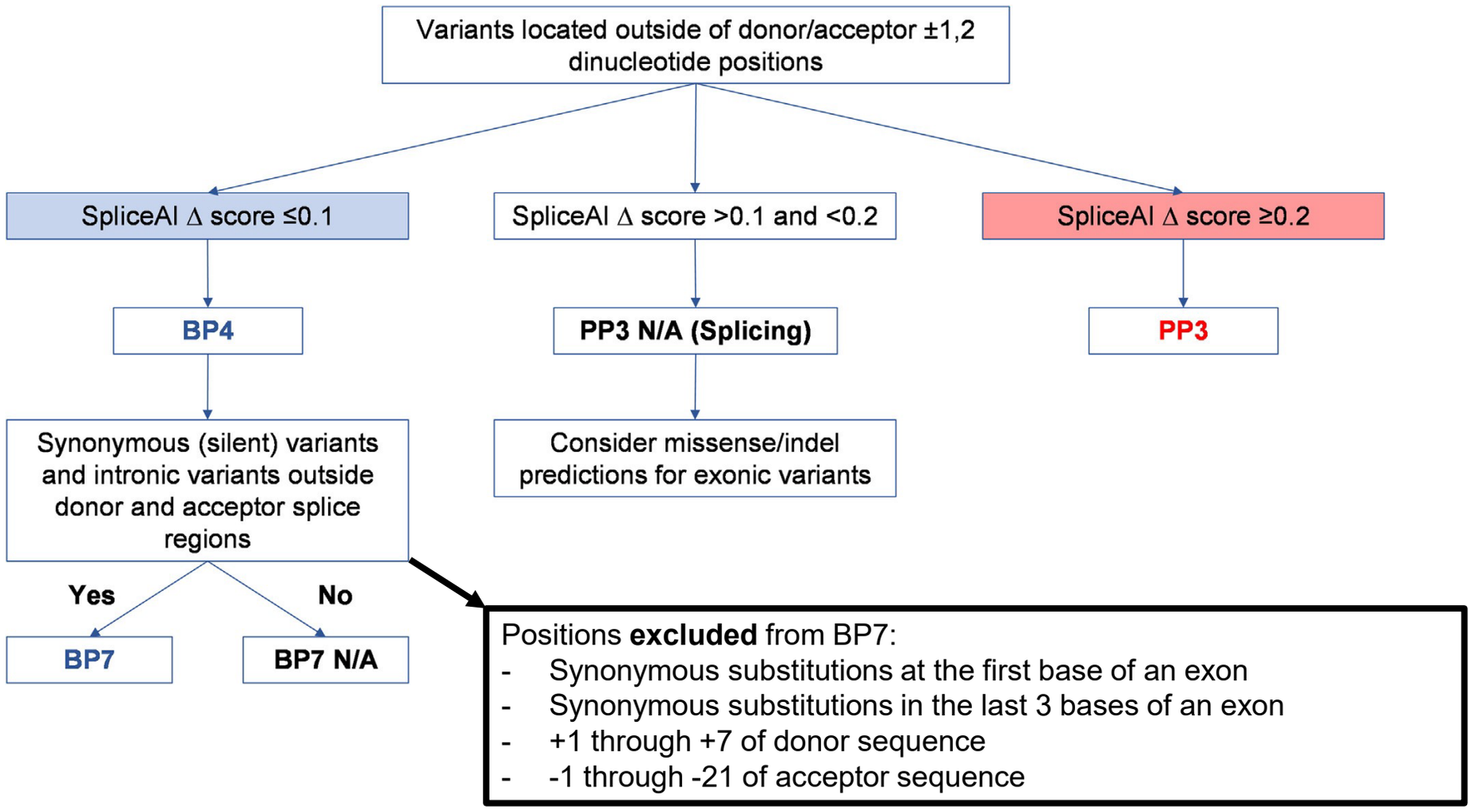


Gene-specific modifications for AIPL1 (additional details on following pages):

(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) AIPL1 rule table for PVS1 – based on Walker et al. but AIPL1-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)

AIPL1 PVS1 rule table (for +- 1,2 changes and RNA splicing assays):

Based on proposal by Walker et al 2023 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
3. No potential “rescue isoforms” are known

Use this table to assign appropriate PVS1 code:

	3' acceptor position	5' donor position	exon skipping leads to preserved reading frame or frameshift (fs) with nmd?	PVS1 code for +- 1,2 dinucleotide change and rationale from Walker et al	exon known to be critical to protein function
exon 1	NA	96	in frame/no nmd	PVS1	yes - FKBP
exon 2	97	276	in frame/no nmd	PVS1	yes - FKBP
exon 3	277	465	in frame/no nmd	PVS1	yes - FKBP
exon 4	466	642	in frame/no nmd	PVS1	yes - TPR
exon 5	643	784	fs/no nmd	PVS1	yes - TPR
exon 6	785	*1721	NA	PVS1	yes - TPR, PRD
FKBP = FK506-binding protein-like domain; TPR = Tetratricopeptide repeat domain; PRD = Proline-rich domain					

AIPL1 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 2+3 is in frame, del of 4+5 is out of frame)