

The flowchart details the criteria for PVS1 classification across five main categories:

- Nonsense or Frameshift:**
 - Predicted to undergo NMD ^b → PVS1
 - Not predicted to undergo NMD → Truncated/ altered region is critical to protein function → PVS1
- GT→AG 1,2 splice sites ^a:**
 - Exon skipping or use of a cryptic splice site disrupts reading frame and is predicted to undergo NMD ^b → PVS1
 - Exon skipping or use of a cryptic splice site disrupts reading frame and is **NOT** predicted to undergo NMD ^b → Truncated/altered region is critical to protein function) ^c → PVS1
 - Exon skipping or use of a cryptic splice site preserves reading frame (exons 1, 4, 5, 8, 9, 10) → Truncated/altered region is critical to protein function) ^c → PVS1
- Deletion (Single exon to full gene):**
 - Full gene deletion → PVS1 ^d
 - Single to multi exon deletion – Disrupts reading frame and is predicted to undergo NMD ^b → PVS1
 - Single to multi exon deletion – Disrupts reading frame and is **NOT** predicted to undergo NMD ^b → Truncated/altered region is critical to protein function ^c → PVS1
 - Single to multi exon deletion – Preserves reading frame → Truncated/altered region is critical to protein function ^c → 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
 - Presumed in tandem → No or unknown impact on reading frame and NMD → N/A
 - Proven not in tandem → Reading frame presumed disrupted and NMD predicted to occur → PVS1_Strong
- Initiation Codon:**
 - No known alternative start codon in other transcripts → No pathogenic variant(s) upstream of closest potential in-frame start codon → PVS1_Supp

(a) This criterion should not be applied in combination with in silico splicing predictions (PP3). Additionally, splice site variants must have no detectable nearby (+/- 20 nts) strong consensus splice sequence that may reconstitute in-frame splicing. (b) NMD prediction based on the premature termination codon not occurring in the 3' most exon or the 3'-most 50 bp of the penultimate exon. (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. (d) Given that GCK is a known haploinsufficient gene, a pathogenic classification is warranted for a full gene deletion (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). NMD, nonsense-mediated decay; LoF, loss of function.