Nonsense or Frameshift

- Predicted to undergo NMD [b]
  - Exon is present in biologically-relevant transcript(s) → PVS1
  - Exon is absent from biologically-relevant transcript(s) → N/A
- Not predicted to undergo NMD [b] (i.e. premature stop codon in the last exon or the last 50 nucleotides of the penultimate exon [c.826 (codon 276) in exon 7])
  - Truncated/altered region is critical to protein function – causes truncation of the transmembrane domain (which begins at amino acid 240) or any distal region (i.e. cytoplasmic domain) → PVS1_Strong
  - Role of region in protein function is unknown
    - LoF variants in this exon are frequent in the general population and/or exon is absent from biologically-relevant transcript(s) → N/A
    - LoF variants in this exon are not frequent in the general population and exon is present in biologically-relevant transcript(s)
      - Variant removes >10% of protein
        - 1+ pathogenic variant present downstream → PVS1_Strong
        - No known downstream pathogenic variants → PVS1_Moderate
      - Variant removes <10% of protein → PVS1_Moderate

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]
  - Exon is present in biologically-relevant transcript(s) → PVS1
  - Exon is absent from biologically-relevant transcript(s) → N/A
- Exon skipping or use of a cryptic splice site disrupts reading frame and is **NOT** predicted to undergo NMD [b] (i.e. premature stop codon in the last exon or the last 50 nucleotides of the penultimate exon [c.826 (codon 276) in exon 7])
  - Truncated/altered region is critical to protein function – causes truncation of the transmembrane domain (which begins at amino acid 240) or any distal region (i.e. cytoplasmic domain) → PVS1_Strong
  - Role of region in protein function is unknown
    - LoF variants in this exon are frequent in the general population and/or exon is absent from biologically-relevant transcript(s) → N/A
    - LoF variants in this exon are not frequent in the general population and exon is present in biologically-relevant transcript(s)
      - Variant removes >10% of protein
        - 1+ pathogenic variant present downstream → PVS1_Strong
        - No known downstream pathogenic variants → PVS1_Moderate
      - Variant removes <10% of protein → PVS1_Moderate
- Exon skipping or use of a cryptic splice site preserves reading frame
  - Role of region in protein function is unknown
    - LoF variants in this exon are frequent in the general population and/or exon is absent from biologically-relevant transcript(s) → N/A
    - LoF variants in this exon are not frequent in the general population and exon is present in biologically-relevant transcript(s)
      - Variant removes >10% of protein
        - 1+ pathogenic variant present within deleted region → PVS1_Strong
        - No known pathogenic variants within deleted region → PVS1_Moderate
      - Variant removes <10% of protein → PVS1_Moderate
  - Truncated/altered region is critical to protein function – causes truncation of the transmembrane domain (which begins at amino acid 240) or any distal region (i.e. cytoplasmic domain) → PVS1_Strong

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]
  - Exon is present in biologically-relevant transcript(s) → PVS1
  - Exon is absent from biologically-relevant transcript(s) → N/A
- Single to multi exon deletion – Disrupts reading frame and is **NOT** predicted to undergo NMD [b] (i.e. premature stop codon in the last exon or the last 50 nucleotides of the penultimate exon [c.826 (codon 276) in exon 7])
  - Truncated/altered region is critical to protein function – causes truncation of the transmembrane domain (which begins at amino acid 240) or any distal region (i.e. cytoplasmic domain) → PVS1_Strong
  - Role of region in protein function is unknown
    - LoF variants in this exon are frequent in the general population and/or exon is absent from biologically-relevant transcript(s) → N/A
    - LoF variants in this exon are not frequent in the general population and exon is present in biologically-relevant transcript(s)
      - Variant removes >10% of protein
        - 1+ pathogenic variant present within deleted region → PVS1_Strong
        - No known pathogenic variants within deleted region → PVS1_Moderate
      - Variant removes <10% of protein → PVS1_Moderate
- Single to multi exon deletion – Preserves reading frame
  - Truncated/altered region is critical to protein function – causes truncation of the transmembrane domain (which begins at amino acid 240) or any distal region (i.e. cytoplasmic domain) → PVS1_Strong

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
- Presumed in tandem
  - Reading frame disrupted and NMD predicted to occur → PVS1_Strong
  - No or unknown impact on reading frame and NMD → N/A
- 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 → PVS1_Moderate
  - No pathogenic variant(s) upstream of closest potential in-frame start codon → PVS1_Supp
- Different functional transcript uses alternative start codon → N/A