⚠️Pathogenic / Likely Pathogenic Criteria

🔴**Very Strong (PVS1)** - **PVS1**: Null variant (nonsense, frameshift, canonical ±1/2 splice sites, initiation codon, gene deletion) in a gene where loss-of-function (LoF) is a known disease mechanism. Variant must be predicted to undergo nonsense-mediated decay (NMD).

🔴**Strong (PS1–PS4)** - **PS1**: Same amino acid change as a previously established pathogenic variant, caused by a different nucleotide change. Must have similar or stronger functional impact. - **PS2**: De novo occurrence (confirmed maternity and paternity) in a patient with no family history. - **PS3**: Well-established functional studies (e.g., in vitro/in vivo assays) show damaging impact (e.g., <50% enzyme activity or disrupted splicing). - **PS4**: Increased prevalence of the variant in affected individuals vs. controls. Must be statistically significant, with evidence of specificity and without benign allele prevalence.

🟠**Moderate (PM1–PM6)** - **PM1**: Located in a mutational hotspot or functional domain with low benign variation. - **PM2**: Absent or extremely rare in large population databases (e.g., <0.001% allele frequency). - **PM3**: Detected in trans with a pathogenic variant in autosomal recessive disease, or part of a validated scoring system. - **PM4**: In-frame indel or length-changing variant not in a repetitive region. - **PM5**: Novel missense change at a residue where a different pathogenic missense change has been seen. Can be downgraded if effect is uncertain. - **PM6**: Assumed de novo (no family history), but without confirmed maternity and paternity.

🟡**Supporting (PP1–PP5)** - **PP1**: Co-segregation with disease in multiple affected family members. Strength varies with number of meioses. - **PP2**: Missense variant in a gene where such variants are a common disease mechanism and benign missense variants are rare. - **PP3**: Multiple computational tools predict deleterious effect (e.g., conservation analysis, splice impact predictors). - **PP4**: Patient's phenotype is highly specific to a known monogenic disorder caused by the gene. - **PP5**: Reported pathogenic by a reputable source, but no publicly available evidence.

🗓️**Combining Criteria for Pathogenic Classification** - 1 Very Strong (PVS1) + ≥1 Strong (PS) - ≥2 Strong (PS) - 1 Strong (PS) + ≥3 Moderate (PM) - 1 Strong (PS) + 2 Moderate (PM) + ≥2 Supporting (PP) - ≥3 Moderate (PM) + ≥2 Supporting (PP) - 1 Very Strong (PVS1) + 1 Moderate (PM) + 1 Supporting (PP)

✅Benign / Likely Benign Criteria

🟢**Stand-Alone (BA1)** - **BA1**: Allele frequency >1% in a large, well-characterized population database with sufficient sample size.

🟢**Strong (BS1–BS4)** - **BS1**: Allele frequency is higher than expected for disorder but <1%. - **BS2**: Observed in homozygous state in healthy individuals (must rule out penetrance or late onset). - **BS3**: Functional studies show no damaging effect on protein or splicing. - **BS4**: Lack of segregation with disease in affected family members.

🟢**Supporting (BP1–BP7)** - **BP1**: Missense variant in a gene where only LoF variants cause disease. - **BP2**: Variant found in trans with a pathogenic variant in dominant condition or in cis with multiple pathogenic variants. - **BP3**: In-frame indel in a repetitive region without known functional relevance. - **BP4**:

Computational tools predict no impact (e.g., benign conservation profile, REVEL ≤0.15). - **BP5**: Found in individuals with alternative molecular cause for disease. - **BP6**: Reported benign by reputable source without public evidence. - **BP7**: Synonymous variant without predicted splice impact, located outside conserved splice region.

Combining Criteria for Benign Classification - **Benign**: BA1 alone OR ≥2 Strong (BS) - **Likely Benign**: 1 Strong (BS) + 1 Supporting (BP), OR ≥2 Supporting (BP)