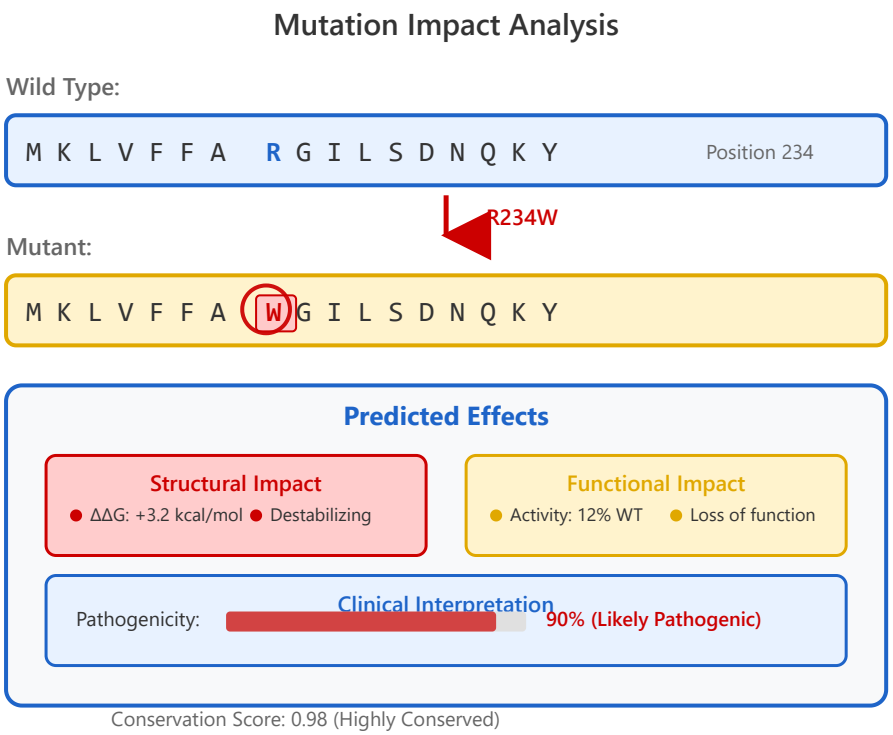
Mutation Effects



Pathogenicity prediction
Disease association scoring

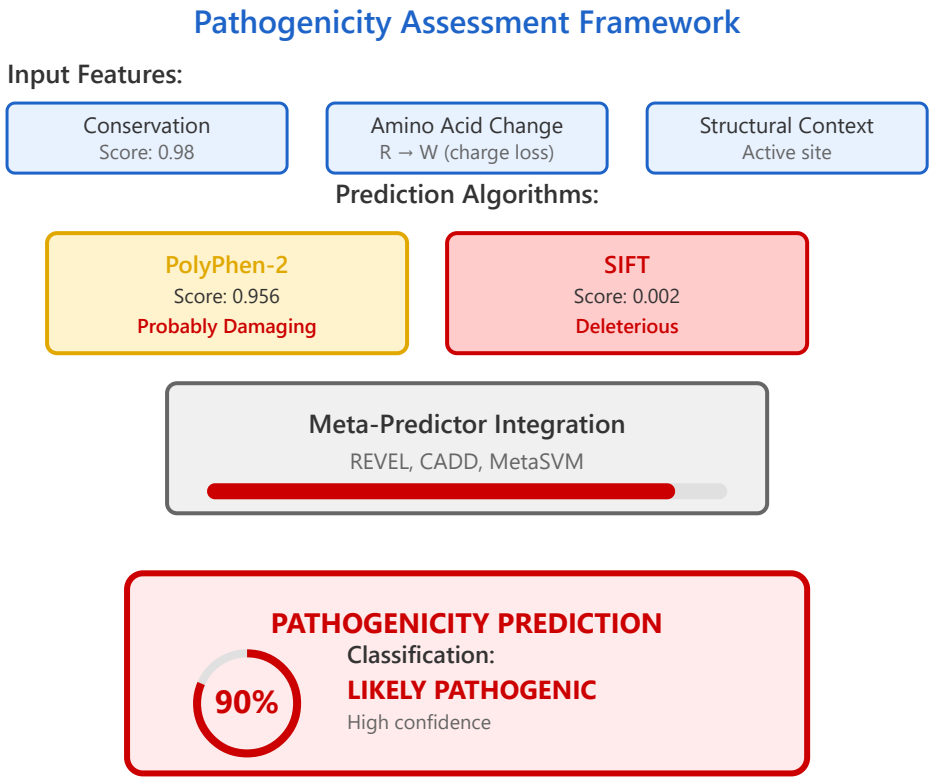
Stability changes
ΔΔG calculation

Function impact
Activity & binding changes

Evolutionary constraints
Conservation analysis

Clinical interpretation
Variant classification

1. Pathogenicity Prediction



Overview

Pathogenicity prediction assesses whether a genetic variant is likely to cause disease. This computational approach integrates multiple lines of evidence to estimate the probability that a mutation contributes to pathology.

Key Prediction Tools

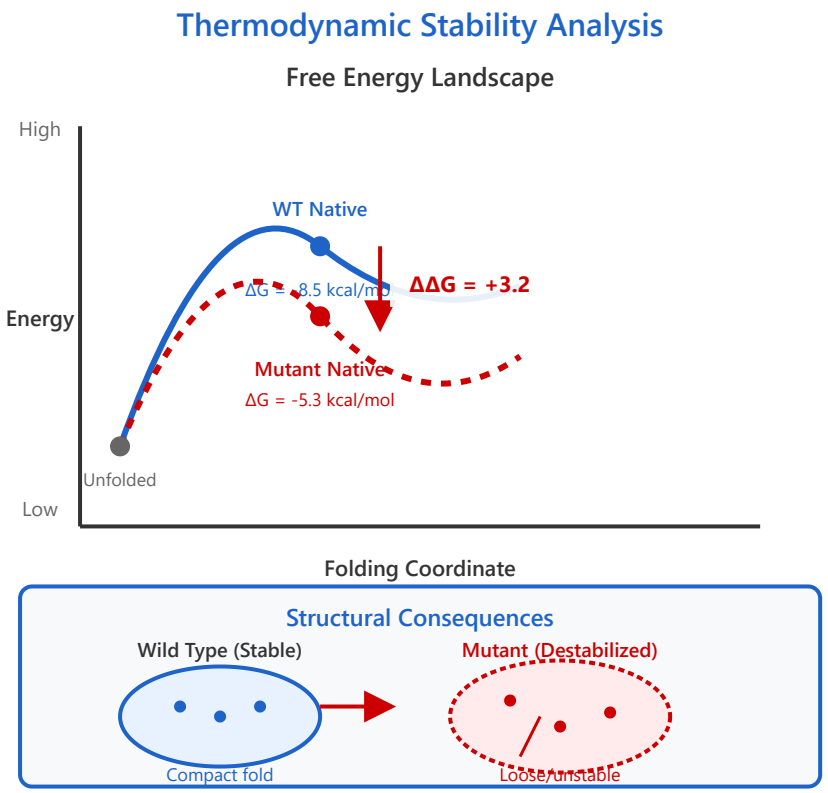
- **PolyPhen-2:** Uses sequence conservation and structural information to predict impact on protein function
- **SIFT:** Predicts whether an amino acid substitution affects protein function based on sequence homology
- **CADD:** Integrates diverse annotations into a single deleteriousness score
- **REVEL:** Ensemble method combining 13 individual tools for improved accuracy

> **Clinical Application:** These predictions help prioritize variants for experimental validation and guide clinical decision-making when interpreting genetic test results.

Interpretation Guidelines

- Scores > 0.8: Likely pathogenic
- Scores 0.4-0.8: Uncertain significance
- Scores < 0.4: Likely benign

2. Protein Stability Changes (ΔΔG)



Overview

The change in Gibbs free energy (ΔΔG) quantifies how a mutation affects protein stability. Positive ΔΔG values indicate destabilization, while negative values suggest stabilization of the protein structure.

Calculation Methods

- **FoldX:** Empirical force field-based approach using high-resolution structures
- **Rosetta:** Energy function combining physics-based and knowledge-based terms
- **DynaMut:** Considers protein dynamics and flexibility changes
- **I-Mutant:** Machine learning approach trained on experimental data

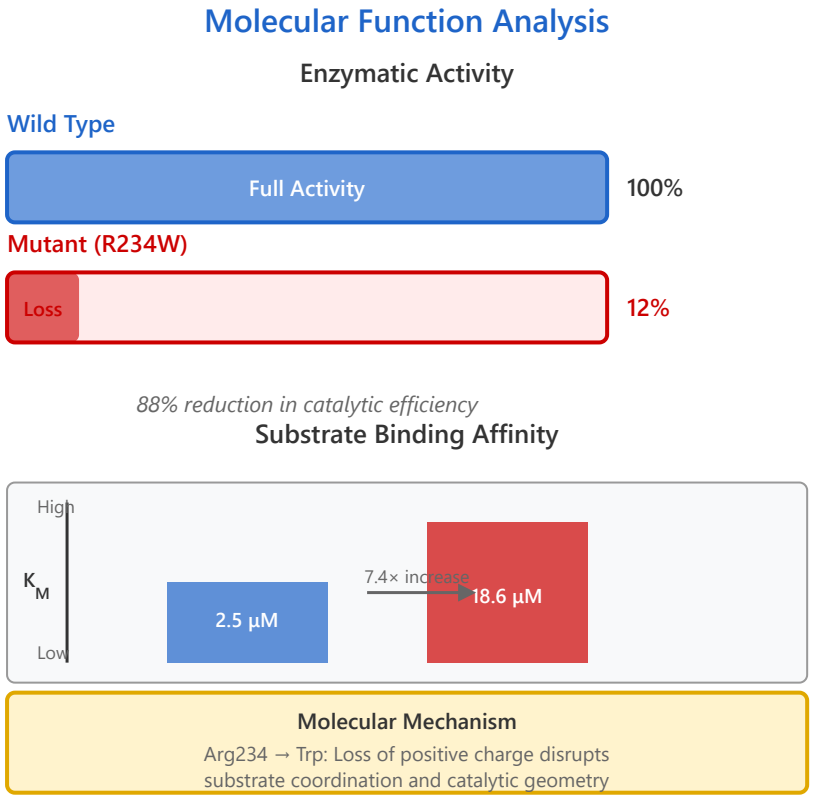
> **Formula:** $\Delta\Delta G = \Delta G_{mutant} - \Delta G_{wild\text{-}type}$
> A value of +3.2 kcal/mol indicates significant destabilization, often leading to protein misfolding or degradation.

Biological Consequences

- **ΔΔG > +2 kcal/mol:** Significant destabilization, likely protein degradation
- **ΔΔG +0.5 to +2:** Moderate instability, temperature-sensitive phenotypes
- **ΔΔG -0.5 to +0.5:** Minimal impact on stability

- **ΔΔG < -0.5:** Stabilizing mutation

3. Functional Impact Assessment



Overview

Functional impact assessment evaluates how a mutation affects the molecular activities of a protein, including catalytic activity, binding affinity, and interaction with other biomolecules.

Key Functional Parameters

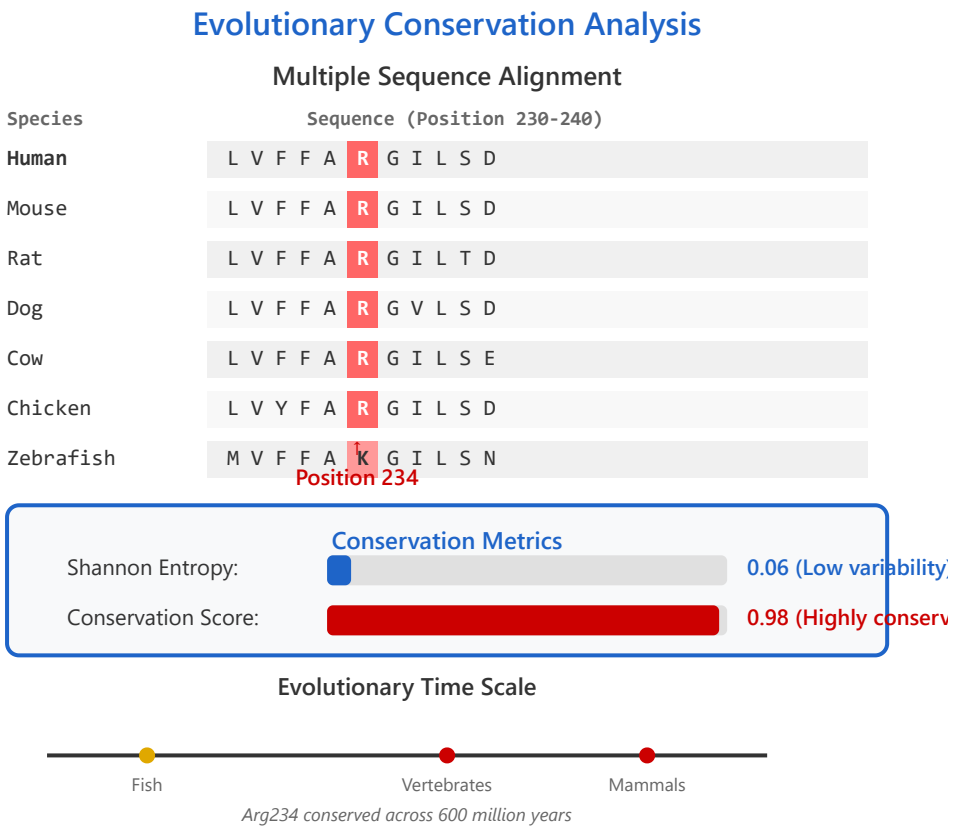
- **Catalytic Efficiency (k_{cat}/K_M):** Overall measure of enzyme performance
- **Binding Affinity (K_D or K_M):** Strength of protein-ligand interactions
- **V_{max}:** Maximum reaction velocity, reflects enzyme concentration
- **Protein-Protein Interactions:** Changes in binding to partner proteins

Example R234W Impact:
• Activity reduced to 12% of wild-type
• K_M increased 7.4-fold (weaker substrate binding)
• Charge loss at position 234 disrupts active site geometry

Experimental Approaches

- **Enzyme kinetics:** Measure k_{cat}, K_M, V_{max}
- **Binding assays:** SPR, ITC, fluorescence polarization
- **Cell-based assays:** Functional readouts in cellular context
- **Structural studies:** X-ray crystallography, cryo-EM

4. Evolutionary Constraints & Conservation



Overview

Evolutionary conservation analysis examines how well a protein position is preserved across species. Highly conserved positions are typically functionally important, and mutations at these sites are more likely to be deleterious.

Conservation Metrics

- **Shannon Entropy:** Measures amino acid variability at each position (0 = identical, higher = variable)
- **Conservation Score:** Quantifies evolutionary constraint (0-1 scale)
- **GERP Score:** Identifies positions under strong selective pressure
- **PhyloP:** Measures evolutionary conservation based on multiple alignments

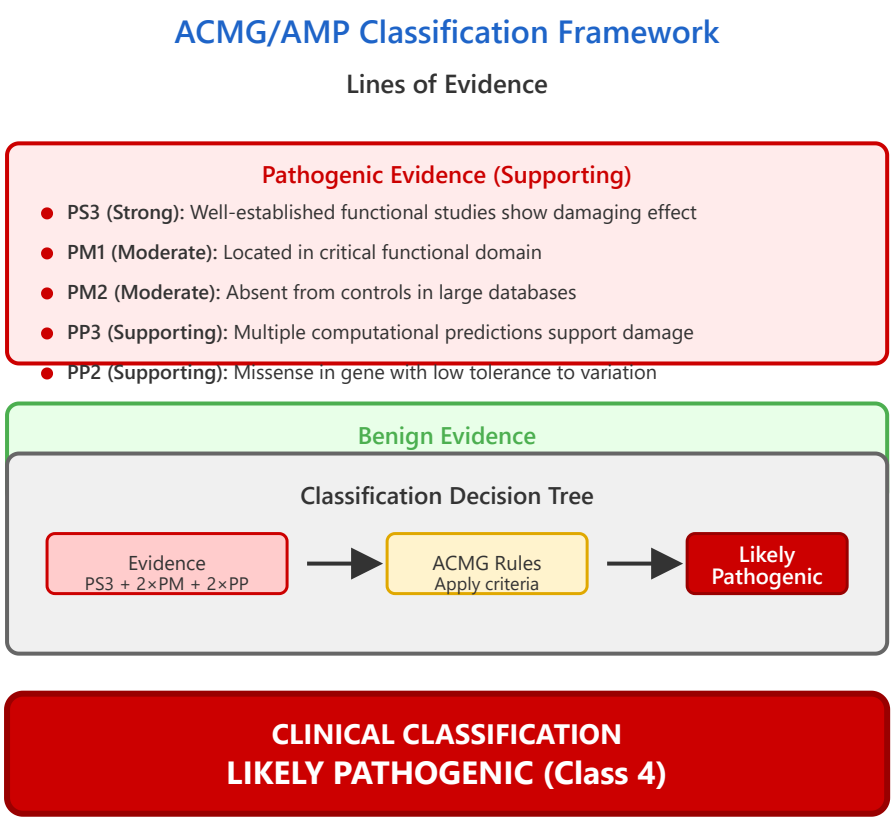
Position 234 Analysis:
• Conserved as Arginine in all mammals
• Only conservative substitution (Lysine) in distantly related species
• Conservation score: 0.98/1.00
• Indicates critical functional role

Interpretation

- **Score > 0.9:** Extremely conserved, mutations likely deleterious
- **Score 0.7-0.9:** Well conserved, mutations often harmful
- **Score 0.4-0.7:** Moderately conserved, variable tolerance

- **Score < 0.4:** Poorly conserved, mutations often tolerated

5. Clinical Interpretation & Variant Classification



Overview

Clinical interpretation follows standardized guidelines from the American College of Medical Genetics (ACMG) and Association for Molecular Pathology (AMP) to classify variants into five categories based on their likelihood of causing disease.

ACMG Classification Tiers

- **Pathogenic (Class 5):** Sufficient evidence of disease causation
- **Likely Pathogenic (Class 4):** Strong but not conclusive evidence
- **Uncertain Significance (Class 3):** Insufficient or conflicting evidence
- **Likely Benign (Class 2):** Strong evidence against pathogenicity
- **Benign (Class 1):** Established as non-pathogenic

Evidence Strength Levels:
• **Very Strong (PVS):** Null variants, proven functional effects
• **Strong (PS):** Well-established in vitro/in vivo

studies
• **Moderate (PM):** Computational predictions, location
• **Supporting (PP):** Conservation, multiple algorithms

Clinical Action

- **Genetic counseling:** Discuss implications with patients
- **Cascade testing:** Test family members for variant
- **Management:** Enhanced surveillance or preventive measures
- **Research:** Further functional validation if needed

Databases & Resources

- **ClinVar:** Repository of variant interpretations
- **gnomAD:** Population frequency data
- **OMIM:** Gene-disease relationships
- **HGMD:** Human gene mutation database