

# Mutation Effects

## Mutation Impact Analysis

### Wild Type:

M K L V F F A R G I L S D N O K Y Position 234

### Mutant:

M K L V F F A W G I L S D N Q K Y

## Predicted Effects

## Structural Impact

- $\Delta\Delta G$ : +3.2 kcal/mol ● Destabilizing

## Functional Impact

- Activity: 12% WT
  - Loss of function

Pathogenicity:

## Clinical Interpretation

on 90% (Likely Pathogenic)

Conservation Score: 0.98 (Highly Conserved)

## Pathogenicity prediction

## Disease association scoring

## Stability changes

## $\Delta\Delta G$ calculation

## Function impact

## Activity & binding changes

## Evolutionary constraints

## Conservation analysis

## Clinical interpretation

## Variant classification

## 1. Pathogenicity Prediction

# Pathogenicity Assessment Framework

## Input Features:

Conservation  
Score: 0.98

Amino Acid Change  
R → W (charge loss)

Structural Context  
Active site

## Prediction Algorithms:

**PolyPhen-2**

Score: 0.956  
Probably Damaging

**SIFT**

Score: 0.002  
Deleterious

## Meta-Predictor Integration

REVEL, CADD, MetaSVM

## PATHOGENICITY PREDICTION

Classification:

**LIKELY PATHOGENIC**

High confidence

90%

## 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

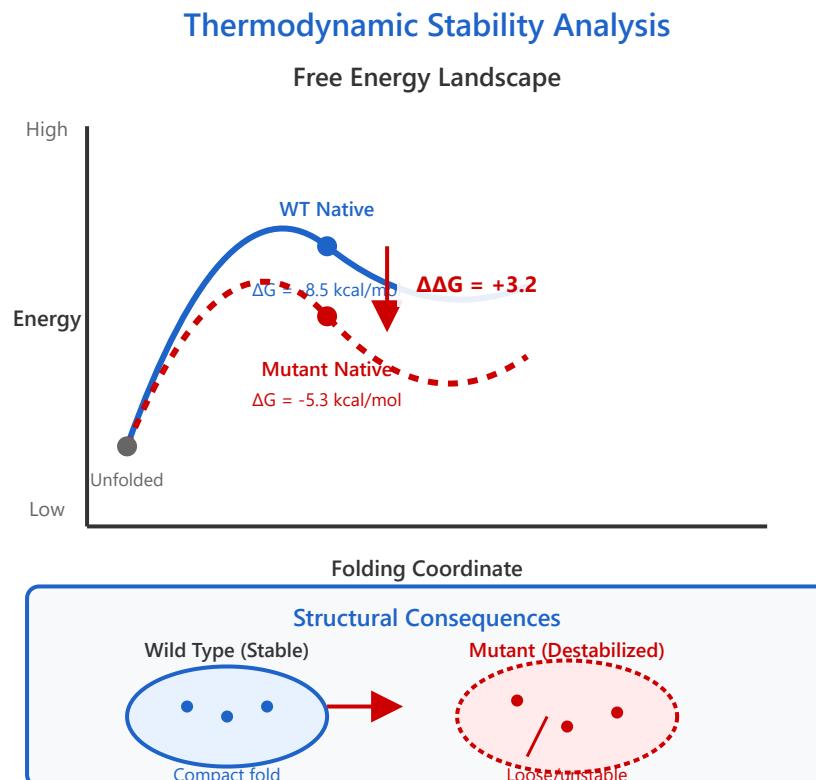
- **PolyPhen-2:** Uses sequence conservation and structural information to predict impact on protein function
- **SIFT:** Predicts whether an amino acid substitution affects protein function based on sequence homology
- **CADD:** Integrates diverse annotations into a single deleteriousness score
- **REVEL:** 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 ( $\Delta\Delta G$ )



### Overview

The change in Gibbs free energy ( $\Delta\Delta G$ ) quantifies how a mutation affects protein stability. Positive  $\Delta\Delta G$  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:**  $\Delta\Delta G = \Delta G_{\text{mutant}} - \Delta G_{\text{wild-type}}$   
A value of +3.2 kcal/mol indicates significant destabilization, often leading to protein misfolding or degradation.

### Biological Consequences

- **$\Delta\Delta G > +2 \text{ kcal/mol}$ :** Significant destabilization, likely protein degradation
- **$\Delta\Delta G +0.5 \text{ to } +2$ :** Moderate instability, temperature-sensitive phenotypes
- **$\Delta\Delta G -0.5 \text{ to } +0.5$ :** Minimal impact on stability

### 3. Functional Impact Assessment

#### Molecular Function Analysis

##### Enzymatic Activity

Wild Type

Full Activity

100%

Mutant (R234W)

Loss

12%

88% reduction in catalytic efficiency

##### Substrate Binding Affinity

$K_M$

High  
Low

2.5  $\mu M$

7.4x increase

18.6  $\mu M$

##### Molecular Mechanism

Arg234 → Trp: Loss of positive charge disrupts substrate coordination and catalytic geometry

#### 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

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# Evolutionary Conservation Analysis

## Multiple Sequence Alignment

Species	Sequence (Position 230-240)									
Human	L	V	F	F	A	R	G	I	L	S D
Mouse	L	V	F	F	A	R	G	I	L	S D
Rat	L	V	F	F	A	R	G	I	L	T D
Dog	L	V	F	F	A	R	G	V	L	S D
Cow	L	V	F	F	A	R	G	I	L	S E
Chicken	L	V	Y	F	A	R	G	I	L	S D
Zebrafish	M	V	F	F	A	K	G	I	L	S N

### Conservation Metrics

Shannon Entropy:	<div style="width: 20px; height: 10px; background-color: blue;"></div>	0.06 (Low variability)
Conservation Score:	<div style="width: 90%; height: 10px; background-color: red;"></div>	0.98 (Highly conserved)

### Evolutionary Time Scale



Arg234 conserved across 600 million years

## 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

### ACMG/AMP Classification Framework

#### Lines of Evidence

##### Pathogenic Evidence (Supporting)

- PS3 (Strong): Well-established functional studies show damaging effect
- PM1 (Moderate): Located in critical functional domain
- PM2 (Moderate): Absent from controls in large databases
- PP3 (Supporting): Multiple computational predictions support damage
- PP2 (Supporting): Missense in gene with low tolerance to variation

##### Benign Evidence

#### Classification Decision Tree

Evidence  
PS3 + 2xPM + 2xPP

ACMG Rules  
Apply criteria

Likely  
Pathogenic

**CLINICAL CLASSIFICATION  
LIKELY PATHOGENIC (Class 4)**

### 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