Color Figures for Xu et al.

Integrated functional and structural analysis of eleven *CYP21A2* variants of uncertain significance resolves their pathogenic role in congenital adrenal hyperplasia.

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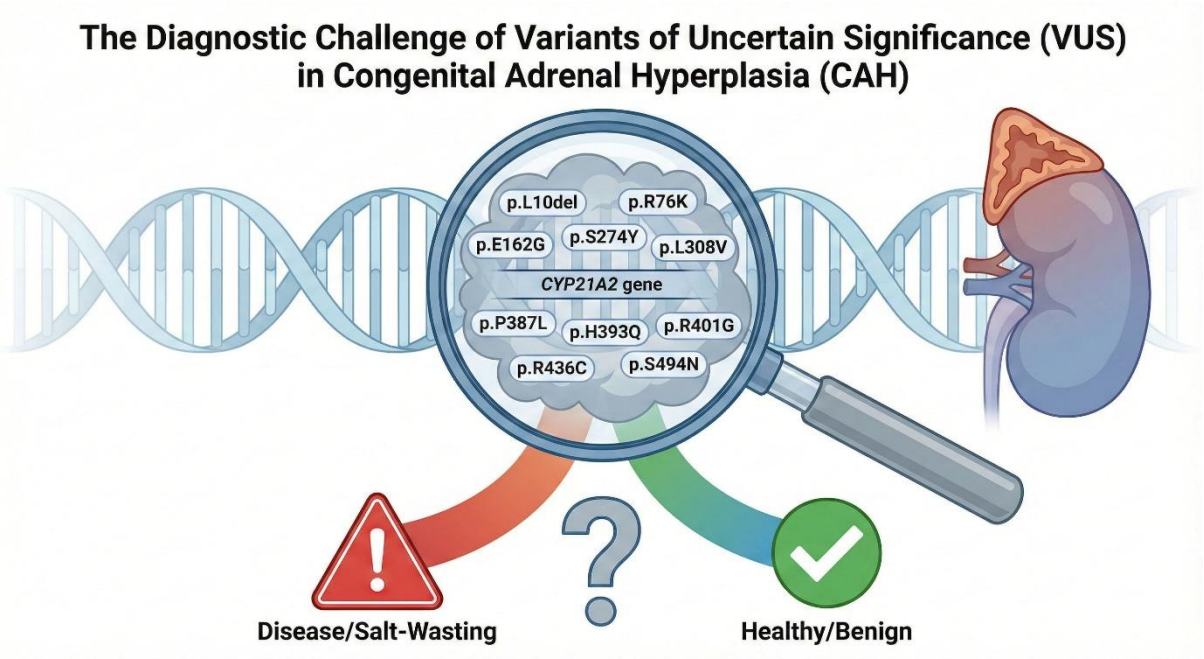


Figure 1

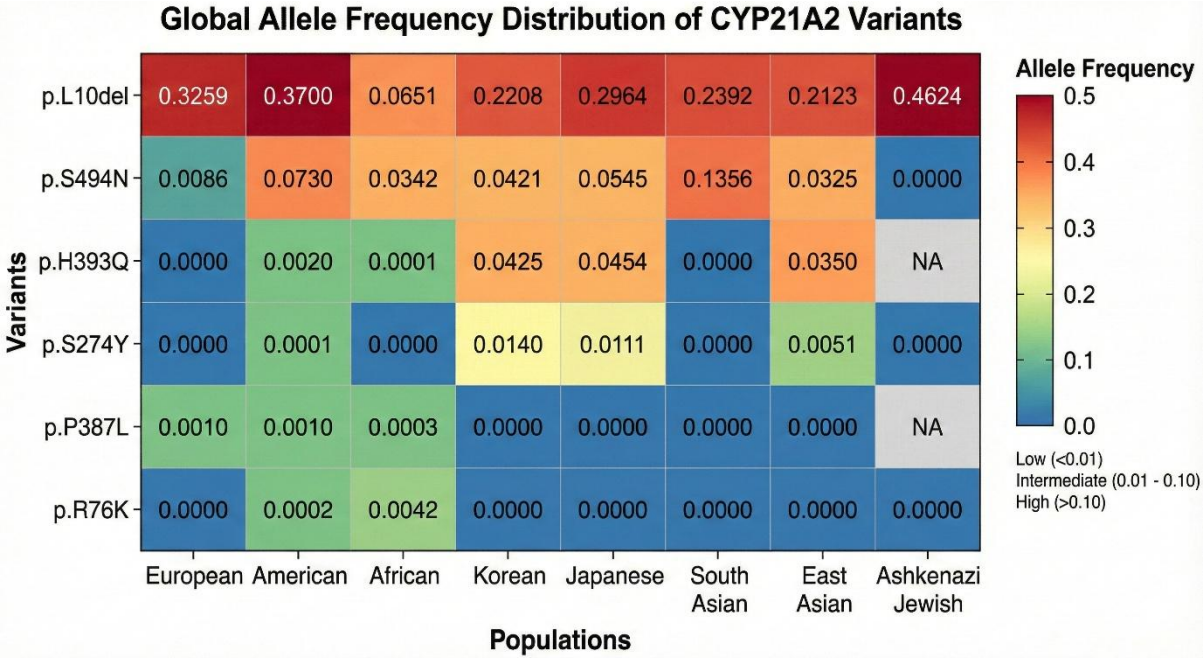


Figure 2

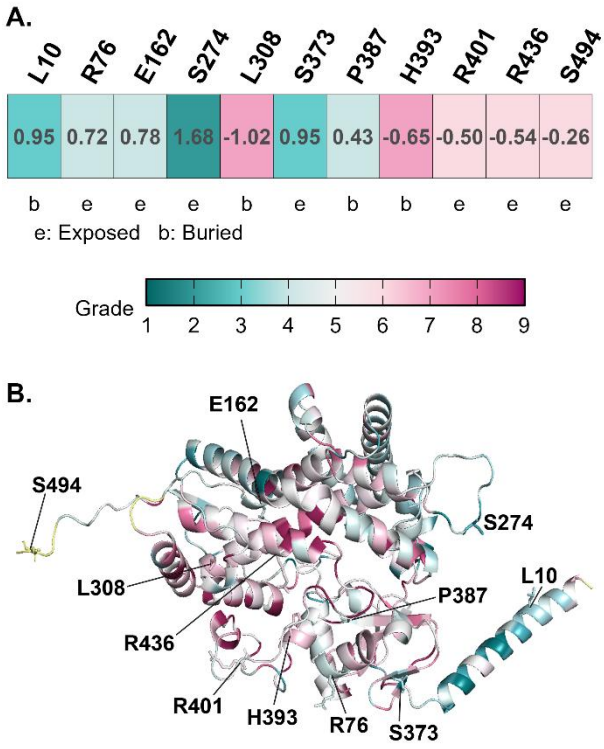


Figure 3

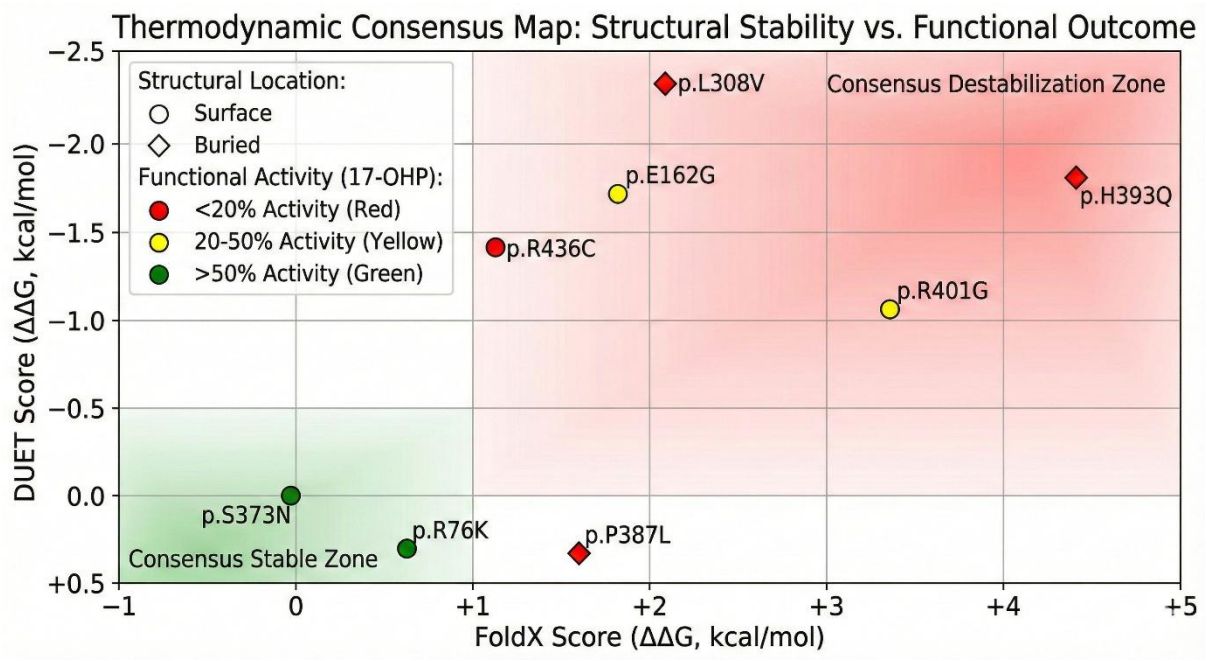
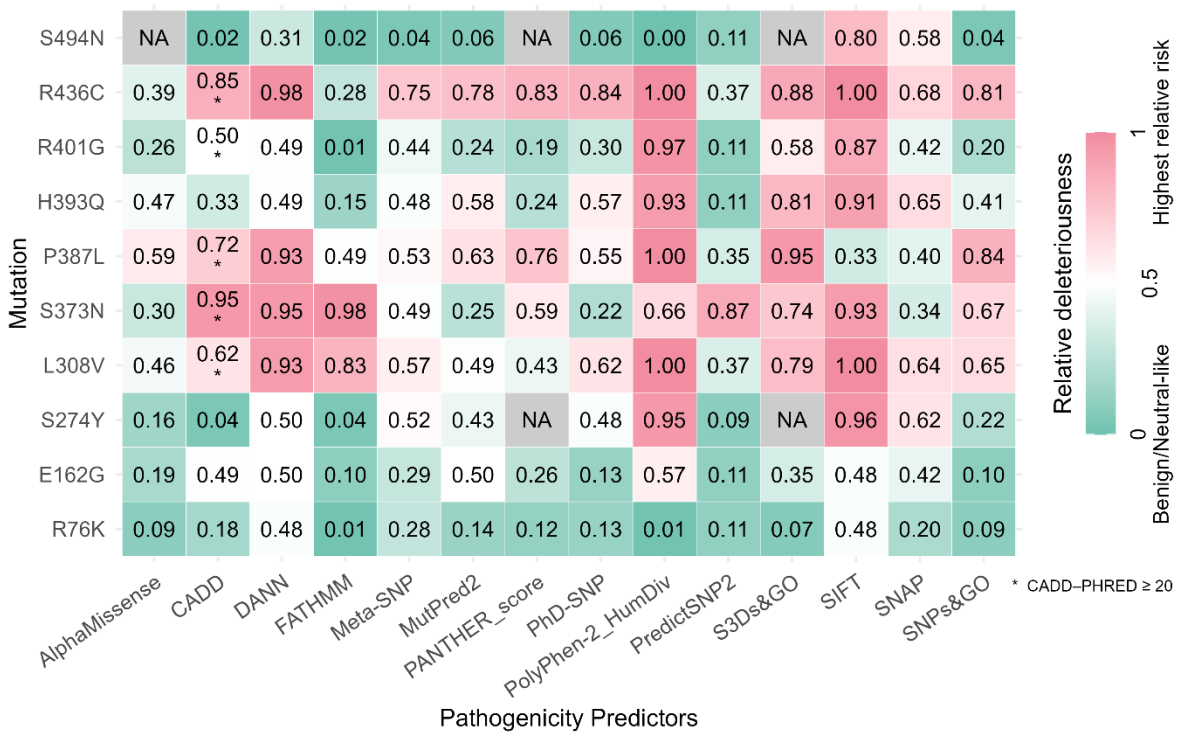


Figure 4


Mutation
S494N NA 0.02 0.31 0.02 0.04 0.06 NA 0.06 0.00 0.11 NA 0.80 0.58 0.04
R436C 0.39 0.85 * 0.98 0.28 0.75 0.78 0.83 0.84 1.00 0.37 0.88 1.00 0.68 0.81
R401G 0.26 0.50 * 0.49 0.01 0.44 0.24 0.19 0.30 0.97 0.11 0.58 0.87 0.42 0.20
H393Q 0.47 0.33 0.49 0.15 0.48 0.58 0.24 0.57 0.93 0.11 0.81 0.91 0.65 0.41
P387L 0.59 0.72 * 0.93 0.49 0.53 0.63 0.76 0.55 1.00 0.35 0.95 0.33 0.40 0.84
S373N 0.30 0.95 * 0.95 0.98 0.49 0.25 0.59 0.22 0.66 0.87 0.74 0.93 0.34 0.67
L308V 0.46 0.62 * 0.93 0.83 0.57 0.49 0.43 0.62 1.00 0.37 0.79 1.00 0.64 0.65
S274Y 0.16 0.04 0.50 0.04 0.52 0.43 NA 0.48 0.95 0.09 NA 0.96 0.62 0.22
E162G 0.19 0.49 0.50 0.10 0.29 0.50 0.26 0.13 0.57 0.11 0.35 0.48 0.42 0.10
R76K 0.09 0.18 0.48 0.01 0.28 0.14 0.12 0.13 0.01 0.11 0.07 0.48 0.20 0.09
AlphaMissense
CADD
DANN
FATHMM
Meta-SNP
MutPred2
PANTHER_score
PhD-SNP
PolyPhen-2_HumDiv
PredictSNP2
S3Ds&GO
SIFT
SNAP
SNPs&GO
Pathogenicity Predictors
* CADD–PHRED ≥ 20
Relative deleteriousness
Highest relative risk
Benign/Neutral-like
1
0.5
0

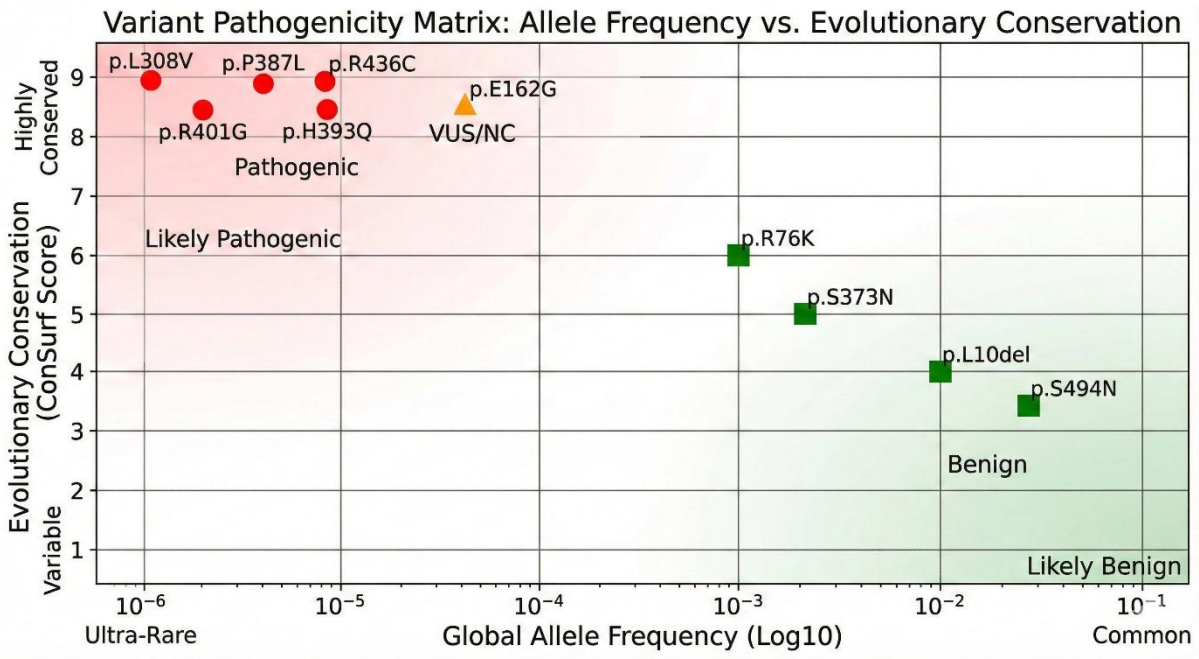

Variant Pathogenicity Matrix: Allele Frequency vs. Evolutionary Conservation
Evolutionary Conservation (ConSurf Score)
Highly Conserved
Variable
p.L308V
p.P387L
p.R436C
p.E162G
p.R401G
p.H393Q
VUS/NC
Pathogenic
Likely Pathogenic
p.R76K
p.S373N
p.L10del
p.S494N
Benign
Likely Benign
Ultra-Rare
Global Allele Frequency (Log10)
Common

Figure 6

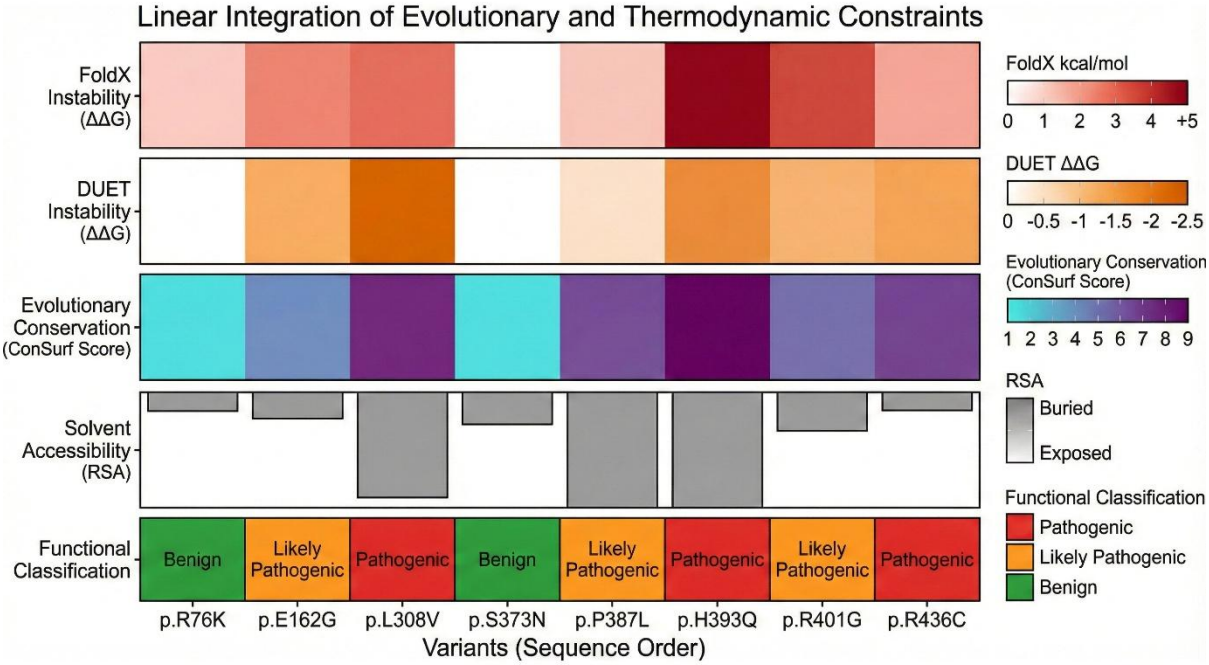


Figure 8

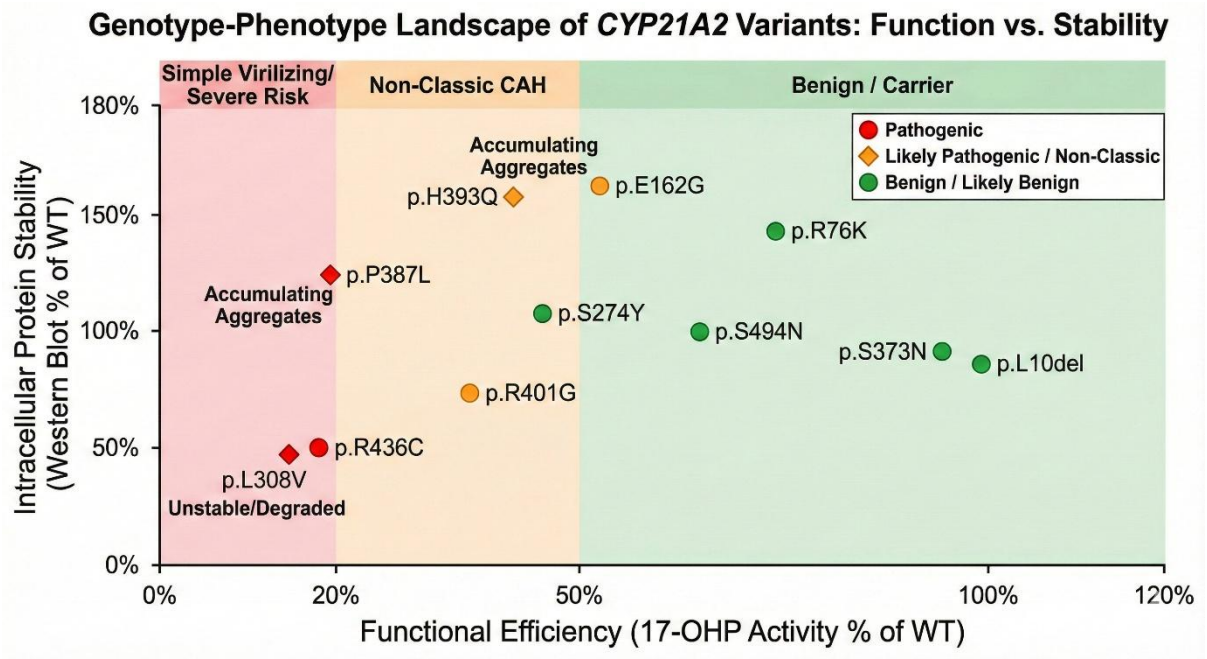


Figure 9

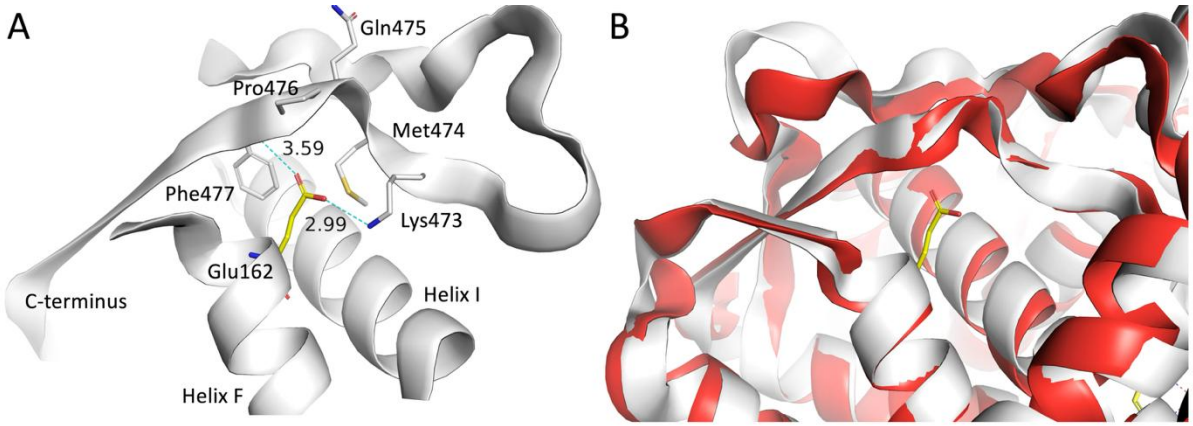

A
Gln475
Pro476
Met474
3.59
Phe477
2.99
Lys473
Glu162
C-terminus
Helix I
Helix F
B

Figure 10

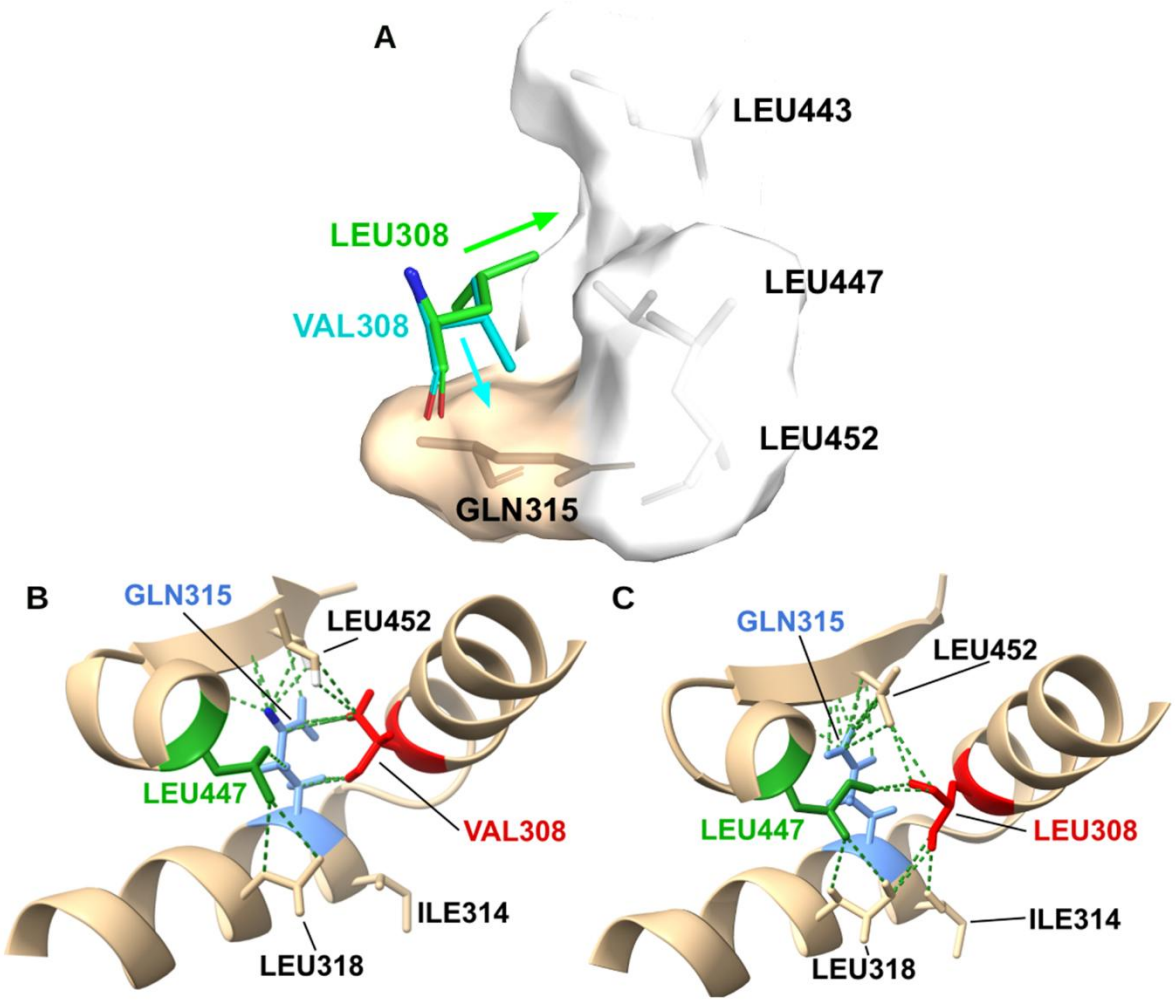


Figure 11

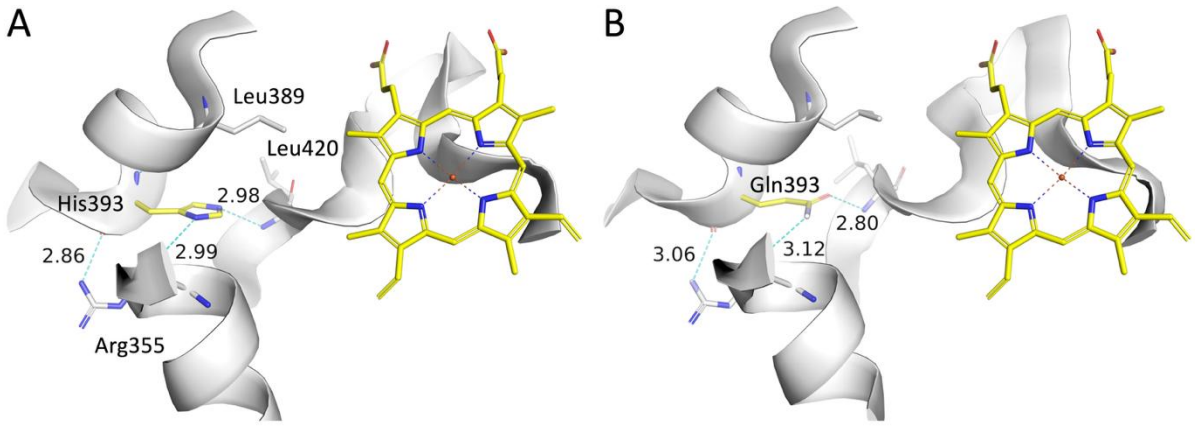


Figure 12

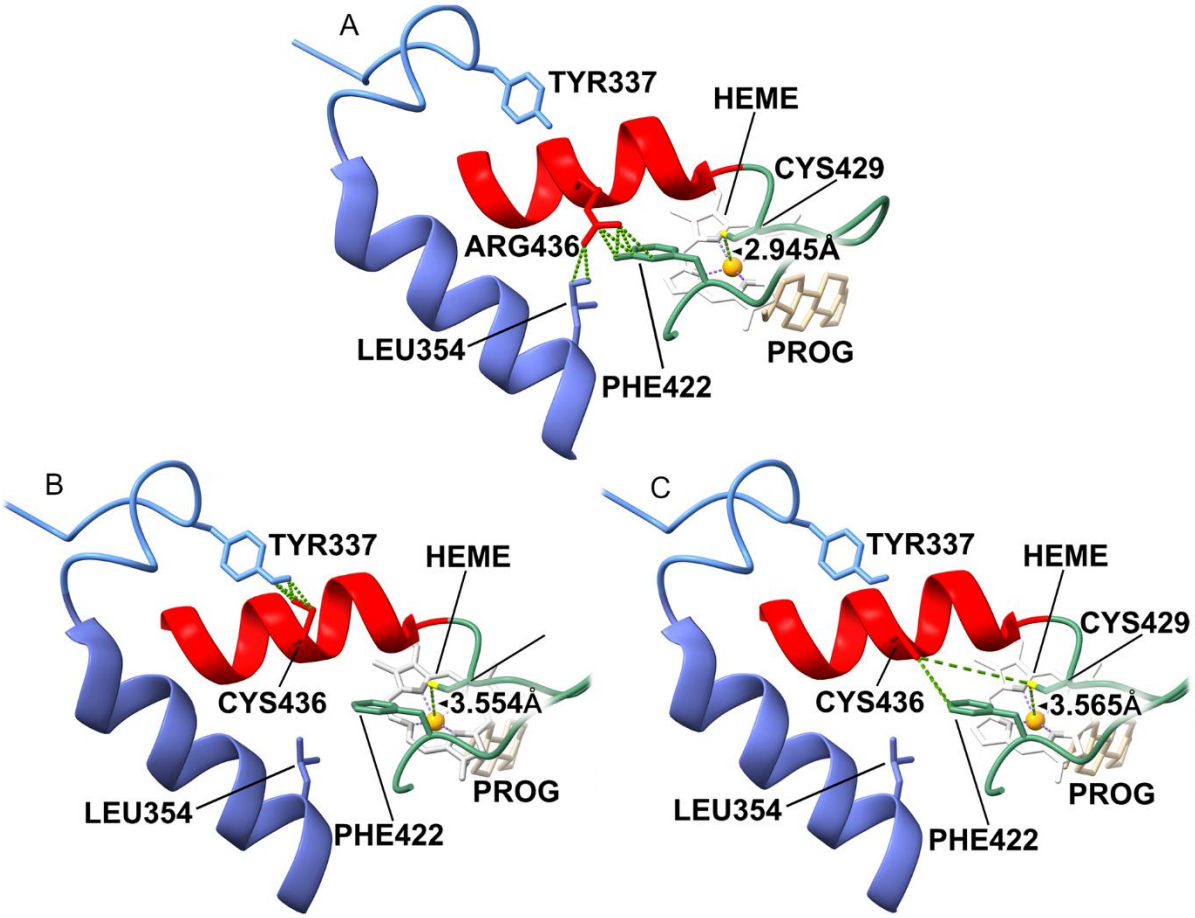

A
TYR337
HEME
CYS429
ARG436
2.945Å
LEU354
PHE422
PROG
B
TYR337
HEME
CYS436
3.554Å
PROG
LEU354
PHE422
C
TYR337
HEME
CYS429
CYS436
3.565Å
PROG
LEU354
PHE422

Figure 13

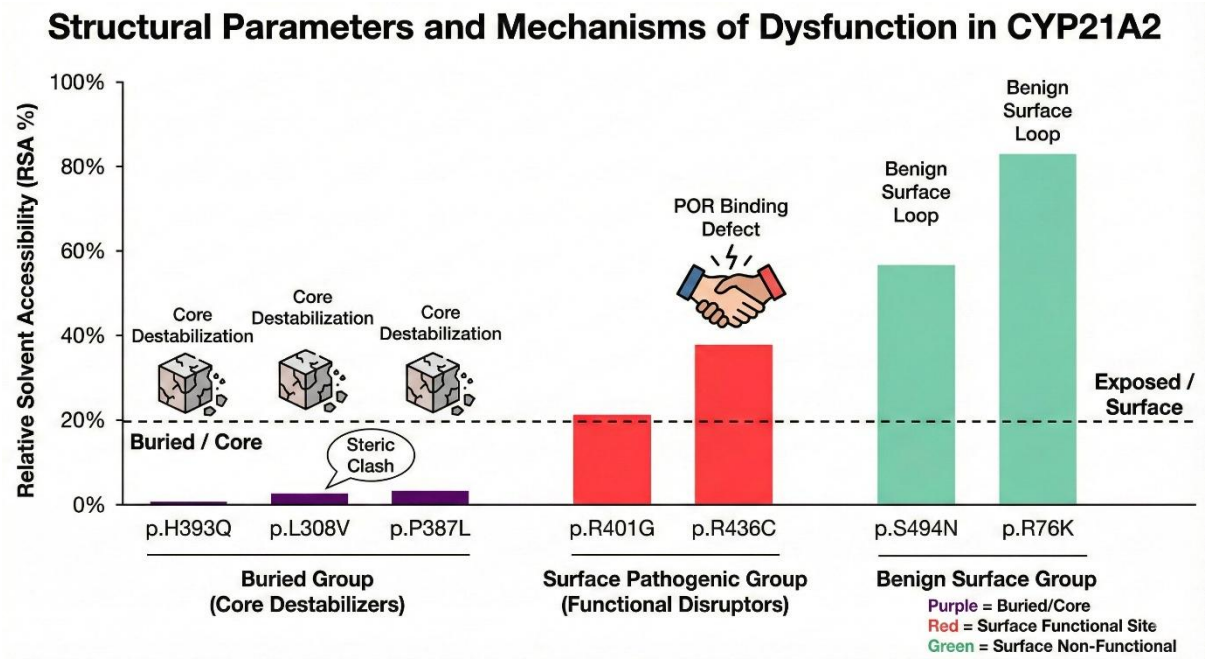


Figure 14

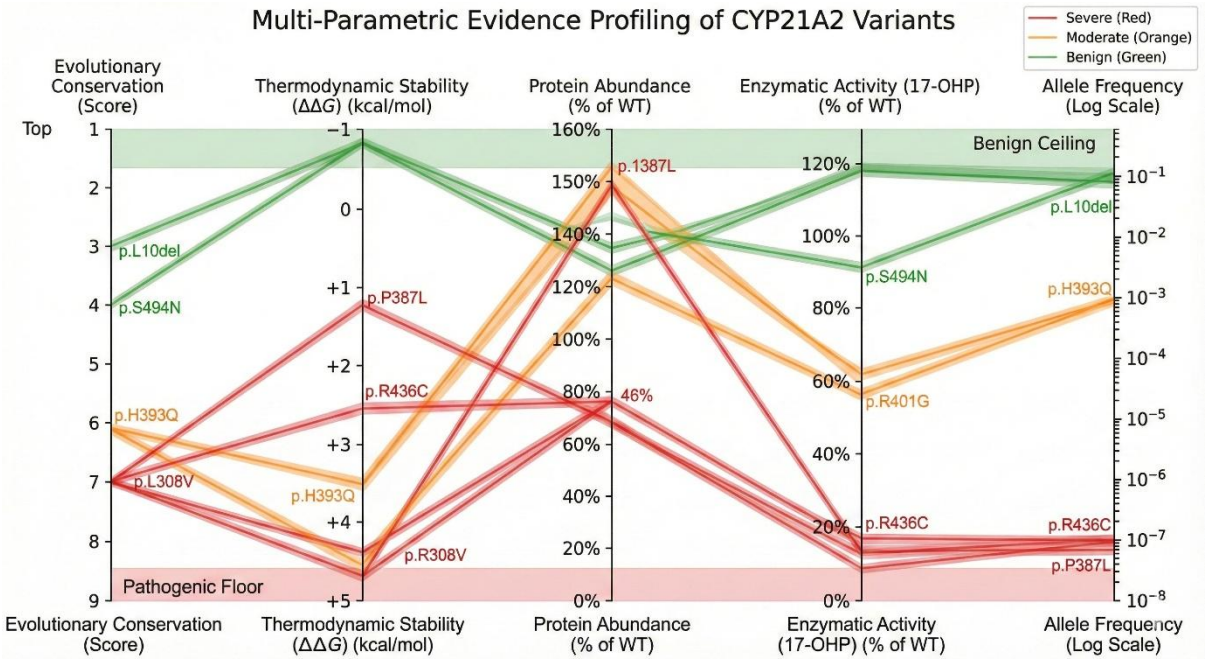


Figure 15

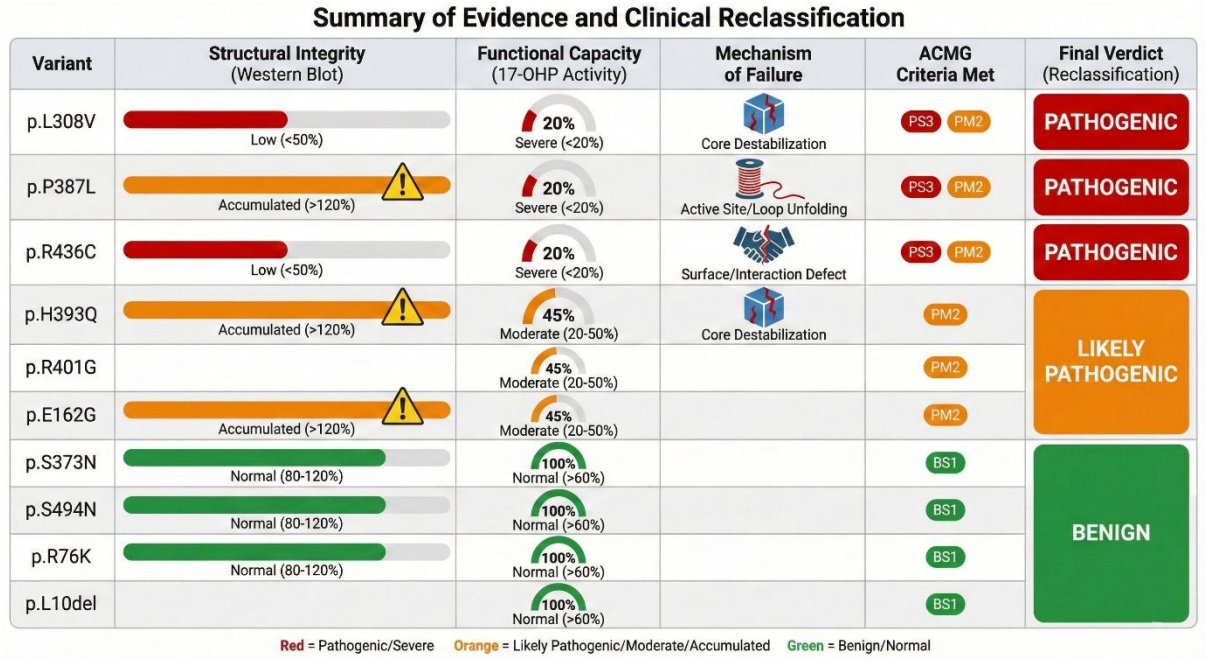

Summary of Evidence and Clinical Reclassification
Variant
Structural Integrity (Western Blot)
Functional Capacity (17-OHP Activity)
Mechanism of Failure
ACMG Criteria Met
Final Verdict (Reclassification)
p.L308V
Low (<50%)
20%
Severe (<20%)
Core Destabilization
PS3
PM2
PATHOGENIC
p.P387L
Accumulated (>120%)
20%
Severe (<20%)
Active Site/Loop Unfolding
PS3
PM2
PATHOGENIC
p.R436C
Low (<50%)
20%
Severe (<20%)
Surface/Interaction Defect
PS3
PM2
PATHOGENIC
p.H393Q
Accumulated (>120%)
45%
Moderate (20-50%)
Core Destabilization
PM2
LIKELY PATHOGENIC
p.R401G
45%
Moderate (20-50%)
PM2
p.E162G
Accumulated (>120%)
45%
Moderate (20-50%)
PM2
p.S373N
Normal (80-120%)
100%
Normal (>60%)
BS1
BENIGN
p.S494N
Normal (80-120%)
100%
Normal (>60%)
BS1
p.R76K
Normal (80-120%)
100%
Normal (>60%)
BS1
p.L10del
100%
Normal (>60%)
BS1
Red = Pathogenic/Severe
Orange = Likely Pathogenic/Moderate/Accumulated
Green = Benign/Normal

Figure 16