**Supplementary Table 3. Structural parameters of CYP21A2 variant residues.**

Description: This table lists the physicochemical properties, solvent accessible surface area (SASA), and percentage of total SASA contributed by side chains for both wild-type (WT) and mutant residues. SASA values were calculated in PyMOL using the crystal structure of CYP21A2 (PDB ID: 4Y8W, chain A), with a probe radius of 1.4 Å. Relative solvent accessibility (RSA) was derived by normalizing total SASA against the theoretical residue SASA values proposed by Tien et al. (2013). ΔRSA represents the difference in relative solvent accessibility between mutant and wild-type residues (ΔRSA = MUT_RSA − WT_RSA).

All SASA values are expressed in Å².

| Identifiers | Location | WT/Mutant | Physicochemical Properties | SASA (Angstrom^2) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | RSA (%) | Total SASA | Main-chain | Side-chain | Percentage of total SASA contributed by side chain (%) | ΔRSA=MUT_RSA-WT_RSA |
| p.R76K | Surface | p.R76 | Basic; Hydrophilicity<br>Secondary structure: α-helix | 69.05 | 189.19 | 12.16 | 177.03 | 93.57 | -1.66 |
| | | p.K76 | Basic | 67.38 | 159.02 | 13.09 | 145.93 | 91.77 | |
| p.E162G | Surface | p.E162 | Acidic; Near-neutral hydropathy<br>Secondary structure: α-helix | 19.20 | 42.81 | 3.51 | 39.30 | 91.79 | 10.40 |
| | | p.G162 | Nonpolar | 29.60 | 30.74 | 30.74 | 0.00 | 0.00 | |
| p.L308V | Buried | p.L308 | Nonpolar hydrophobic aliphatic amino acid; Hydrophobicity<br>Secondary structure: α-helix | 2.53 | 5.08 | 0.71 | 4.34 | 85.41 | 1.47 |
| | | p.V308 | Nonpolar | 4.00 | 7.28 | 0.70 | 6.58 | 90.42 | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| p.S373N | Surface | p.S373 | Neutral polarity; Hydrophilicity Secondary structure: Sheet at N-terminal | 24.29 | 37.65 | 0.67 | 36.98 | 98.21 | 15.71 |
| | | p.N373 | Uncharged polar | 40.00 | 62.31 | 2.24 | 60.07 | 96.40 | |
| p.P387L | Buried | p.P387 | Aromatic; Hydrophobicity Secondary structure: β7-sheet near heme-binding site | 2.71 | 4.30 | 0.00 | 4.30 | 100.00 | -0.77 |
| | | p.L387 | Nonpolar | 1.94 | 3.90 | 0.00 | 3.90 | 100.00 | |
| p.H393Q | Buried | p.H393 | Basic; Hydrophilicity Secondary structure: α-helix | 0.35 | 0.80 | 0.00 | 0.80 | 100.00 | 0.02 |
| | | p.Q393 | Uncharged polar | 0.38 | 0.85 | 0.00 | 0.85 | 100.00 | |
| p.R401G | Surface | p.R401 | Basic; Hydrophilicity Secondary structure: unmodeled in 4Y8W; no defined secondary structure in 5VBU | 18.19 | 49.85 | 19.32 | 30.53 | 61.24 | 22.97 |
| | | p.G401 | Nonpolar | 41.17 | 42.81 | 42.81 | 0.00 | 0.00 | |
| p.R436C | Surface | p.R436 | Basic; Hydrophilicity Secondary structure: α-helix near heme-binding site | 32.04 | 87.79 | 5.28 | 82.51 | 93.99 | -9.88 |
| | | p.C436 | Uncharged polar | 22.16 | 37.01 | 4.50 | 32.52 | 87.85 | |

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