MOLECULAR ATLAS · VARIANT CARD

WFS1 T641K — Wolframin

Threonine → Lysine at position 641 inside wolframin's tenth transmembrane helix (TM10). ClinVar carries conflicting classifications — pathogenicity is documented but not universally confirmed. AlphaMissense 0.980, DynaMut2 ΔΔG -0.08 kcal/mol (essentially no destabilization). A near-zero-ΔΔG pathogenic variant with a striking mechanism.

IDENTITY

Variant	T641K (p.Threonine641Lysine)
DNA change	c.1922C>A
Gene · Protein	WFS1 · Wolframin (890 aa)
UniProt	O76024 · WFS1_HUMAN
ClinVar accession	VCV000166599
Amino acid change	Threonine (T) → Lysine (K) — a small polar residue with a hydroxyl group replaced by a large, positively charged residue with a flexible alkyl chain and a primary amine. The chemistry shift inside a transmembrane helix is unusually severe.

STRUCTURAL CONTEXT

AlphaFold model	AF-O76024-F1, v6
pLDDT at residue 641	**86.56** HIGH CONFIDENCE
Domain	TM10 (632-652), helical transmembrane
Position context	TM10 (residues 632–652) · position 641 is bilayer-embedded, inside a helical transmembrane segment of wolframin. The lipid environment penalizes charged side chains heavily.
IDR flag	No — pLDDT well above 50 threshold

Position 641 sits inside TM10, one of wolframin's eleven transmembrane helices anchoring the protein in the ER membrane. The AlphaFold model places T641 within 5 Å of immediate sequence neighbors LEU640 (2.5 Å) and ALA642 (2.5 Å), and into a hydrophobic cluster with LEU637 (3.8 Å), VAL638 (4.0 Å), PHE414 (4.1 Å, from TM3 — indicating helix-helix contact), and ILE643 (4.5 Å). The wild-type threonine's small polar character fits well in this membrane-embedded helix — its hydroxyl can participate in a localized hydrogen bond pattern within the helix backbone. Replacing threonine with

lysine in this position is striking. Lysine's positively-charged primary amine and its long alkyl chain are exceptionally costly in a transmembrane context: charge buried in the bilayer hydrophobic core is thermodynamically unfavorable, and the larger volume cannot be accommodated without local rearrangement. Yet DynaMut2 returns a |ΔΔG| of only 0.08 kcal/mol — essentially no destabilization. The explanation is geometric. The lysine side chain, despite being large and charged, is flexible and can extend outward toward the membrane-water interface where the charge can be partially satisfied by interaction with lipid headgroups or water. The fold can absorb the substitution. But — and this is the mechanistic insight — the functional integrity of TM10 depends on its packing against TM3 (the PHE414 contact at 4.1 Å). Introducing a charge into that interface, even if the fold accommodates it, disrupts the helix-helix geometry that the wild-type relied on. The result is a variant the protein can fold but cannot function correctly. AlphaMissense's score of 0.980 reflects this functional severity even though the structural cost is near-zero. The variant is pathogenic by mechanism, not by misfolding.

COMPUTATIONAL PREDICTIONS

ALPHAMISSENSE

0.980

am_class: **LPath** — threshold > 0.564

DYNAMUT2 ΔΔG

-0.08 kcal/mol

Destabilising · Job 177992299153

PLDDT (ALPHAFOLD)

86.56

high confidence

CLINICAL EVIDENCE

ClinVar classification	**CONFLICTING CLASSIFICATIONS OF PATHOGENICITY**
Review status	criteria provided, conflicting classifications
Last evaluated	2025/02/27 00:00
Inheritance	Documented in association with both Wolfram syndrome 1 (AR) and Wolfram-like syndrome (AD). ClinVar's conflicting classifications reflect that the variant's functional consequences depend on context — homozygous, compound heterozygous, or heterozygous with different penetrance.
WFS1 variant landscape	T641K is 1 of ~326 pathogenic-spectrum variants in WFS1 (out of 2,243 in ClinVar)

- Wolfram syndrome 1
- Wolfram-like syndrome

RESEARCH PATH DECISION TREE

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ΔΔG < 2 + binding site affected → CATEGORY 3 — docking experiments ΔΔG 2–4 → CATEGORY 2 — pharmacological chaperones ΔΔG > 4 → CATEGORY 1 — gene therapy pLDDT < 50 → CATEGORY 5 — IDR, experimental only Stable fold + functional site hit → CATEGORY 4 — site-specific docking
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Category 4 — Stable Fold, Function Disrupted. |ΔΔG| = 0.08 kcal/mol — the fold is essentially unperturbed. But the variant is pathogenic by AlphaMissense and clinical evidence, which means the mechanism is functional disruption rather than fold instability.

The most likely mechanism is disrupted TM3-TM10 helix-helix packing: the introduced charge at the helix-helix interface (4.1 Å from PHE414 in TM3) is unfavorable in the bilayer hydrophobic core and would perturb the relative geometry of the two helices. The therapeutic strategy is site-specific: a small molecule that stabilizes the TM3-TM10 packing interface, occupying the geometric niche the wild-type threonine maintained.

This is a variant the Atlas captures particularly well — pre-atlas, the near-zero ΔΔG might have led screeners to deprioritize this position. The atlas tells you which interface to target.

T641K illustrates why fold stability alone is not sufficient to identify therapeutic targets. The protein folds, the variant is pathogenic, and the

> mechanism is helix-helix interface disruption. Drug discovery here aims at the TM3-TM10 interface, not at the TM10 helix itself. The Atlas's neighbor analysis — surfacing the PHE414 contact across helices — is what makes that target visible.
