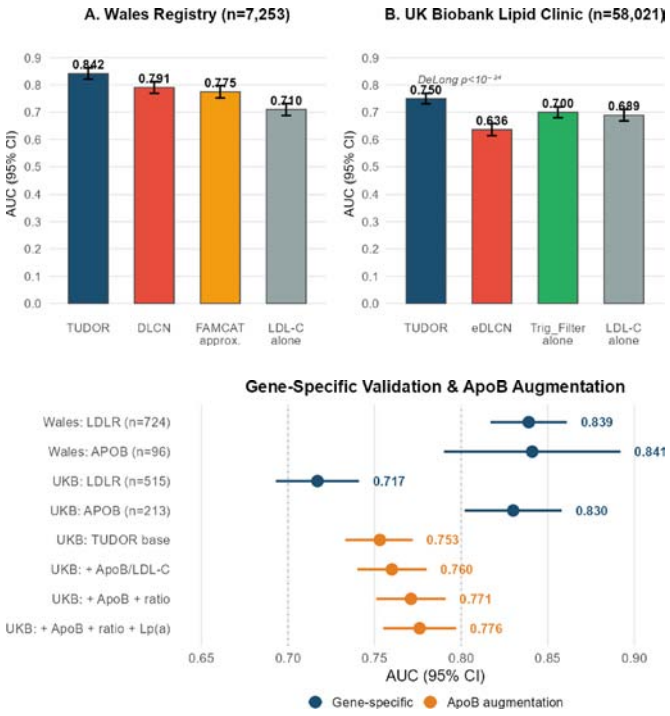

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CALON-FH: AlphaFold3-Derived Structural Severity Score Enables Domain-Specific ASCVD Penetrance Prediction and Mutation-Guided Treatment Selection in Familial Hypercholesterolaemia

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Background: Current familial hypercholesterolaemia (FH) management applies uniform treatment algorithms regardless of mutation location, despite the LDL receptor (LDLR) comprising functionally distinct domains with different mechanisms of dysfunction. The ligand-binding repeats (LA1–LA7) capture LDL particles, the EGF-A domain mediates PCSK9 binding, the beta-propeller enables receptor recycling at endosomal pH, and the cytoplasmic NPXY motif signals clathrin-mediated endocytosis. Disruption of each mechanism has distinct therapeutic implications, yet no framework exists to translate mutation location into treatment selection. We developed the Structural Severity Score (SSS) to classify LDLR mutations by mechanism of receptor dysfunction and predict domain-specific ASCVD penetrance and treatment response.

Methods: We constructed the SSS by integrating 14 independent data sources across all 860 LDLR residue positions: AlphaFold3 structure prediction (pLDDT confidence), FoldX 5.0 thermodynamic stability (ΔΔG) for 359 clinical variants and saturation mutagenesis, Rosetta energy decomposition, AlphaFold3 mutant LDLR–PCSK9 complex modelling, interface distance to the LDL binding surface derived from the Reimund *et al.* (*Nature* 2025) cryo-EM structure, and Islam *et al.* (2024) cell-surface LDLR functional assay (234 variants, 200 positions). ClinVar pathogenicity classifications were incorporated. Clinical validation used three independent cohorts: the Wales FH Registry (n=7,253; 2,405 genetically positive; 323 unique LDLR mutations; 1,515 ASCVD events), the DRAGON3 clinical cohort (n=1,362; 424 with identified mutations), and UK Biobank whole-exome sequencing (n=426,732; 1,623 FH carriers). Kaplan–Meier survival analysis with time-to-first-ASCVD methodology was used for domain-level penetrance estimation. ASCVD prediction used logistic regression adjusted for age, sex, LDL-C, statin use, diabetes, smoking, hypertension, and tendon xanthomata. An interactive clinical atlas with 3D AlphaFold structure visualisation was deployed at https://nadergenedy.github.io/calon-fh-atlas/

Results: Domain-level SSS correlated with ASCVD penetrance across 19 LDLR domains (Spearman ρ=0.644, P=0.003), with a 5.7-fold range: ligand-binding R4 (21.4%, n=42) versus ligand-binding R3 (3.8%, n=26). SSS was significantly higher in patients with tendon xanthomata (0.343 vs 0.297, $P=2.7\times10^{-4}$). In 1,095 patients with longitudinal LDL-C data, treatment response varied significantly by domain: ligand-binding R4 achieved 25.6% LDL-C reduction (n=21), EGF-A 21.9% (n=31), versus cytoplasmic domain 4.1% (n=19) — consistent with the mechanistic prediction that NPXY internalisation defects are refractory to conventional lipid-lowering therapy. Twenty-eight patients on PCSK9 inhibitors spanning 14 LDLR domains and 23 unique variants achieved 52% mean LDL-C reduction, demonstrating efficacy across all mutation types through wild-type allele rescue in heterozygous FH. VUS carriers (n=232) demonstrated ASCVD rates comparable to pathogenic carriers (18.1% vs 15.6% vs 3.5% in mutation-negative). FoldX thermodynamic predictions were independently validated against the Islam *et al.* functional assay: variants classified as decreased binding/uptake had mean ΔΔG=3.37 kcal/mol versus 0.06 kcal/mol for undetermined variants (56-fold difference). A fully adjusted ASCVD prediction model incorporating SSS with conventional risk factors and xanthomata achieved AUC=0.85 (n=350, 39 events).

Conclusions: CALON-FH demonstrates that LDLR domain architecture determines both ASCVD penetrance and treatment response in heterozygous FH. The 5.7-fold variation in domain-specific ASCVD risk and the 6.2-fold variation in treatment response (25.6% vs 4.1%) support a paradigm shift from uniform genotype-aware management to structure-informed, mutation-guided precision treatment. PCSK9 inhibitor efficacy across all 14 tested domains provides mechanistic reassurance for early use through wild-type allele protection, while the identification of cytoplasmic NPXY mutations as treatment-refractory supports early referral for LDLR-independent therapies including evinacumab.

Table 1. Domain-Specific ASCVD Penetrance, Treatment Response, and Structural Classification Across 3,064 LDLR Mutation Carriers

LDLR Domain	n	ASCVD Rate (%)	LDL-C Reduction on LLT (%)	Mean SSS	Mechanistic Class	Recommended Approach
Ligand-binding R4	42	21.4	25.6	0.303	Binding failure (BS1)	Early PCSK9i + ezetimibe
Transmembrane	37	21.6	—	0.629	Trafficking defect	High-intensity statin + ezetimibe
Cytoplasmic (NPXY)	48	18.8	4.1	0.447	Internalisation defect	Evinacumab /apheresis
EGF-A	102	15.7	21.9	0.397	Dual: BS1 + PCSK9 binding	Statin + ezetimibe → monitor PCSK9i
Ligand-binding (null)†	328	14.3	20.3	0.539	Loss-of-function	PCSK9i (WT rescue)
Linker (EGF-C/O-linked)	161	13.0	12.2	0.330	O-glycosylation defect	High-intensity statin
EGF-B	158	12.7	15.2	0.274	Recycling pivot	Standard escalation
EGF-precursor homology	258	10.9	15.6	0.409	Recycling /structural	Standard escalation
Beta-propeller	894	10.7	11.6	0.132	Recycling (BS2)	Standard escalation
Ligand-binding R2	214	8.4	16.9	0.191	Binding (BS1)	Statin + ezetimibe
Ligand-binding R3	26	3.8	25.3	0.314	Binding (BS1)	Standard care

Data from Wales FH Registry (n=3,064 LDLR carriers; 1,095 with longitudinal LDL-C). ASCVD = composite of MI, ACS, PCI, CABG, angina, TIA, PVD. LDL-C reduction = percentage change between first and second clinic visit on lipid-lowering therapy. SSS = Structural Severity Score integrating 14 data sources (AlphaFold3, FoldX 5.0, Rosetta, Islam et al. functional assay, UK Biobank WES outcomes, ClinVar). BS1 = primary LDL binding site (LA3–LA7 + EGF-A, Reimund et al. Nature 2025); BS2 = secondary binding site (beta-propeller). WT = wild-type. ASCVD column colour-coded: red ≥18%, amber 12–17.9%, green <12%. †Splice-site and frameshift mutations assigned SSS based on predicted loss-of-function. — = insufficient longitudinal data.