POSITION MATTERS: FUNCTIONAL EFFECTS OF *LDLR* NONSENSE VARIANTS

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BACKGROUND

Familial hypercholesterolaemia (FH) is a common monogenic lipid disorder predominantly caused by pathogenic variants in *LDLR*, leading to impaired clearance of low-density lipoprotein cholesterol (LDL-C). Nonsense variants are traditionally assumed to result in complete loss of receptor function through nonsense-mediated mRNA decay (NMD). However, emerging evidence suggests that their molecular and functional impact may vary depending on their position within the gene.

AIM

To functionally characterise eleven *LDLR* nonsense variants localized through the LDLR gene using a flow cytometry–based assay assessing the full LDLR cycle (expression, LDL binding, and uptake) to better understand the effect of variants in the 3' end.

METHODS

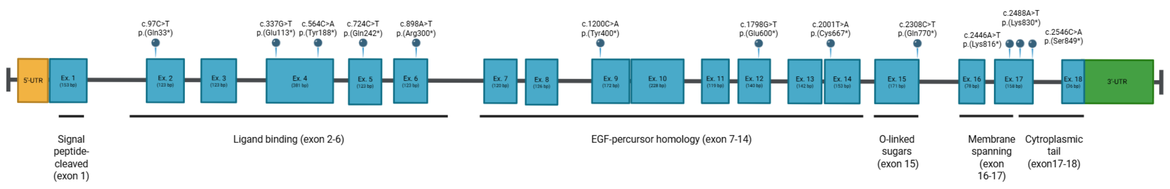
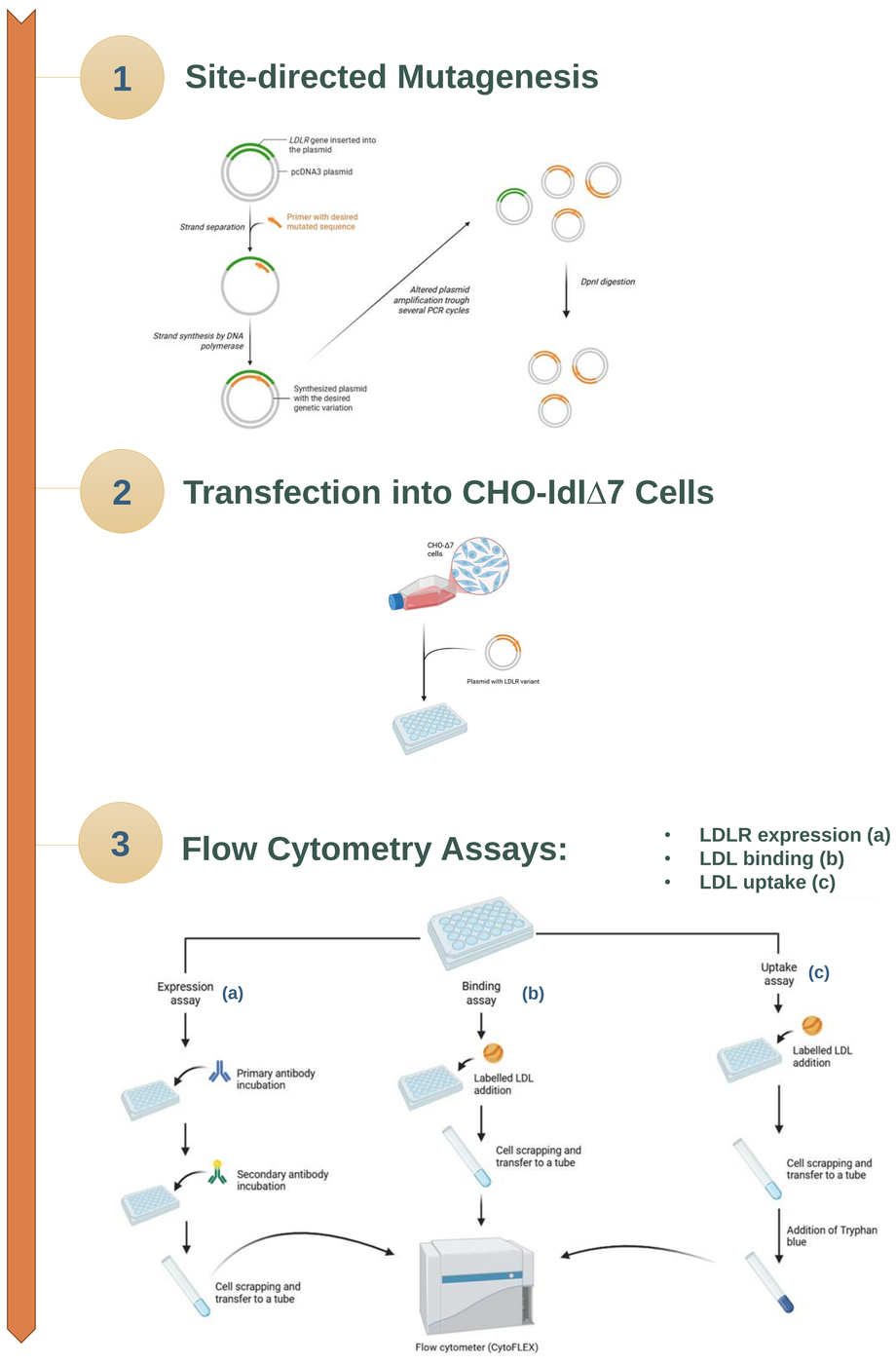


Figure 1. Schematic representation of the *LDLR* gene showing the nonsense variants analysed in this work.



RESULTS

- ✓ Eleven nonsense (stop) variants distributed along the *LDLR* gene were analysed (Figure 1), revealing that functional activity varies according to variant position within the gene.
- ✓ Variants **p.(Gln33*), p.(Glu113*), p.(Tyr188*), p.(Gln242*)** showed severely impaired activity across all assays: expression, binding, and LDL uptake (<10%) (Figure 2).
- ✓ The **p.(Glu600*)** variant exhibited an intermediate functional profile, with reduced receptor expression (47%), binding (16%), and markedly impaired LDL uptake (9%), consistent with a partial loss-of-function phenotype (Figure 2).
- ✓ The **p.(Lys816*)** variant exhibited normal receptor expression and binding, but reduced LDL uptake (40%), consistent with an internalization defect (Figure 2).
- ✓ In contrast, **p.(Lys830*)** and **p.(Ser849*)** displayed expression, binding, and uptake levels comparable to those of wild-type controls, suggesting that truncations near the C-terminal domain may escape nonsense-mediated decay (NMD) and retain most functional receptor activity (Figure 2).

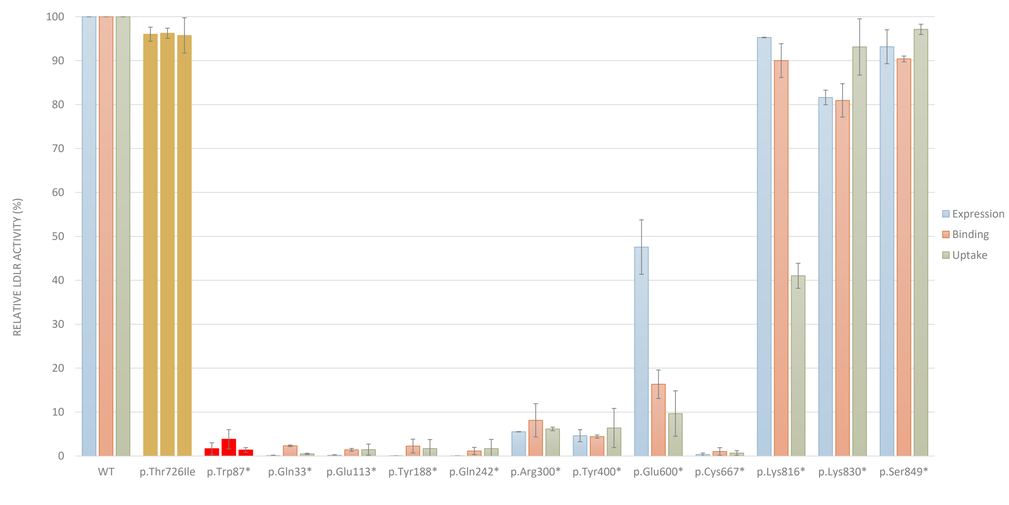


Figure 2. Results of flow cytometry from the three functional assays performed for 11 *LDLR* variants. First column (**blue**) represents **LDLR expression** after incubation with primary antibody (anti-LDL receptor mouse monoclonal, IgG-C7) and secondary antibody (Alexa Fluor™ 488 goat anti-mouse IgG). Second column (**orange**) represents **LDL receptor's binding** capacity after 3h incubation at 4°C with FITC-labelled LDL. Third column (**green**) represents **LDL receptor's uptake** capacity after 3h incubation at 37°C with FITC-labelled LDL and treatment with Trypan Blue. Variant p.(Trp87*) (**red**) represents a positive control and variant p.(Thr726Ile) (**light golden**) represents a normal control.

CONCLUSIONS

These findings challenge the assumption that all nonsense variants abolish LDLR function, showing that their molecular and functional impact varies with position. While the majority of nonsense variants undergo NMD, some variants may escape NMD and this can not be determined by variant type alone, underscoring the need for functional validation. Notably, very early truncating variants such as p.(Gln33*) have been reported and characterized as escaping NMD (1). Ongoing work will further characterise these 11 *LDLR* nonsense variants and others around the C terminal, including targeted assays to assess NMD susceptibility.

Functional characterization is essential to accurately interpret *LDLR* nonsense variants and improve FH diagnosis

(1) Wang K, Hu T, Tai M, Shen Y, Lin S, Guo Y, Chen X. Pathogenicity of the LDLR c.97C>T (p.Gln33Ter) Mutation in Familial Hypercholesterolemia. Mol Genet Genomic Med. 2024 Nov;12(11):e70030. doi: 10.1002/mgg3.70030. PMID: 39600113; PMCID: PMC11599428.

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