FROM SCREENING TO PRECISION CARE: VALIDATING BORDERLINE *LDLR* VARIANTS THROUGH FLOW CYTOMETRY

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BACKGROUND

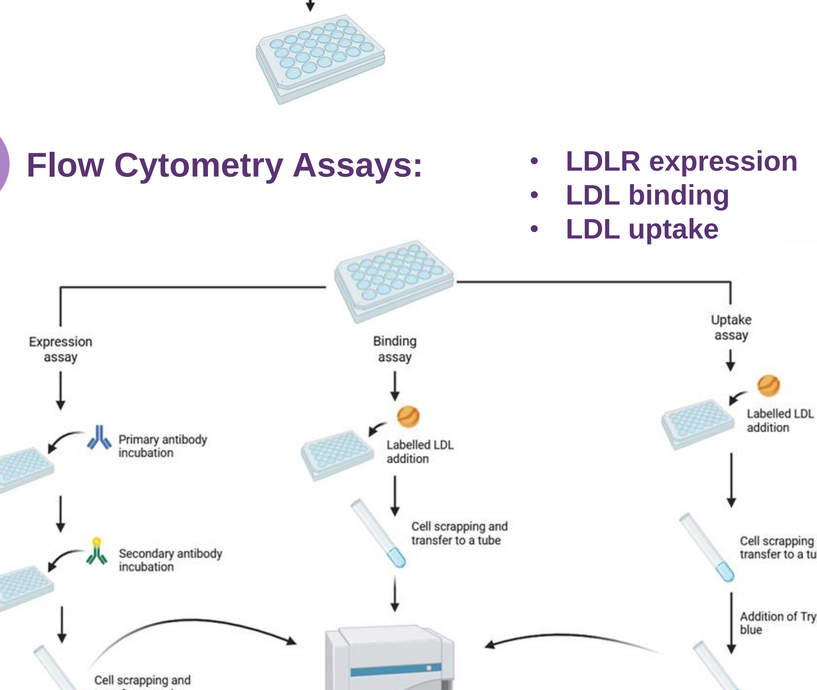
- Familial Hypercholesterolaemia (FH) is a semidominant disorder primarily caused by variants in *LDLR*, leading to elevated LDL-cholesterol levels. Functional characterisation is essential for accurate diagnosis and clinical management.
- High-throughput assays start to emerge as screening tools (e.g., PerMedFH), but they provide an overall measure of LDLR activity and may mask defects in specific steps of the LDLR cycle.
- Variants with borderline activity (70–90%) remain particularly challenging, as current FH VCEP guidelines do not consider these results as definitive functional evidence.

AIM

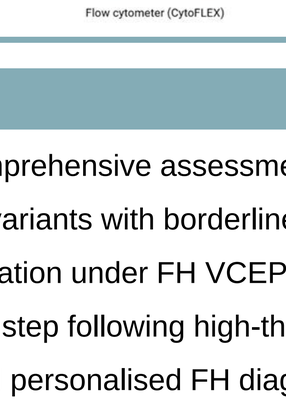
To determine whether LDLR variants with borderline activity (70–90%) exhibit functional defects when assessed by flow cytometry.

METHODS

1 Site-directed Mutagenesis



2 Transfection into CHO-ldlΔ7 Cells



3 Flow Cytometry Assays:

- **LDLR expression**
- **LDL binding**
- **LDL uptake**

RESULTS

- ✓ Most variants (15/16) showed normal LDLR expression, binding, and uptake (Figure 1), meaning that these variants don't affect the LDL metabolism.
- ✓ Variant **c.1190C>T/p.(Ser397Phe)** showed:
  - Normal expression
  - Normal binding
  - Impaired LDL uptake (<5%)
    - ❑ This variant is responsible for an increase in LDL cholesterol levels.
- ✓ Flow cytometry revealed functional defects not detected by high-throughput screening.
- ✓ Functional classification may differ depending on the assay used, particularly for variants with borderline activity (70–90%).
- ✓ p.(Ser397Phe) shows a selective defect in LDL uptake (<70%), supporting pathogenic classification (FH VCEP).
- ✓ Even in the absence of detectable defects, flow cytometry provides high-level evidence that strengthens variant classification (FH VCEP).

Flow cytometry reveals a functional defect in p.(Ser397Phe) not detected by screening assays

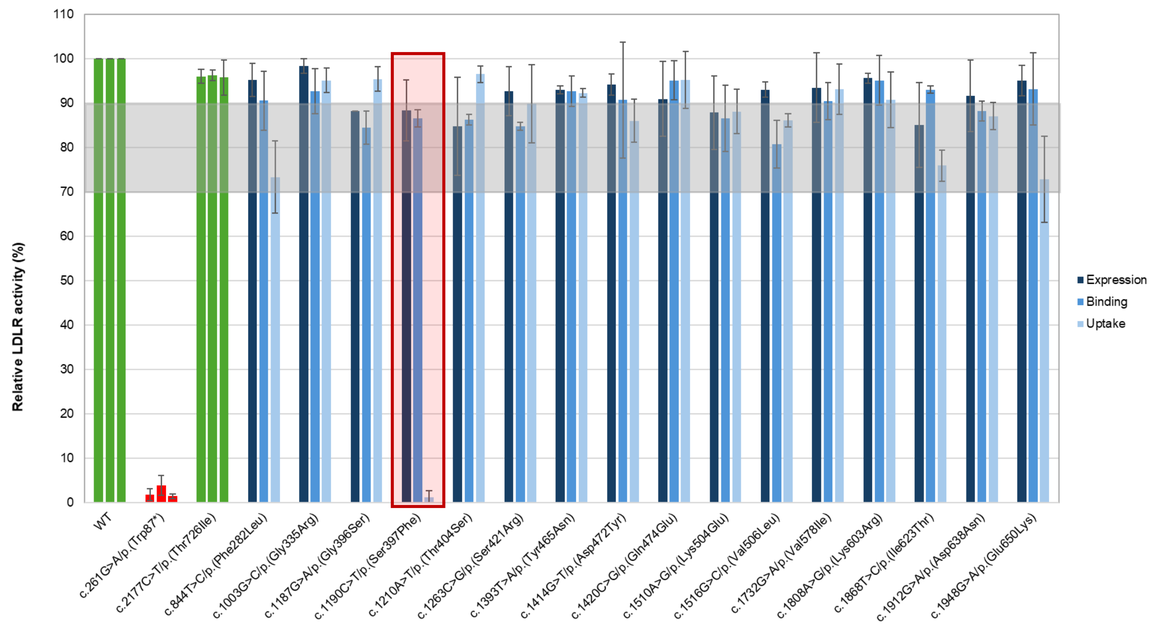


Figure 1. Results of flow cytometry from the three functional assays performed for 16 *LDLR* variants. First column (**dark blue**) represents LDLR expression after incubation with primary antibody (anti-LDL receptor mouse monoclonal, IgG-C7) and secondary antibody (Alexa FluorTM 488 goat anti-mouse IgG). Second column (**medium blue**) represents LDL receptor's binding capacity after 3h incubation at 4°C with FITC-labelled LDL. Third column (**light blue**) 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 defective control and variant p.(Thr726Ile) (**green**) represents a normal control.

CONCLUSIONS

- Flow cytometry enables comprehensive assessment of LDLR function
- Identifies hidden defects in variants with borderline activity
- Strengthens variant classification under FH VCEP guidelines
- Provides a critical validation step following high-throughput screening
- Supports more accurate and personalised FH diagnosis

IMPACT

Integrating targeted functional assays into screening pipelines improves diagnostic accuracy and helps refine clinical decision-making in FH.

PerMedFH project funded by La Caixa Foundation in partnership with:
Fundação para a Ciência e a Tecnologia