Functional Studies in *LDLR* to Improve Genetic Diagnosis in Familial Hypercholesterolemia

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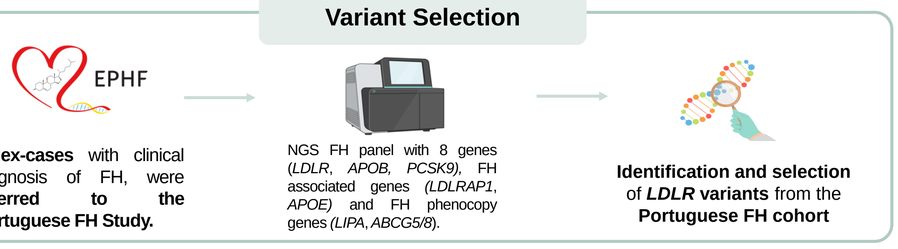
BACKGROUND AND AIMS

Familial hypercholesterolemia (FH) is the most common inherited disorder of lipid metabolism, affecting approximately 1 in 300 individuals. FH is an autosomal semidominant disorder and was the first genetic lipid metabolism disorder to be molecularly characterized. Individuals with FH present elevated blood cholesterol levels from birth, leading to a high cumulative risk of developing cardiovascular disease. Pathogenic variants in the low-density lipoprotein receptor (LDLR) gene are the primary cause of FH, with more than 4,000 variants identified to date. However, only 15% of these have been functionally characterized in vitro, demonstrating their impact (or lack of) on LDL receptor function.

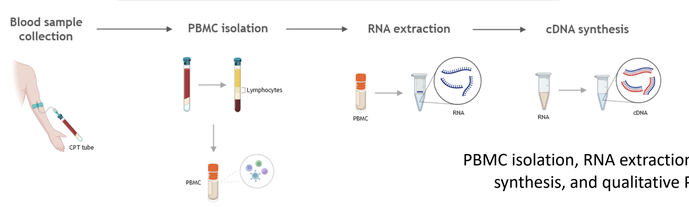
This study aims to **highlight the role of functional studies in genetic diagnosis and personalized medicine**, using the Portuguese Familial Hypercholesterolemia Study (EPHF) as an example of how these studies can have a clinical impact. Functional studies of *LDLR* are crucial for understanding how the LDL receptor activity regulates cholesterol clearance.

METHODS

Variant Selection

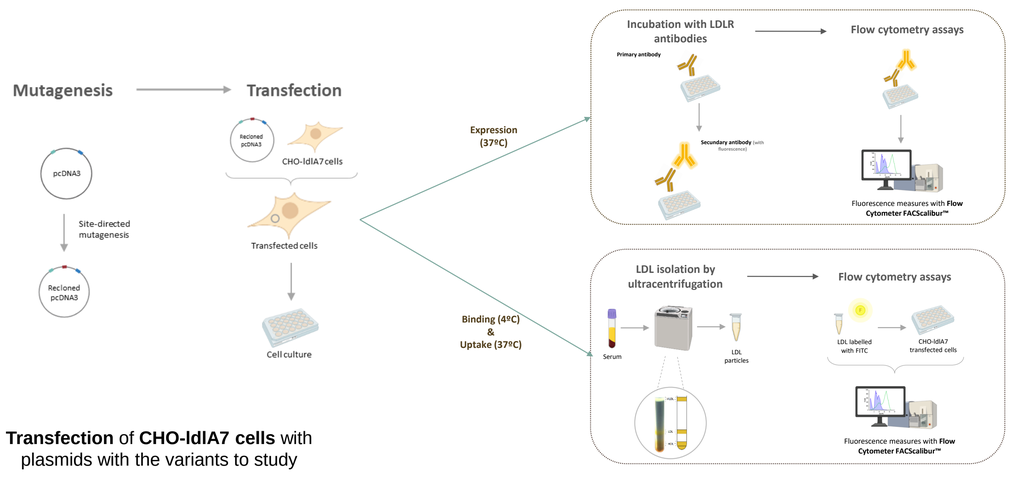


Functional Studies - splicing variants



PBMC isolation, RNA extraction, cDNA synthesis, and qualitative PCR

Functional Studies - missense variants



Transfection of **CHO-IdlA7 cells** with plasmids with the variants to study

Expression, LDL binding and **uptake** determination with **Flow Cytometer FACScalibur™**

Key Information Overview

Table 1. Functional characterization of *LDLR* variants: key information overview.

LDLR residual activity	allele type	clinical significance	Most common type variant	impact
<10%	null allele	very severe phenotype (LDL ↑↑↑↑)	stop variant, frameshift or large deletions	complete lack of LDLR expression or an inability to bind to LDL cholesterol
>10-70%	defective allele	variable phenotype (LDL ↑↑)	missense variants/ splicing/	can lead to partial defects in various stages of the LDLR cycle, including expression, binding, internalization, and recycling – a complete inability of binding to LDL cholesterol
>90%	normal allele	does not cause the phenotype (do not cause FH)	missense variants/ intronic/ Synonymous	no impact

RESULTS & DISCUSSION

- A total of 3579 individuals were referred to the Portuguese Familial Hypercholesterolemia Study (EPHF), including 1291 index cases and 2288 relatives (**Figure 1**).
- In a cohort of 1088 genetically confirmed FH cases (1073 heterozygotes and 15 homozygotes), a total of 164 distinct *LDLR* variants were identified (**Figure 1**).

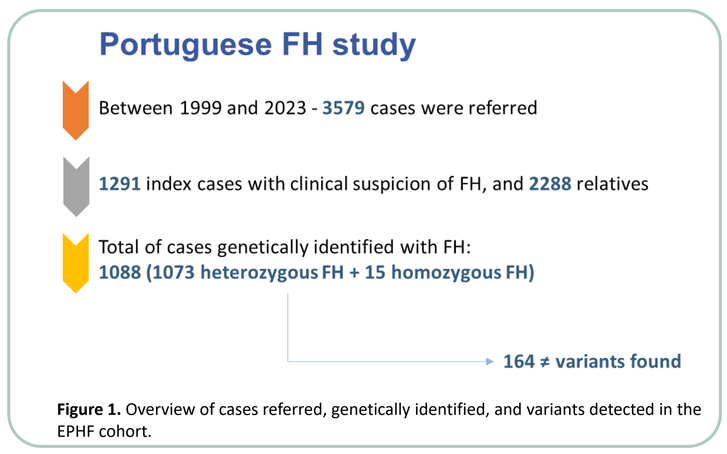


Figure 1. Overview of cases referred, genetically identified, and variants detected in the EPHF cohort.

- To date, **70 of these variants** have been functionally studied within the EPHF and 38 have been characterized by other labs (**Figure 2**).

164 variants in the EPHF	variants	patients	with FS	FS by EPHF
LARGE DELETIONS	7	79	4	0
NONSENSE	10	29	3	0
MISSENSE	81	681	66	54
FRAMESHIFT	20	64	2	0
IN FRAME	5	40	5	0
5'UTR/PROMOTER	3	51	3	0
SPLICING/INTRONIC	28	142	20	12
SYNONYMOUS	10	29	4	1

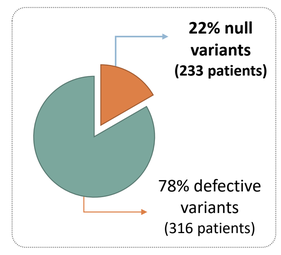


Figure 2. Type of variants identified in the EPHF cohort. Twenty two percent of all missense variants studied were shown to be null alleles due to no receptor function. Eighty-two percent of the missense variants were functionally characterized in our laboratory, with the majority (78%) classified as defective. FS- Functional studies

- The functional characterization of variants is essential, as it provides key information for the ACMG classification system, particularly supporting the application of the PS3 criterion. Following functional assays, 47 variants (127 individuals) were reclassified from VUS to likely pathogenic according to ACMG criteria.

CONCLUSIONS

This functional characterization contributed to obtain a **definitive genetic diagnosis for 847 individuals**, marking a significant step towards personalized medicine and the effective management of FH.
These studies not only improve the classification of *LDLR* variants but also directly influence treatment decisions and patient outcomes. By advancing the functional characterization of these variants, we contribute to more specialized and precise diagnostics, leading to more tailored therapeutic approaches for individuals with FH.

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