EAS 93rd Congress
4-7 May 2025 | Glasgow, UK
eas-congress.com/2025

UNVEILING THE ROLE OF *APOB* VARIANTS IN FAMILIAL HYPERCHOLESTEROLEMIA: FUNCTIONAL INSIGHTS

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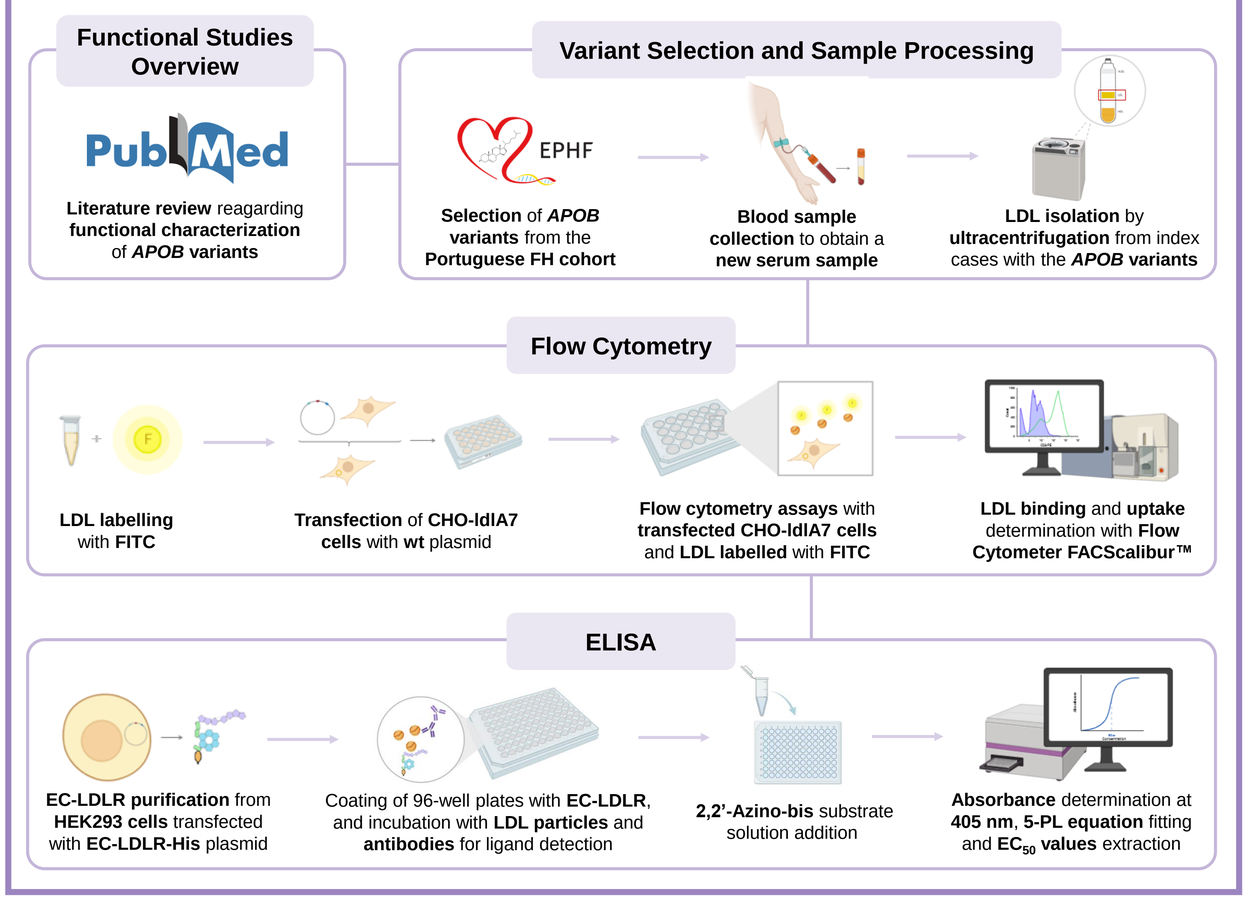
BACKGROUND

Familial hypercholesterolemia (FH) is a condition characterized by increased LDL cholesterol levels with *APOB* variants accounting for about 5-10% of FH cases. However, variants in this gene may be more common than initially estimated since the entire *APOB* gene has only recently started to be sequenced. Although most of the alterations are missense, nonsense variants and small indels in exon 29 were also identified in individuals with FH phenotype and can be the cause of disease.

AIM

This work aimed to characterize *APOB* variants identified in individuals with clinical diagnosis of FH. Moreover, we intended to do an overview of the *APOB* variants presenting functional studies.

METHODS



RESULTS

APOB FUNCTIONAL STUDIES OVERVIEW

- ✓ PubMed repository was consulted to collect publications regarding functional characterization of *APOB* variants.
- ✓ There are **22 *APOB* variants** functional characterized in the literature, **six** of which characterized by our group (variants in bold below).
- ✓ **13 variants** affect binding of the apoB to the LDL receptor and **nine variants** do not affect apoB binding.

Normal Function

c.2981C>T/p.(Pro994Leu) (1)
c.3337G>C/p.(Asp1113His) (2)
c.3740A>G/p.(Tyr1247Cys) (2)
c.5741A>G/p.(Asn1914Ser) (3)
c.5768A>G/p.(His1923Arg) (3)
c.10193C>T/p.(Ala3398Val) (4)
c.10294C>G/p.(Gln3432Glu) (4)
c.11401T>A/p.(Ser3801Thr) (5)
c.13441G>A/p.(Ala4481Thr) (3)

Defective Function

c.148C>T/p.(Arg50Trp) (6)
c.2863C>T/p.(Pro955Ser) (7)
c.3491G>C/p.(Arg1164Thr) (2)
c.9175C>T/p.(Arg3059Cys) (8)
c.10030A>G/p.(Lys3344Glu) (5)
c.10182G>T/p.(Lys3394Asn) (8)
c.10519C>T/p.(Arg3507Trp) (9)
c.10579C>T/p.(Arg3527Trp) (10)
c.10580G>A/p.(Arg3527Gln) (11)
c.10629C>G/p.(Asn3543Lys) (12)
c.10672C>T/p.(Arg3558Cys) (13)
c.11477C>T/p.(Thr3826Met) (1)
c.13480_13482del/p.(Gln4494del) (2)

RESULTS

FLOW CYTOMETRY ASSAYS

- ✓ Flow cytometry assays were performed to assess LDL binding and uptake.
- ✓ The variants **p.(Ala1393Val)**, **p.(Asp1456Asn)**, **p.(Met2042Thr)**, **p.(Asp2213del), p.(Ile3374Thr), p.(Val4295Leu)** and **p.(Arg4519Thr)** do not affect the binding of apoB to the LDL receptor (**Figure 1A, B**).
- ✓ In contrast, the **p.(Gln4316*)** variant exhibited reduced affinity for the LDLR, impairing apoB's ability to bind to LDL receptor (**Figure 1A, B**).
- ✓ Here, we present only the findings from the index cases studied; however, analyses of their relatives (both with and without phenotype) revealed similar results.

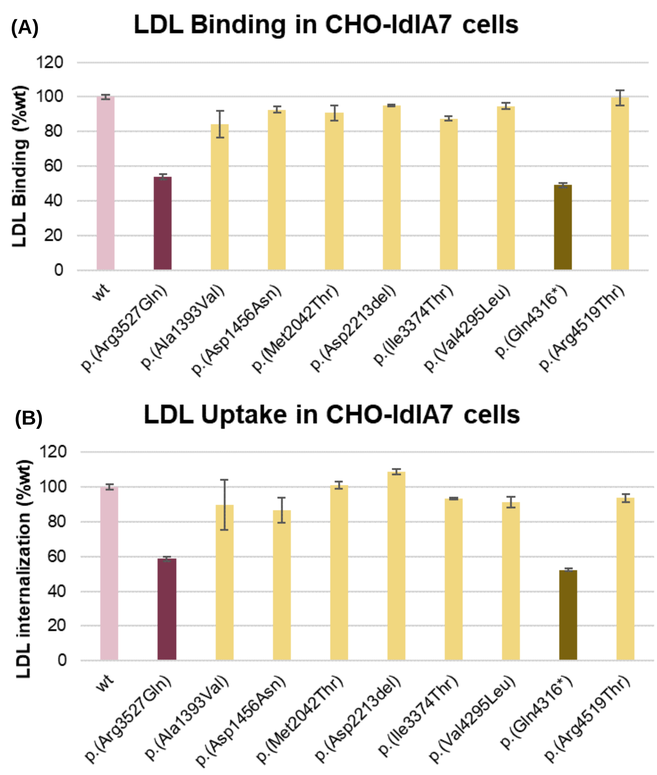


Figure 1. Results of flow cytometry assays for the eight variants analysed. **(A)** LDL binding efficiency after 3h incubation at 4°C in CHO-IdlA7 cells with FITC-LDL from each the index case. **(B)** LDL internalization efficiency after 3h incubation at 37°C in CHO-IdlA7 cells with FITC-LDL from each index case.

ELISA ASSAYS

- ✓ For **p.(Gln4316*)** variant, ELISA assays were also performed to determine the binding affinity of apoB to the LDLR.
- ✓ Preliminary results showed reduced affinity for the LDLR compared to wildtype (wt), consistent with findings from flow cytometry. These results suggest that this variant impairs the normal function of apoB (**Figure 2**).
- ✓ The characterization of the remaining variants by ELISA is ongoing.

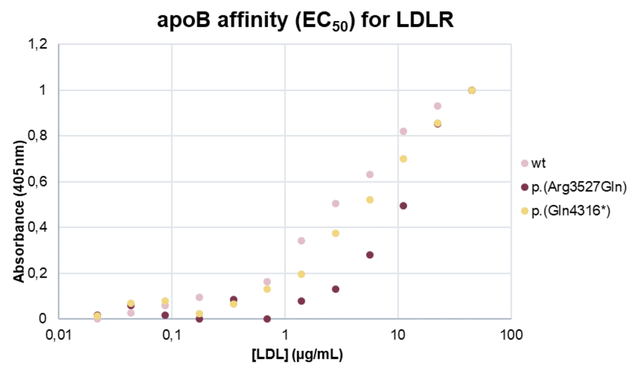


Figure 2. Results of ELISA assays for p.(Gln4316*) variant. Affinity curves representing the binding affinity of *APOB* variant for the LDLR determined by solid-phase immunoassay at pH 7,4. Data represents the mean of three independent experiments. *p<0,01 compared to wt.

CONCLUSION

Here we report the functional study of 8 *APOB* variants. Seven missenses do not alter apoB function but **one variant that introduces a stop codon in exon 29, was shown to affect apoB binding to the LDL receptor, being the first stop variant in *APOB* characterized as causing FH**. Functional studies play a critical role in assessing the pathogenicity of genetic variants and are among the key criteria for variant classification. This is particularly important for *APOB* variants because of their low penetrance and high number of silent variants. These functional analyses confirm clinical diagnosis and provide essential insights for developing personalized treatment strategies. In the future, we aim to increase the number of studied variants, starting with 15 more variants from the Portuguese FH Study.

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

With thanks to the EAS for support in the form of a Young Investigator Fellowship