Functional characterization of two *APOB* variants from exon 29 found in individuals with clinical diagnosis of Familial Hypercholesterolemia

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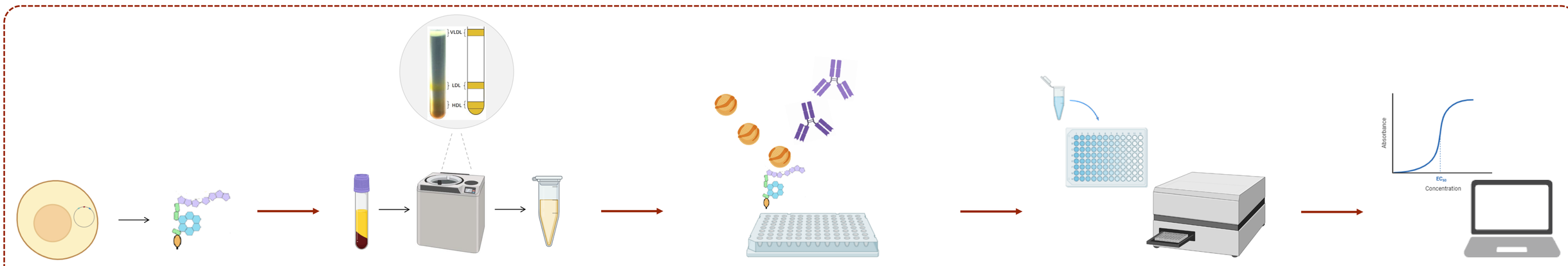
Introduction

Familial hypercholesterolemia (FH) is an inherited lipid disorder characterized by increased levels of LDL cholesterol. About 5-10% of FH cases occur due to variants in the *APOB* gene, but these alterations can be a more common cause of FH than expected since most of *APOB* variants identified is still unknown their effect on the metabolism. The majority of the variants are missense but there are a few nonsense variants and small indels in exon 29 identified in individuals with hypercholesterolemia phenotype that can cause FH.

The aim of this project was to functional characterize *APOB* variants from exon 29 identified in individuals referred to the Portuguese FH Study to assess if these are the genetic cause of disease.

Methodologies



ED-LDLR purification from **HEK293 cells** transfected with **EC-LDLR-His** plasmid

LDL isolation by **ultracentrifugation** from index cases and relatives with the ***APOB* variants**

Coating of 96-well plates with **ED-LDLR**, and incubation with **LDL particles** and **antibodies** for ligand detection

2,2'-Azino-bis substrate solution addition and **absorbance** determination at **405 nm**

5-parameter logistic equation fitting and **EC_{50} values** extraction

- The **N-terminal extracellular ectodomain of the LDLR (ED-LDLR)** carrying both c-myc and His tag was purified from HEK293 cells transfected with the pcDNA3.1-EC-LDLR-His plasmid, and LDL from individuals with the alterations was separated by ultracentrifugation.
- Purified ED-LDLR fragments were coated onto 96-well plates at a fixed concentration and incubated with a serial dilution of each LDL sample; antibodies were used for ligand detection.
- Antibody binding was determined by adding **2,2'-Azino-bis substrate solution** and the color change was measured at **405 nm**.
- Absorbance values were corrected for unspecific binding relative to maximum absorbance and **EC_{50} values** were extracted from curves after fitting the data to a **5-parameter logistic (5-PL) equation** using **SigmaPlot 13.0 software**.

Results

- ✓ We aimed to functionally assessed two variants located in exon 29: **p.(Gln4316*)** and **p.(Glu4387Asnfs*7)**. Until now, we only have results for the variant **p.(Gln4316*),** the functional characterization of the frameshift variant is ongoing.
- ✓ Both variants are located closest to the region responsible for apoB:LDLR binding, had never been found in a normolipidemic panel and are not present in GnomAD
- ✓ The variant **p.(Gln4316*)** was found in an index case (II:1) with FH phenotype and it appears to **co-segregate** in the family with the phenotype. Individuals II:2 and I:1 present the same variant as individual II:1, as well as FH phenotype (**Figure 1**).
- ✓ *In vitro* analysis of **p.(Gln4316*)** showed reduced affinity for the LDL receptor compared to wildtype (wt), meaning that p.(Gln4316*) variant affects apoB normal function (**Table 1** and **Figure 2**).
- ✓ Flow cytometry assays to determine LDL binding for and uptake for **p.(Gln4316*)** are ongoing, and preliminary results demonstrate similar results to those obtained by ELISA.

Table 1. Results of functional assays for p.(Gln4316*) variant. EC_{50} values of wt, p.(Arg3527Gln) *APOB* variant, and from the index case and respective relatives representing the binding affinity of *APOB* variants to the LDLR determined by solid-phase immunoassay at pH 7,4.

Sample	EC_{50}
wt	100 ± 0,39
p.(Arg3527Gln)	272,3 ± 0,38*
II:1	214,0 ± 0,09*
II:2	208,6 ± 0,56*
I:1	181,7 ± 0,75*
I:2	113,6 ± 0,075*

*p < 0,01 compared to wt

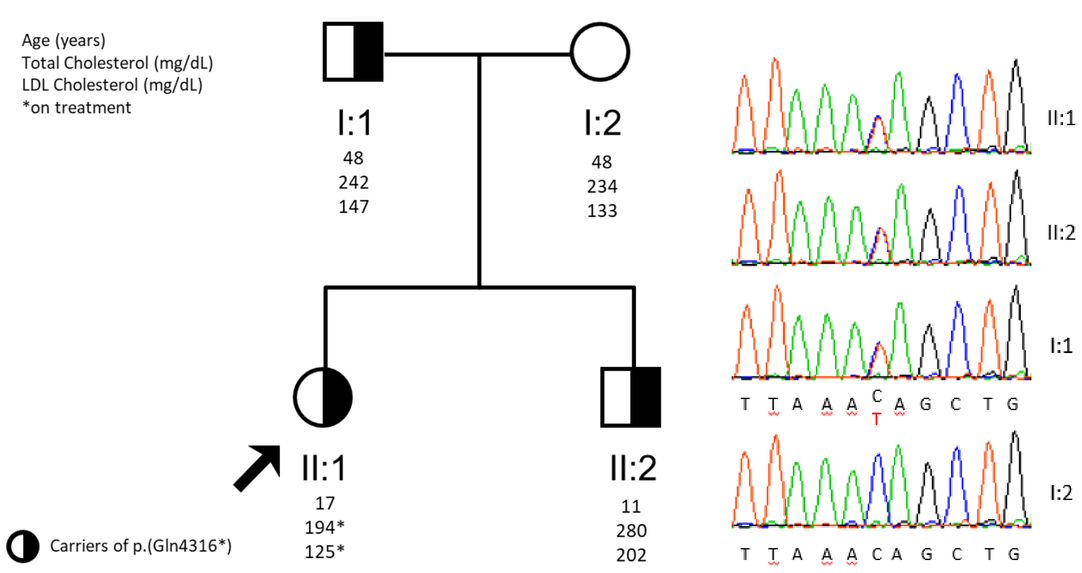


Figure 1. Pedigree of the family and partial sequence of exon 29 of *APOB* gene of the index case and respective relatives, showing the alteration C→T located in position c.12946, resulting in the amino acid change from a Glutamine to a stop codon at protein position 4316. Index case is indicated by an arrow.

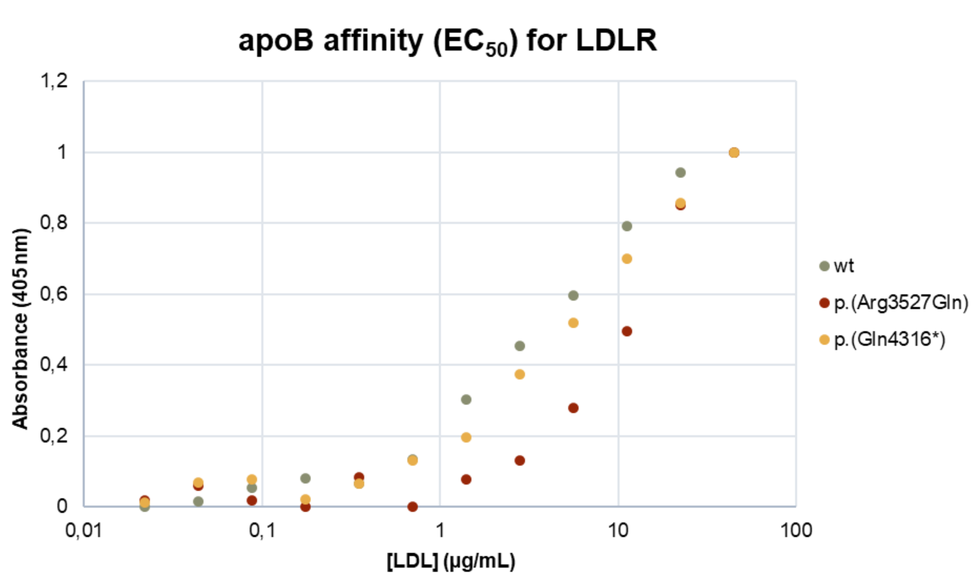


Figure 2. Results of functional assays for p.(Gln4316*) variant. Affinity curves representing the binding affinity of *APOB* variants for the LDLR determined by solid-phase immunoassay at pH 7.4. Data represents the mean of three independent experiments. EC_{50} values are shown in Table 1.

Discussion

APOB variants studied in this work produce truncated forms of apoB that are unlikely to lead to nonsense-mediated decay processes due to their location near the end of the gene. The variants in exon 29 that produce truncated forms of apoB should all be studied, since they may be the cause of hypercholesterolemia in patients with a clinical diagnosis of FH and without an identified variant. Functional studies can provide important evidence for variant pathogenicity assessment being these essential to provide an accurate diagnosis. These assays can confirm the clinical diagnosis by highlighting the cause of disease an contribute to a personalized treatment, also allowing for a better patient cardiovascular risk stratification.