Estudios de Análisis funcional de variantes de significado incierto

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HOW TO UPGRADE VARIANT CLASSIFICATION

- Share data between labs to increase evidence level
  - **ClinGen FHVCEP**

- Functional studies (several techniques)
  - *LDLR* Missense, splicing, promotor and 5'UTR – construct of in vitro mutants
  - *APOB* Missense - **Need patient sample to isolate LDL**
  - *PCSK9* Missense promotor and 5'UTR – construct of in vitro mutants

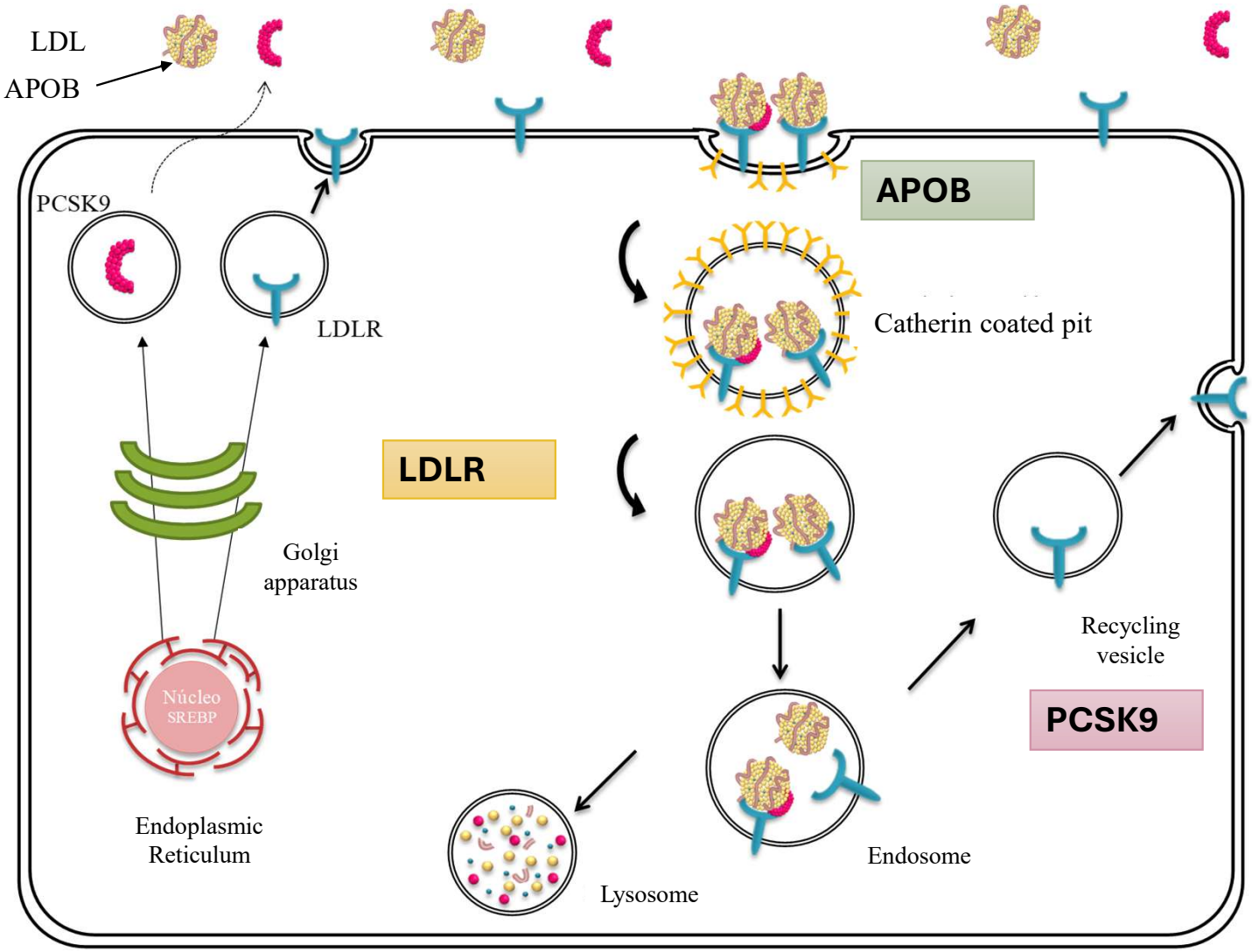
LDLR Cycle

3 genes causing FH

LDLR **- >90%**

APOB **– 5-10%**

PCSK9 **– 1-3%**



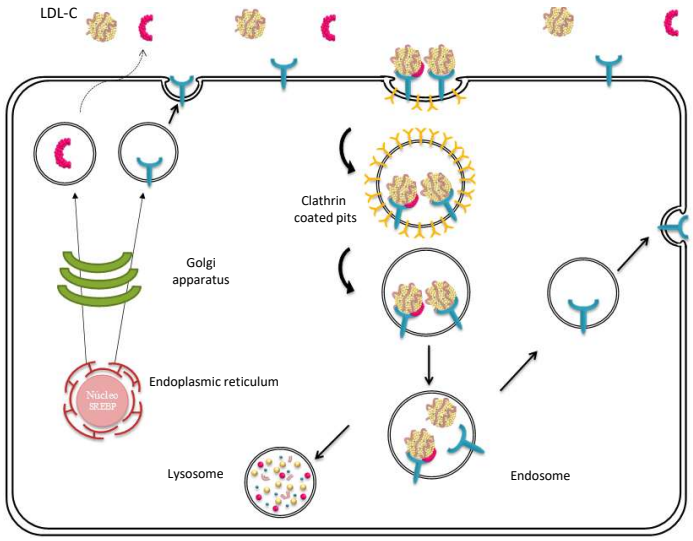
Type of functional assays

LDLR

- **Expression defects**
  - Promotor and 5' UTR variants
  - Missense variants
  - Nonsense, large deletions
- **Binding (+ uptake) defects**
  - Missense variants (eg in exons 2-6)
- **Uptake defects**
  - Missense variants (eg in exons 17 and 18)
- **Recycling defects**
  - Missense variants

APOB

- **Binding defects**
  - Missense variants
  - Stop variants exon 29???



PCSK9

- **Recycling defects / increased LDLR degradation**
  - Missense variants
- **Expression defects / increased number of PCSK9 at cell surface**
  - promotor variants
  - Duplications of all gene
  - 5'UTR variants???

??? Under investigation

Type of functional assays

LDLR and *PCSK9* – variant cloned in a vector (*in vitro* mutagenesis) and transfected into specific cells. Analysis of the whole *LDLR* cycle

Missense variants (>50% of *LDLR* variants)

Quantification of LDLR Activity by high throughput microscopy (DiI-LDL)

- Expression
- Binding + internalization

Quantification of LDLR Activity by Flow Cytometry (FITC-LDL)

- Expression – 37ºC
- Binding – 4ºC
- Internalization – 37ºC

OTHER STUDIES

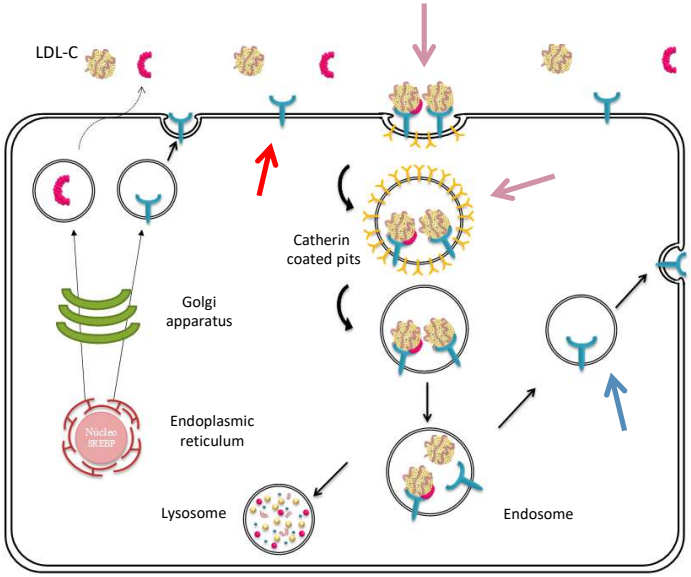
APOB – serum from the patients. Binding assay.

LDLR and *PCSK9* – Promotor assay with Luciferase

LDLR - Recycling assays. Degradation studies (pH dependent)

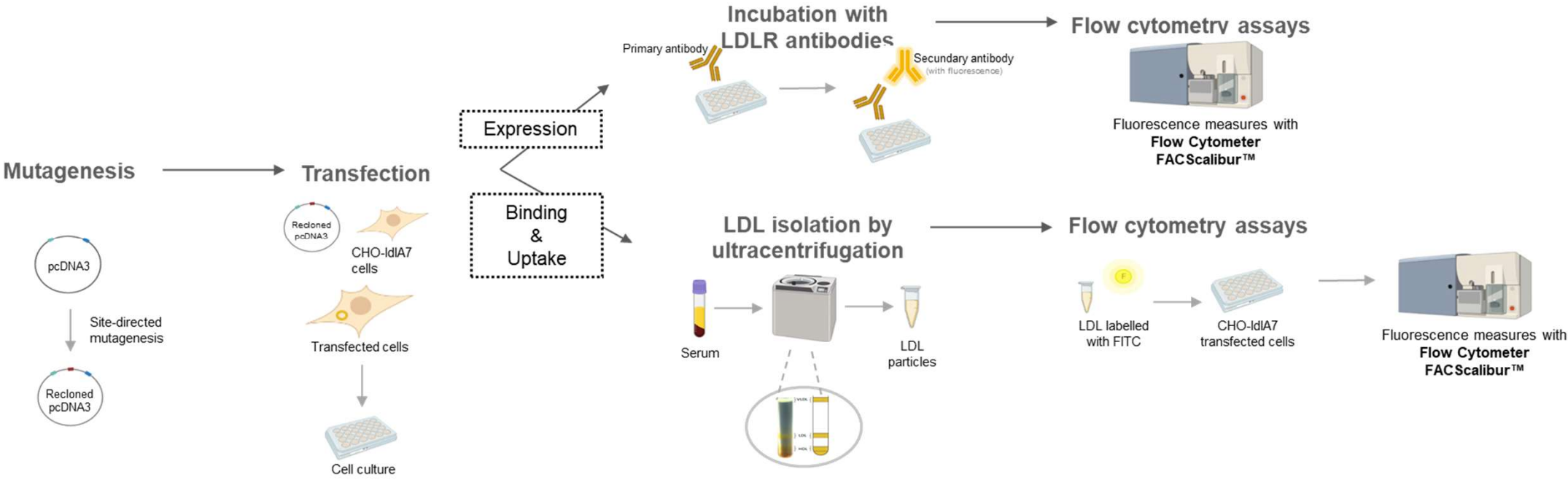
LDLR – splicing assays. Mini genes. Transcript quantification

Different *LDLR* variants



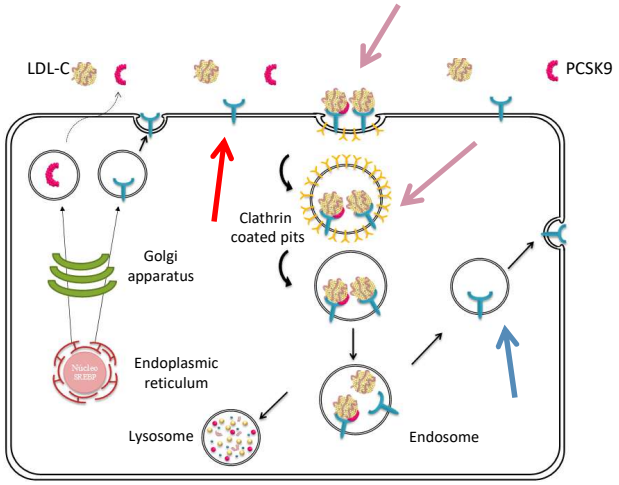
LDLR residual activity	allele type	clinical significance	Most common type variant	impact
<10%	null allele	very severe phenotype	stop variant, frameshift or large deletions	complete lack of LDLR expression or an inability to bind to LDL cholesterol
>10-70%	defective allele	variable phenotype	missense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

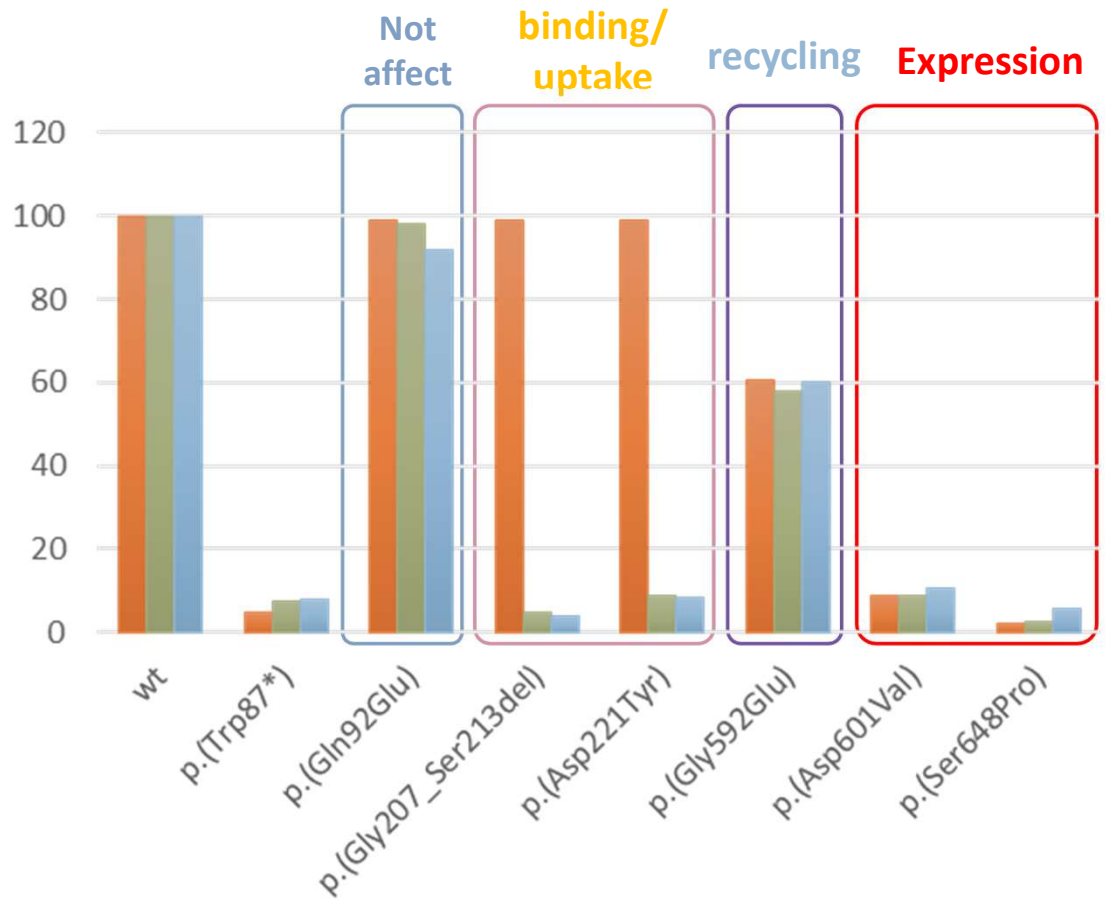
Functional Studies - Missense



Functional Studies - Missense

In vitro model to study the *LDLR* cycle

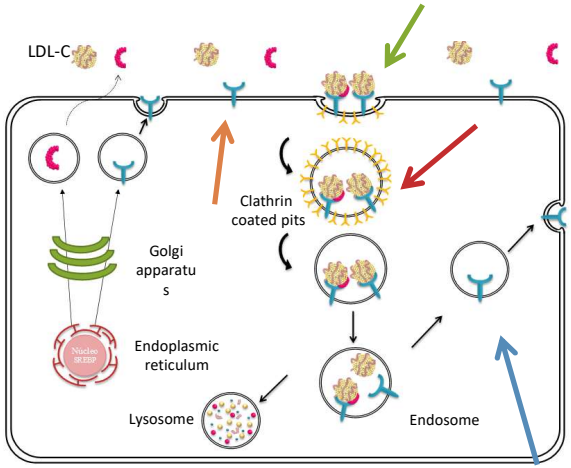


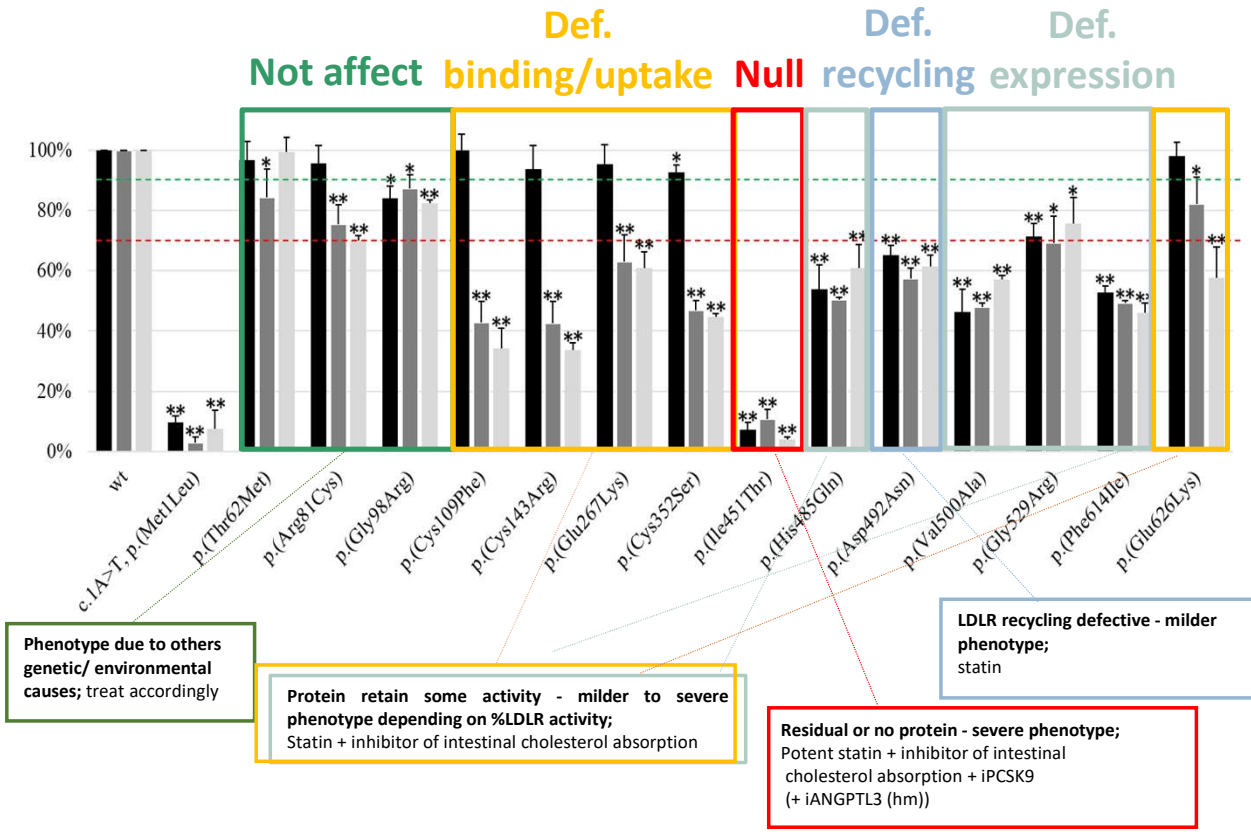


PRECISION MEDICINE FOR FH PATIENTS

Disease mechanism known for more than 30 years

In vitro model to study the LDLR cycle

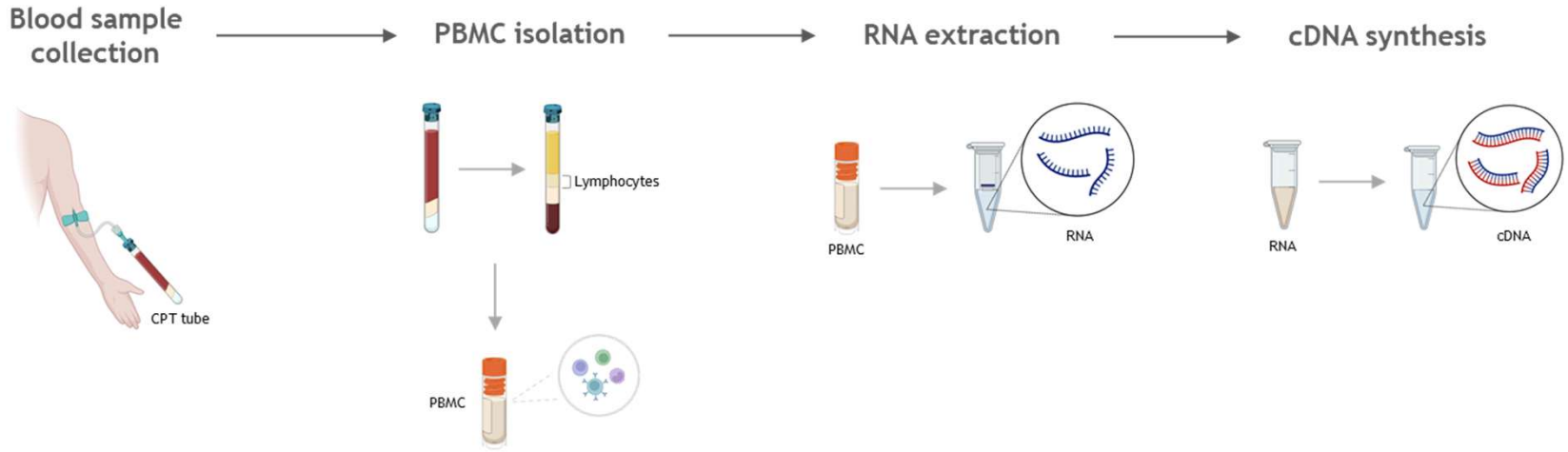




- Treatment optimization considering LDLR activity
- increase adherence to treatment
- decrease of side effects

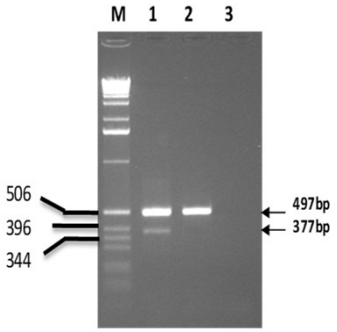
Graça R, *et al.* 2022, JCL

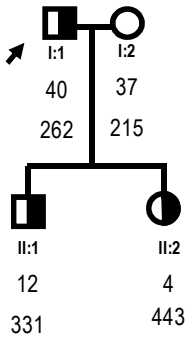
Methods – splicing variants

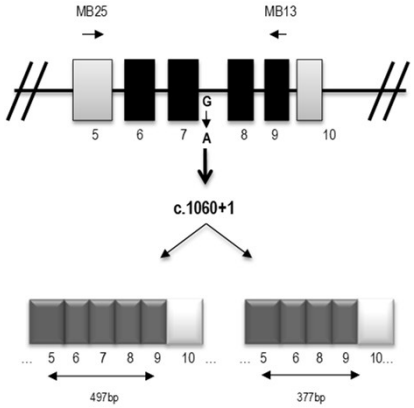


Functional Studies - Splicing

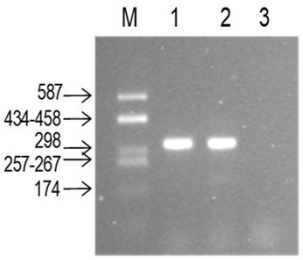
c. 1060+1 G>A – skipping ex7

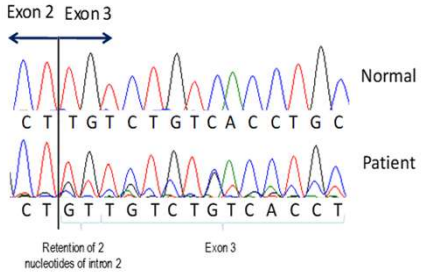


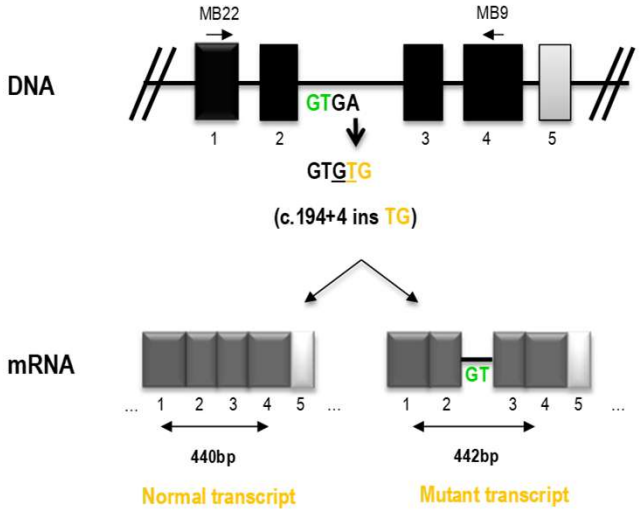




c.192+4 ins TG - Retention 2 nucleotides in intron 2

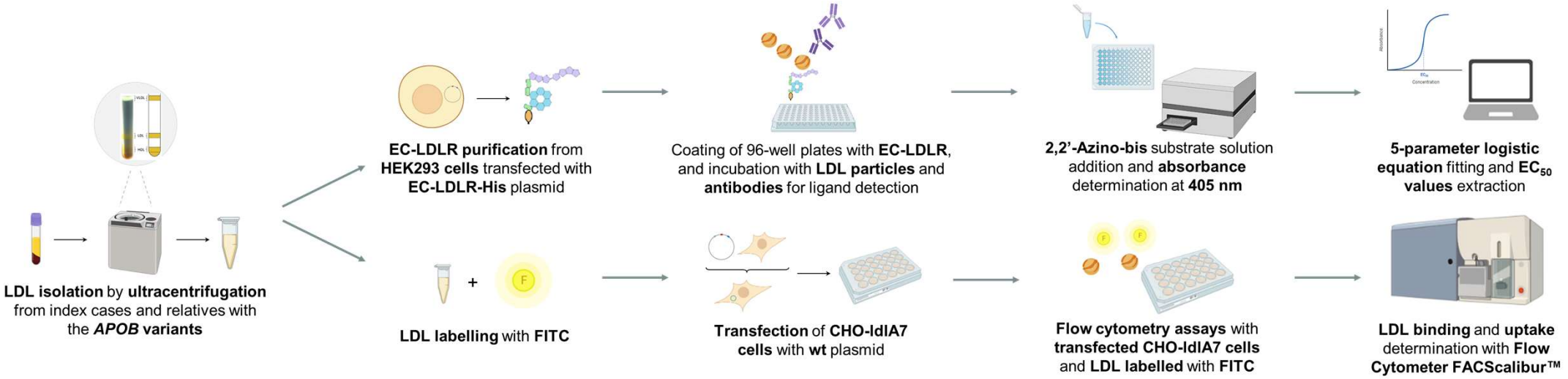






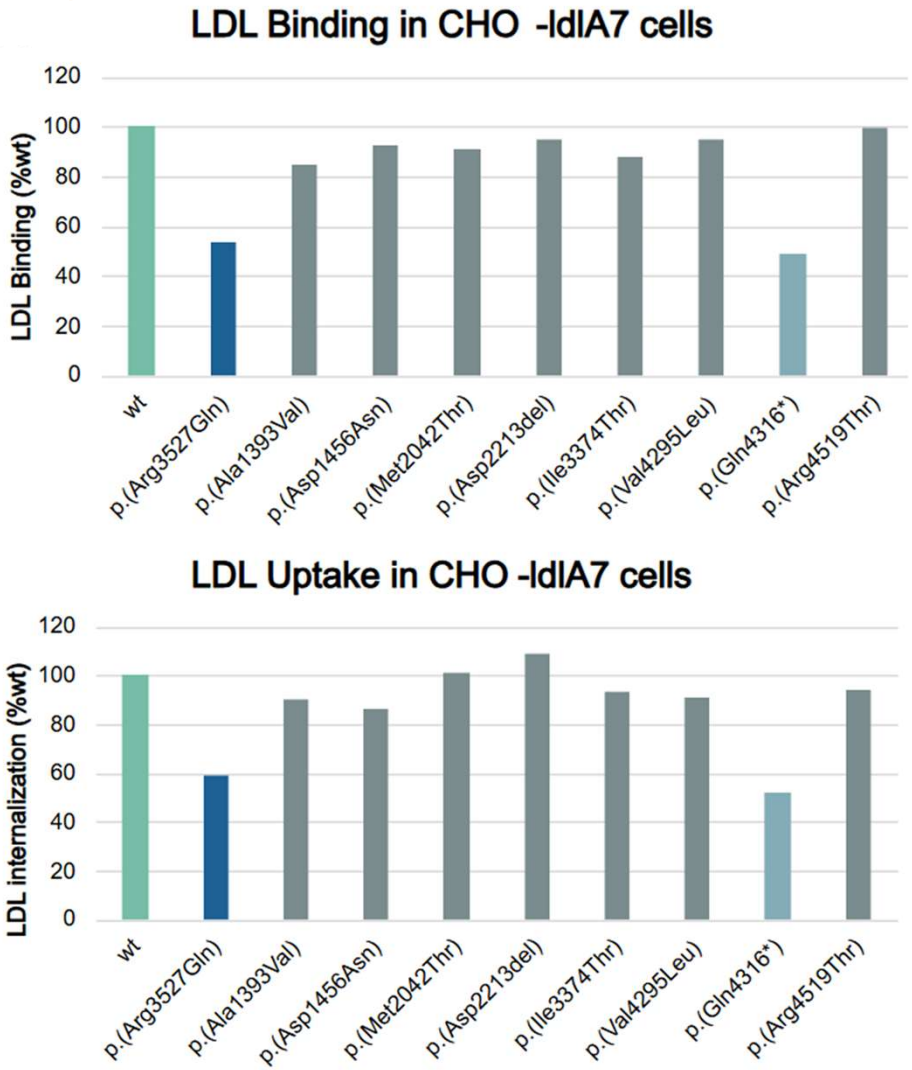
Bourbon *et al*, J Med Genet 2009;46:352–357 and Medeiros *et al*, 2010 Atherosclerosis

Functional Studies - *APOB*

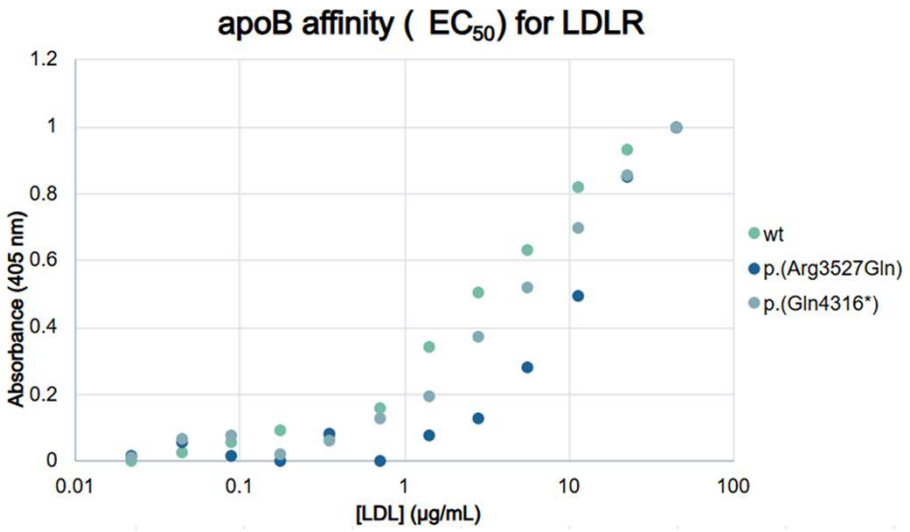


Functional Studies - *APOB*

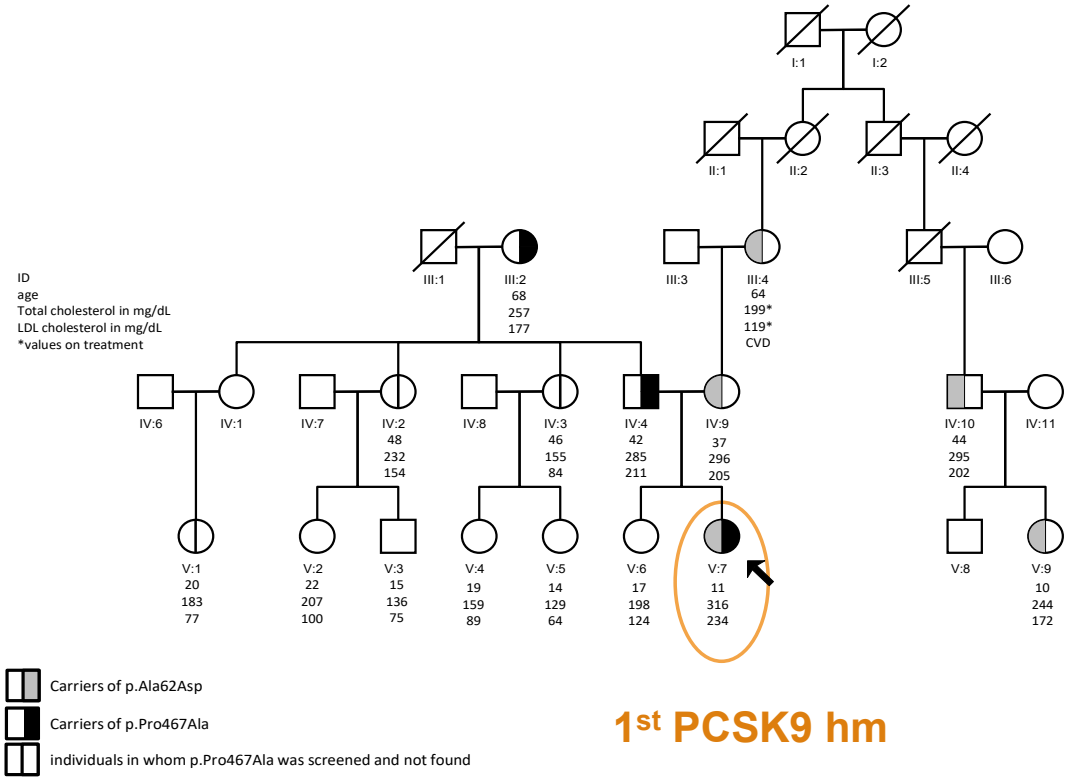
Flow cytometry



ELISA



Functional Studies – *PCSK9*



1st PCSK9 hm

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Letters

Characterization of the First *PCSK9* Gain of Function Homozygote

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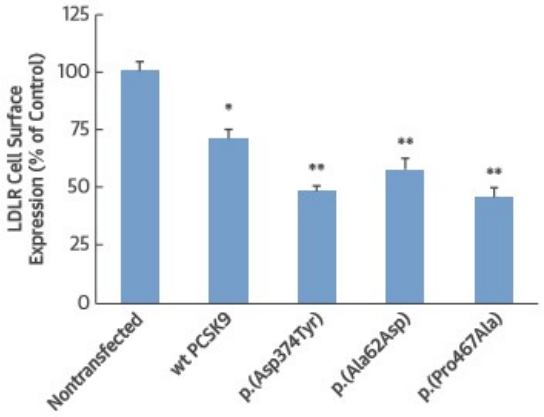
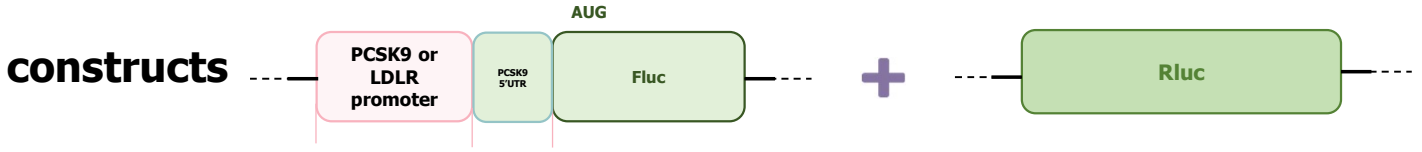
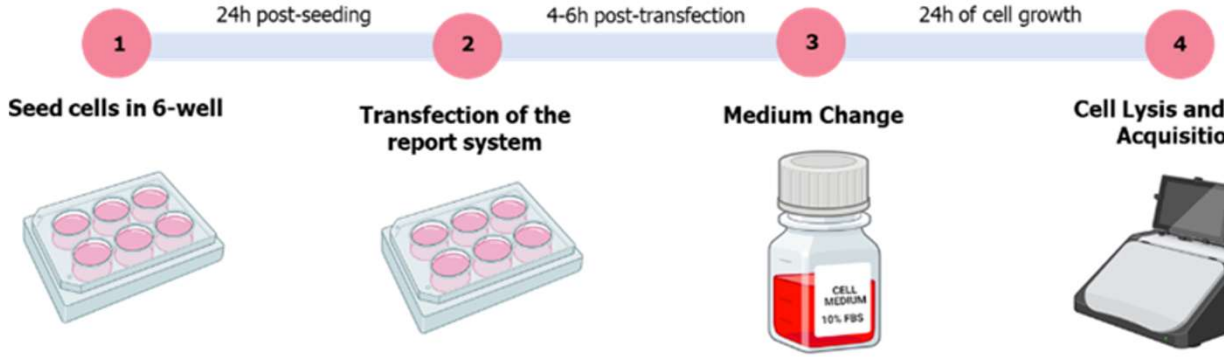


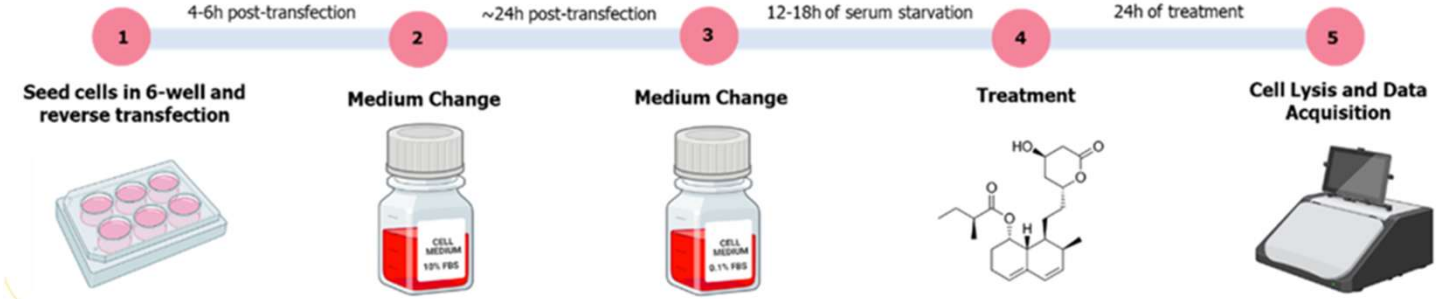
Alves AC, *et al*. 2015 JACC

FUNCTIONAL CHARACTERIZATION OF VARIANTS IN *LDLR* AND *PCSK9* REGULATORY REGIONS



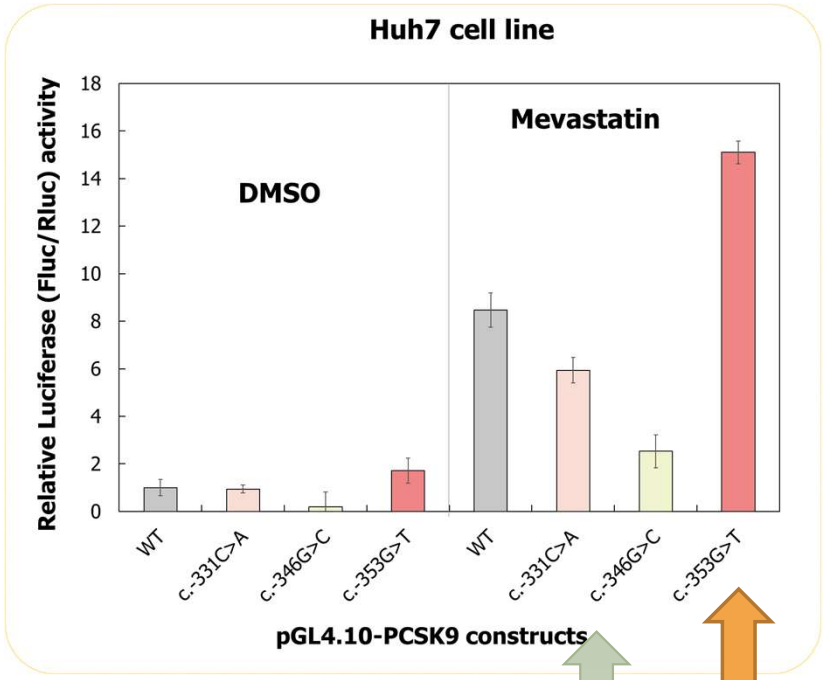


LDLR
~50 variants



PCSK9
~30 variants

PROMOTOR *PCSK9* VARIANTS



- c.-346G>C decreased promoter activity 30% in Huh7
- c.-353G>T increased it by178% in Huh7

FH causing

Hypo variant?

Graça *et al*, 2025 in preparation

Summary

- Type of variant matters
- Type of residual activity matters

For treatment efficacy and optimization

But…

- Only 10% of all variants are functionally characterized
- About 20% of all variant are known null alleles

70% of all variants we do not know LDLR residual activity

New projects
PerMedFH
FH EARLY

Thank you!

Grupo de Investigação Cardiovascular
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EPHF
Estudo Português de Hipercolesterolemia Familiar

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