FUNCTIONAL STUDIES OF LDLR MUTATIONS

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Estudo Português de Hipercolesterolemia Familiar

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Ministério da Saúde

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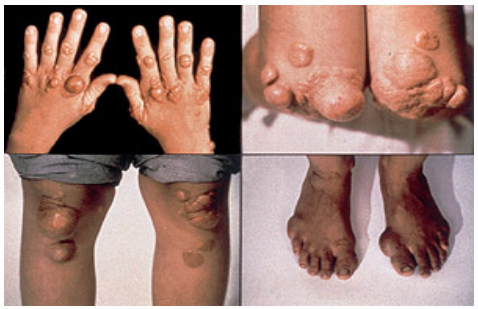
Familial Hypercholesterolemia (FH)

Causative genes:

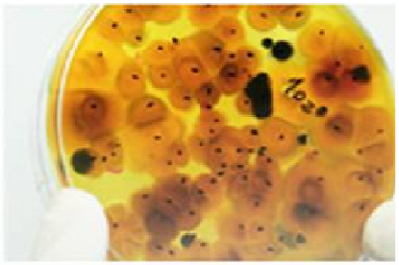
1. *LDLR*
2. *APOB*
3. *PCSK9*



- Autosomic dominant
- High prevalence (1/500)
- **Increased total and LDL cholesterol levels TC> 290 mg/dl and LDL-c >190 mg/dl)**
- Elevated levels of Apo B (>120 mg/dL)
- Triglycerides levels within normality (TG <150 mg/dl)
- **Family history of hypercholesterolaemia in all generations**
- **Family history of premature CHD**
- Presence of tendon xanthomas
- Therapeutics: 2nd generation statins and selective inhibitor of cholesterol absorption (combined therapeutic) associated to modification of life habits as diet, regular exercise and tobacco cessation

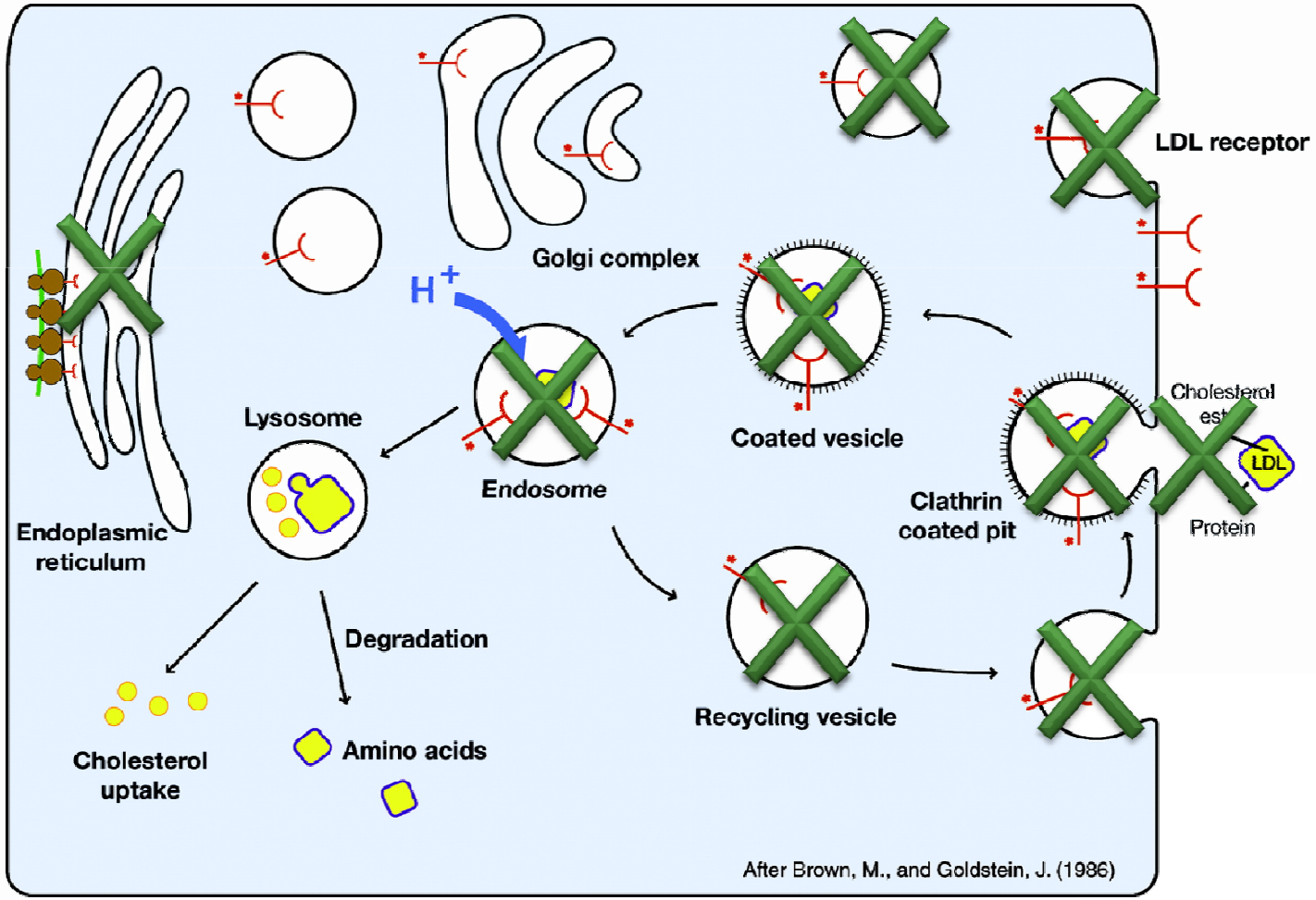
Clinical relevance

→ high cardiovascular risk



LDLR cycle

LDLR



Type of mutations

Nonsense, large rearranjments and frameshift (**severe mutations**).

Splicing and missense (**less severe mutations**) – the protein retains some activity (**defective alleles**) – increased levels of cholesterol but lower than cases with null alleles.

Type of studies

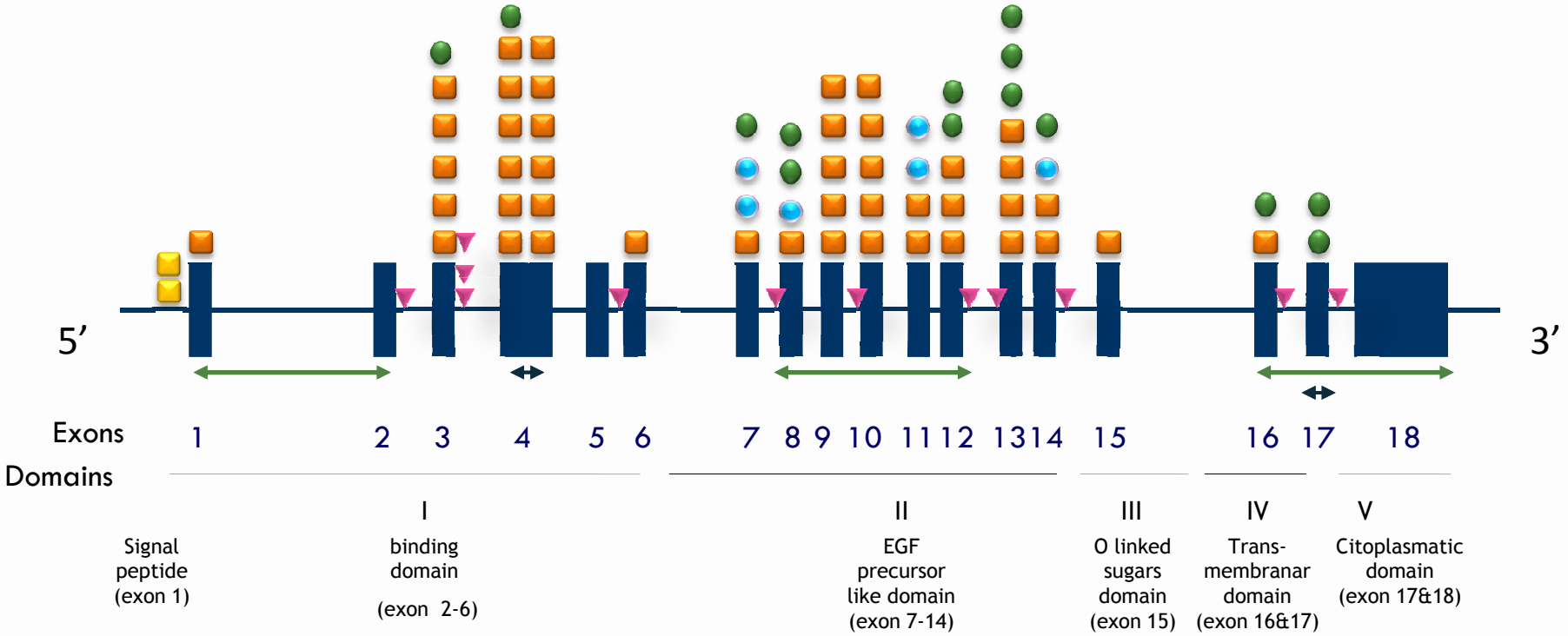
No need for functional studies
non production of protein (**null alleles**) – increased levels of cholesterol – **major CHD risk.**

Missense mutations – binding, internalization and degradation studies of labelled LDL (^{125}I-LDL) or flow cytometry.

Splicing mutations – RT PCR study of the mRNA extracted from patients fresh blood mononuclear cells and Real Time PCR.

Bourbon *et al*, J Med Genet 2009;46:352–357

Mutations in LDLR gene



Aims

To characterize LDLR variants not previously described in other population, or that have not been functionally characterized before.

To determine mutation pathogenicity, important for cardiovascular risk stratification since FH has a much higher cardiovascular risk than other dyslipidaemias.

METHODS

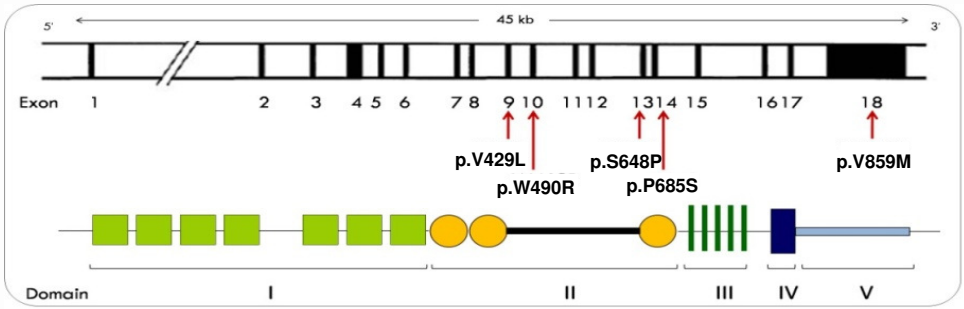
pcDNA3-LDLR
In vitro mutagenesis

Transfection CHO cells (Lipofectamin)

Western blot

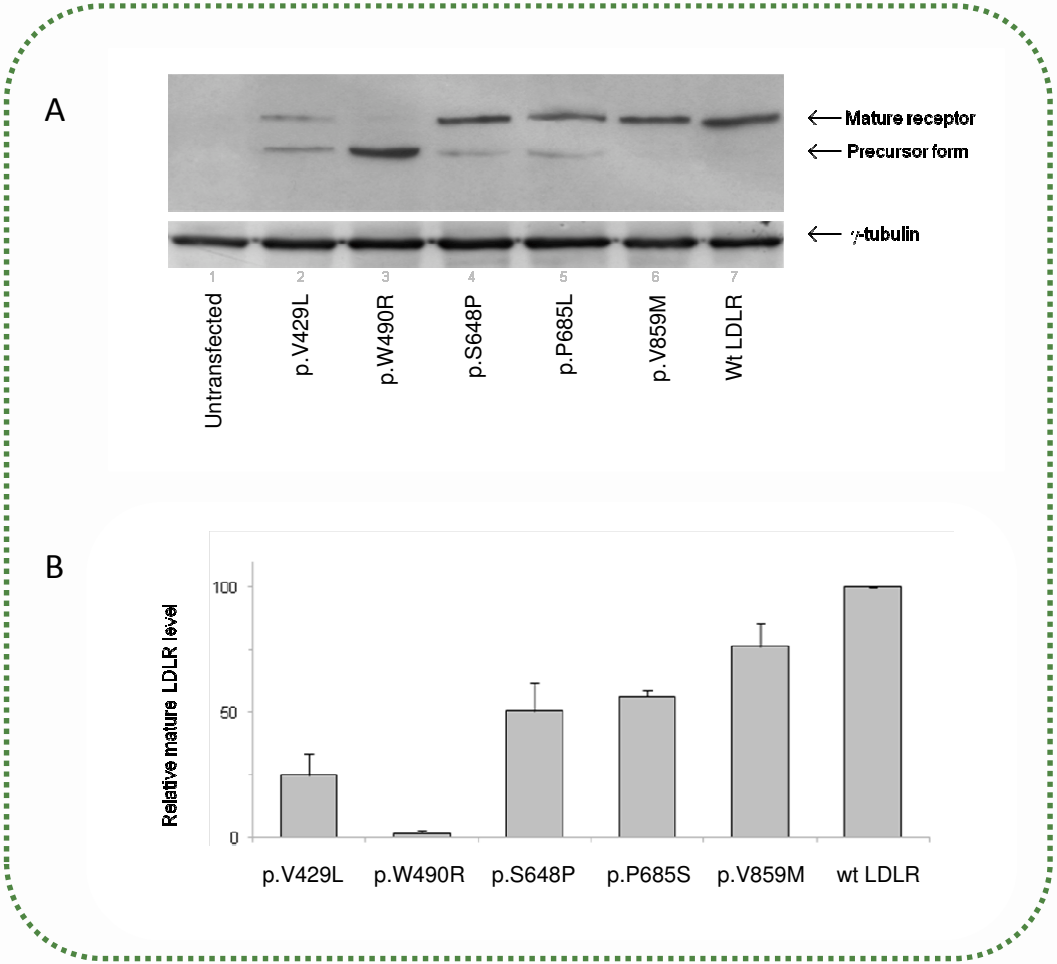
Immunofluorescence assays

Uptake and Degradation of 125I-LDL (37ºC)



exon	cDNA	Protein
Ex 9	c.1285G>C	p.V429L in EGF precursor homology domain
Ex 10	c.1468T>C	p.W490R in EGF precursor homology domain
Ex 13	c.1942T>C	p.S648P in EGF precursor homology domain
Ex 14	c.2053C>T	p.P685S in EGF precursor homology domain
Ex 18	c.2575G>A	p.V859M in internalisation signal domain

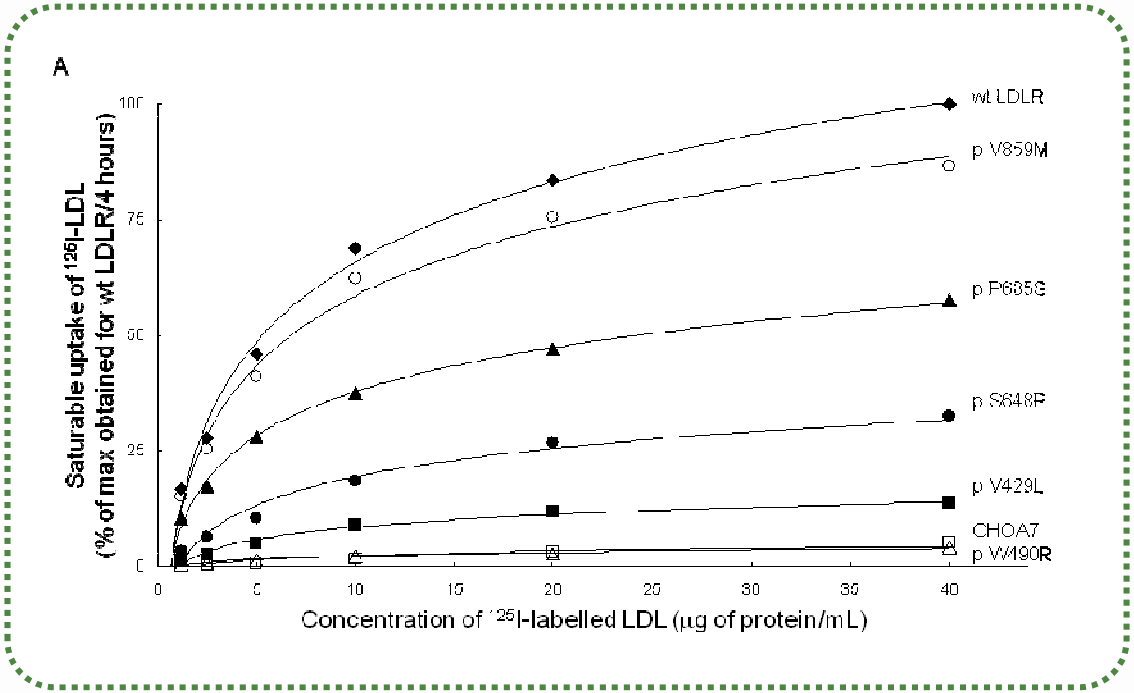
Detection of LDLR expression in transfected CHOA7 cells

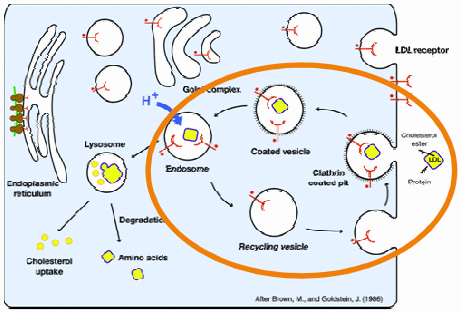


p.V429L and p.W490R variants might have a defective mature form?

Silva S *et al*, submitted Hum Mut

Uptake of ^{125}I-LDL @ 37ºC (binding + internalization)

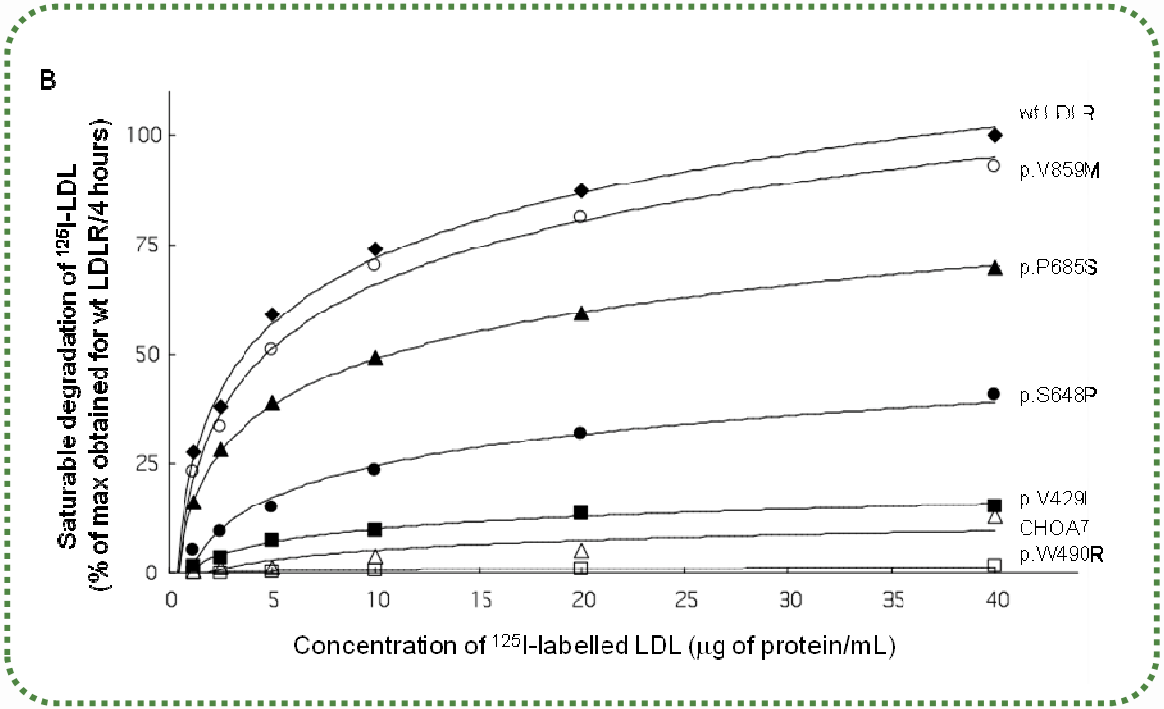




- p.V429L and p.W490R variants severely impair LDLR ability to uptake LDL;
- p.S648P, LDLR retains only ~25% of normal capacity for uptake of LDL;
- p.P685S retains ~50% of normal LDLR activity;
- p.V859M variant of LDLR has only ~10% less capacity for uptake of LDL than the native receptor protein.

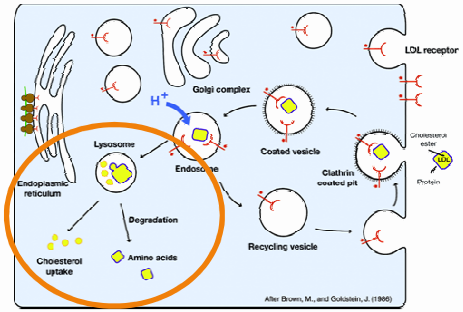
Silva S *et al*, submitted Hum Mut

Degradation of ^{125}I-LDL @ 37ºC



- No significant variation of degradation compared to uptake rates

- Impaired binding rather than internalization or release of ligand at the endosomes



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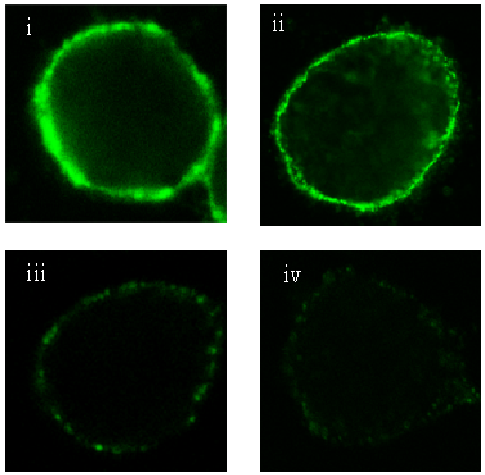
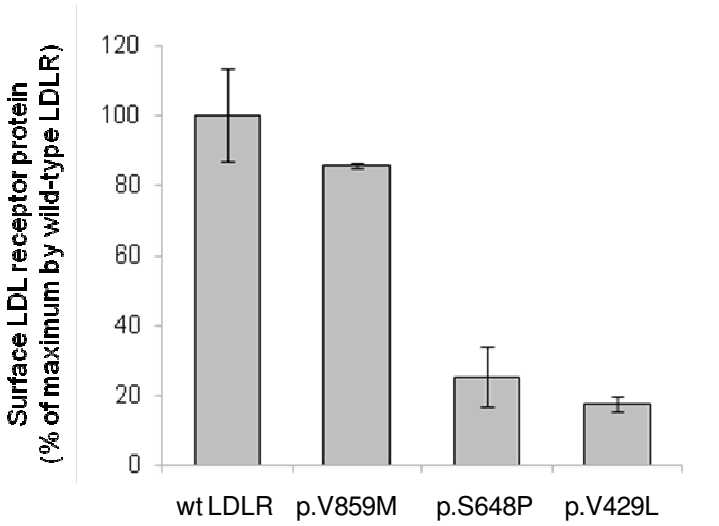
Immunofluorescence assays

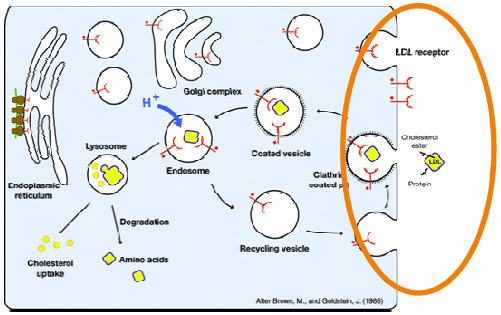
A



(i) Expressing normal, (ii) p.V859M (iii), p.S648P (iv), and p.V429L

B





Putative mutation

Putative mutation	Polyphen Prediction	Polyphen-2	SIFT Prediction
p.V429L	Benign	Probably damaging	Not tolerated
p.W490R	Probably damaging	Probably damaging	Tolerated
p.S648P	Benign	Probably damaging	Tolerated
p.P685S	Possibly damaging	Probably damaging	Not tolerated
p.V859M	Benign	Probably damaging	Tolerated

Putative mutation	Polyphen Prediction	Polyphen-2	SIFT Prediction
p.V429L	X	√	√
p.W490R	√	√	X
p.S648P	X	√	X
p.P685S	√	√	√
p.V859M	√	X	√

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Conclusions

• *In vitro* studies are of great importance to determine the actual cause of hyperlipidaemia and distinguish pathogenic mutations from rare silent variants. In silico analysis is not sufficient to predict mutation pathogenecity.

• Four of the five LDLR missense variants studied (p.V429L, p.W490R, p.S648P,) (p.P664L) are mutations causing disease and p.V859M was characterized as non pathogenic.

• Functional studies allows the correct identification of pathogenic mutations and should be always performed to avoid false positive results:

Δ co-segregation family studies
Δ *in vitro* studies

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Estudo Português de Hipercolesterolemia Familiar

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