FUNCTIONAL STUDIES OF LDLR MUTATIONS

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Familial Hypercholesterolemia (FH)

Causative genes:
1. LDLR
2. APOB
3. PCSK9

- Autosomal 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

**Type of mutations**

Nonsense, large rearrangements 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.

Mutations in LDLR gene

Exons

Domains

1

Signal peptide (exon 1)

2

I binding domain (exon 2-6)

3

4

5

6

7

8

9

10

11

12

13

14

15

16

17

18

II

EGF precursor like domain (exon 7-14)

III

O linked sugars domain (exon 15)

IV

Transmembranar domain (exon 16-17)

V

Citoplasmatic domain (exon 17&18)

2 Promotor

43 Missense

6 Nonsense

12 Splicing

14 Frameshift

3 large deletions

2 Short deletion
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 (37°C)

<table>
<thead>
<tr>
<th>Exon</th>
<th>cDNA</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ex 9</td>
<td>c.1285G&gt;C</td>
<td>p.V429L in EGF precursor homology domain</td>
</tr>
<tr>
<td>Ex 10</td>
<td>c.1468T&gt;C</td>
<td>p.W490R in EGF precursor homology domain</td>
</tr>
<tr>
<td>Ex 13</td>
<td>c.1942T&gt;C</td>
<td>p.S648P in EGF precursor homology domain</td>
</tr>
<tr>
<td>Ex 14</td>
<td>c.2053C&gt;T</td>
<td>p.P685S in EGF precursor homology domain</td>
</tr>
<tr>
<td>Ex 18</td>
<td>c.2575G&gt;A</td>
<td>p.V859M in internalisation signal domain</td>
</tr>
</tbody>
</table>
Detection of LDLR expression in transfected CHOA7 cells

A

B

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

Silva S et al, submitted Hum Mut
• 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.
Degradation of $^{125}$I-LDL @ 37°C

- No significant variation of degradation compared to uptake rates
- Impaired binding rather than internalization or release of ligand at the endosomes

Silva S et al, submitted Hum Mut
**Immunofluorescence assays**


**B**

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<th>Surface LDL receptor protein (% of maximum by wild-type LDLR)</th>
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<tbody>
<tr>
<td>wt LDLR</td>
<td>100</td>
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<tr>
<td>p.V859M</td>
<td>80</td>
</tr>
<tr>
<td>p.S648P</td>
<td>30</td>
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<tr>
<td>p.V429L</td>
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## Putative mutation

<table>
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<tr>
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<th>Polyphen Prediction</th>
<th>Polyphen-2</th>
<th>SIFT Prediction</th>
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<tr>
<td>p.V429L</td>
<td>Benign</td>
<td>Probably damaging</td>
<td>Not tolerated</td>
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<tr>
<td>p.W490R</td>
<td>Probably damaging</td>
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<td>Tolerated</td>
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<tr>
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<td>X</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>p.W490R</td>
<td>√</td>
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Silva S et al, submitted Hum Mut
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 pathogenicity.

• 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:

  △ co-segregation family studies
  △ *in vitro* studies
Acknowledgements

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