Functional Role of Lipoprotein Receptors in Alzheimer’s Disease

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Genetic factors associated with AD

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome</th>
<th>Age of onset</th>
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Alzheimer's Disease (AD)

- Amyloid Plaque
- Neurofibrilar Tangles

Alzheimer's Disease and Trisomie 21

- Trisomie 21
- 3 copies of the APP-gene
- Onset of Alzheimer’s Disease starts at age 40!
APP Processing

A) Amyloidogenic

B) Non-Amyloidogenic

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Apolipoprotein E

- ApoE is a soluble secreted protein of 299 aa
- Main function: Triglyceride, cholesterol transport
- ApoE gene is localized on chromosome 19
- ApoE polymorphism: ApoE2, ApoE3, ApoE4
  - Single aa exchange at position 112 or 158
- Carriers of ApoE4 show an increased risk in developing Alzheimer’s Disease (Alan Roses).
  - ApoE3/ApoE4 or ApoE4/ApoE4

Binding partners of LRP1
Increased risk to develop AD for ApoE4

- ApoE4: Chromosome 19
- >50 years

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Low Density Lipoprotein Receptor Family

- LDL receptor gene family
- Main functions: scavenger receptor for lipoproteins + neuronal signaling receptor
- 600kDa type I transmembrane protein
- cleavage by furin in the Golgi-compartement generates α- and β-chain
- four extracellular ligand-binding domains
- ubiquitously expressed, ↑ liver + brain

-reviewed in Dieckmann M 2010 Biol Chem
**LRP1 Biosynthesis and Secretion**

- **Asparagine – Proline – any amino acid – Tyrosine**
  
- conserved domain of transmembrane proteins (e.g. lipoprotein receptors and integrins)

**Low Density Lipoprotein Receptor Related Protein 1 (LRP1)**

- 2.5-fold Increase of APPs secretion in LRP-/- cells.
- 4-6-fold Reduction of APP-CTF Level in LRP-/- cells.

**LRP1 Mediated Processing of APP**

APP Internalization and Aβ-Secretion

Endosomal compartments

Internalization from the cell surface


LRP1 and APP in Alzheimer’s Disease

- N-terminal interaction through KPI-domain?
- C-terminal interaction through NPXY domains?

LRP (α) ~515 kDa

LRP (β) ~85 kDa

Dab-1

X11 / mint

Truncated LRP1 constructs (LRP-CT)

LRP-/-
LRP+/-
LRP-/- LRPCT

MW kDa

200
97.4
63

Strept.
Lysate

APP-FE65-LRP1 Complex Formation

GST-pull down of the APP-FE65-LRP Complex

APP-FE65-LRP1 Complex Formation

GST-pull down of in vitro translated Proteins

MW kDa

[35S]-APP695

LRP-ST-GST

FE65

in vitro Translation

FE65∆PID2

[35S]-APP695

LRP-ST-GST

GST

[35S]-APP695

LRP-ST-GST

GST

FE65

MW kDa

97.4

+++ ++ + + + + + + + + +

+++ ++ + + + + + + + + +

FE65 and APP Processing

Overexpression of FE65 constructs and its effect on APP Processing.

MW kDa

APPs-secretion

7 WD10

FE65

FE65∆PID2

APPs

Aβ

APPs-secretion

FE65

FE65∆PID2


Function of FE65 on APP Processing

Expression of human FE65 in amyloid precursor protein transgenic mice is associated with a reduction in β-amyloid load

Retention of LRP-CT-KKAA in the ER

LRP1 interacts with APP as early as in the secretory compartments

Reduced surface expression of retained LRP-CT-KKAA construct

Reduced surface expression of APP after LRP-CT retention
**LRP1: Friend or Foe**

- Increased LRP expression → Increased Aβ-clearance → Increased Aβ-production → LRP expression

**LRP1 mutant mice**

- Complete LRP1 knock-out (KO) is embryonic lethal at E10
  - Herz J 1992 Cell
- Conditional LRP1 forebrain knock-out mice (LRP1 floxP x CaMKII-Cre)
  - Liu Q 2010 J Neurosci
- LRP1 knock-in mice: NPxY → AAxA

- NPxY1 embryonic lethal E15-19
- NPxY2 vital + fertile
- NPxY1+2 embryonic lethal E10-13
LRP1 KO leads to neuronal disturbance

- LRP1 is highly expressed in brain.
- A full LRP1 knock-out is lethal and shows a severe neuronal phenotype.
- A CaMKII-Cre/LRP1 knock-out leads to morphological and behavioural changes.

Spine degeneration

Rotarod

Open field

from Liu et al., J. Neuroscience 2010

LRP1 ligands activate NMDA receptors

- Tissue-type plasminogen activator (tPa)
- serine protease
- in vivo: highly expressed in neurons
- main ligand of LRP1
- internalized by LRP1 via receptor-mediated endocytosis
- FDA-approved medication for patients with acute ischemic stroke

Nicole et al., 2002, Nat Med
Medina et al., 2005, EMBO

Actilyse® (Tissue-type plasminogen activator)

- Tissue-type plasminogen activator (tPa)
- FDA-approved medication for patients with acute ischemic stroke
- recombinant tissue plasminogen activator (r-PA)

Nicole et al., 2002, Nat Med
Medina et al., 2005, EMBO

Proposed function of Actilyse®

Blood clot in an artery

MRI scan of brain after ischemic stroke

The image on the left shows a right hemisphere area where blood flow has been reduced due to ischemia caused by the occlusion of a cerebral artery. The image on the right shows the restoration of blood flow following treatment with Actilyse®.
LRP1 ligands induce Erk signaling in neurons

LRP1 Biosynthesis and Secretion

tPA induces MEK-mediated Erk activation

LRP1 knock-down reduces Erk 1/2 activation

*shRNA gene silencing of LRP1 in HT22:*
NMDA receptor inhibitors prevent tPA-induced Erk 1/2 activation

Cooperation of LRP1 and NMDA receptors

tPa mediated NMDAR activity in LRP1 knock-in animals

Does an LRP1-KI affect NMDR subunit expression?
LRP1 influences NMDAR surface expression

- LRP1 endocytosis is reduced in LRP1ΔNPxY neurons.
- NR1 and NR2B internalization is reduced in LRP1ΔNPxY neurons.
- NR2A internalization is normal in LRP1ΔNPxY neurons.

Maier et al. Mol. Neurodegeneration 2013

Surface biotinylation with cleavable biotin in primary neurons

- LRP1, NR1 and NR2B internalization is LRP1 mediated and time dependent.
- NR2A internalization is normal in LRP1ΔNPxY neurons.

Surface biotinylation in primary neurons

LRP1ΔNPxY2 knock-in has no effect on neuron survival or synaptic markers

Does LRP1 interact with NMDAR subunits?

NR1 does not interact with LRP1
Behavioral test in mouse models for AD

Learning in the Morris water maze

LRP1ΔNPxY2 mice perform better on Rotarod

Fear conditioning of wt and LRP1ΔNPxY2 mice
Aβ binding to LRP1 may influence the NMDAR?

Kimberly Moore Olsen & Morgan Sheng 2012: Scientific Reports 2, Article number: 225

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