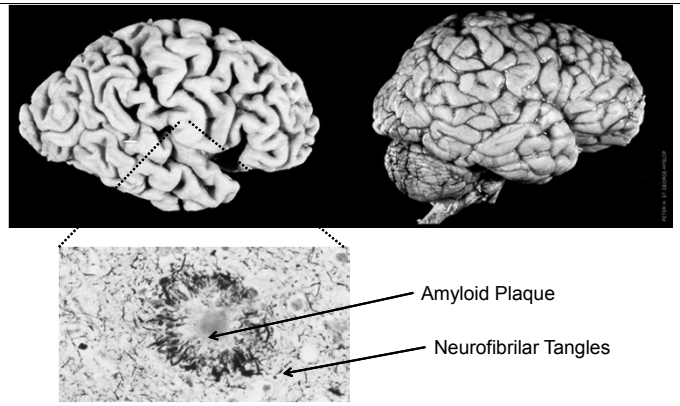
UNIVERSITÄTSmedizin. MAINZ

Functional Role of Lipoprotein Receptors in Alzheimer's Disease

Claus U. Pietrzik, PhD
Professor for Pathobiochemistry
University Medical Center of the
Johannes Gutenberg-University Mainz

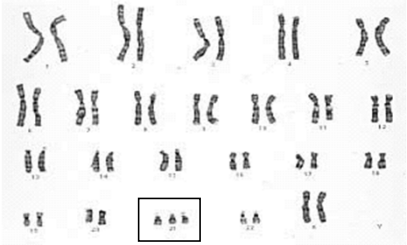
Alzheimer's Disease (AD)



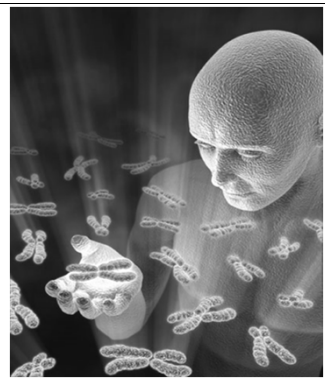
Genetic factors associated with AD

Gene		Age of onset
Familial early onset genes		
APP	Chromosome 21	40-65 years
Presenilin 1	Chromosome 14	17-60 years
Presenilin 2	Chromosome 1	45-84 years
Late onset genes (sporadic and senile familial AD)		
ApoE4	Chromosome 19	>50 years
α2-Macroglobulin	Chromosome 12	>50 years
LRP1	Chromosome 12	>50 years

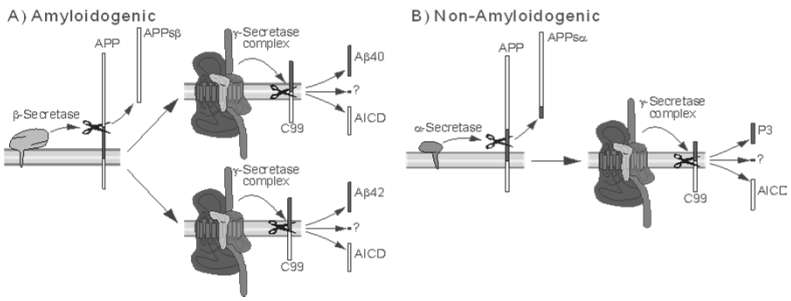
Alzheimer's Disease and Trisomie 21



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



APP Processing



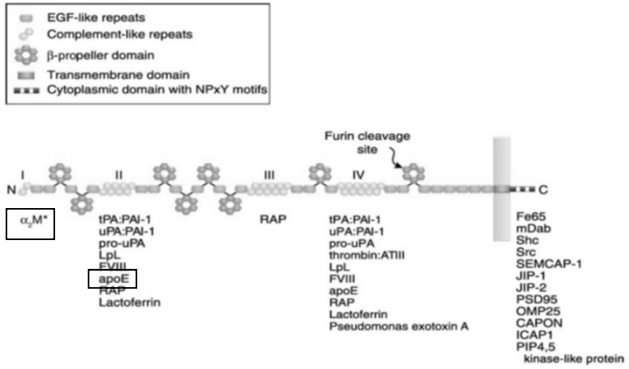
Genetic factors associated with AD

Gene		Age of onset
Familial early onset genes		
APP	Chromosome 21	40-65 years
Presenilin 1	Chromosome 14	17-60 years
Presenilin 2	Chromosome 1	45-84 years
Late onset genes (sporadic and senile familial AD)		
ApoE4	Chromosome 19	>50 years
α2-Macroglobulin	Chromosome 12	>50 years
LRP1	Chromosome 12	>50 years

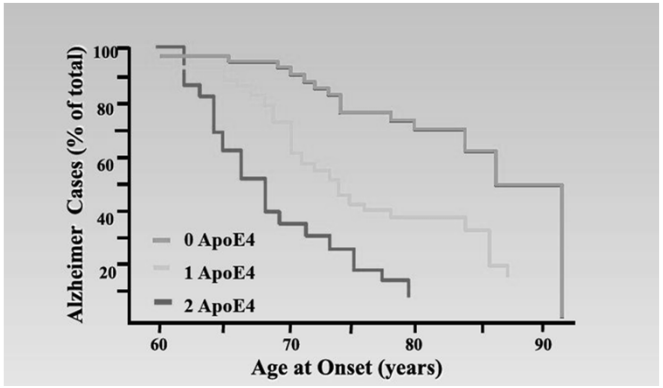
Apolipoprotein E

- 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



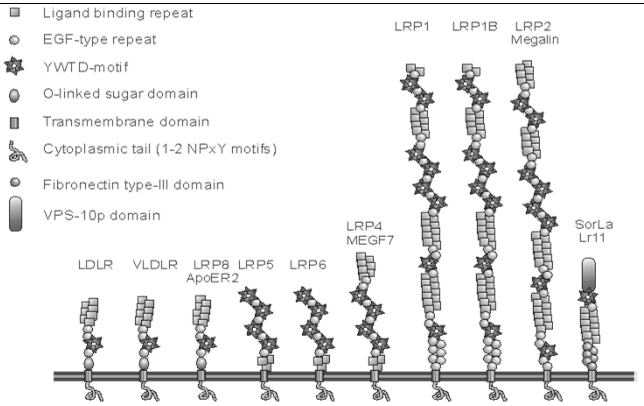
Increased risk to develop AD for ApoE4



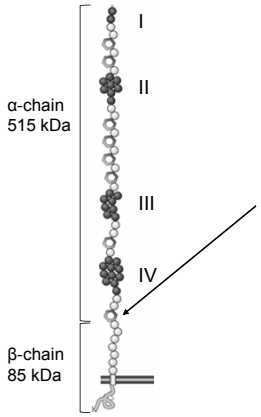
Genetic factors associated with AD

Gene		Age of onset
Familial early onset genes		
APP	Chromosome 21	40-65 years
Presenilin 1	Chromosome 14	17-60 years
Presenilin 2	Chromosome 1	45-84 years
Late onset genes (sporadic and senile familial AD)		
ApoE4	Chromosome 19	>50 years
α2-Macroglobulin	Chromosome 12	>50 years
LRP1	Chromosome 12	>50 years

Low Density Lipoprotein Receptor Family



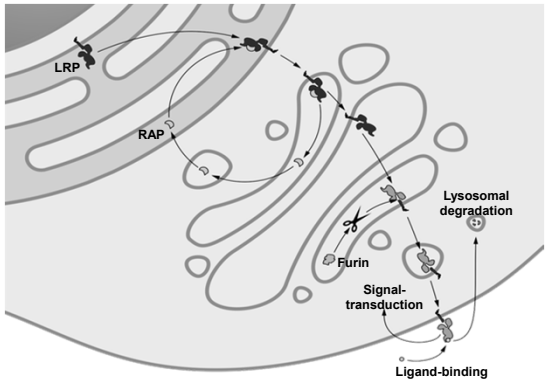
Low Density Lipoprotein Receptor Related Protein 1 (LRP1)



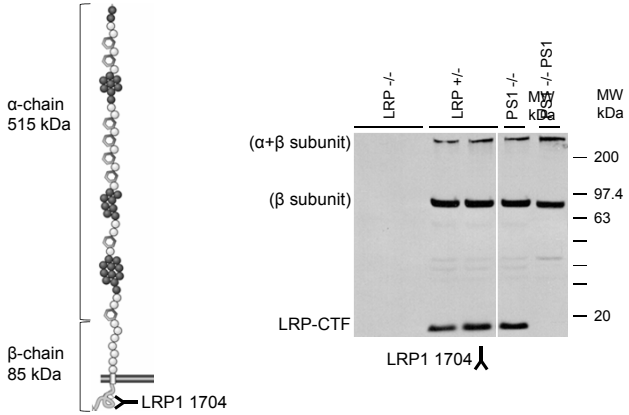
- LDL receptor gene family
- Main functions: scavenger receptor for lipoproteins + neuronal signaling receptor
- 600kDa type I transmembrane protein
- cleavage by furin in the Golgi-compartement generates α- and β-chain
- four extracellular ligand-binding domains
- ubiquitously expressed, ↑ liver + brain

reviewed in *Dieckmann M 2010 Biol Chem*

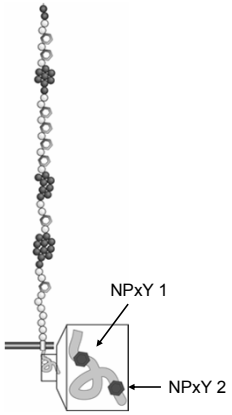
LRP1 Biosynthesis and Secretion



Low Density Lipoprotein Receptor Related Protein 1 (LRP1)



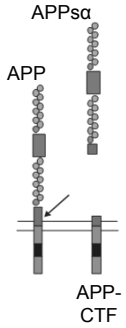
Low Density Lipoprotein Receptor Related Protein 1 (LRP1)

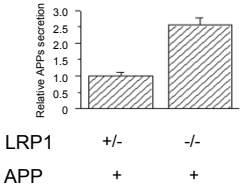


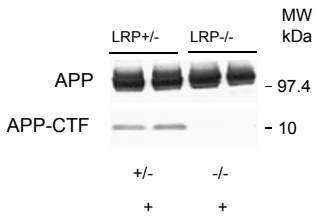
The Nobel Prize in Physiology or Medicine 1985

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

LRP1 Mediated Proccessing of APP



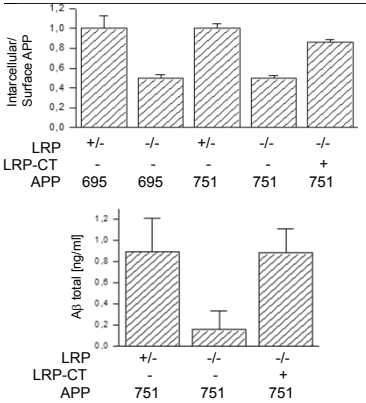


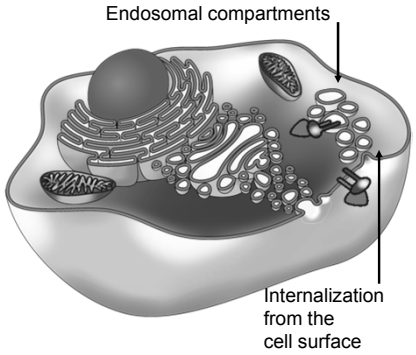


- 2.5-fold Increase of APPs secretion in LRP-/- cells.
- 4-6-fold Reduction of APP-CTF Level in LRP-/- cells.

Pietrzik et al. 2002: EMBO J Nov 1;21(21):5691-700.

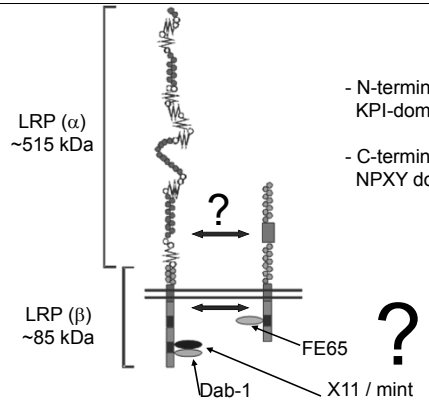
APP Internalization and Aβ-Secretion





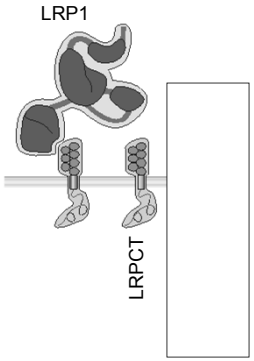
Pietrzik et al. 2002: EMBO J Nov 1;21(21):5691-700.

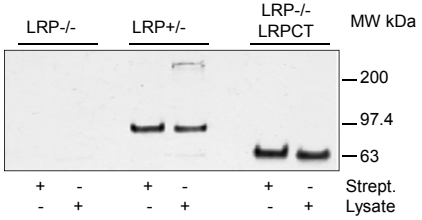
LRP1 and APP in Alzheimer's Disease



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

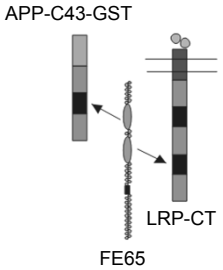
Truncated LRP1 constructs (LRP-CT)

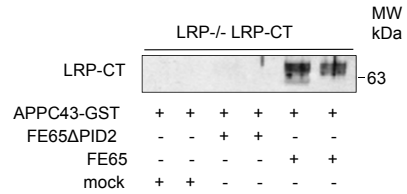




APP-FE65-LRP1 Complex Formation

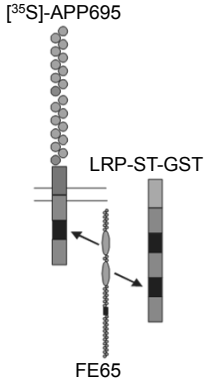
GST-pull down of the APP-FE65-LRP Complex



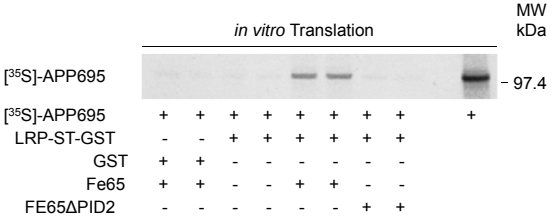


Pietrzik et al. 2004: J Neuroscience Apr 28;24(17):4259-65.

APP-FE65-LRP1 Complex Formation



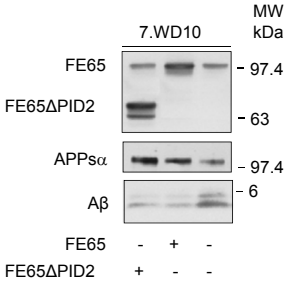
GST-pull down of *in vitro* translated Proteins

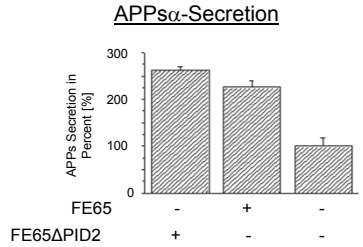


Pietrzik et al. 2004: J Neuroscience Apr 28;24(17):4259-65.

FE65 and APP Processing

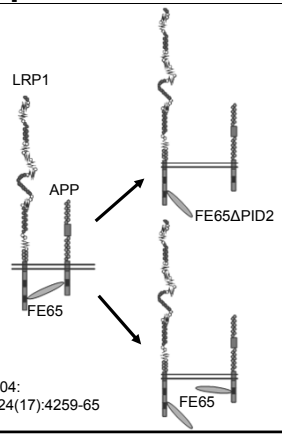
Overexpression of FE65 constructs and its effect on APP Processing.

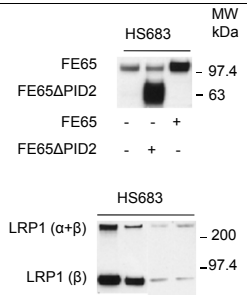




Pietrzik et al. 2004: J Neuroscience Apr 28;24(17):4259-65.

FE65 overexpression and LRP1-APP-complex formation





Pietrzik et al. 2004:
J. Neurosci. 28;24(17):4259-65

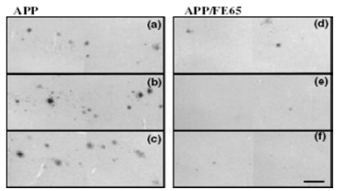
Function of FE65 on APP Processing

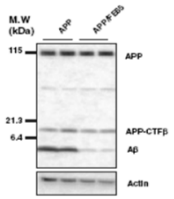
Journal of Neurochemistry, 2005, **93**, 330–338 doi:10.1111/j.1471-4159.2005.03026.x

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

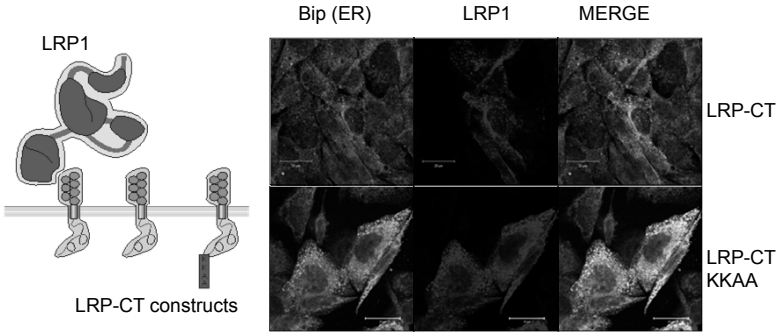
Dominique Santiard-Baron,* Dominique Langui,† Maryse Delehedde,‡ Benoît Delatour,†,1 Brigitte Schombert,§ Nathalie Touchet,§ Günter Tremp,§ Marie-Françoise Paul,§ Véronique Blanchard,* Nicolas Sergeant,‡ André Delacourte,‡ Charles Duyckaerts,† Laurent Pradier* and Luc Mercken[1]

*Neurodegenerative Diseases Group, Aventis, Vitry-sur-Seine, France
†Laboratory of Neuropathology and Inserm U289, La Salpêtrière Hospital, Paris, France
‡INSERM U422, Lille, France
§Functional Genomic, Aventis, Vitry-sur-Seine, France



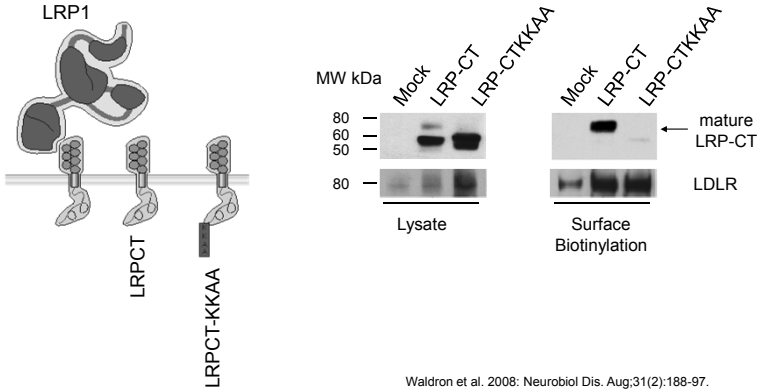


Retention of LRP-CT-KKAA in the ER



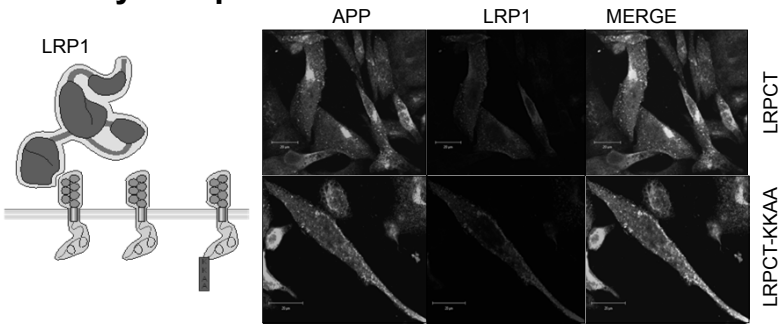
Waldron et al. 2008: Neurobiol Dis. Aug;31(2):188-97.

Reduced surface expression of retained LRP-CT-KKAA construct



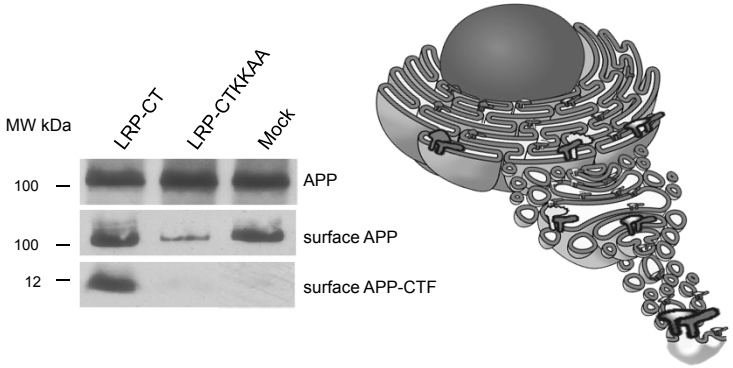
Waldron et al. 2008: Neurobiol Dis. Aug;31(2):188-97.

LRP1 interacts with APP as early as in the secretory compartments



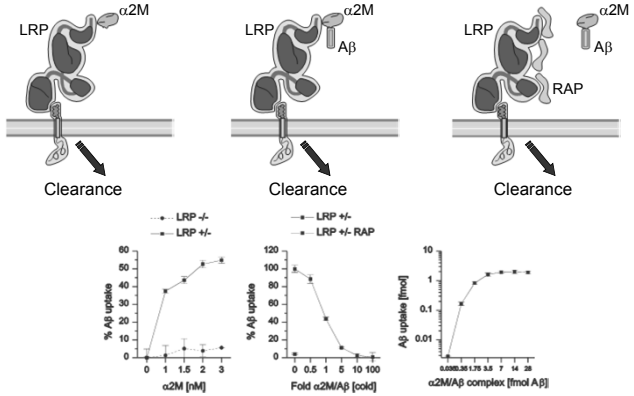
Waldron et al. 2008: Neurobiol Dis. Aug;31(2):188-97.

Reduced surface expression of APP after LRP-CT retention



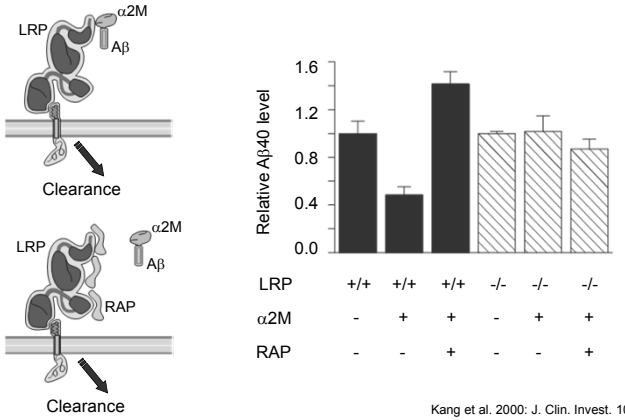
Waldron et al. 2008: Neurobiol Dis. Aug;31(2):188-97.

α-2-Macroglobulin (α2M) mediated Aβ-clearance



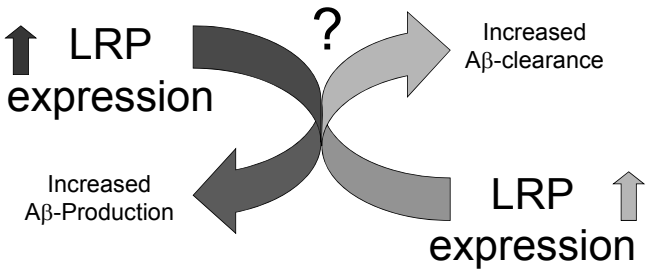
Kang et al. 2000: J. Clin. Invest. 106(9):1159-66

α-2-Macroglobulin (α2M) mediated Aβ-clearance

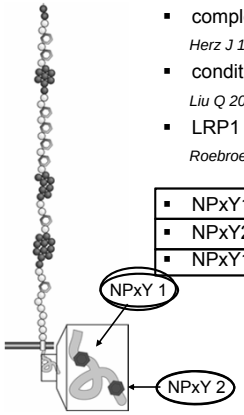


Kang et al. 2000: J. Clin. Invest. 106(9):1159-66

LRP1: Friend or Foe

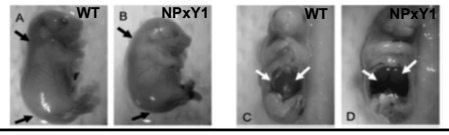


LRP1 mutant mice



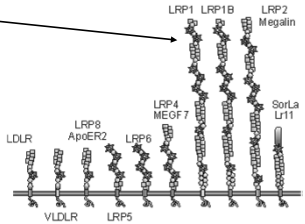
- complete LRP1 knock-out (KO) is embryonic lethal at E10
  Herz J 1992 Cell
- conditional LRP1 forebrain knock-out mice (LRP1 floxP x CaMKII-Cre)
  Liu Q 2010 J Neurosci
- LRP1 knock-in mice: NPxY → AAxA
  Roebroek AJM 2006 Mol Cell Biol; Reekmans SM 2010 Cell Mol Life Sci.

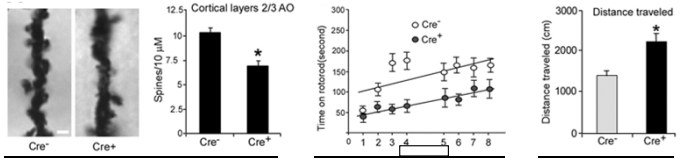
NPxY1	embryonic lethal E15-19
NPxY2	vital + fertile
NPxY1+2	embryonic lethal E10-13



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.



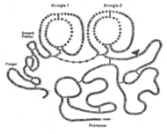


Spine degeneration | Rotarod | Open field

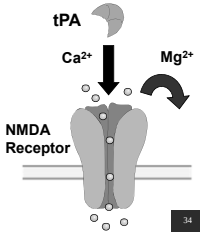
from Liu et al. J. Neuroscience 2010

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



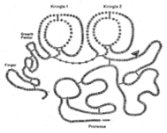
(www.bioweb.uwlax.edu)



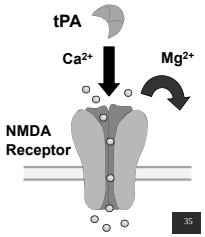
Nicole et al., 2002, Nat Med
Medina et al., 2005, EMBO

Actilyse® (Tissue-type plasminogen activator)

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

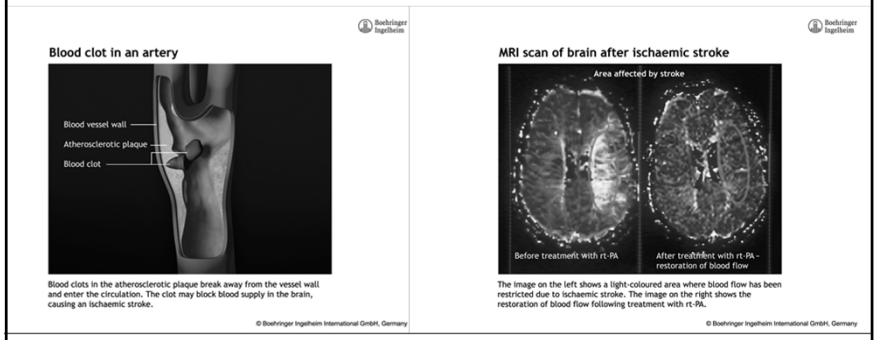


(www.bioweb.uwlax.edu)

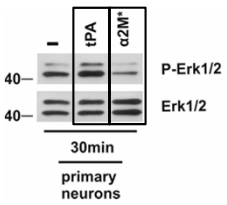


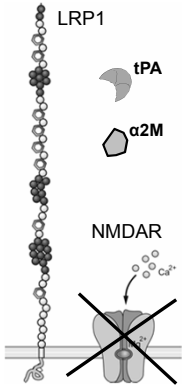
Nicole et al., 2002, Nat Med
Medina et al., 2005, EMBO

Proposed function of Actilyse®



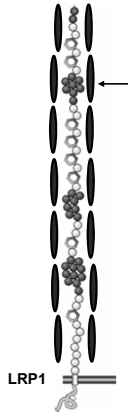
LRP1 ligands induce Erk signaling in neurons

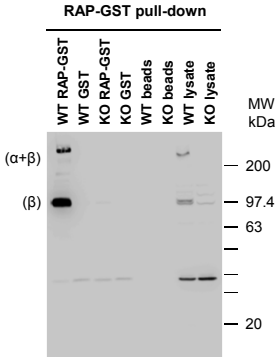


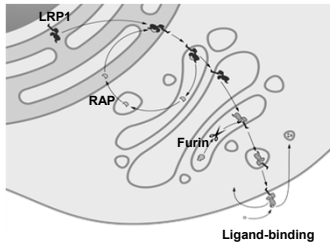


Martin et al. J. Biol. Chem. 2008

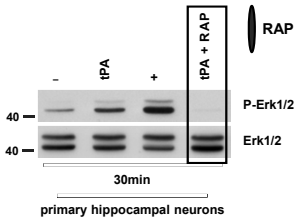
LRP1 Biosynthesis and Secretion

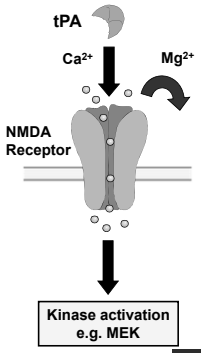






tPA induces MEK-mediated Erk activation

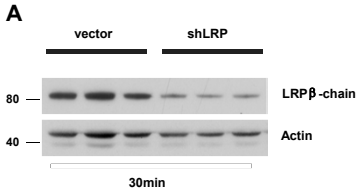


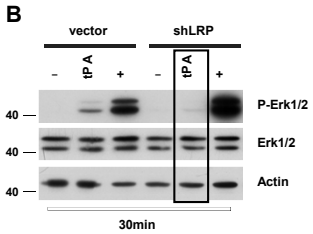


Martin et al. J. Biol. Chem. 2008

LRP1 knock-down reduces Erk 1/2 activation

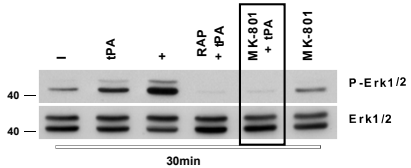
shRNA gene silencing of LRP1 in HT22:

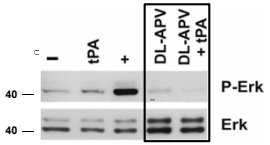




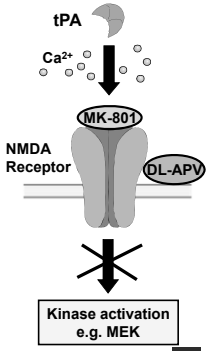
Martin et al. J. Biol. Chem. 2008

NMDA receptor inhibitors prevent tPA-induced Erk 1/2 activation



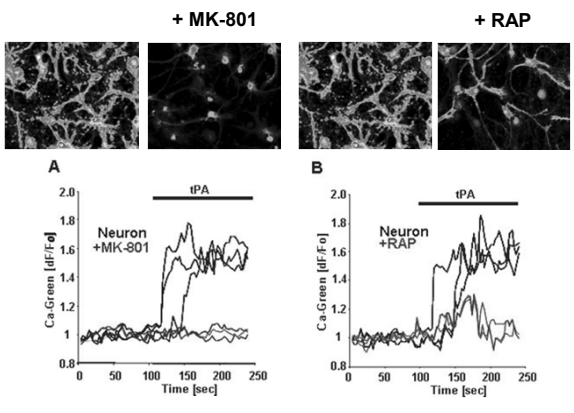


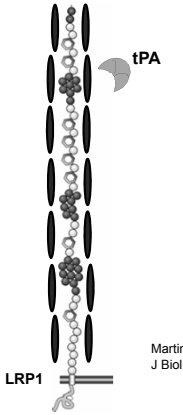
primary hippocampal neurons



Martin et al. J. Biol. Chem. 2008

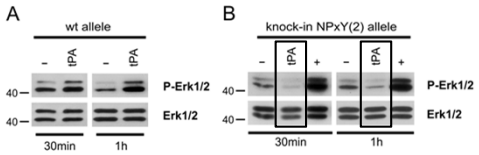
Cooperation of LRP1 and NMDA receptors

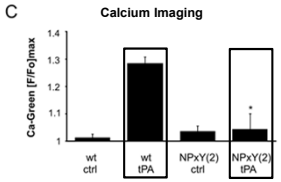


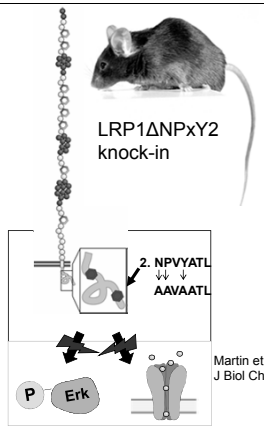


Martin et al.
J Biol Chem. 2008

tPa mediated NMDAR activity in LRP1 knock-in animals

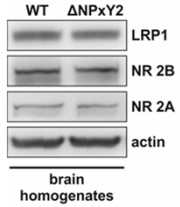


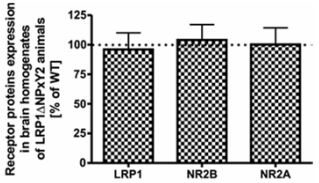


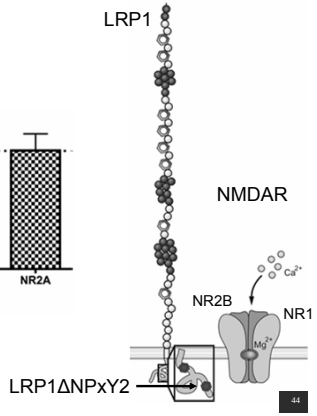


Martin et al.
J Biol Chem. 2008

Does an LRP1-KI affect NMDR subunit expression?

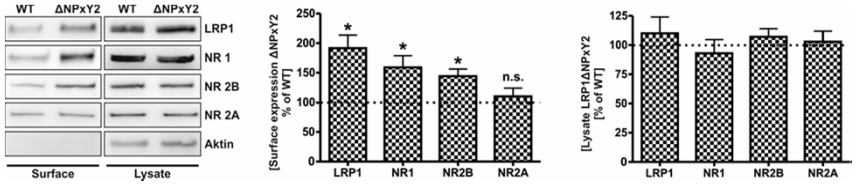






LRP1 influences NMDAR surface expression

Surface biotinylation in primary neurons

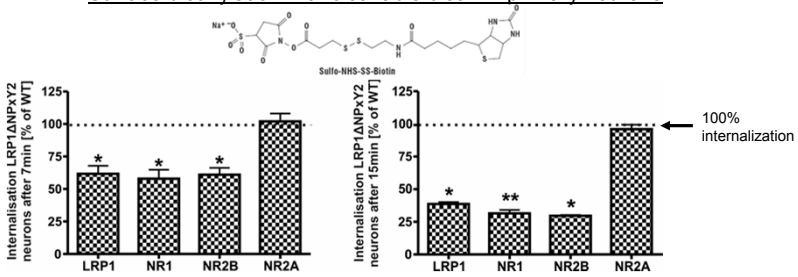


- LRP1 endocytosis is reduced in LRP1ΔNPxY neurons.
- NR1 and NR2B internalization is reduced in LRP1ΔNPxY neurons.
- NR2A internalization is normal in LRP1ΔNPxY neurons.

Reekmans et al. CMLS 2010
Maier et al. Mol. Neurodegeneration 2013

LRP1 influences NMDAR surface expression

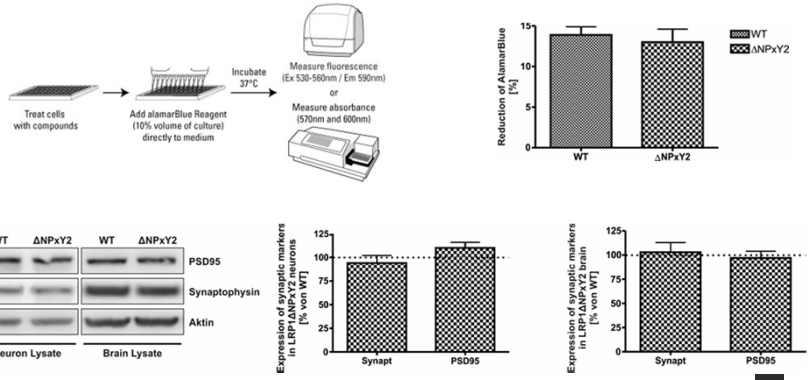
Surface biotinylation with cleavable biotin in primary neurons



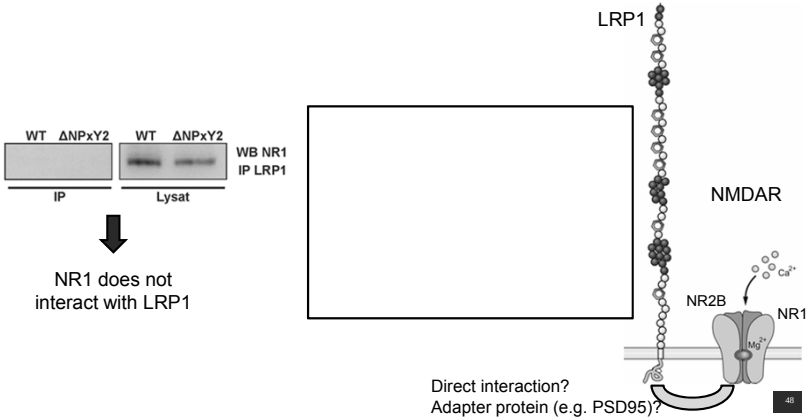
- LRP1, NR1 and NR2B internalization is reduced in LRP1ΔNPxY neurons.
- NR1 and NR2B internalization is LRP1 mediated and time dependent.
- NR2A internalization is normal in LRP1ΔNPxY neurons.

Maier et al. Mol. Neurodegeneration 2013

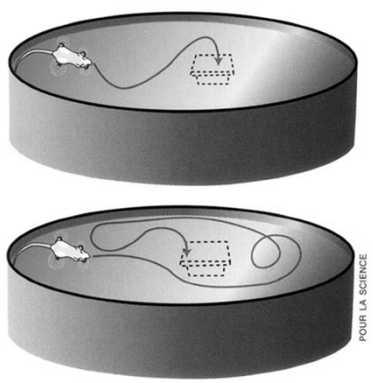
LRP1ΔNPxY2 knock-in has no effect on neuron survival or synaptic markers

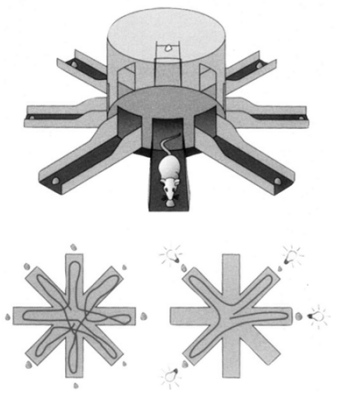


Does LRP1 interact with NMDAR subunits?

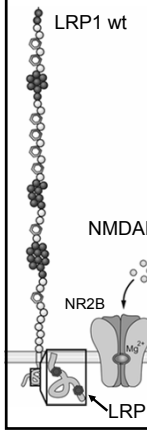


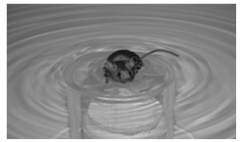
Behavioral test in mouse models for AD

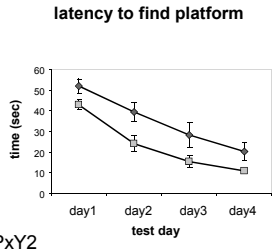


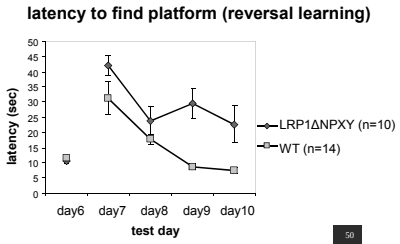


Learning in the Morris water maze





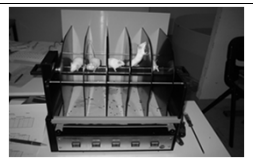


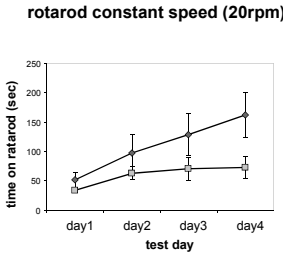


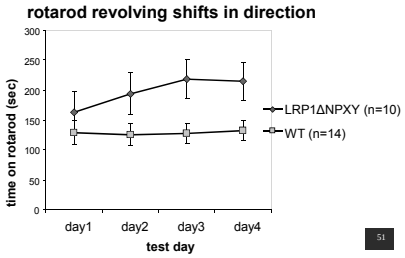
Maier et al. Mol. Neurodegeneration 2013

LRP1ΔNPxY2 mice perform better on Rotarod

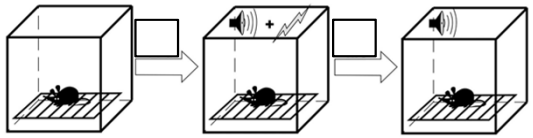
Evaluating balance and coordination

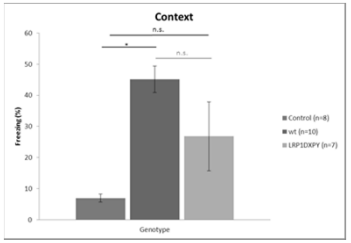




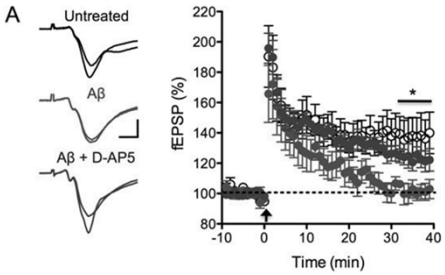


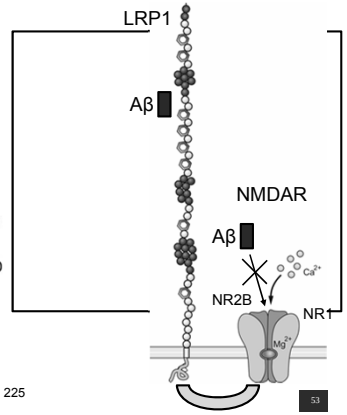
Fear conditioning of wt and LRP1ΔNPxY2 mice





Aβ binding to LRP1 may influence the NMDAR?





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

Steffen Storck, Sabrina Meister, Roswitha Nehrbaß, Erik Hameister, Johanna Wesselowski, Caroline Schönherr, Jessica Bien, Uta Herr, Verena Wolf
(Former lab members: Anne Martin, Wladislaw Maier, Elaine Waldron, Simone Isbert)

- Volkmar Lessmann (University Magdeburg, Germany)
- Ulrich Schmitt (University Medical Center Mainz, Germany)
- Anton Roebroek (University Leuven, Belgium)

- Funding Organizations:

Deutsche Forschungsgemeinschaft DFG

neuron Network of European Funding for Neuroscience Research

Bundesministerium für Bildung und Forschung

Kompetenznetz Degenerative Demenzen

Stiftung Rheinland-Pfalz: für Innovation