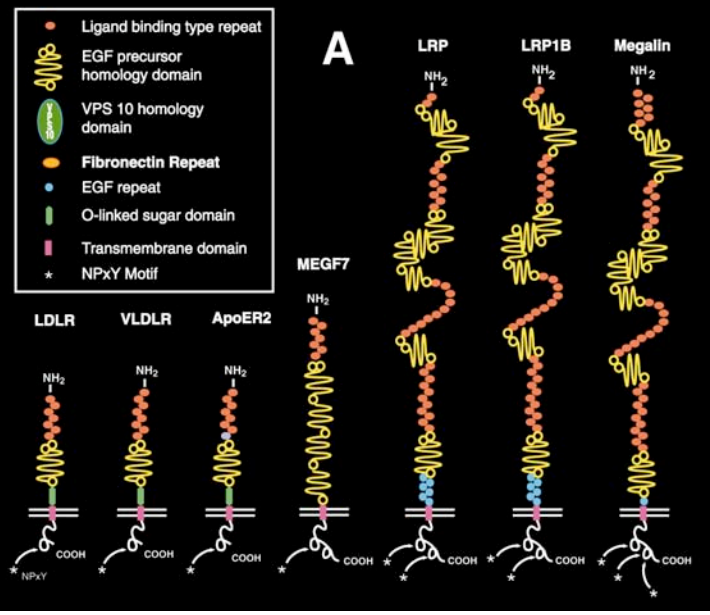
Analysis of Animal Models - Expect the Unexpected

Using Targeted Mutations in a Signaling Pathway for the Mapping of Modifiers

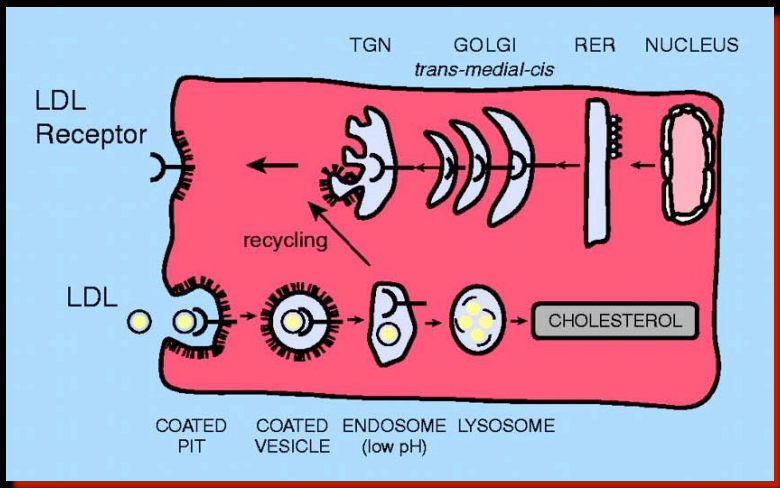
September 29, 2005

Lecture 2

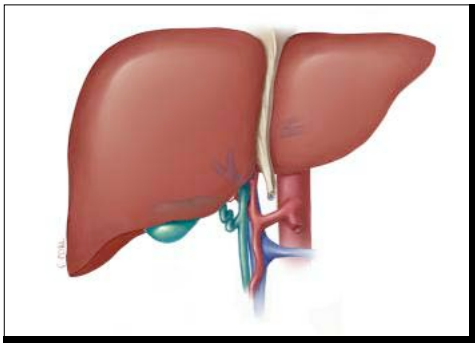
The LDL Receptor Gene Family

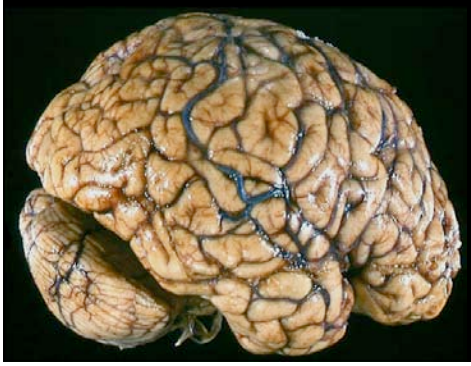


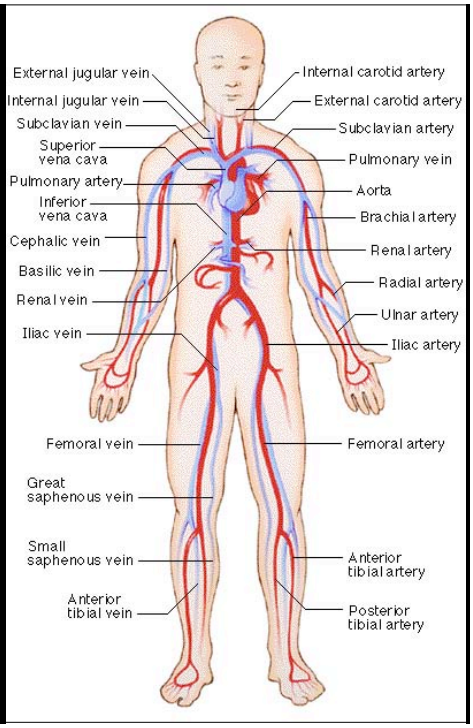
Cholesterol Transfer By LDL Receptor Mediated Endocytosis



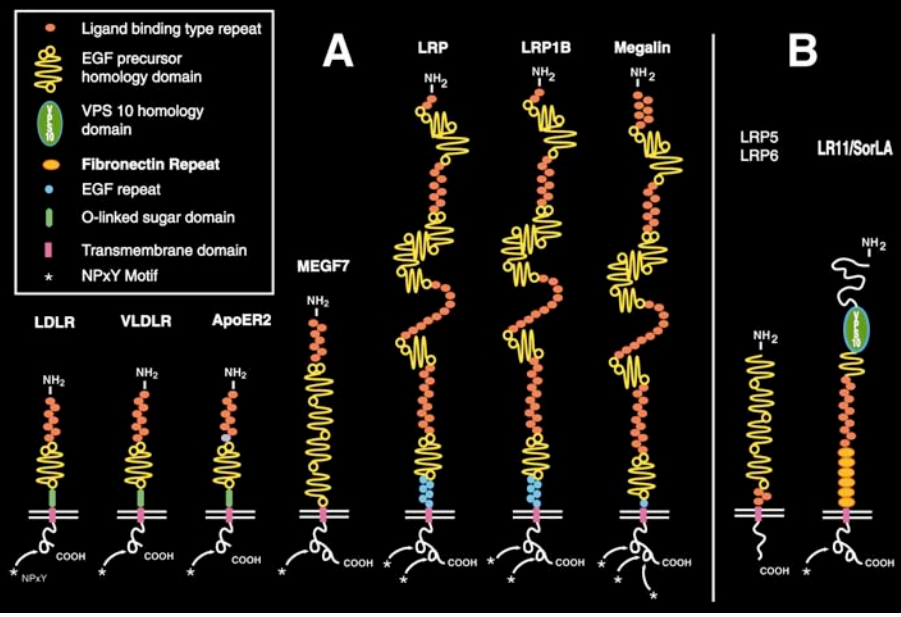
From Monty Krieger







The LDL Receptor Gene Family



Expanding Functions of ApoE Receptors

- ApoE Receptor Signaling Regulates τ-Phosphorylation
- Mapping of Genes that Modify τ-Phosphorylation
- ApoE Receptors Modulate Neurotransmission
- Tyrosine-Phosphorylation of ApoE Receptors and Control of Vascular Wall Integrity

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Tyrosine-phosphorylated Low Density Lipoprotein Receptor-related Protein 1 (LRP1) Associates with the Adaptor Protein SHC in SRC-transformed Cells*

Received for publication, December 19, 2000, and in revised form, February 28, 2001
Published, JBC Papers in Press, March 20, 2001, DOI 10.1074/jbc.M011437200

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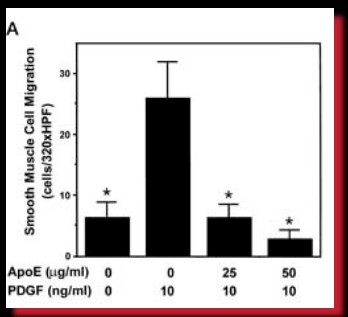
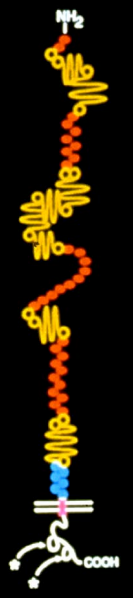
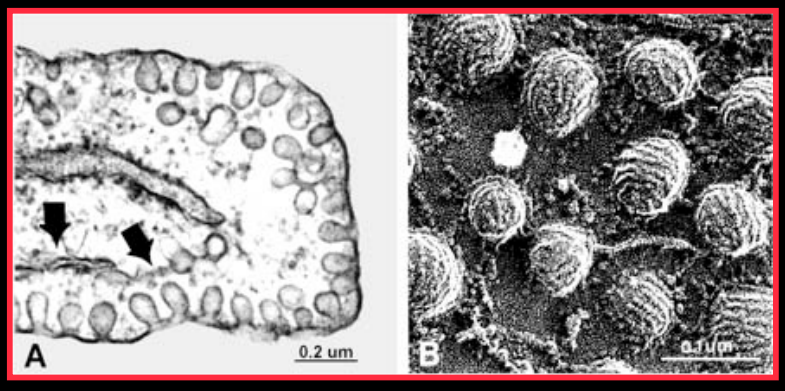




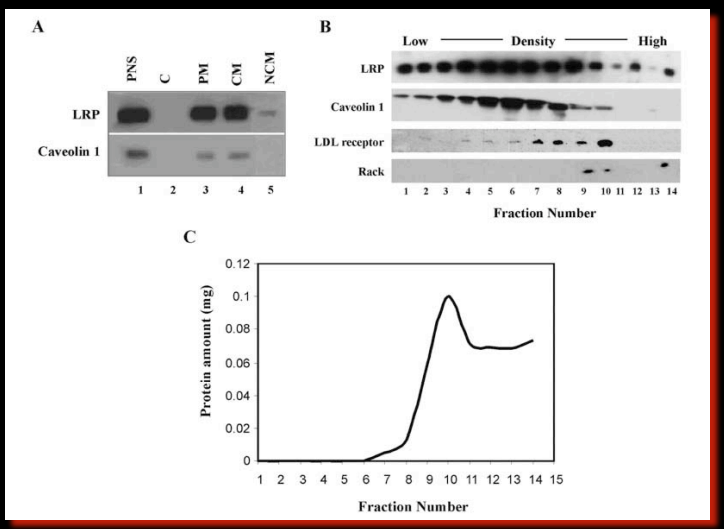
Ishigami M. et al. *J Biol Chem*, 273, 20156-20161, 1998

THE CAVEOLAE MEMBRANE SYSTEM



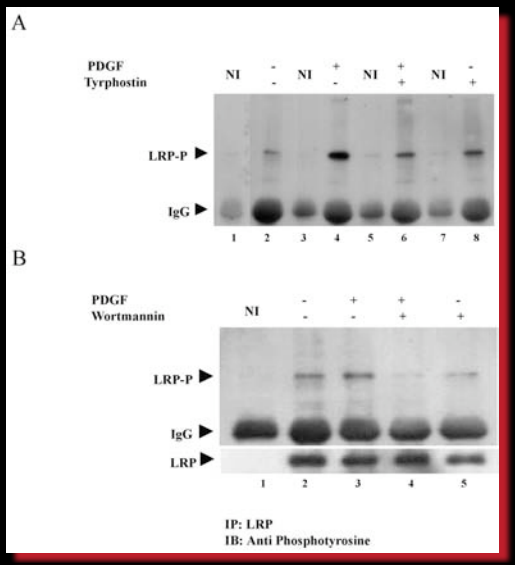
Anderson, RGW., *Annu. Rev. Biochem.*, 1998, 67, 199-225

LRP Is Enriched in Caveolae in Human Fibroblasts



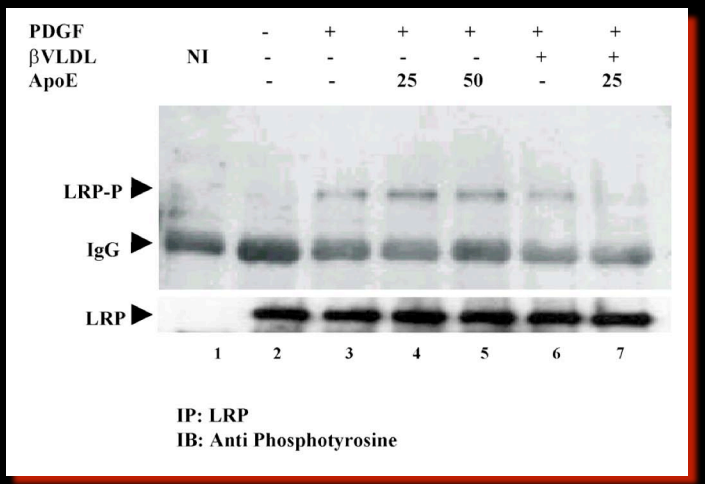
Philippe Boucher 2001

PDGF-BB Induces LRP-Tyrosine Phosphorylation Through the PDGF Receptor β in Caveolae



Philippe Boucher 2001

ApoE Lipoproteins Prevent PDGF-Mediated Tyrosine Phosphorylation of LRP



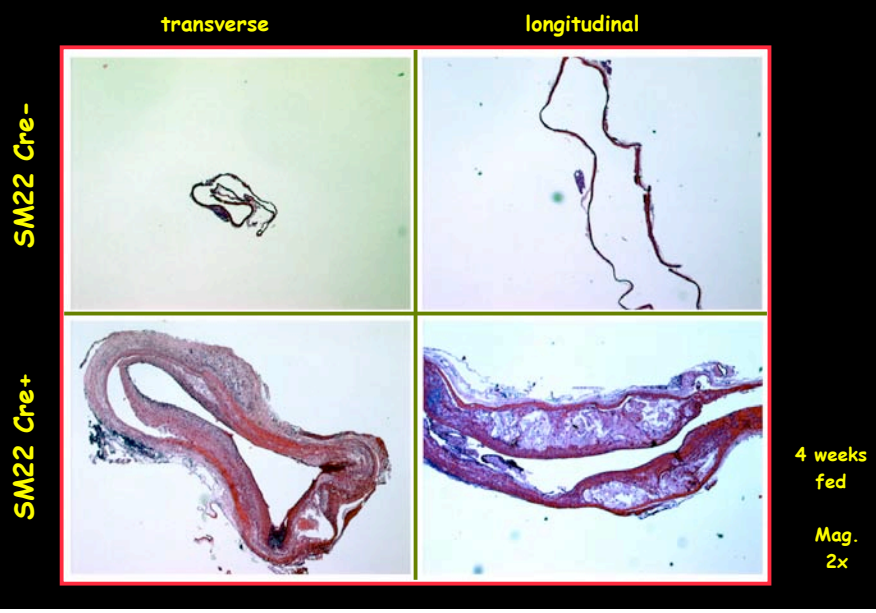
Generation of LRP Tissue-Specific Knockout Mice

Smooth Muscle Cell Specific LRP Defect on LDL Receptor Knockout Background

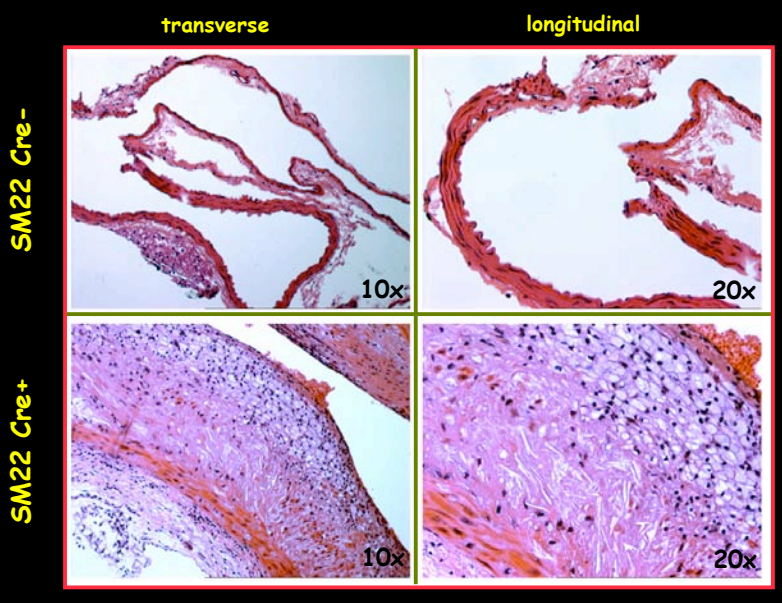
Cholesterol-Induced Atherosclerosis and Aneurysms in the Aorta of LDLR-/-;LRP$^{flox/flox}$ Mice

♂ 8 weeks fed ♀ 4 weeks fed ♂ 8 weeks fed

Histology of the Abdominal Aorta of Cholesterol-Fed LDLR$^{-/-}$;LRP$^{flox/flox}$ Mice

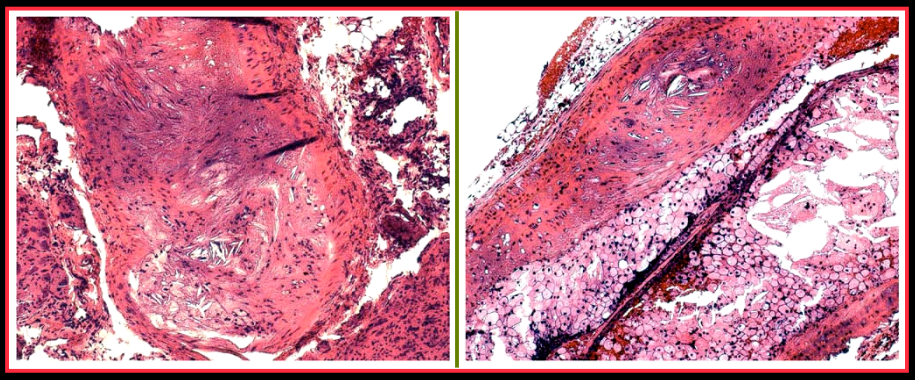


Histology of the Aorta of Cholesterol-Fed LDLR$^{-/-}$;LRP$^{flox/flox}$ Mice



4 weeks fed

Histology of Abdominal Aneurysm in Cholesterol-Fed SM22Cre+;LDLR$^{-/-}$;LRP$^{flox/flox}$ Mice



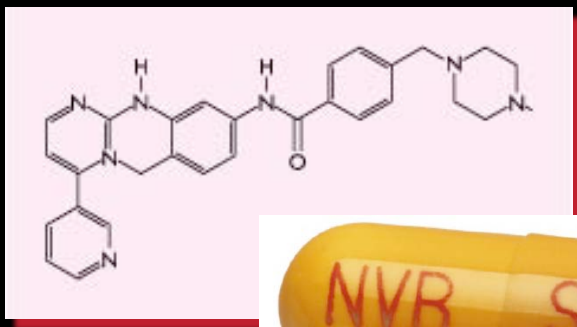
Mag. 40x

Philippe Boucher, 2001

Does PDGF Receptor Signaling Play A Role?

Gleevec

An Inhibitor of Tyrosine Kinases



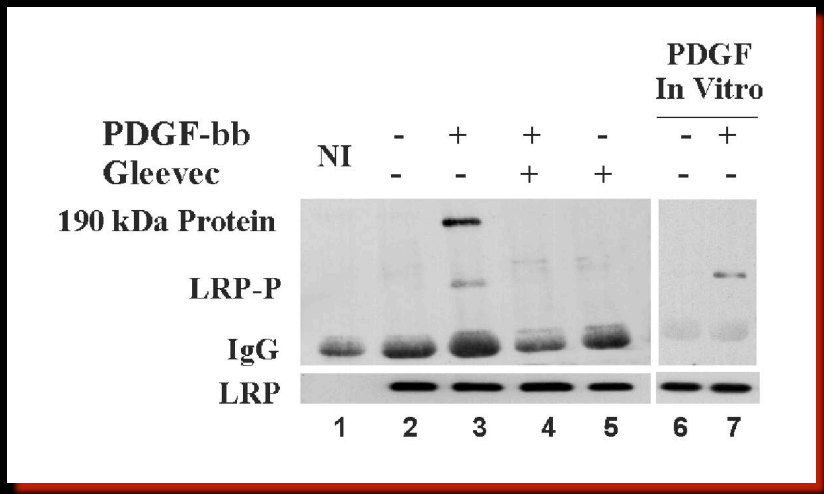
Known Targets

Bcr-Abl (CML)
c-Kit (GIST)
PDGFR

Proven Clinical Effectiveness

Chronic Myelogenous Leukemia
GastroIntestinal Stromal Tumor

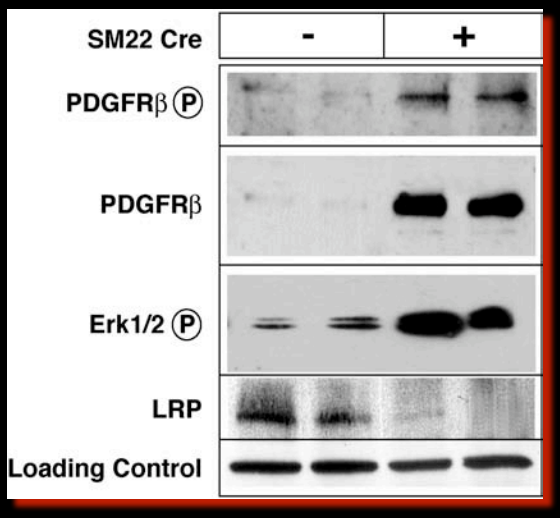
Gleevec Blocks PDGF-Induced LRP Phosphorylation
PDGFR coimmunoprecipitates with LRP



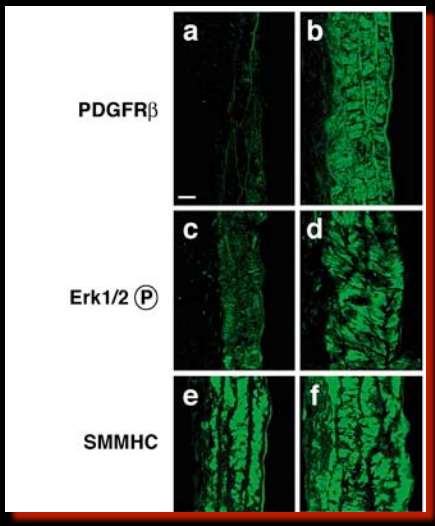
Effect of Gleevec on Atherosclerotic Lesion Development in Cholesterol-Fed LDLR$^{-/-}$;LRP$^{flox/flox}$ Mice

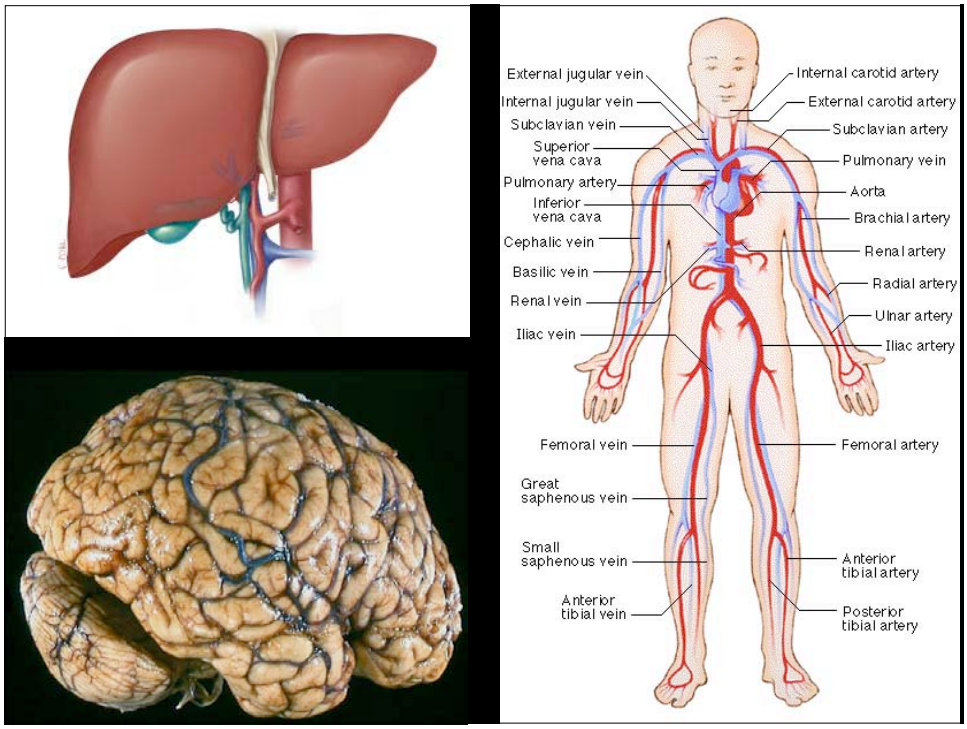
Philippe Boucher, 2002

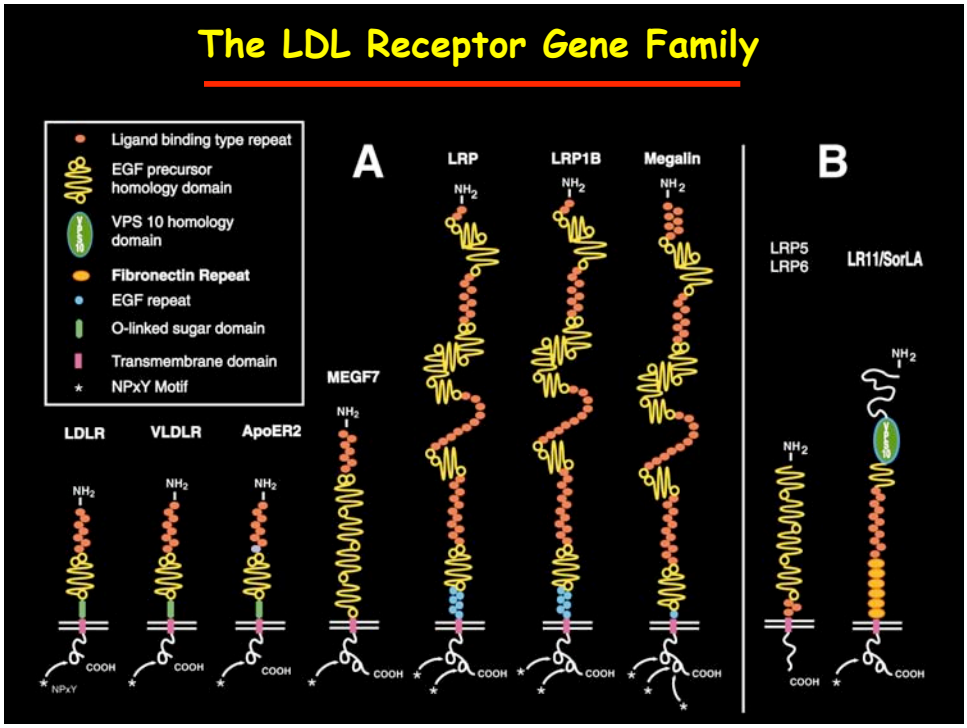
PDGFRB expression and activation and Erk activation in LRP-deficient mouse aorta



PDGFRB expression and activation and Erk activation in LRP-deficient mouse aorta

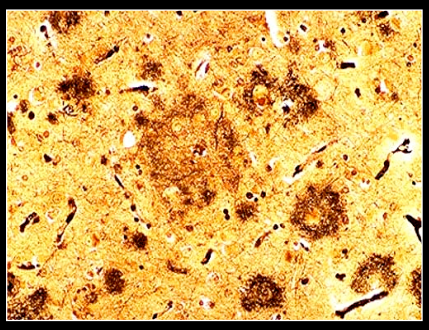



External jugular vein
Internal jugular vein
Subclavian vein
Superior vena cava
Pulmonary artery
Inferior vena cava
Cephalic vein
Basilic vein
Renal vein
Iliac vein
Internal carotid artery
External carotid artery
Subclavian artery
Pulmonary vein
Aorta
Brachial artery
Renal artery
Radial artery
Ulnar artery
Iliac artery
Femoral vein
Femoral artery
Great saphenous vein
Small saphenous vein
Anterior tibial vein
Anterior tibial artery
Posterior tibial artery

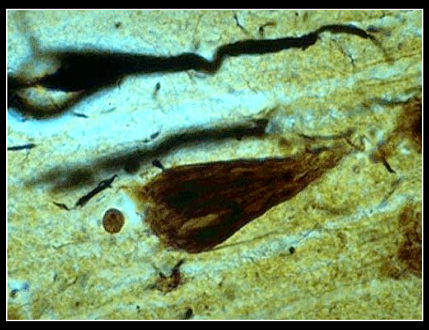

The LDL Receptor Gene Family
Ligand binding type repeat
EGF precursor homology domain
VPS 10 homology domain
Fibronectin Repeat
EGF repeat
O-linked sugar domain
Transmembrane domain
NPxY Motif
LDLR
VLDLR
ApoER2
A
LRP
LRP1B
Megalin
MEGF7
B
LRP5
LRP6
LR11/SorLA
NH2
COOH
NPxY

The Hallmarks of Alzheimer Disease

Amyloid Plaques



Neurofibrillary Tangles



From: Medical Library of Utah

Apolipoprotein E

Is a component of lipoproteins and mediates their binding to LDL receptor family members

In 1993 Allan Roses and his group report that the ApoE4 isoform predisposes its carriers to late-onset Alzheimer Disease

Effect of ApoE4 Dose On Late-Onset Alzheimer's Disease

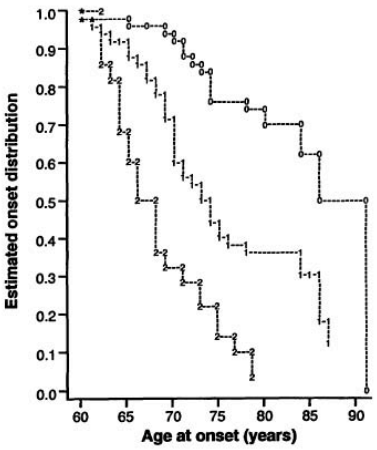
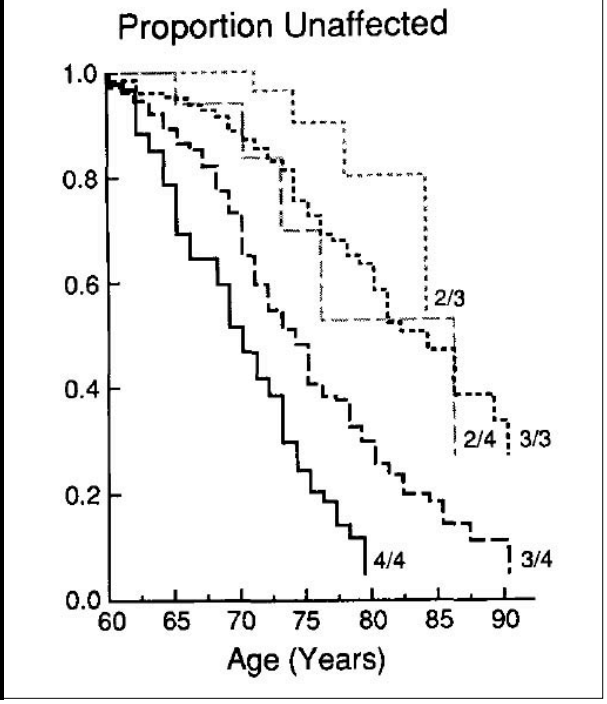
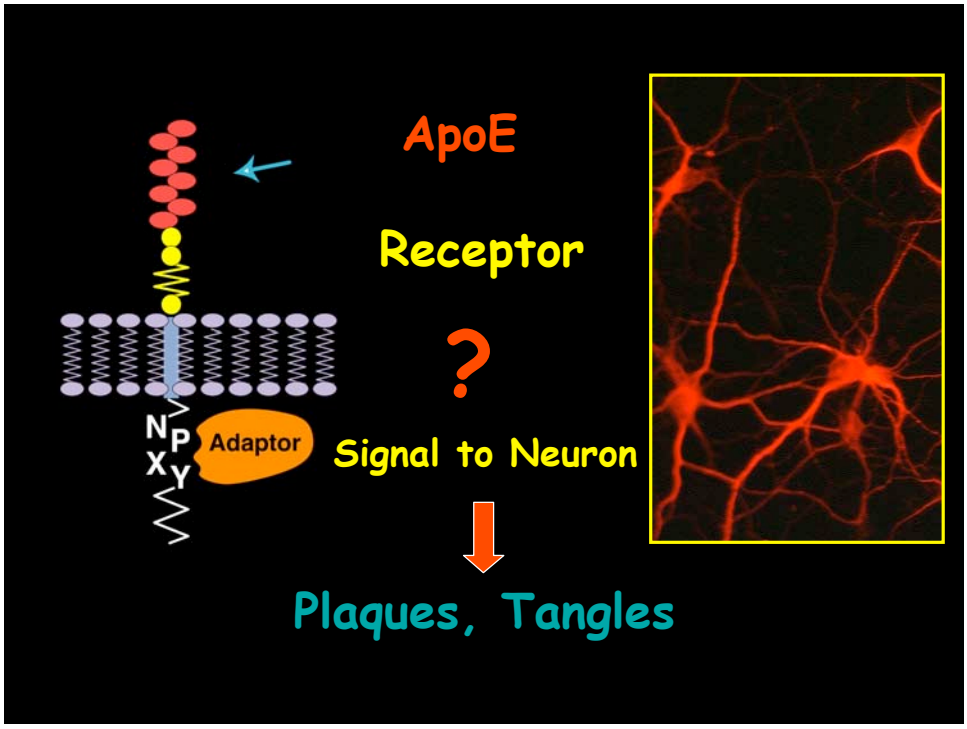
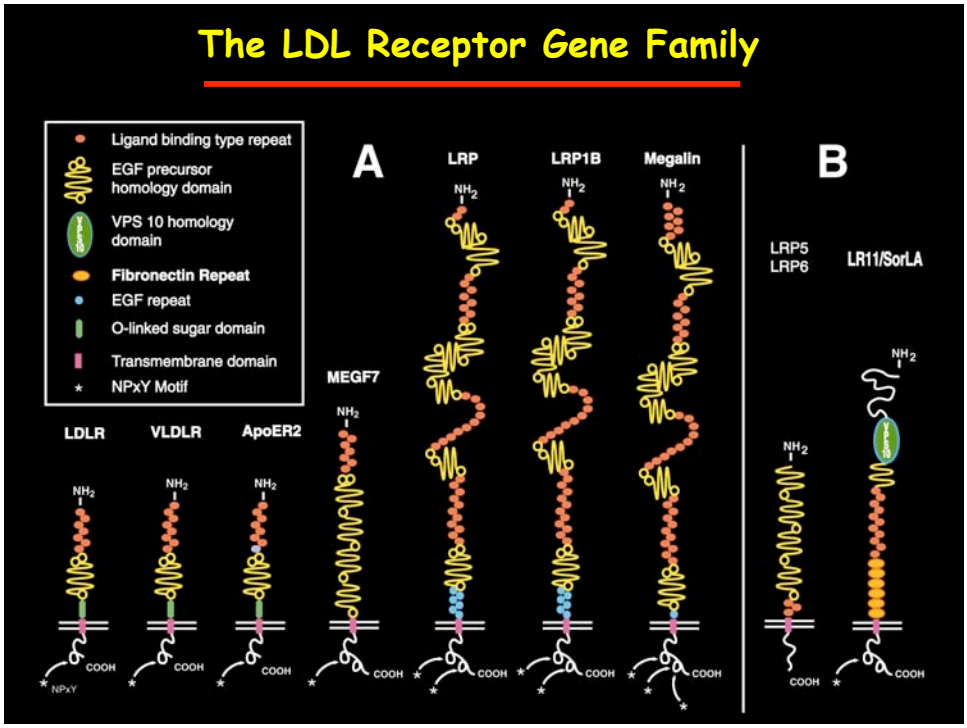


Fig. 1. Age at onset for subjects with 0, 1, and 2 *APOE*-ε4 alleles. Each curve is labeled by the number of *APOE*-ε4 alleles. An asterisk indicates multiple diagnoses within a short interval. Onset curves were estimated by Kaplan-Meier product limit distributions (*14, 15*). For example, at age 75, an estimated 24% of subjects without *APOE*-ε4 were diagnosed with AD compared to 61% of subjects with one *APOE*-ε4 allele and 86% of subjects with two *APOE*-ε4 alleles.

ApoE2 Is Protective




ApoE
Receptor
?
Signal to Neuron
Plaques, Tangles
N
P
X
Y
Adaptor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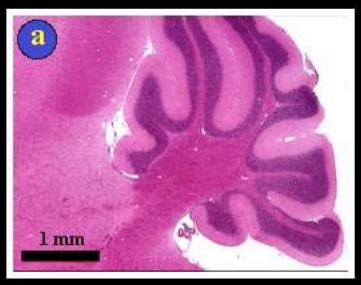
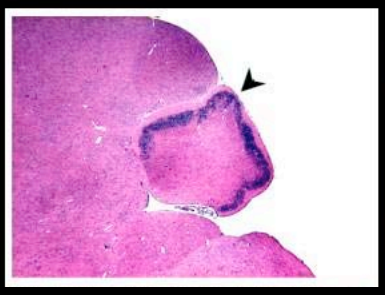
O-linked sugar domain
Transmembrane domain
NPxY Motif
A
B
LDLR
VLDLR
ApoER2
MEGF7
LRP
LRP1B
Megalin
LRP5
LRP6
LR11/SorLA
NH2
COOH
NPxY

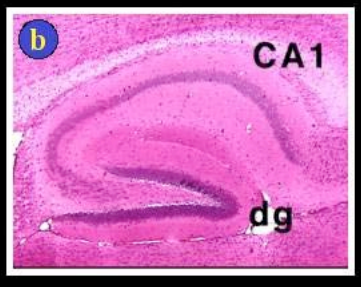
Ataxia in VLDLR/ApoER2 Double Knockout Mice

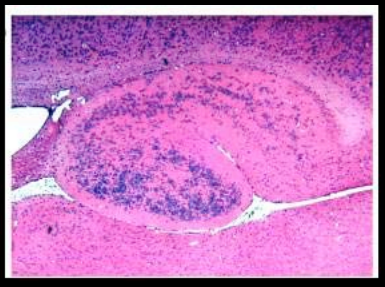
Normal

Mutant

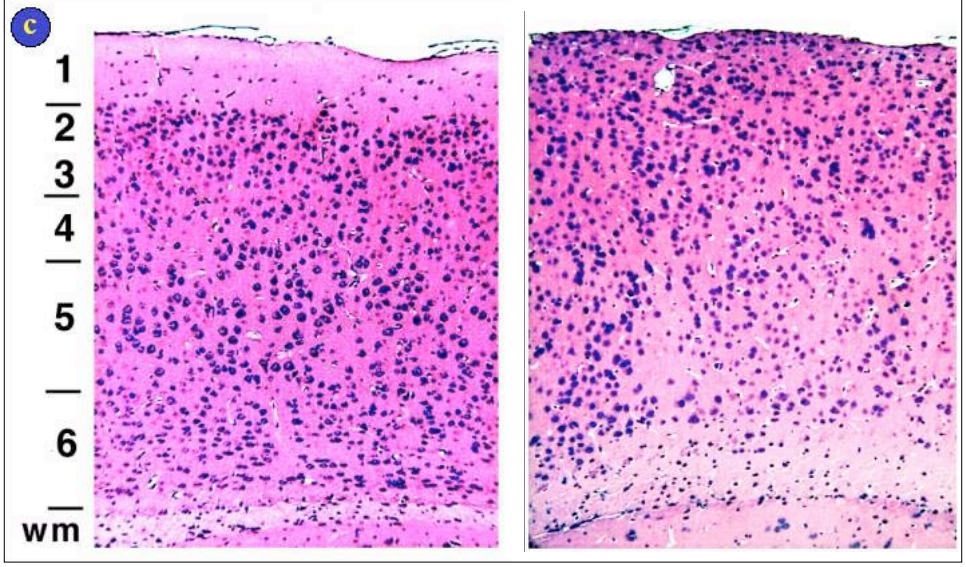








Normal Mutant

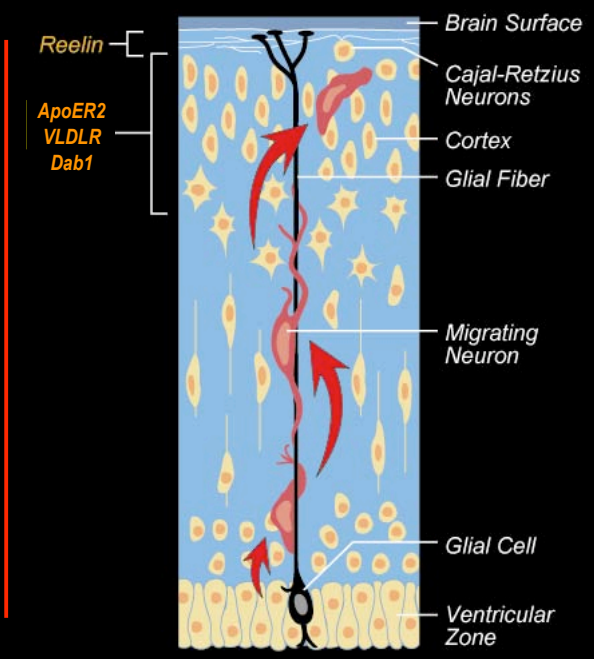


The identical phenotype has been observed in two independent strains of mice:

reeler: **Loss of function mutation in a gene encoding a large secreted signaling molecule (Reelin)**

scrambler: **Loss of function mutation in a gene encoding a cytoplasmic adaptor protein (mammalian Disabled-1; Dab1)**

Radial Neuronal Migration Along Glial Guidance Fibers



Am. J. Hum. Genet. 77:477–483, 2005

Report

Homozygous Deletion of the Very Low Density Lipoprotein Receptor Gene Causes Autosomal Recessive Cerebellar Hypoplasia with Cerebral Gyral Simplification

Kym M. Boycott,[1] Shauna Flavelle,[1] Alexandre Bureau,[1,4] Hannah C. Glass,[2] T. Mary Fujiwara,[5] Elaine Wirrell,[2] Krista Davey,[1] Albert E. Chudley,[6] James N. Scott,[3] D. Ross McLeod,[1] and Jillian S. Parboosingh[1]

[1]Department of Medical Genetics and [2]Division of Pediatric Neurology, Alberta Children's Hospital and University of Calgary, and [3]Department of Radiology, Foothills Hospital, Calgary; [4]School of Health Sciences, University of Lethbridge, Lethbridge, Alberta, Canada; [5]Departments of Human Genetics and Medicine, McGill University, Montreal; and [6]Section of Genetics and Metabolism, Children's Hospital and the Department of Pediatrics and Child Health, University of Manitoba, Winnipeg

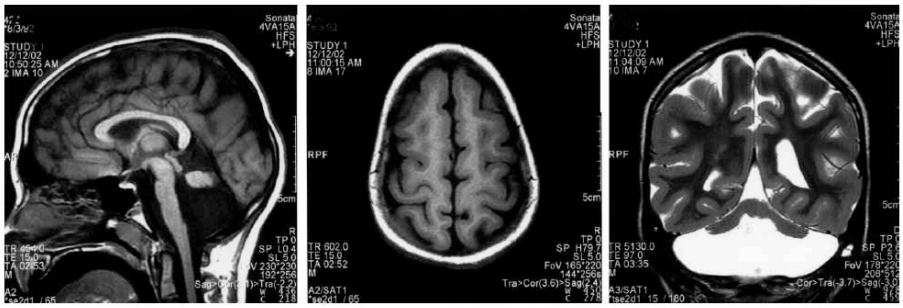


Figure 1 MRI demonstrating characteristic brain malformations seen in patients with DES-H. *A*, Sagittal T1W. *B*, Axial T1W. *C*, Coronal T2W spin echo images showing a small cerebellum in a fluid-filled but normal-sized posterior fossa. The superior half of the vermis is formed, but the inferior half is not. The pons is abnormally small. The cerebral cortex is simplified. Reprinted with permission (Glass et al., in press).

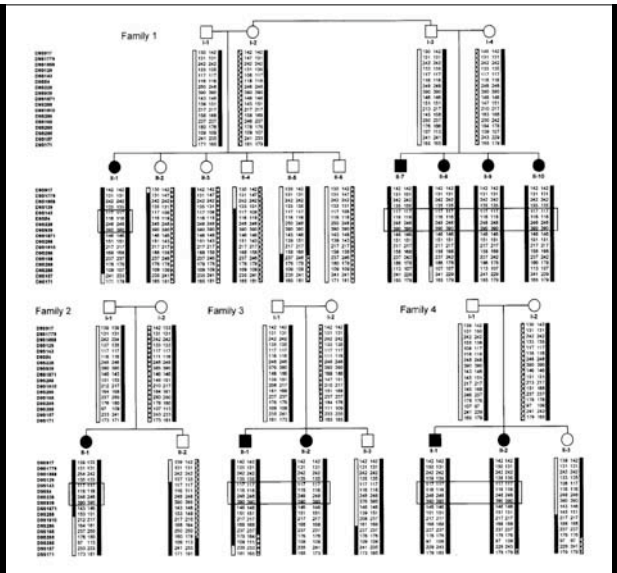
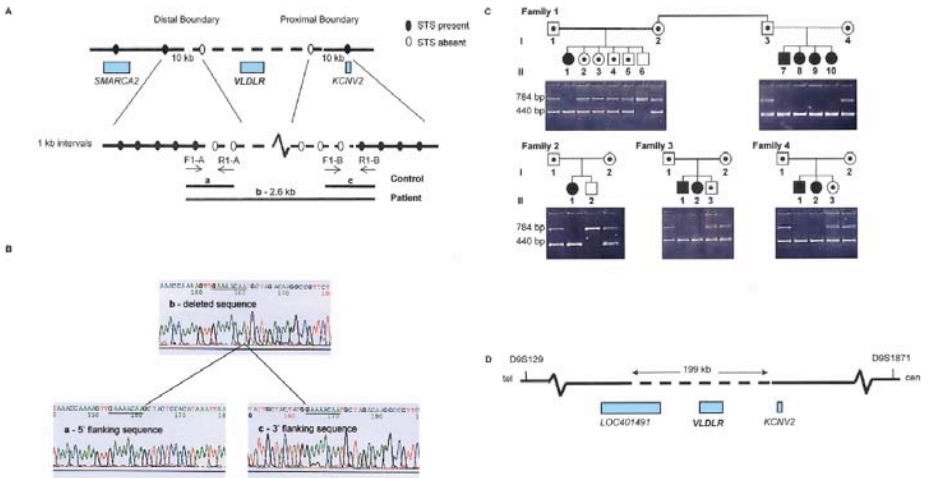
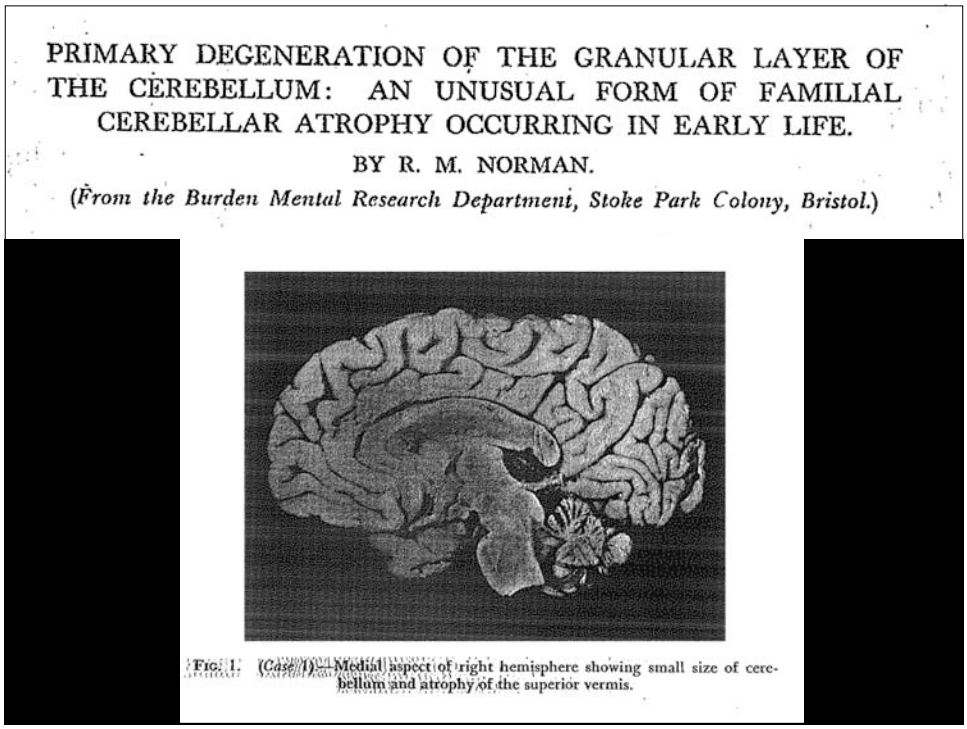


Figure 2 Haplotype analysis of the Hutterite families. Affected individuals are identified by blackened symbols and unaffected family members by unblackened symbols. In family 1, individuals I-2 and I-3 are full siblings. Haplotypes were constructed by visual inspection, with the assumption of a minimal number of recombination events. The marker order was determined from the physical map of the region. For the children in each family, the allele on the left was inherited from the father and the allele on the right from the mother. A patterned bar indicates haplotypes for chromosome 9 markers, a black bar indicates the alleles associated with the DES-H disease allele, and a gray region indicates a region in which a recombinant event occurred. An observed paternal recombination event in unaffected sibling II-3 from family 4 indicates a proximal limit of *D9S286* for the DES-H locus. A recombinant defining the distal boundary for the DES-H locus was not identified. The boxed markers correspond to the smallest region of shared homozygosity and the minimal region for the DES-H gene. *D9S129* was predicted to be the distal limit, on the basis of inferred ancestral recombination events that resulted in different *D9S129* alleles in affected individuals II-1 from family 2 and the siblings II-1 and II-2 from family 4. *D9S1871* was predicted to be the proximal limit on the basis of an inferred recombinant in one of the ancestors of the father in family 2, who carried a different allele on the disease chromosome at this locus.





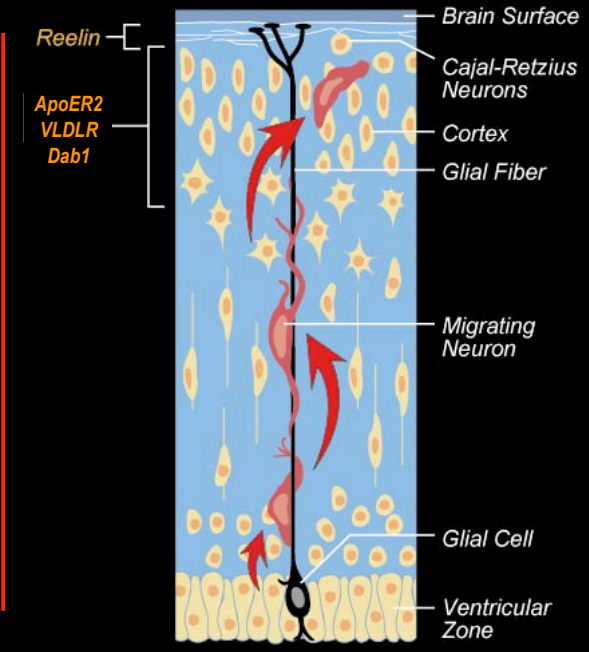
PRIMARY DEGENERATION OF THE GRANULAR LAYER OF THE CEREBELLUM: AN UNUSUAL FORM OF FAMILIAL CEREBELLAR ATROPHY OCCURRING IN EARLY LIFE.

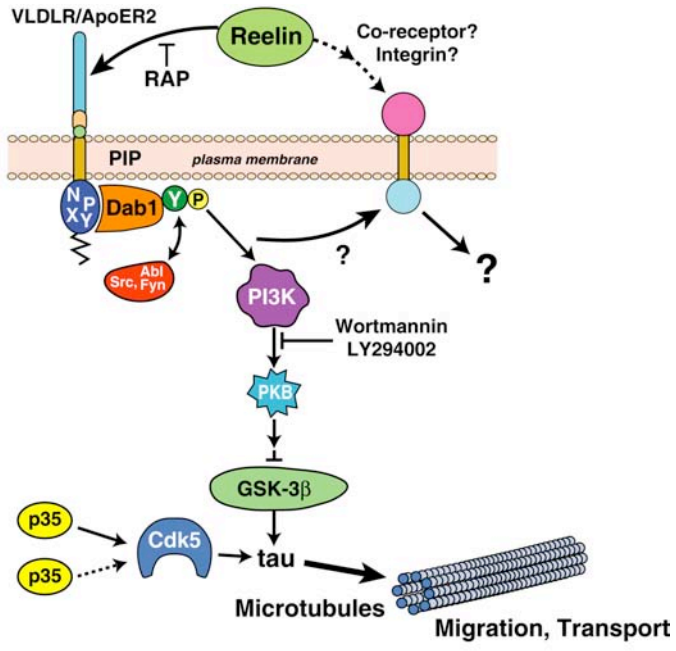
BY R. M. NORMAN.

(From the Burden Mental Research Department, Stoke Park Colony, Bristol.)

FIG. 1. (Case 1).—Medial aspect of right hemisphere showing small size of cerebellum and atrophy of the superior vermis.

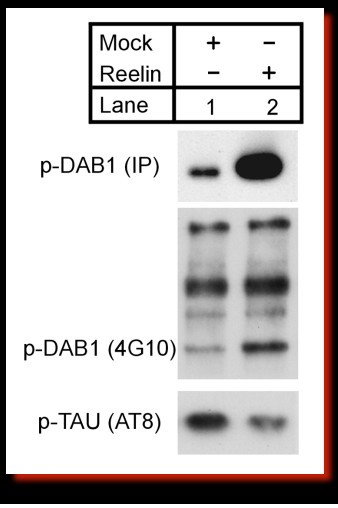
Radial Neuronal Migration Along Glial Guidance Fibers





Hans Bock, Uwe Beffert

Reelin Suppresses τ-Phosphorylation in Primary Embryonic Neurons



(Hans Bock, 2002)

Tau Hyperphosphorylation in *reeler*, *apoer2*, and *vldlr* Knockout Mice