

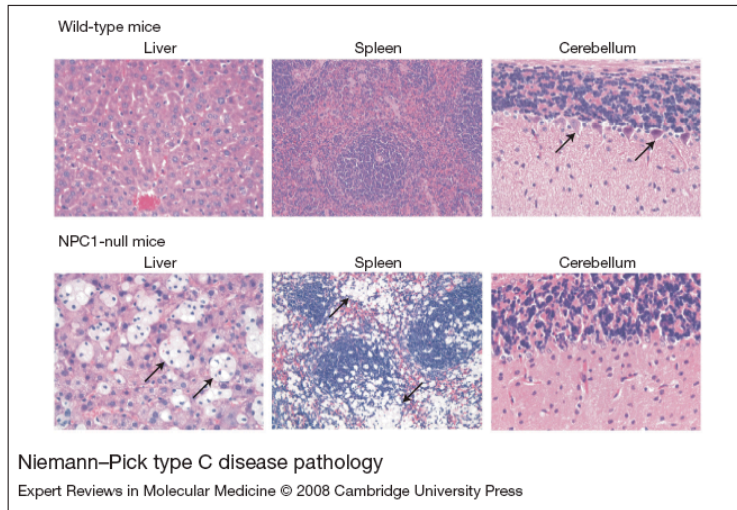
## Disorders of Lipid Trafficking: Niemann-Pick type C

- lysosomal storage disorder characterized by a defect in lipid transport
- cellular trafficking of exogenous cholesterol is defective resulting in the accumulation of unesterified cholesterol; others lipids such as the gangliosides GM2 and GM3 also build up
- Niemann-Pick types A and B, caused by defects in the enzyme acid sphingomyelinase (ASM), are phenotypically related storage disorders characterized by the accumulation of sphingomyelin in lysosomes
- the pathological hallmark of all NP disorders is the so-called "Niemann-Pick" cell, a lipid-laden foam cell of the macrophage/monocyte lineage
- incidence is rare (1:150,000) but in some areas of Nova Scotia, the incidence is almost 1% and 10-25% of population are carriers

Build-up of cholesterol is problematic in organs that are enriched in this molecule  
*(common theme in lysosomal storage disorders)*

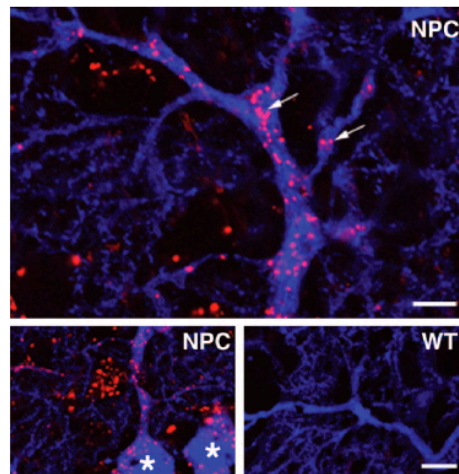
Brain: 5% of body mass, contains 25% of cholesterol (mostly in myelin)

- cholesterol homeostasis in brain is maintained by export of cholesterol as 24-hydroxy-cholesterol into plasma
- cholesterol accumulates in all tissues except brain likely due to masking effect of age-induced demyelination



- loss of Purkinje cells in cerebellum
- presence of foam cells and enlargement of Kupffer cells in liver

**FIG. 1. Cholesterol accumulation in Purkinje neurons of NPC1 mice at post-natal day 8.** Cerebellar brain sections from PND 8 NPC1 (BALB/c NPC1<sup>0/0</sup>) mice and WT mice were stained with the cholesterol binding agent BC-theta (red) and anti-Calbindin antibodies (a Purkinje cell marker protein; blue). *Main panel*, NPC1 Purkinje dendrite, cholesterol accumulation indicated by arrows; scale bar is 5  $\mu$ m; *lower left panel*, NPC1 Purkinje cell body, indicated by asterisk; *lower right panel*, WT Purkinje dendrite, scale bar is 10  $\mu$ m. Reproduced with permission from Reid *et al.* (53).



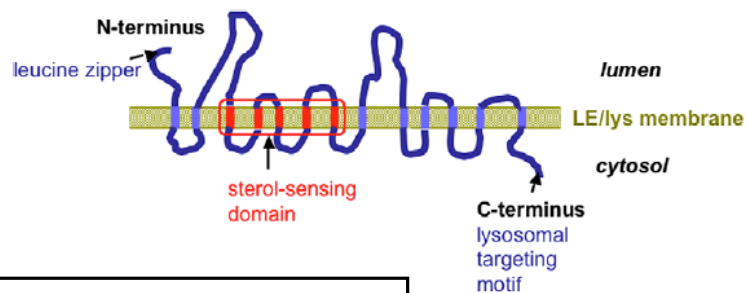
Niemann-Pick type C is caused by mutations in two genes: NPC1 and NPC2

**NPC1:** an integral membrane protein found in late endosomes/lysosomes

- contains a sterol sensing domain similar to SCAP and HMG Co-A reductase
- may act as the cholesterol "sensor"

- in NPC1 deficiency, cholesterol becomes sequestered in the endosomal/lysosomal system but the content of cholesterol at the plasma membrane is reduced
- despite an overall accumulation of cholesterol in NPC1-deficient cells, the ER does not sense the increase and keeps making cholesterol
- both endocytosed and newly-synthesized cholesterol can accumulate in NPC cells

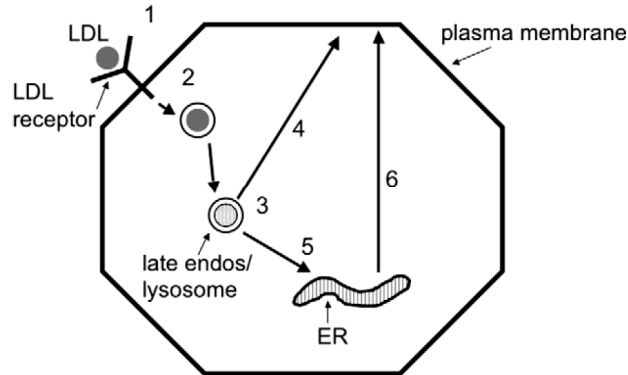
### Topology of NPC1



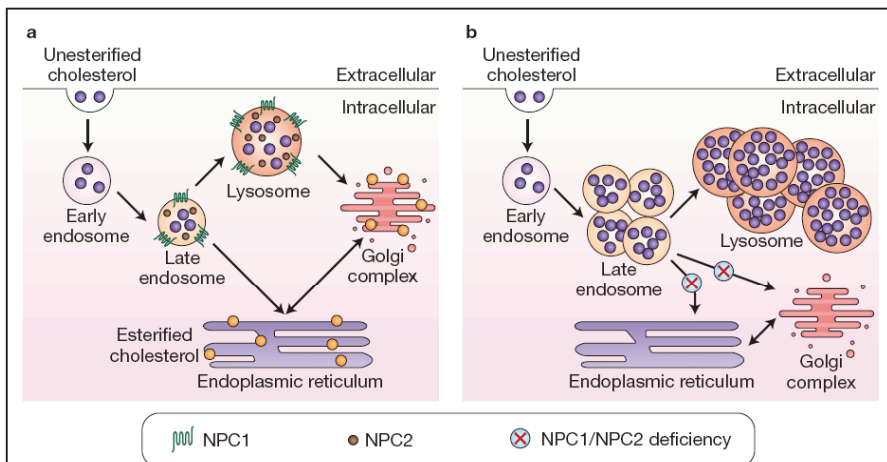
potential functional roles of NPC1...

- 1) a cholesterol "flippase"
- 2) a fatty acid permease
- 3) a ganglioside transporter
- 4) a cholesterol sensor

## Cholesterol transport and homeostasis



- after uptake as LDL, cholesteryl esters are hydrolyzed within these particles into unesterified cholesterol
- this form of cholesterol can then be transported to the plasma membrane (in an NPC1-dependent manner) or transported back to the ER where the cell downregulates new cholesterol and LDL synthesis



### Lipid-trafficking defects in Niemann–Pick type C disease

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NPC2 (or HE1): a soluble lysosomal glycoprotein with M6P residues

- major secretory component of epididymal fluid
- contains hydrophobic pocket and can bind cholesterol
- may act as the cholesterol transporter

*NPC2 was identified by proteomic analysis of the lysosome*

-other lysosomal storage diseases have been identified in this way (TPP: tripeptidyl peptidase I as cause of late-infantile NCL)

Loss of NPC1 has effects on multiple vesicle transport steps

- 1) reduced mobility of endosomal tubulovesicular structures
- 2) impaired trafficking of mannose 6-phosphate receptors
- 3) increased levels of Rab9 due to its impaired degradation

Overexpression of Rab7 or Rab9 in NPC1-deficient cells reduces cholesterol accumulation in late endosomes/lysosomes (Rabs can bypass NPC1)

### The mystery of NPC pathogenesis: who accumulates first?

- besides cholesterol, gangliosides, sphingolipids and bis mono-acylglycerol phosphate

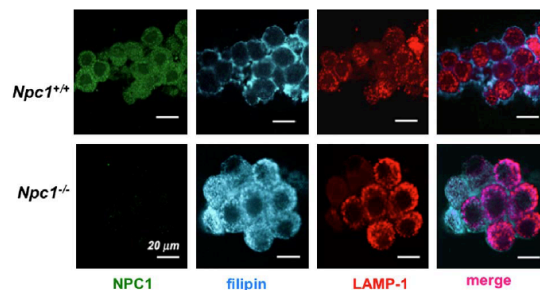
Question #1: Is the accumulation of cholesterol secondary to ganglioside storage or vice versa? (Nat Med paper for next week)

Question #2: Are neurological problems due to excess cholesterol in late endosomes/lysosomes or a deficiency of cholesterol in the ER or plasma membrane

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- although cholesterol accumulates in cell bodies in NPC, it is diminished in axons likely due to impaired forward transport of cholesterol

- NPC1 and 2 are abundant in axons and may play neuron-specific functions in axons



Cell bodies of mouse sympathetic neurons

- NPC1 is also present in recycling endosomes in pre-synaptic nerve terminals
- no change in cholesterol synthesis but altered formation of synaptophysin and synaptobrevin, a complex that plays a role in vesicle recycling, is seen in NPC<sup>-/-</sup> brains
- function of NPC1 in axons could be the regulation of synaptic transmission (loss of NPC1 --> lower PM cholesterol --> impaired synaptic vesicle recycling and neurotransmitter release)

ataxia in NPC: neuronal death (Purkinje cells)

other neurological symptoms: defects in synaptic transmission

- a link with Alzheimer's disease?
  - *levels of A $\beta$ 40 and A $\beta$ 42 are increased by NPC1 deficiency*
  - *neurofibrillary tangles and hyperphosphorylated tau protein are seen in NPC*

## Liver disease in NPC

- hepatomegaly and cholestasis occur perinatally in 50% of NPC patients and NPC1<sup>-/-</sup> mice have signs of liver damage
- NPC1 is highly expressed in liver and flux of LDL-derived cholesterol thru this organ is the highest
- the rate of cholesterol esterification is several fold higher in NPC1-deficient mouse hepatocytes but the rate of cholesterol synthesis is not changed
- # of apo B100-containing vLDL particles is doubled in NPC deficient hepatocytes

*collectively suggest that the profound changes in lipid metabolism in liver may play a role in NPC liver disease (distinct mechanisms from brain)*

## Potential therapies for the treatment of NPC

- blood-brain barrier represents a problem in delivery

### Steroid Therapy

- levels of neurosteroids and biosynthetic enzymes are low in NPC<sup>-/-</sup> mouse brains
- one neurosteroid, allopregnanolone, delayed the onset of neurological symptoms and increased lifespan of the mice from 67 to 147 days

### Substrate Deprivation Therapy

- NPC1<sup>-/-</sup> have been crossed with several null mice (LDL receptor, GM2 synthase) with variable results in lowering cholesterol

LDL receptor - peripheral cholesterol lower but no effect in brain

GM2 synthase - no increase in lifespan (GM2 not important?)

- NB-DNJ: inhibitor of all glycolipid synthesis did extend lifespan (20%) and delayed neurological symptoms