Cholesterol transporters as new therapeutic targets
Physiological roles of cholesterol

Physical role: structure and functions of membranes

- fluidity
- domains (rafts)
- protein function (pumps)
Physiological roles of cholesterol

Biochemical role: precursor of steroid hormones and bile acids

- Cholesterol
  - Bile acids
    - Aldosterone
    - Testosterone
    - Estradiol
    - Cortisol
Pathological roles of cholesterol

- increase in storage
- alteration of cellular fate
- HDL/LDL dysbalance
- increase in absorption
- alteration of excretion
- obesity, dyslipidemia
- Niemann-Pick disease
- atheromatosis, Tangier dis.
- sitosterolemia
- stones

**current therapeutic options... and their limitations**

- lipid adsorbants
- statins
- fibrates

adsorption of liposoluble vitamins
risk of side effects
useful if high triglycerides
### Pathological roles of cholesterol transporters

<table>
<thead>
<tr>
<th>Cholesterol Transporters</th>
<th>Diseases and Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPC1L1</td>
<td>obesity, dyslipidemia</td>
</tr>
<tr>
<td>NPC1-NPC2</td>
<td>Niemann-Pick disease</td>
</tr>
<tr>
<td>ABCA1</td>
<td>atheromatosis, Tangier dis.</td>
</tr>
<tr>
<td>ABCG5-ABCG8</td>
<td>sitosterolemia</td>
</tr>
<tr>
<td>ABCG8</td>
<td>stones</td>
</tr>
</tbody>
</table>

**Cholesterol transporters as new drug targets?**
Sterol fate in the body

Sterol fate in the body

Transport of sterols in intestinal cells

NPC1L1

ABCG5
ABCG8

caveolin

Golgi

NPC1L1, a RND sterol transporter

http://www-biology.ucsd.edu/~msaier/transport/

2.A.6. The Resistance-Nodulation-Cell Division (RND) Superfamily

2.A.6.6. The Eukaryotic (Putative) Sterol Transporter (EST) Family

2.A.6.6.1 Niemann-Pick C1 AND C2 disease proteins (together to form a possible lipid/cholesterol exporter from lysosomes to other cellular sites) (Slat et al., 2004).

Animals

NPC1 and NPC2 of Homo sapiens
NPC1 (AAH63302)
NPC2 (AAH02532)

2.A.6.6.2 Patched (Ptc) segmentation polarity protein

Animals

'Patched' of Drosophila melanogaster

2.A.6.6.3 Yeast membrane protein YPL006w

Protein, yeast

YPL006w of Saccharomyces cerevisiae

2.A.6.6.4 SREBP cleavage-activating protein, SCAP

Animals

SCAP of Cricetulus griseus

2.A.6.6.5 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase

Animals

HMG-CoA reductase of Homo sapiens

2.A.6.6.6 Intestinal enterocyte brush border Niemann-Pick C1 like 1 (NPC1L1) protein; probably responsible for ezetimibe-sensitive absorption of luminal cholesterol (Altman et al., 2004).

Animals

NPC1L1 of Homo sapiens (NP_037521)

NPC1L1 mediates sterol absorption

NPC1L1 reduces cholesterol uptake and processing to the Golgi

Davies et al. (2005) JBC 280:12710-20
NPC1L1 controls lipid endocytosis

NPC1L1 controls caveolin localization

Davies et al. (2005) JBC 280:12710-20
NPC1L1 as a target for treating obesity?

NPC1L1 KO mice are protected from hypercholesterolemia induced by a high fat diet.

Davies et al. (2005) JBC 280:12710-20
Ezetimibe as inhibitor of sterol absorption
Ezetimibe, a dual target inhibitor: \( \gg \text{NPC1L1} \)

Ezetimibe is not efficient in NPC1L1 KO mice

Ezetimibe, a dual target inhibitor: caveolin

Ezetimibe inhibits the formation of the annexin 2 – caveolin 1 complex by binding to caveolin

Smart et al. (2004) PNAS 101:3450-5
Ezetimibe in combination with statins

Reduction of cholesterol absorption stimulates endogeneous synthesis

Smart et al. (2004) PNAS 101:3450-5
Ezetimibe approved in Europe and in USA

10 mg EZ + 10-20 mg simvastatin = 80 mg simvastatin

Sterol fate in the body

NPC1/2, two other RND sterol transporters

http://www-biology.ucsd.edu/~msaier/transport/

Subcellular localization of NPC1 and NPC2

NPC1
‘frequent flyer’

NPC2
late endosomes/lysosomes resident


NPC1 and NPC2 as lipid transporters

NPC1 expressed in *E. coli* transports oleic acid and other lipids but NOT cholesterol!

[Davies et al. (2000) Science 290:2295-8]
Shuttle role of NPC1 is impaired in Niemann-Pick disease.

NPC1 trafficking

normal

pathologic


Zhang et al. (2004) PNAS 98:4466-71
Niemann – Pick disease

Mutations in NPC cause mislocalization of NPC1

...inducing a lipid ‘traffic jam’

LAMP-1

filipin-labeled cholesterol

mutations in the lysosomal targeting signal

### Niemann – Pick disease

**accumulation of cholesterol and polar lipids in lysosomes**

<table>
<thead>
<tr>
<th>Cells</th>
<th>Age at diagnosis (years)</th>
<th>Total lipids (µg/mg cell protein)</th>
<th>Cholesterol (µg/mg cell protein)</th>
<th>Gb3* (µg/mg cell protein)</th>
<th>Cholesterol (% of total lipids)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal controls (n=10)</td>
<td>Range</td>
<td>1–63</td>
<td>340–450</td>
<td>37–54</td>
<td>&lt;1.6–10.9</td>
</tr>
<tr>
<td></td>
<td>Mean±S.D.</td>
<td>11.2±19.6</td>
<td>368±50.2</td>
<td>40.5±7.81</td>
<td>6.18±4.12</td>
</tr>
<tr>
<td>Niemann-Pick type C</td>
<td>No. 1</td>
<td>0.25</td>
<td>775</td>
<td>155</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>No. 2</td>
<td>0.5</td>
<td>850</td>
<td>193</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>No. 3</td>
<td>2.8</td>
<td>660</td>
<td>140</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>No. 4</td>
<td>2.9</td>
<td>801</td>
<td>157</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>No. 5</td>
<td>3.8</td>
<td>1117</td>
<td>199</td>
<td>14.5</td>
</tr>
</tbody>
</table>

gene therapy - NPC:
• restoration of normal traffic in *in vitro* models of Niemann-Pick disease

• no efficient vector for gene delivery to the brain

Niemann – Pick disease: therapeutic strategies

accumulation of cholesterol and polar lipids in lysosomes

statins:
- ↓ cholesterol
- BUT no clinical benefit!

miglustat
inhibitor of glucosylceramide synthase

↓ gangliosides

NPC+/+ NPC-/-
ct mig ct mig

FDA and EMEA approved for Gaucher disease; tested for Niemann-Pick disease

Sterol fate in the body

- **HDL**
- **LDL**
- **VLDL**
- **chylomicrons**

stimulation of favorable transport

cholesterol efflux

ABCA1, a ABC sterol transporter

http://www-biology.ucsd.edu/~msaier/transport/

ABCA1 as lipid transporter

ABCA1 involved in reverse cholesterol transport

ABCA1 activity protects against atheromatosis

<table>
<thead>
<tr>
<th></th>
<th>normal</th>
<th>FHA</th>
<th>TD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CAD Incidence</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ABCA1 Activity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ABCA1 Allele 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>normal</td>
<td>normal</td>
<td>normal</td>
<td>10-15% HDL-C</td>
</tr>
<tr>
<td>&gt;50% HDL-C</td>
<td>~50% HDL-C</td>
<td>&lt;50% HDL-C</td>
<td>10-15% HDL-C</td>
</tr>
</tbody>
</table>

| **ABCA1 Allele 2** |        |     |    |
| normal             | >50% HDL-C | ~50% HDL-C | <50% HDL-C | 10-15% HDL-C | 1-4% HDL-C |

- A255T
- W590S
- T929I
- A937V
- R1680W
- P85L
- R587W
- ΔL693
- R909X
- N935S
- A1046D
- D1099Y
- C1477R
- ΔE,D 1893/94
- R2081W
- 2145X
- 2203X
- M1091T
- A255T
- Q597R
- R1680W
- 635X
- N935S
- N1800H
- 1851X
- 2203X
- C-term deletion

Regulation of ABCA1 expression

Retinoid X receptor; Liver X receptor

LXR agonists more potent than oxysterols

APD
acetyl-podocarpic dimer

Regulation of ABCA1 expression

PPARγ agonists: new target for thiazolidinediones?

BRL (rosiglitazone)

induction of LXRα mRNA expression mediated by PPARγ

increase in cholesterol efflux mediated by PPARγ

Questions for future research

- inhibition of cholesterol absorption
  - Deleterious consequences of inhibiting the formation of the caveolin-annexin complex? (Cohen et al. (2004) Physiol Rev. 84:1341-79; Kim et al. (2002) Front Biosci. 7:d341-8)
    - Development of inhibitors targeting exclusively NPC1L1?

- restoration of NPC trafficking
  - Appropriate vectors for gene delivery in the brain?

- increase in ABCA1 expression
  - Other genes under the control of LXR?
    - Lipid metabolism, carbohydrate metabolism, energy homeostasis, inflammatory response (Steffensen & Gustafsson (2004) Diabetes 53 S1:S36-42)