SLC13A5 variants in epilepsy and developmental delay

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Outline

1. **SLC13 family**
   a. Mammalian
   b. Drosophila INDY
   c. VcINDY structure and mechanism

2. **NaCT/SLC13A5**
   a. Function
   b. Brain: Genetic disorder
   c. Liver: Drug target

3. **Summary**
1. SLC13 family

- SLC13 family: human gene nomenclature
- DASS: divalent anion sodium symporter family, includes bacteria
### SLC13 family: 5 genes in humans

<table>
<thead>
<tr>
<th>Name</th>
<th>Hu Gene</th>
<th>Substrate</th>
<th>Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaDC1</td>
<td>SLC13A2</td>
<td>Dicarboxylates</td>
<td>Kidney, intestine</td>
</tr>
<tr>
<td>NaDC3</td>
<td>SLC13A3</td>
<td>Dicarboxylates</td>
<td>Kidney, brain, liver, placenta</td>
</tr>
<tr>
<td>NaCT</td>
<td>SLC13A5</td>
<td>Citrate, DC</td>
<td>Liver, brain, testis</td>
</tr>
<tr>
<td>NaS1</td>
<td>SLC13A1</td>
<td>Sulfate</td>
<td>Kidney, intestine</td>
</tr>
<tr>
<td>NaS2</td>
<td>SLC13A4</td>
<td>Sulfate</td>
<td>Placenta, endothelial venules, testis</td>
</tr>
</tbody>
</table>
SLC13 transporters

- Sodium coupled
- Electrogenic (net movement of positive charge)
- NaDC1, NaDC3: substrates are dicarboxylates (and citrate$^{2-}$)
- NaCT: substrates are tricarboxylates (citrate$^{3-}$) or dicarboxylates
Transporters for TCA cycle intermediates:

- **NaDC1:**
  - succinate, citrate
  - Prefers 4C
  - Low affinity (Km succinate 600µM)

- **NaDC3:**
  - succinate, citrate, αKG
  - Wider range of structures
  - High affinity (Km succinate 25µM)

- **NaCT:**
  - citrate > succinate, malate
  - Low affinity (Km citrate 300 µM)
1b. Other DASS transporters: Insects

- Drosophila INDY
  - *I’m not dead yet*
- Exchanger, not sodium dependent
- Midgut, oenocyte, fat body
Mutations in *Indy* gene lead to life-span extension

Mimics effects of caloric restriction.

From Helfand and Rogina, Bioessays 25:134, 2003
1c. Other DASS transporters: VcINDY from *Vibrio cholerae*

- Na⁺/dicarboxylate transporter
- 3.2 Å resolution
- Inward-facing conformation
- Citrate and 1 Na⁺
- Homodimer

VcINDY structure

- 11 TM
- Inverted repeat
- Transport domain: binding sites in opposing hairpin loops and unwound helices 5 and 10

SLC13 family elevator mechanism

- Dimer
- Each monomer has cation and substrate binding sites
- Transport domain moves
- Scaffold domain stationary

2. NaCT

- SLC13A5 gene
- $\text{Na}^+/\text{citrate}$ transporter

- Most abundant in liver
- Brain
- Other: testis, tooth (mice)
2a. NaCT in brain

- NaCT in neurons
- Astrocytes NaDC3 and efflux, release citrate

From: Beyond the ion channel blog
http://epilepsygenetics.net/2015/10/26/slc13a5-neuronal-citrate-transport-and-epileptic-encephalopathies/
SLC13A5 deficiency

- Mutations in SLC13A5 gene
- Autosomal recessive
- 26 patients from 14 families reported so far
  - (Thevenon et al., 2014; Hardies et al., 2015; Klotz et al., 2016; Anselm et al., 2016)
- Most are compound heterozygous, 6 homozygous
- Parents not necessarily related
Symptoms

- Early onset epileptic encephalopathy
  - Starts within first week of life
  - Variable seizure frequency: from 1/week to >100/day
  - Severe, prolonged episodes

- Developmental delay, motor difficulty, language difficulty
Symptoms

- Tooth hypoplasia
- Do not respond to most medications
- Ketogenic diet: no effect or makes symptoms worse

From Hardies et al., 2015
Brain 138:3238
NaCT epilepsy mutations

- DelG deletion mutant, premature stop
No citrate transport in mutants

- DelG and G219R activate endogenous activity in HEK cells
Some mutants are on plasma membrane

A. Total

B. Cell surface

- Anti-NaCT antibodies
- Lysates vs cell surface biotinylation
NaCT epilepsy mutants model

- Y82 in dimerization domain
- G219 near substrate binding site, helix packing
- T227 in binding site for citrate and 1 Na
- L488, L492 mutations affect helix structure

Model: Schlessinger and Colas
NaCT in brain

- Mutant transporters have no activity
- No effective treatment yet
- What is the role of citrate in brain?
  - Metabolic?
  - Lipids?
  - Chelation? Extracellular Zn$^{2+}$ inhibits NMDA receptors (Westergaard et al., 1995)
2b. NaCT in liver
SLC13A5 expression affects lipid accumulation in human hepatocytes

- Expression of SLC13A5 in human liver correlates with lipid content
- Pregnane X receptor
- Activated by drugs and xenobiotics (Rifampicin)
- Induces expression of SLC13A5
- Drug induced hepatic steatosis?
- AhR similar

From Li et al., 2015 Mol. Pharm. 87:674
Knockout mouse (Slc13a5\(^{-/-}\))
Birkenfeld et al. 2011 Cell Metabolism 14:184

- Mouse NaCT also called mINDY
- Metabolic changes
- Protection from obesity
- Protection from high fat diet:
  - lower body weight, increased energy expenditure, decreased liver lipids, improved glucose tolerance
Liver specific knockdown has similar effects as whole animal

- Rats, antisense oligonucleotides (Pesta et al., 2015) and mice RNAi (Brachs et al., 2016)

- Knockdown prevents diet-induced hepatic steatosis and improves hepatic insulin sensitivity

- Systemic knockdown not needed
NaCT inhibitor

- Specific for hNaCT:
  - IC$_{50}$: 0.4 µM
- Inhibits citrate transport into human and rat hepatocytes
- Chronic admin in mouse decreases citrate uptake in liver, reverses glucose intolerance after HFD

Huard et al. 2015 Sci. Reports 5:17391
• In human and rat liver, citrate transport from plasma accounts for ~10% of the total
• (Li et al., 2016)
Summary and Conclusions

- SLC13 family: sodium-coupled transporters for TCA cycle intermediates or sulfate
- Non-mammalian: INDY, VcINDY

- NaCT/SLC13A5: transporter for citrate and succinate
- Function in brain: unknown, mutations produce epilepsy, inactive NaCT
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Klotz et al., 2016 Mol. Med. 22:310

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Pajor et al., 2016 Mol. Pharm. 90:755