SLC13A5 variants in epilepsy and developmental delay

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SKAGGS SCHOOL OF PHARMACY AND PHARMACEUTICAL SCIENCES

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

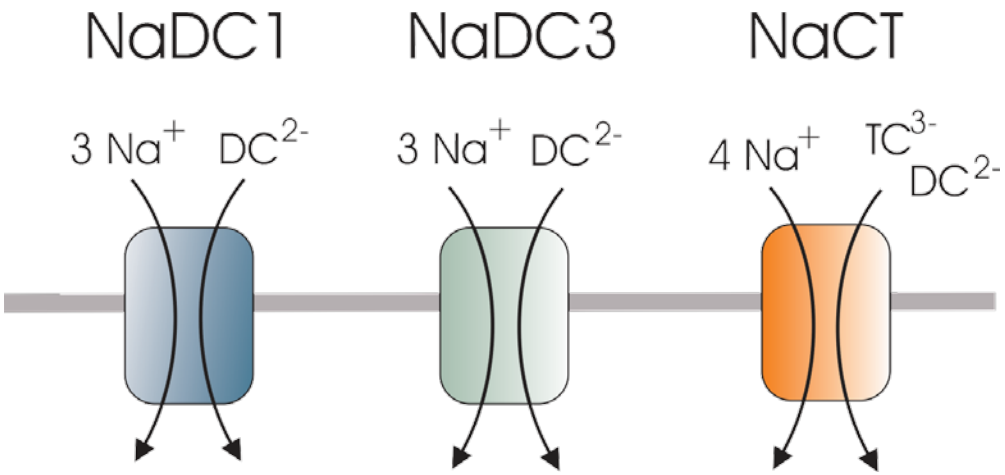
- SLC13 family: human gene nomenclature

- DASS: divalent anion sodium symporter family, includes bacteria

SLC13 family: 5 genes in humans

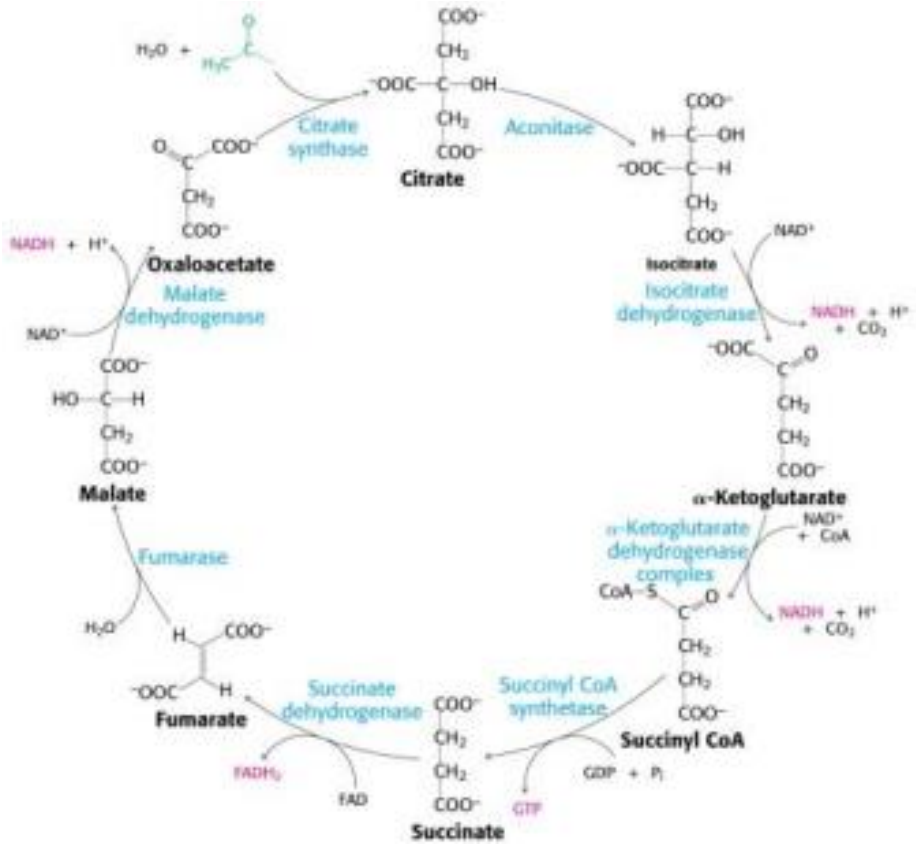
Name	Hu Gene	Substrate	Tissue
NaDC1	SLC13A2	Dicarboxylates	Kidney, intestine
NaDC3	SLC13A3	Dicarboxylates	Kidney, brain, liver, placenta
NaCT	SLC13A5	Citrate, DC	Liver, brain, testis
NaS1	SLC13A1	Sulfate	Kidney, intestine
NaS2	SLC13A4	Sulfate	Placenta, endothelial venules, testis

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

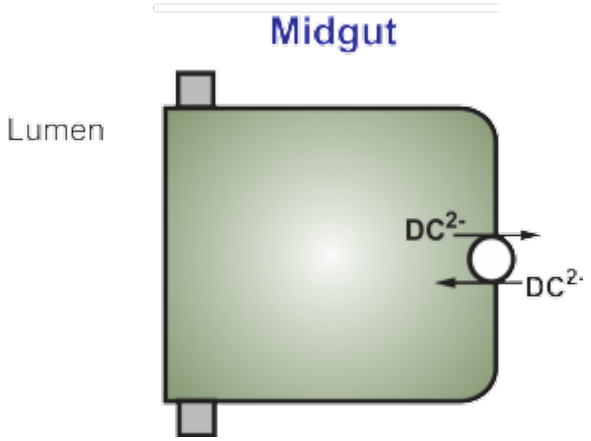
Transporters for TCA cycle intermediates:



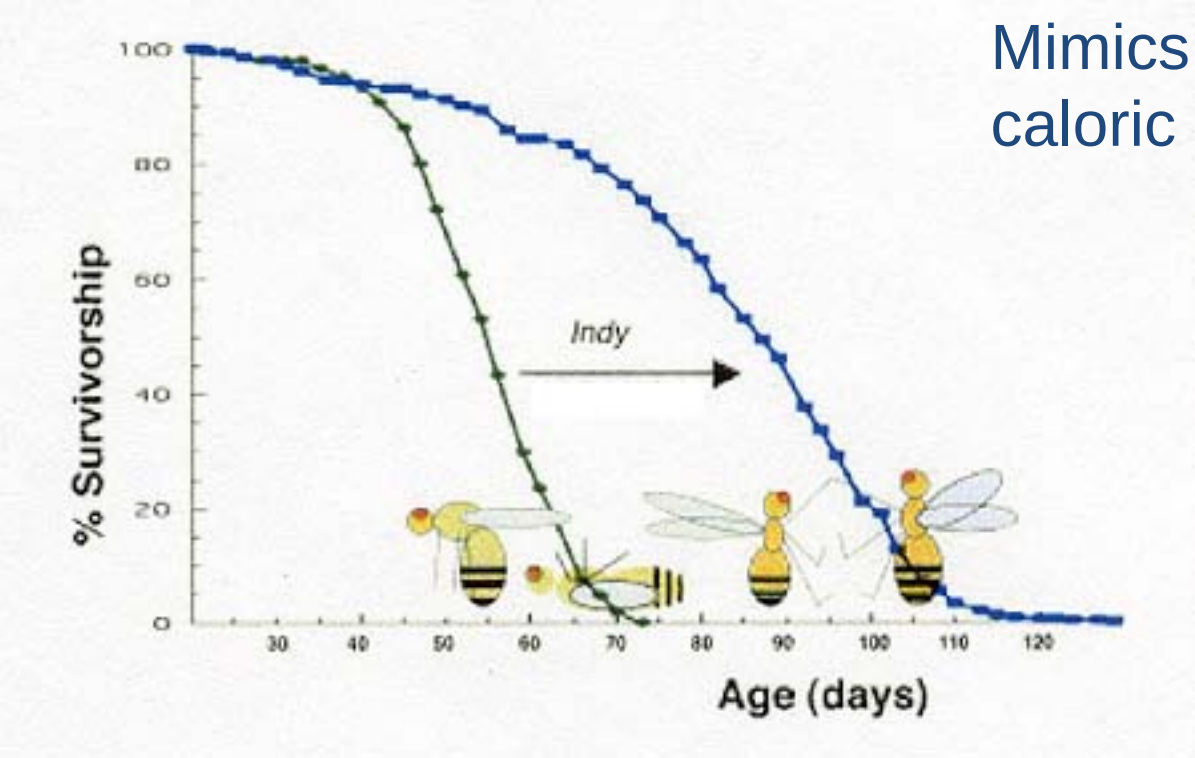
- NaDC1:
  - succinate, citrate
  - Prefers 4C
  - Low affinity (Km succinate 600µM)
- NaDC3:
  - succinate, citrate, αKG
  - Wider range of structures
  - High affinity (Km succinate 25µM)
- NaCT:
  - citrate > succinate, malate
  - Low affinity (Km citrate 300 µM)

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

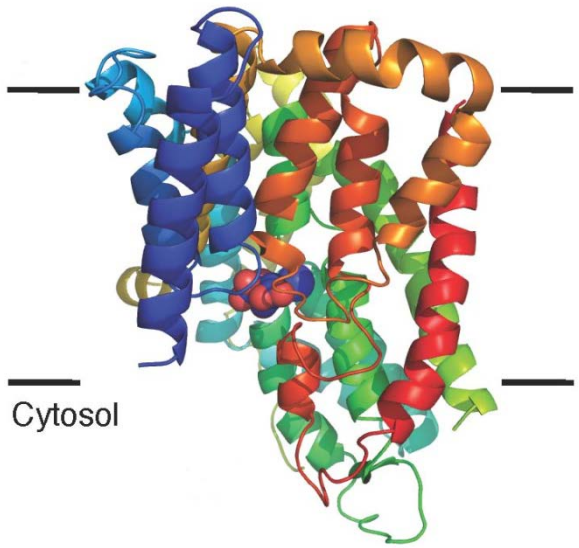


Mimics effects of caloric restriction.

From Helfand and Rogina, Bioessays 25:134, 2003

1c. Other DASS transporters: VcINDY from *Vibrio cholerae*

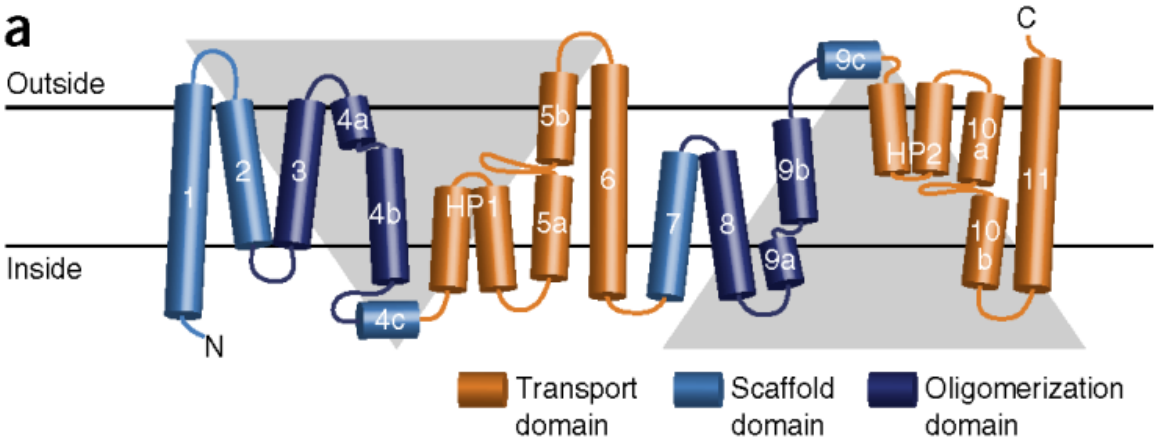
PDB 4F35



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

Mancusso et al. Nature 491: 622, 2012

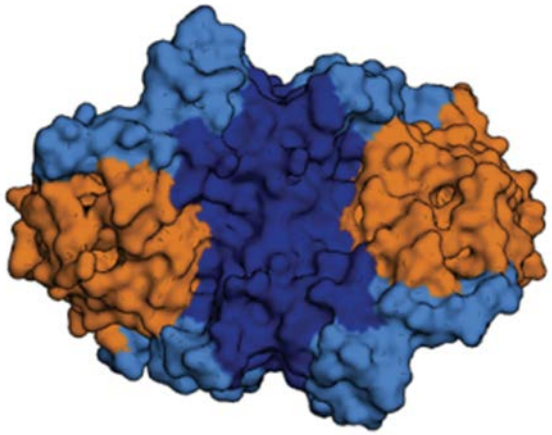
VcINDY structure

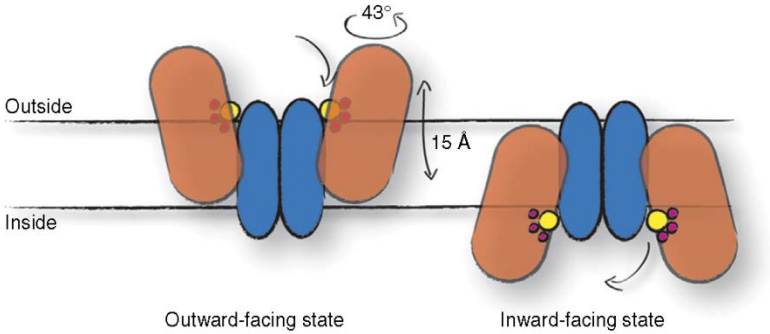


- 11 TM
- Inverted repeat
- Transport domain: binding sites in opposing hairpin loops and unwound helices 5 and 10

From: Mulligan et al. 2016 Nature Struct. Mol. Biol. 23:256

SLC13 family elevator mechanism





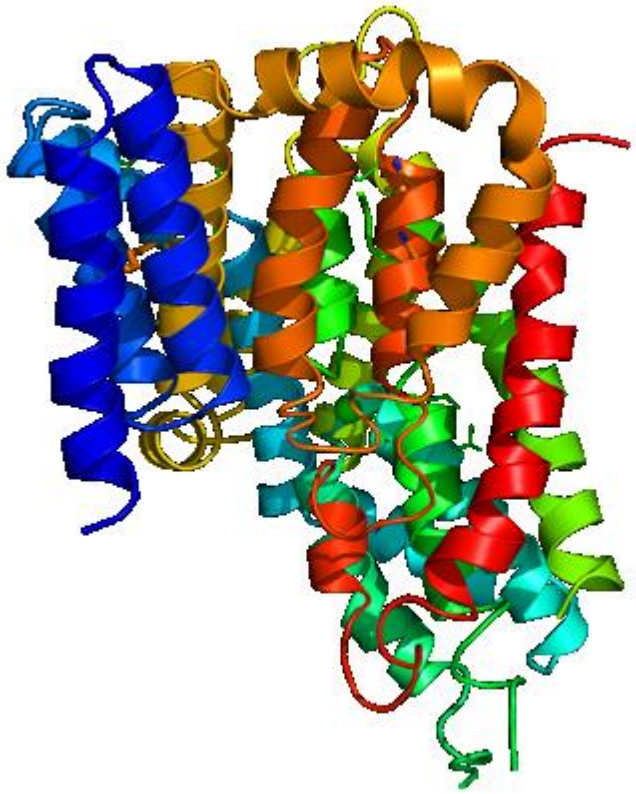
- Dimer
- Each monomer has cation and substrate binding sites
- Transport domain moves
- Scaffold domain stationary

From: Mulligan et al. 2016 Nature Struct. Mol. Biol. 23:256

2. NaCT

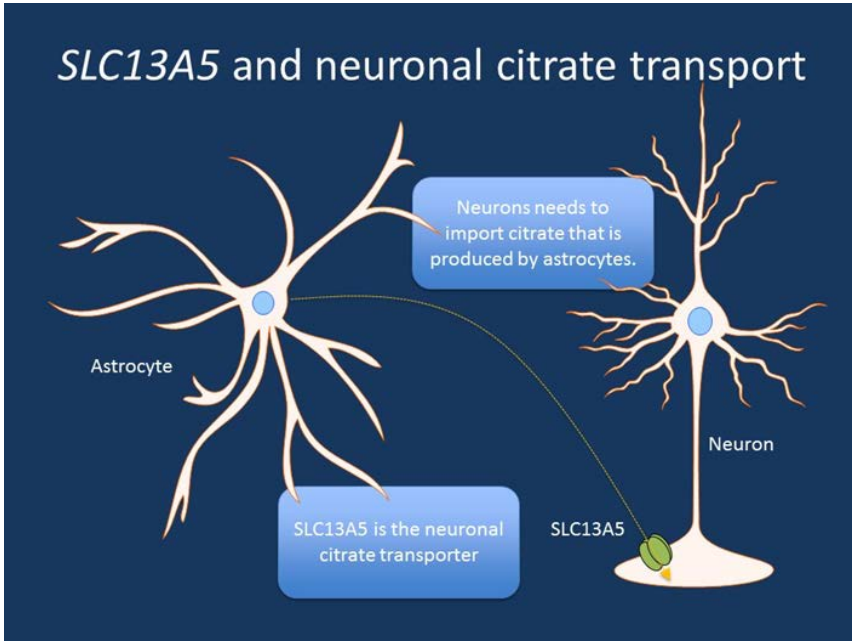
- SLC13A5 gene
- Na^+/citrate transporter

- Most abundant in liver
- Brain
- Other: testis, tooth (mice)



NaCT model Colas and Schlessinger

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/

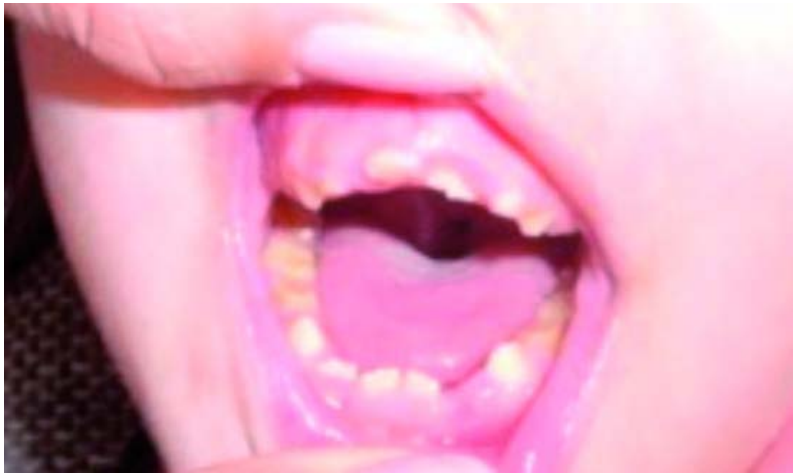
SLC13A5 deficiency

- 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

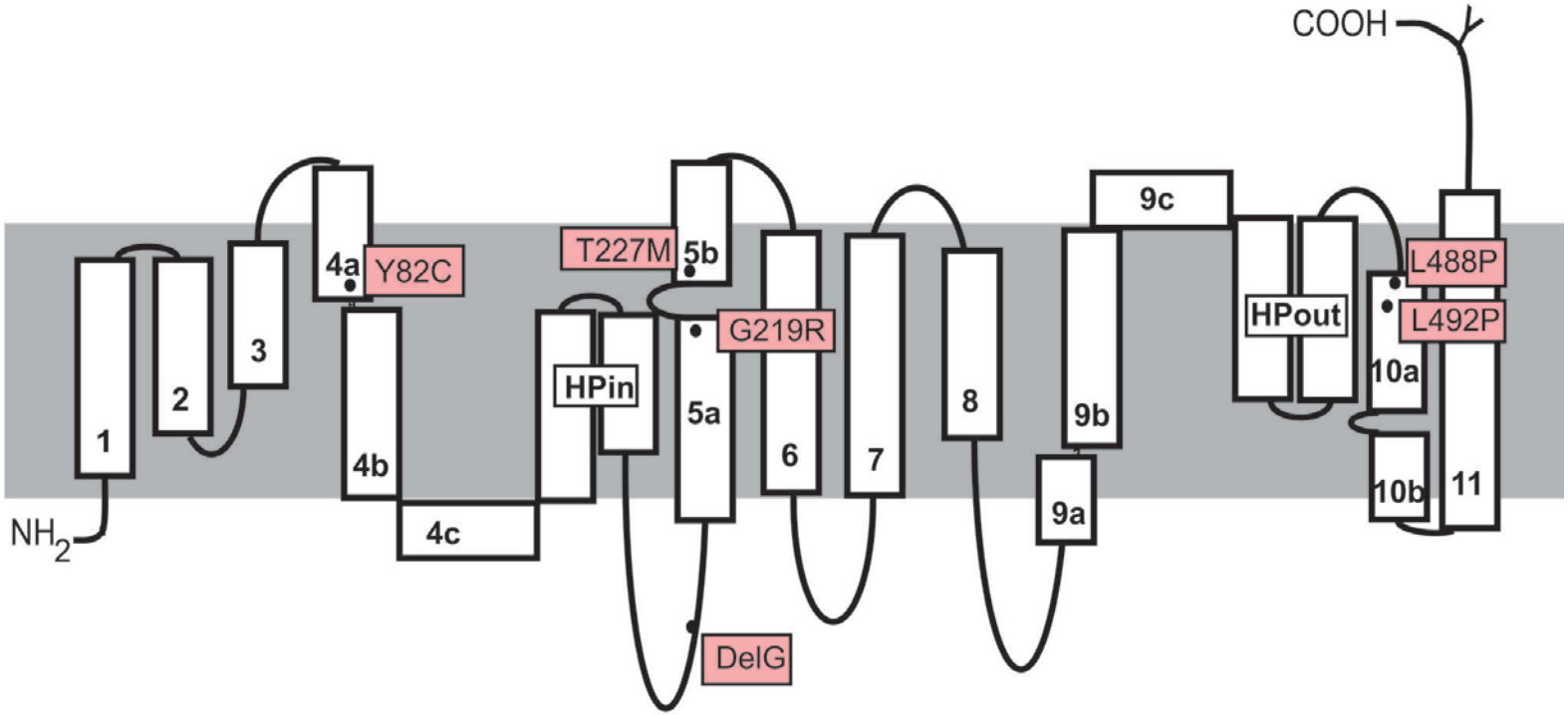
Symptoms



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

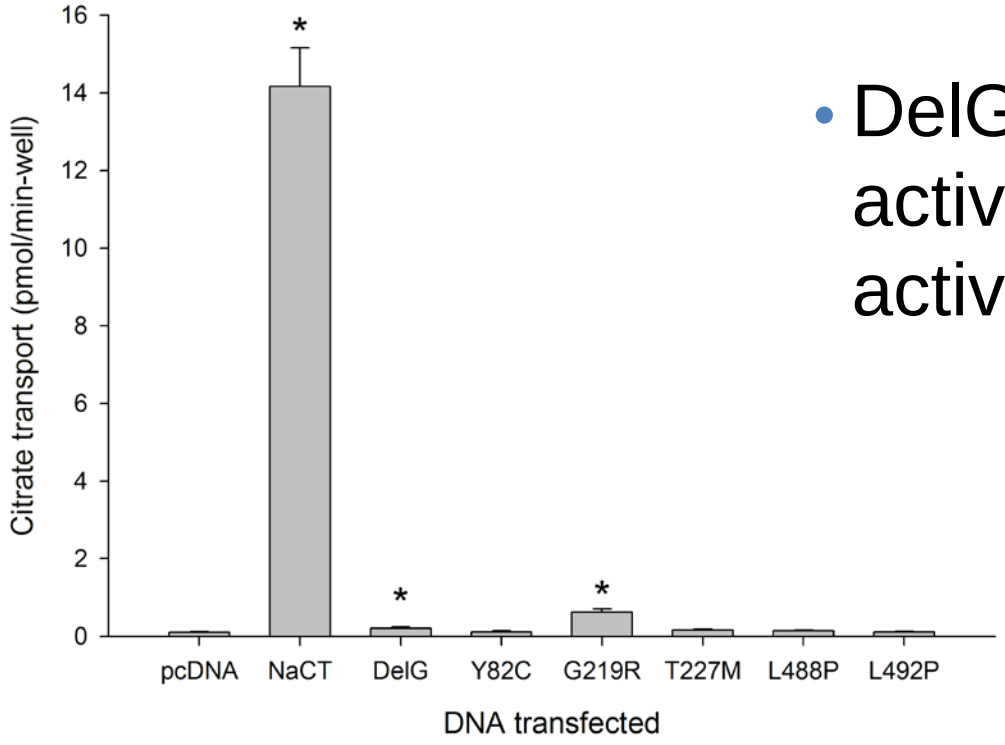
From Hardies et al., 2015
Brain 138:3238

NaCT epilepsy mutations



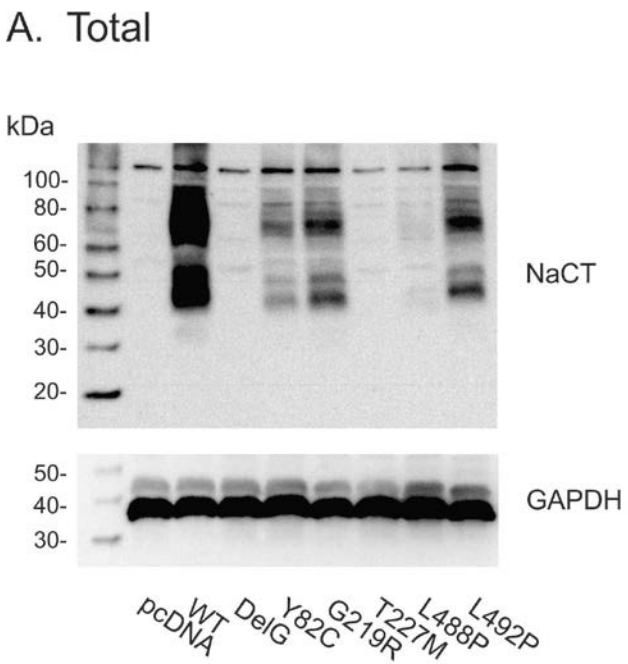
- DelG deletion mutant, premature stop
- From Klotz et al. 2016 J. Mol. Med. 22:310

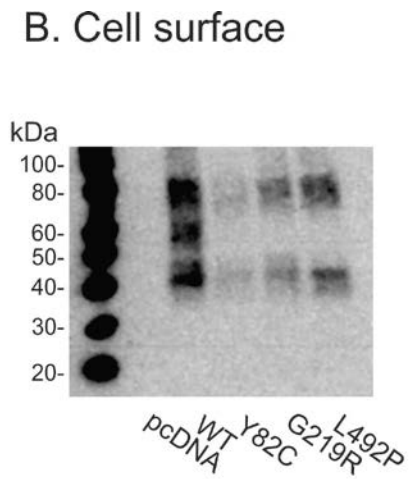
No citrate transport in mutants



- DelG and G219R activate endogenous activity in HEK cells

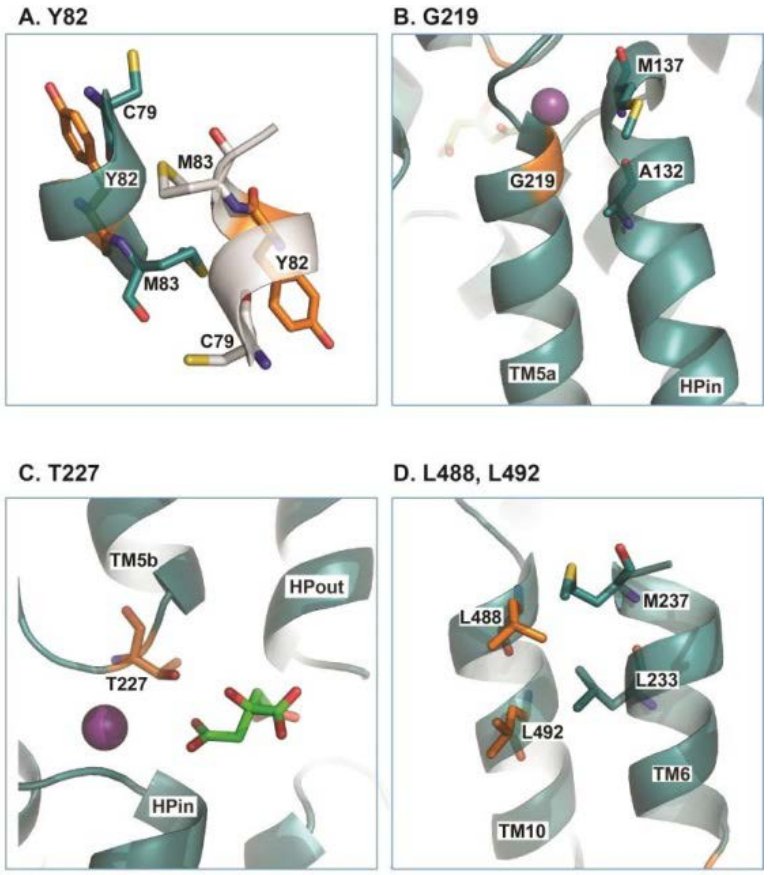
Some mutants are on plasma membrane





- Anti-NaCT antibodies
- Lysates vs cell surface biotinylation

NaCT epilepsy mutants model



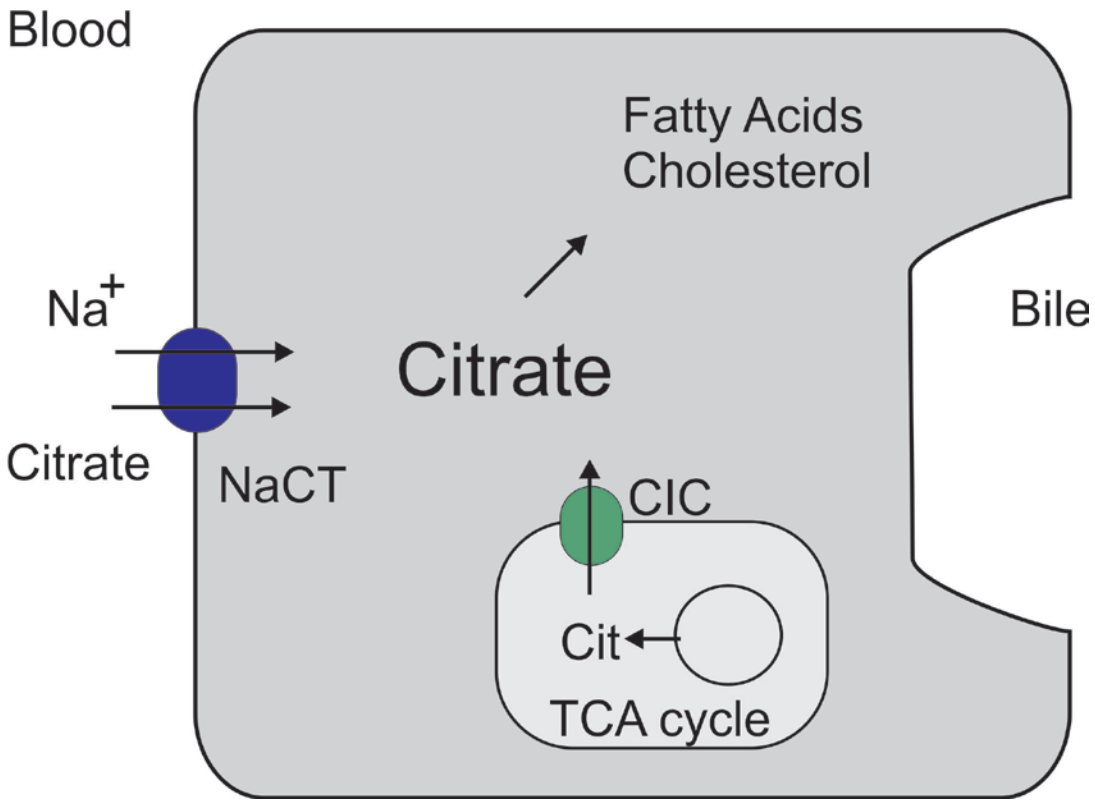
- Y82 in dimerization domain
- G219 near substrate binding site, helix packing
- T227 in binding site for citrate and 1 Na
- L488, L492 mutations affect helix structure

Model: Schlessinger and Colas

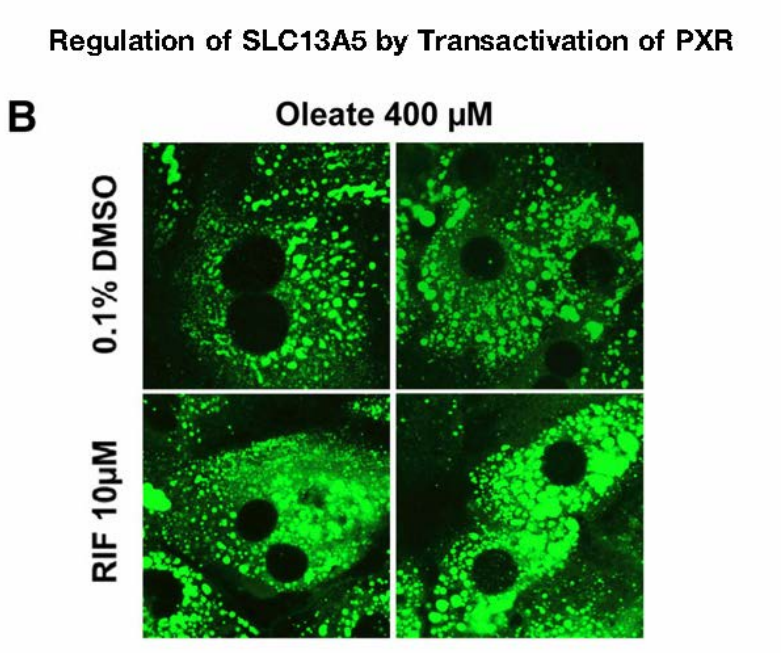
NaCT in brain

- 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



From Li et al., 2015 Mol.Pharm. 87:674

- 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

Knockout mouse (*Slc13a5*$^{-/-}$)

Birkenfeld et al. 2011 Cell Metabolism 14:184

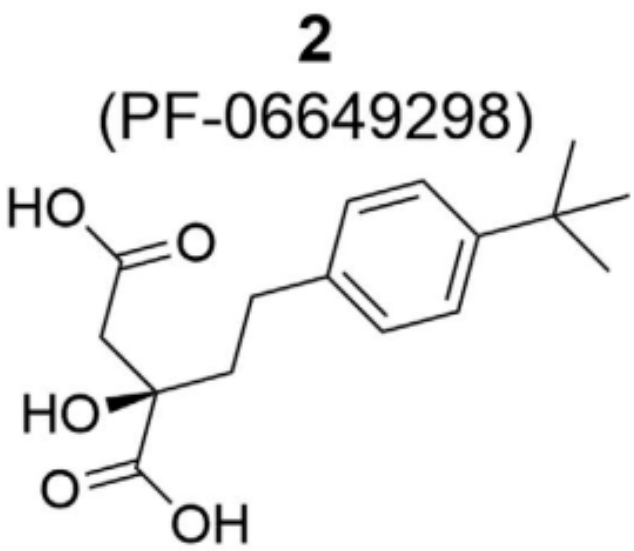
- Mouse NaCT also called mINDY
- Metabolic changes
- Protection from obesity
- Protection from high fat diet:
  - lower body weight, increased energy expenditure, decreased liver lipids, improved glucose tolerance

mINDY$^{+/+}$ mINDY$^{-/-}$

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

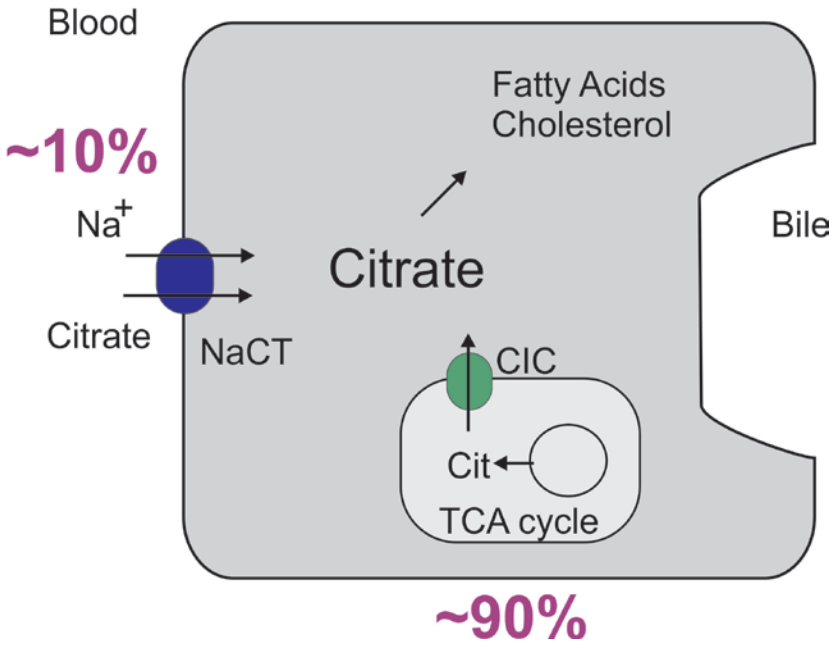
NaCT inhibitor



- 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

Mechanism?



- 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

- Function in liver: metabolic, lipid synthesis. Knockdowns beneficial.

Acknowledgments

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Klotz et al., 2016 Mol. Med. 22:310

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

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