Expanding the Universe of SLC6A19 Substrates: SLC Inhibition Protects Kidney from Nephrotoxic Metabolite Accumulation

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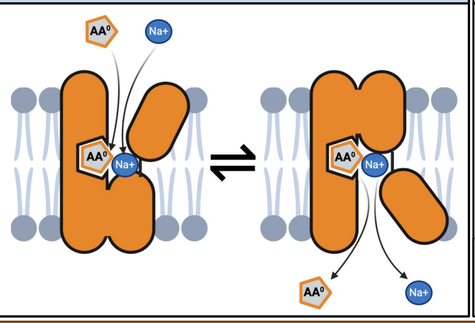
Background

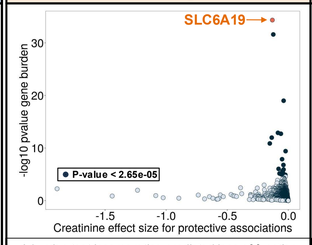
SLC6A19 (B^0AT1)

Sodium (Na^+) dependent neutral amino acid (AA^0) transporter that reabsorbs nutrients from the diet (intestine) and filtered blood (kidney)



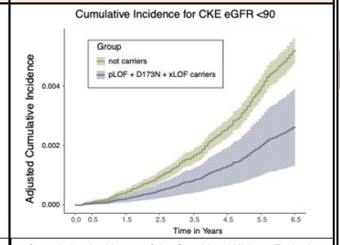
SLC6A19 Loss of Function Is Associated With Nephroprotection In Humans

Among the strongest improved kidney function effects in the genome



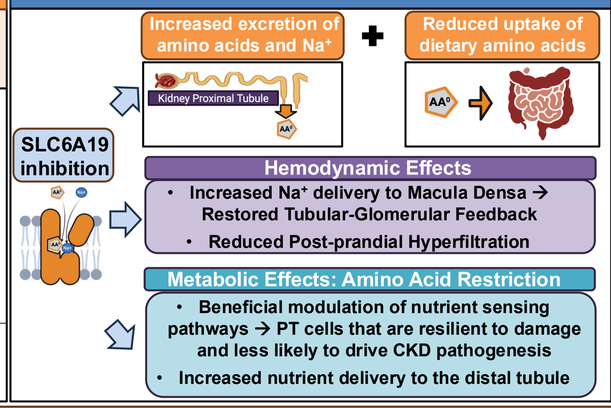
A burden test incorporating predicted loss of function (pLOF) and rare missense variants (MAF <1%) previously identified SLC6A19 as among the strongest effects in the genome for improved kidney function in UK Biobank (plasma creatinine).

SLC6A19 loss of function reduces progression to kidney outcomes (CKE)

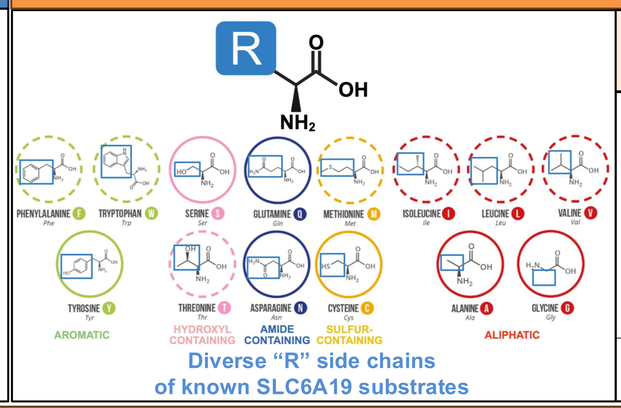


Cumulative incidence of the Combined Kidney Endpoint (CKE) over 6.5 years in carriers of SLC6A19 LoF variants (combined pLOF, D173N, and xLOF carriers) relative to non-carriers in matched UKB participants.

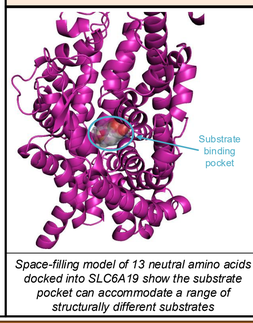
SLC6A19 Inhibition: Proposed Nephroprotective Mechanisms



SLC6A19 Transports A Structurally Diverse Set Of Neutral Amino Acids



Substrate binding pocket of SLC6A19 accommodates diverse "R" groups



Space-filling model of 13 neutral amino acids docked into SLC6A19 show the substrate pocket can accommodate a range of structurally different substrates

Scientific Question: Can SLC6A19 transport more than neutral amino acids?

Approach

Strategy to identify additional SLC6A19 substrates

>400 candidate substrates
- Metabolites altered in plasma of SLC6A19 variant carriers
  - Biologically active amino acid metabolites
  - "Neutral Amino Acid-sized" nephrotoxins

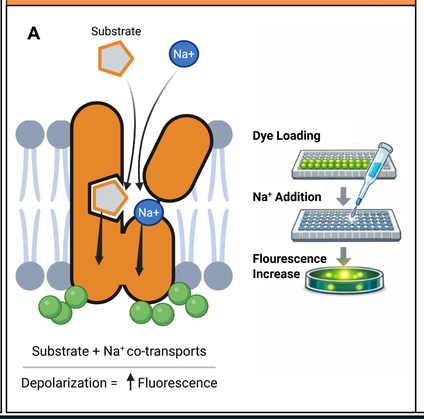
Candidate prioritization
- substrate docking model
- known nephrotoxins
- biologically active metabolites

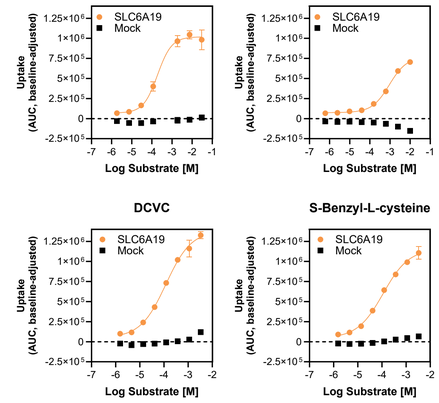
>40 candidate substrates tested

Results

Novel SLC6A19 Substrates Include Biologically Active Amino-Acid Metabolites And Nephrotoxic Cysteine-S-Conjugates

SLC6A19 FLIPR Uptake Assay

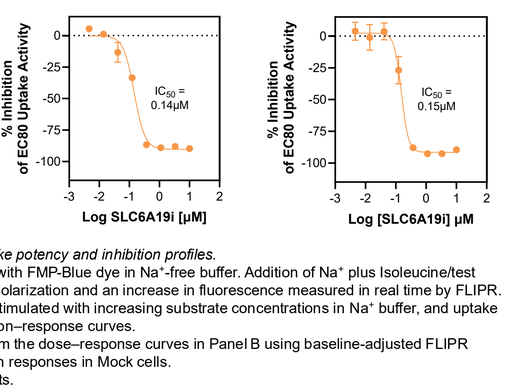




C Uptake EC_{50} Values for SLC6A19 and Mock Cells

Compound	Class	SLC6A19 (mM)	Mock (mM)
L-Isoleucine	Neutral Amino Acid	0.19	>30
Kynurenine	Amino Acid Metabolite	0.93	>10
Homocysteine	Amino Acid Metabolite	0.13	>30
Allo-Isoleucine	Amino Acid Metabolite	0.05	>30
DCVC	Cysteine-S-Conjugate	0.12	>3.3
S-Benzyl-L-cysteine	Cysteine-S-Conjugate	0.11	>3.3

D



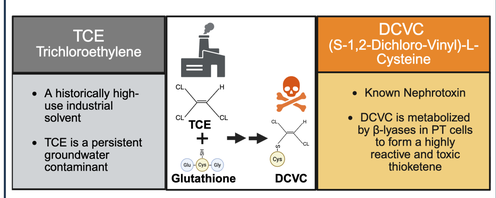
Novel SLC6A19 substrates and their uptake potency and inhibition profiles.

A) CHO cells expressing human SLC6A19 and CLTRN or Mock control were loaded with FMP-Blue dye in Na^+-free buffer. Addition of Na^+ plus Isoleucine/test substrate triggered SLC6A19-mediated Na^+/AA^0 co-transport, causing membrane depolarization and an increase in fluorescence measured in real time by FLIPR.
B) Substrate-evoked uptake dose–response curves. SLC6A19 and Mock cells were stimulated with increasing substrate concentrations in Na^+ buffer, and uptake activity was quantified from the FLIPR fluorescence response to generate concentration–response curves.
C) Uptake EC_{50} values for SLC6A19 and Mock cells. EC_{50} values were calculated from the dose–response curves in Panel B using baseline-adjusted FLIPR signals and reflect the potency of substrate-evoked SLC6A19 transport compared with responses in Mock cells.
D) %Inhibition of EC_{80} uptake and IC_{50} values determined from 4-parameter logistic fits.

Results

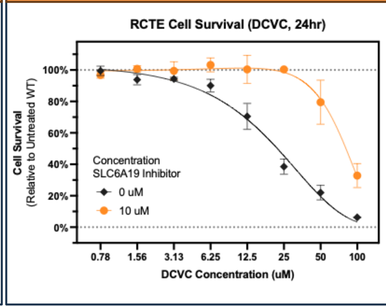
SLC6A19 inhibition protects kidney cells from DCVC-mediated toxicity *in vitro*

DCVC: A pollutant derived cysteine-s-conjugate



Key References for TCE Metabolism and Health Impacts
Lash LH, Fisher JW, Lipscomb JC, Parker JC. Metabolism of trichloroethylene. Environmental Health Perspectives. 2000 May;108 Suppl 2:177-200. DOI: 10.1289/ehp.00108s2177
Cooper AJL, Hanigan MH. Enzymes Involved in Processing Glutathione Conjugates. Comprehensive Toxicology. 2010:323–66. doi: 10.1016/B978-0-08-046884-6.00417-6. Epub 2010 Jul 12. PMCID: PMC7173439
Chui, W. et al. (2013) Human health effects of trichloroethylene: key findings and scientific issues. Environ. Health Perspect. 121, 303–311.

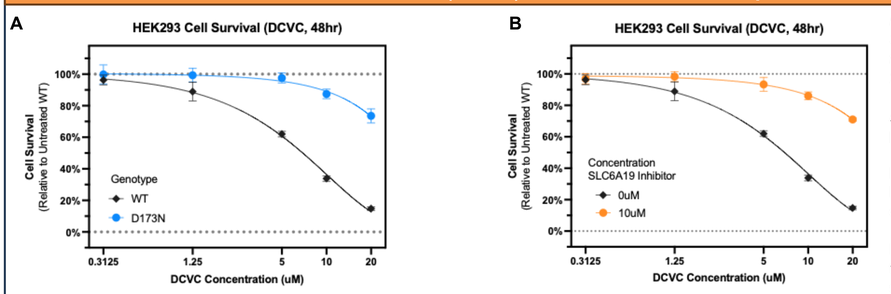
SLC6A19 inhibition protects RCTE cells from DCVC



Immortalized Renal Cortical Tubular Epithelial (RCTE) cells overexpressing CLTRN and SLC6A19 were treated with DCVC for 24H, and cell viability was quantified using CellTiter Glo 2.0 (Promega).

SLC6A19 inhibitor MZ-404 protects against DCVC toxicity. Survival = viability readouts normalized to untreated WT SLC6A19 controls, error bars show mean+/-SD, n=3, and curves show data fit to a 4-parameter logistic regression.

SLC6A19 Loss of Function (D173N) and SLC6A19 inhibition protects HEK293 cells from DCVC



HEK293 cells overexpressing CLTRN and SLC6A19 were treated with DCVC for 48H, and then cell viability was quantified using CellTiter Glo 2.0 (Promega).

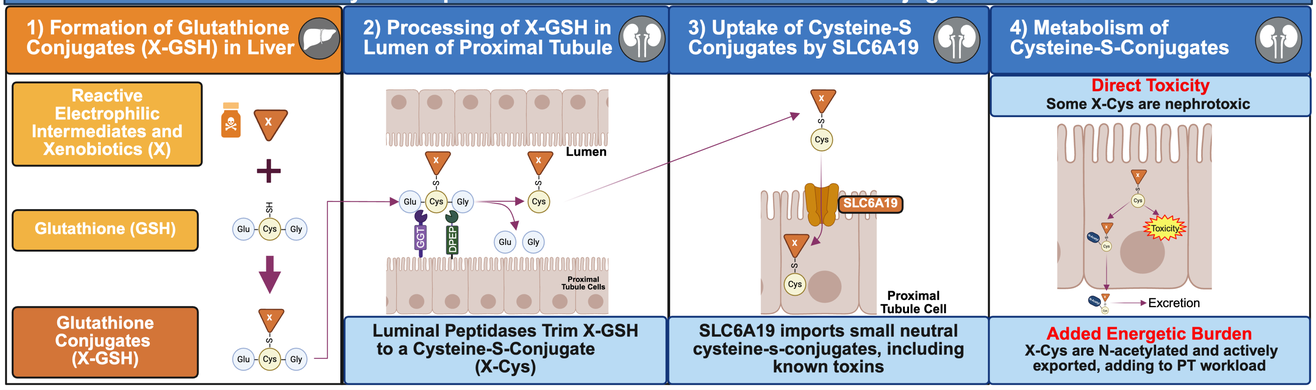
A) The SLC6A19 loss-of-function variant D173N protects against DCVC toxicity.

B) SLC6A19 inhibitor MZ-404 protects against DCVC toxicity in a manner similar to SLC6A19 loss-of-function.

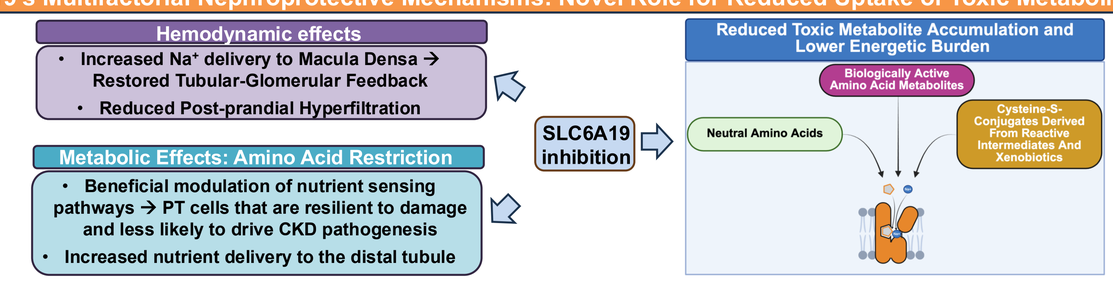
For all plots, survival = viability readouts normalized to untreated WT SLC6A19 controls, error bars show mean+/-SD, n=3, and curves show data fit to a 4-parameter logistic regression.

Implications

SLC6A19 May Participate In Glutathione And Glutathione-Conjugate Metabolism



SLC6A19's Multifactorial Nephroprotective Mechanisms: Novel Role for Reduced Uptake of Toxic Metabolites?



Summary

- **SLC6A19 can transport other substrates beyond neutral amino acids, including biologically active amino acid derivatives and nephrotoxic metabolites.**
- **We propose a multifactorial model for the nephroprotection of SLC6A19 inhibition: *hemodynamic and metabolic benefits + reduced tubular uptake of toxins.***
- **An investigational small molecule inhibitor of SLC6A19, MZE782, is currently being evaluated as a potential therapy for CKD and Phenylketonuria (PKU).**