Objectives

Genetic bases of cystinuria
Relevance of mouse models for cystinuria
Limitations of current therapies for cystinuria
New pharmacologic approach for cystinuria
Cysteine and cystine

Cysteine: Reduced form, intracellular, biologically active

Cystine: Oxidized form, extracellular, disulfide bridge in proteins

Cystinuria: Defect in cystine transport in kidney and intestine
Cystinuria

Is:
- An old disease
- A rare genetic cause of urolithiasis

Has contributed to:
- Understanding of inborn errors of metabolism
- Understanding of renal transport disorders

Continues to:
- Provide insight into urinary tract stone diseases
- Act as a model system for new therapeutic modalities
First description of cystine stones

On Cystic Oxide, a New Species of Urinary Calculus

William Hyde Wollaston


Cystine crystals in urine, 100-400 µm

Cystine stones in bladder

International Cystinuria Foundation
Location of stones in urinary tract

Cystine stones (compared to a quarter) from kidney of a young woman

MedicineNet.com
## Cystinuria types

<table>
<thead>
<tr>
<th>Classification</th>
<th>Type A</th>
<th>Type B</th>
<th>Type AB</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Frequency (%)</strong></td>
<td>45</td>
<td>53</td>
<td>2</td>
</tr>
<tr>
<td><strong>Gene (Protein)</strong></td>
<td>$SLC3A1$ (rBAT)</td>
<td>$SLC7A9$ (b0,+AT)</td>
<td>$SLC3A1$, $SLC7A9$</td>
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<td><strong>Chromosome</strong></td>
<td>2</td>
<td>19</td>
<td>2, 19</td>
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<tr>
<td><strong>Prevalence (USA)</strong></td>
<td>1/2,500 to 1/100,000</td>
<td>(1/15,000, ~20,000 affected)</td>
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</table>
Cystine transport in the kidney

Same transporter for cystine, ornithine, lysine, arginine (COLA or COAL)

Same transport system in intestine

Goldfarb DS, Cystinuria Support Network
Urinary excretion of cystine

(1 mM ≈ 250 mg/l)

Cystinuria in animals

Cystinuria found in many animals, including

• Dogs, cats, wolves

Canine cystinuria

• First reported in 1823
• Over 60 breeds of dog
• Males more severely affected
• Model for testing therapeutic approaches
• Mutation in Slc3a1 in Newfoundlands

Limitations

• Large animals not amenable for lab study
Knockout mouse models

Mouse models provide information on:

- Changes in kidney structure/function during progression from crystalluria to urolithiasis
- Effects on other organs or tissues
- Contribution of diet to disease process
- Effects of pharmacological intervention
Slc3a1 knockout mice

Same transporter for cystine, ornithine, lysine, arginine (COLA or COAL)

Same transport system in intestine

Slc3a1 disrupted in mice with cystinuria

Goldfarb DS, Cystinuria Support Network
Creation of $Slc3a1$ knockout mouse
Comparison of Urine Amino Acid Levels in Cystinuria Male and Female Mice

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
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<tbody>
<tr>
<td>Arginine</td>
<td>24521</td>
<td>26732</td>
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<tr>
<td>Cystine</td>
<td>3884</td>
<td>6292</td>
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<tr>
<td>Lysine</td>
<td>20271</td>
<td>56112</td>
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<tr>
<td>Ornithine</td>
<td>9285</td>
<td>6416</td>
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Fold Increase of Basic Amino Acids in Urine of Cystinuria Mice Over the Wild Type

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<tr>
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<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine</td>
<td>135</td>
<td>535</td>
</tr>
<tr>
<td>Cystine</td>
<td>38</td>
<td>73</td>
</tr>
<tr>
<td>Lysine</td>
<td>49</td>
<td>213</td>
</tr>
<tr>
<td>Ornithine</td>
<td>21</td>
<td>21</td>
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</table>
Typical hexagonal cystine crystals in Slc3a1-/- mouse urine
(A). Male with stones in kidney, ureter, and bladder
(B). Male with stones in bladder, but no stones in kidneys or ureters
(C). Female with no stones
Computed tomography of mice with stones
Bladder hypertrophy and stone formation in $S/lc3a1^{-/-}$ male mice

(A). Enlargement of the bladder, kidneys, and ureters

(B). Bladder with numerous uroliths
Bladder stones in $Slc3a1^{-/-}$ male mice

Bladder stones from male mice. The big stones here are about 1 cm in longest dimension.
Current treatment modalities for cystinuria

Current drugs: mode of action

MPG (Tiopronin) and others have free SH groups

Cystine + MPG → Cysteine-MPG mixed disulfide

Mixed disulfide more soluble than cystine

Reduction in cystine concentration

Dissolution of cystine stones over time
Problems with current therapy

Cystine stones difficult to treat medically/surgically

High recurrence rate and repeated surgical interventions can lead to renal insufficiency

Medical management has low efficacy due to adverse drug effects and poor compliance

Need for safer and more effective therapies
Need for new drugs (example)

Female patient asymptomatic up to age 10; then developed urinary tract obstruction due to cystine stones.

Irreversible loss of renal function despite medical and surgical treatment.

Toxicity of current drugs makes it difficult to use these therapies in young asymptomatic patients.

Need for new drugs that inhibit cystine stone formation without high toxicity.
New drugs for cystinuria

Rare Kidney Stone Consortium

- Department of Genetics, Rutgers University
- Department of Chemistry, NYU
- School of Medicine, NYU

Cystine analogs as crystal growth inhibitors

- Cystine dimethyl ester (CDME)
- Cystine methyl ester (CME)
- New mimics
# Cystine and some analogs

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
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<tbody>
<tr>
<td>Cysteine (biologically active amino acid)</td>
<td><img src="image" alt="Cysteine Structure" /></td>
</tr>
<tr>
<td>Cystine (amino acid found in body fluids)</td>
<td><img src="image" alt="Cystine Structure" /></td>
</tr>
<tr>
<td>Cysteine methyl ester (CysME)</td>
<td><img src="image" alt="Cysteine Methyl Ester Structure" /></td>
</tr>
<tr>
<td>Cystine methyl ester (CME)</td>
<td><img src="image" alt="Cystine Methyl Ester Structure" /></td>
</tr>
<tr>
<td>Cystine dimethyl ester (CDME)</td>
<td><img src="image" alt="Cystine Dimethyl Ester Structure" /></td>
</tr>
</tbody>
</table>
Inhibition of cystine crystal growth *in vitro*

With increasing CDME or CME concentration, crystal growth is inhibited. CDME more potent inhibitor.
Cystine crystal shapes under different conditions

A = Cystine + CDME
B = Cystine + CDME
C = Cystine + CME
D = Cystine + CME

A = Smaller hexagonal crystals (10 µm)
B = Small amounts of tetragonal crystals
C = Tapered hexagonal crystals
D = Growth of tapered needles

A new pharmacological approach

Cystine analogs inhibit cystine crystal growth *in vitro*

In presence of inhibitor, smaller hexagonal crystals and change in crystal shape from hexagonal to tetragonal

Cystine dimethyl ester (CDME) most effective inhibitor

Can *in vitro* results be replicated in mice with cystinuria?

Inhibition of crystal growth may provide new strategy for treatment of cystinuria
L-Cystine dimethyl ester (CDME), structural mimic of cystine

CDME inhibits cystine crystal growth by attaching to crystal surfaces, blocking growth

Can CDME prevent cystine crystal growth, and hence stone formation, in mice with cystinuria?
Mice with cystinuria

$Slc3a1$-/- male/females have cystine crystals in urine from birth

Cystine stones in bladder and, to lesser extent, in kidney

Bladder stones apparent in male mice from age 2-3 months, in female mice from age 12 months

Disease more severe in male mice

2-3 months old males used in this study
Treatment protocol

Slc3a1-/- mice

Cystine analog
(4 week treatment)

Yes

Palatable?

No

Water bottle

Stomach tube

Urine

Feces

Stone

Blood

Tissue

LC-MS
HPLC

LC-MS

LC-MS SEM

LC-MS HPLC

Histology
Micro CT analysis of bladder after CDME treatment.
Scanning electron microscopy

Water treated

CDME treated
Variation in stone size and number with CDME

Size distribution:
CDME: 0.5-3 mm
Water: 0.5-9 mm

CDME:
9152
9127
9134

Water:
9125
9128
Water v CDME in mice with stones

**Water**
- Blue bars: Bladder wt (mg)
- Red bars: Stone wt (mg)
- Green bars: Stone number

1. 100
2. 80
3. 60
4. 40
5. 20
6. 0

**CDME**
- Blue bars: Bladder wt (mg)
- Red bars: Stone wt (mg)
- Green bars: Stone number

1. 120
2. 100
3. 80
4. 60
5. 40
6. 20
7. 0
Water vs CDME treatment in mice

Y axis = Number of stones
X axis = Size range of stones (mm)
Summary

Normal bladder size in Slc3a1-/- mice without stones

Bladder hypertrophy and large stones in untreated mice

Reduction in bladder and stone weight/size in treated mice

Smaller stones suggest inhibition of cystine crystal growth/aggregation, similar to in vitro data

Cystine analogs may have therapeutic potential
Future direction

- Evaluate organ specific toxicity
- Test other analogs with improved efficacy
- Biomarker validation for disease monitoring
- Phase I studies in humans
## Acknowledgements

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<thead>
<tr>
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NIH Rare Kidney Stone Consortium