How does cystinosin deficiency leads to cell damage & how can it be repaired?

Francesco Emma

Division of Nephrology and Dialysis
Bambino Gesù Children’s Hospital, IRCCS
Rome, Italy
Cystinosis

Kidney: 200 - 400 x normal
Liver: 80 - 1000 x normal
Muscle: 40 - 70 x normal
Brain: 5 - 20 x normal


Anikster et al, Chest 2001
Monogenic disease mutations in the *CTNS* gene (17p13) that encodes for cystinosin

Molecular basis of cystinosis

Cystinosin LKG

pHrodo™ Red dextran

Taranta et al, Histochem Cell Biol 2012
Mechanisms of cell damage in cystinosis

- Storage disease?
- Cell oxidation?
- Apoptosis / Autophagy?
- Impairment intracellular trafficking?
- Impaired intracellular signaling?
- Inflammation?

Possibly a variable combination of the above in different tissues
Cystine is spontaneously converted into cysteine by GSH

\[ \text{GSSG} \xrightarrow{\text{NADPH}} \text{GSH} \xrightarrow{\text{ATP}} \text{cysteine} \xrightarrow{\text{lyosyme}} \text{cystine} \]
Oxidation in cystinosis

Conflicting results:
Wilmer et al, BBA 2011
Vaisbich et al, Nephron Extra 2011

Are these the result of differing systems and assays?
How does stored cystine affect the cytosolic redox state?

Wilmer et al, BBA 2011
Increased apoptosis (fibroblasts and RPTE cells)

Apoptosis may be promoted by:
Cysteinylation of PKCδ (fibroblasts and RPTE cells)
Activation of AMP kinase (RPTE cells)
Overexpression of caspase-4 (human kidney)
Taub M, Cutuli F. Biochem Biophys Res Commun, 2012

Mitophagy (fibroblasts and RPTE cells)

Gene expression profiling: oxidative phosphorylation, apoptosis, mitochondrial genes, ER stress, oxidative stress
Sansanwal P et al, J Inherit Metab Dis, 2010
Lysosomal membrane permeabilization

Increased cytosolic cystine → cysteine

Cysteinylation of protein kinase C delta

Increased activity of protein kinase C delta

Increased apoptosis rate
Lysosomes and cell survival

Repnic et al. BBA 2012

Sansanwal et al. Autophagy 2010
Autophagosome accumulation in cystinosis

LC3 accumulation in cystinotic PT cells

Sansanwal et al. JASN 2010
Coordinated Lysosomal Expression and Regulation (CLEAR)

- Most lysosomal genes, including CTNS, are regulated by the transcription factor EB (TFEB)
- Under lysosomal storage conditions, TFEB translocates from to the nucleus
- TFEB overexpression in cultured cells induces lysosomal biogenesis, lysosomal exocytosis and autophagy

Sardiello et al, Science 2009
Sardiello and Ballabio, Cell Cycle 2009
Palmieri et al, Hum Mol Genet 2011
TFEB overexpression

![Graph showing TFEB overexpression](image)

**Cystine levels (nmol/mg protein)**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Cystine</th>
<th>GFP</th>
<th>TFEB-GFP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>0.6</td>
<td>0.5</td>
<td>0.4</td>
</tr>
<tr>
<td>Cystamine</td>
<td>0.0</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>TFEB-GFP</td>
<td>0.3</td>
<td>0.6</td>
<td>0.5</td>
</tr>
</tbody>
</table>

**Size of Lamprop-positive structures (µm)**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctrl</td>
<td>0.5</td>
</tr>
<tr>
<td>Cystinotic</td>
<td>0.3</td>
</tr>
<tr>
<td>Cystinotic + GFP</td>
<td>0.2</td>
</tr>
<tr>
<td>Cystinotic + TFEB</td>
<td>0.1</td>
</tr>
</tbody>
</table>

**Number of Lamprop-positive structures (nr/field)**

- Ctrl: 4
- Cystinotic: 8
- Cystinotic + GFP: 8
- Cystinotic + TFEB: 4

**Erysop1pm markers**

- Ctrl: 600
- Cystinotic: 800
- Cystinotic + GFP: 800
- Cystinotic + TFEB: 600

*Significance levels: *p < 0.05, **p < 0.01, ***p < 0.001, ns = not significant.
Increased apoptosis is associated with increased proliferation rate and dedifferentiation of cystinotic PT

Ctns -/- mice

Gaide Chevrornnay et al. JASN 2014

Raggi et al. HMG 2014
Fanconi syndrome, decreased expression of endocytotic receptors and Na-dependent transporters in cystinotic PT

Lesion progression: S1 → S2 → S3

Changes occur **before** structural damage and in the absence of renal failure!

Gaide Chevronnay et al. JASN 2014

Raggi et al, Hum Mol Genet 2013
Impaired recycling of megalin to the cell surface and delayed albumin degradation in cystinotic PT cells

**Megalin expression: PTEC**

- **Cystinosis**
- **Control**
- Cysteamine 24h

**Megalin expression: HK2**

- **Control**
- **CTNS KD**
- After 48 hrs CTNS down-regulation by siRNA

**Albumin processing: PTEC**

- **Control**
- **Cystinosis**
- Cysteamine treatment partially rescued impaired albumin degradation

Ivanova et al. Under revision
Vesicular transport defects and increased ER stress in cystinotic cells

Upregulation of the Rab27a-Dependent Trafficking and Secretory Mechanisms Improves Lysosomal Transport, Alleviates Endoplasmic Reticulum Stress, and Reduces Lysosome Overload in Cystinosis

Jennifer L. Johnson, a Gennaro Napolitano, a Jlenia Monfregola, a Celine J. Rocca, b Stephanie Cherqui, b Sergio D. Catz a

Ctns -/- murine kidney proximal tubular cells:

• Downregulation of Rab27
• Impaired lysosomal transport
• ER expansion
• Increase expression of Grp78 and Grp94 (unfolded protein response)

Upregulation of the Rab27a-dependent vesicular trafficking mechanisms:

• rescues defective lysosomal transport
• reduces ER stress
• decreases cystine content

Mol Cel Biol 2013
High content drug screening for cystinosis
## Low-molecular weight proteinuria

<table>
<thead>
<tr>
<th>Disease</th>
<th>Locus</th>
<th>Protein</th>
<th>Age at CRF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cystinosis</td>
<td>CTNS</td>
<td>Cystinosin</td>
<td>&lt; 10 yrs</td>
</tr>
<tr>
<td>Dent 1</td>
<td>CLCN5</td>
<td>CLC-5</td>
<td>&gt; 20 yrs</td>
</tr>
<tr>
<td>Lowe &amp; Dent 2</td>
<td>OCRL1</td>
<td>PI-4,5-biphosphate-phosphatase</td>
<td>&gt; 20 yrs</td>
</tr>
<tr>
<td>Imerslund-Gräsbeck syndrome</td>
<td>AMN</td>
<td>Amnionless Cubulin</td>
<td>Rare</td>
</tr>
<tr>
<td>Imerslund-Gräsbeck syndrome</td>
<td>CUBN</td>
<td>Cubulin</td>
<td>Rare</td>
</tr>
<tr>
<td>Donnai–Barrow syndrome</td>
<td>LRP2</td>
<td>Megalin</td>
<td>Rare</td>
</tr>
<tr>
<td>Mitochondrial cytopathies</td>
<td>~</td>
<td>~</td>
<td>&lt; 10 yrs</td>
</tr>
</tbody>
</table>


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**Diagram:**

- **Axes:**
  - Retinol-binding protein, mg/mmol creatinine
  - Albumin, mg/mmol creatinine

- Key areas:
  - Normal
  - Tubular proteinuria
  - Glomerular proteinuria
  - Hematuria

KDIGO
Cystine crystals activate the inflammosome

Prencipe et al, JASN 2014
BMT for cystinosis

**GFP transgenic mice**

- Ctns⁻/⁻ mice
- BMC
- HSC (SCA1+)
- Irradiation

**Analysis**
- 2 months
- 4 months
- 15 months post-transplantation

Kidney: WT BMC or HSC-treated Ctns⁻/⁻

**Syres et al 2009**
**BMT for cystinosis**

Confocal Microscopy 4 months post-transplantation

**Brain**

**Liver**

**Muscle**

**Spleen**

**Eye (Dr. Jester)**

Objective X 60

GFP+ cells

Actin (Phalloidin)

Endothelial cells (vWF)

Syres et al 2009
BMT for cystinosis

Syres et al. 2009

Graph showing nmol half cystine/mg protein over months for different tissues:
- Spleen
- Liver
- Kidney
- Heart
- Muscle
- Eye
- Brain

KDIGO
Possible transfer of material from WT to CTNS -/- cells

Iglesias et al., PlosOne, 2012

Thoene et al., Mol Genet Metabol, 2013

Naphade et al, Stem Cell, 2014
BMT for cystinosis (15-17 months)

Wildtype

Treated Ctns-/-

High level of donor-derived blood cell engraftment expressing Ctns

Ctns-/-

Treated Ctns-/-

Low level of donor-derived blood cell engraftment expressing Ctns

Yeagy et al
Kidney Intl 2011
BMT for cystinosis (15-17 months)

Ctns-/-

Treated Ctns-/- (low engraftment)

Number of Cystine Crystals

KO

BMSC/HSC

*p<0.00001

Yeagy et al
Kidney Intl 2011
Preclinical proof of concept in the Ctns-/- mice

Lentivirus vector (safe version of HIV)
From Dr. Donald Kohn (UCLA)

Ctns-/- mice
Tail Vein Injection

Ctns-/- mice

New CTNS

KDIGO
Impact of Ctns-/- HSC-transduced with pCCL-CTNS on kidney: Study in males at 8 months post-transplantation

### Kidney cystine content

![Graph showing kidney cystine content for KO and pCCL-CTNS genotypes.]

### Renal function in males 8 months post-transplantation

**Table 1. Serum and urine analyses for renal function**

<table>
<thead>
<tr>
<th></th>
<th>Wildtype n=6</th>
<th>Control Ctns-/- n=9</th>
<th>pCCL-CTNS Treated Ctns-/- n=8</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>serum</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.27 ± 0.03</td>
<td>0.31 ± 0.08</td>
<td>0.22 ± 0.11^b</td>
</tr>
<tr>
<td>Creatinine clearance (ml/mi/kg)</td>
<td>4.44 ± 0.39</td>
<td>3.86 ± 1.42</td>
<td>4.89 ± 5.56</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>14.55 ± 1.87</td>
<td>28.29 ± 16.11^a</td>
<td>24.10 ± 7.32^a</td>
</tr>
<tr>
<td>Phosphate (mg/dL)</td>
<td>12.25 ± 2.38</td>
<td>13.20 ± 2.90</td>
<td>13.16 ± 2.21</td>
</tr>
<tr>
<td><strong>urine</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphate (mmol/24h)</td>
<td>6.82 ± 2.90</td>
<td>8.84 ± 4.60</td>
<td>4.78 ± 3.87^b</td>
</tr>
<tr>
<td>Volume (ml)</td>
<td>1.05 ± 0.51</td>
<td>1.26 ± 0.54</td>
<td>0.70 ± 0.60^b</td>
</tr>
</tbody>
</table>

^a P<0.05 compared to wildtype mice
^b P<0.05 compared to Ctns-/-

**Cystine crystals quantification**

- **Non-treated Ctns-/- mice**
- **pCCL-CTNS-treated mice**

![Graph showing cystine crystal quantification for Non-treated Ctns-/- and pCCL-CTNS-treated mice.]

Harisson et al., Mol. Ther., 2012
Renal phenotype in Ctns -/- mice is dependent on the genetic background

congenic C57BL/6 and FVB/N Ctns -/- mice

CRF > 9 months

Fig. 2. Cystine levels in congenic C57BL/6 and FVB/N Ctns +/- mice. (A) Relative cystine levels in different tissues of 9-month-old C57BL/6 (n=4) and FVB/N (n = 4) Ctns +/- mice. (B) Evolution of cystine levels in different tissues in 3-, 6-, 9- and 12-month-old C57BL/6 Ctns +/- mice (n = 4 for each group). K=kidney, S=spleen, H=heart, Li=liver, Lu=lung, M= muscle, E= eye and B= brain.

Fig. 3. Histological lesions in C57BL/6 Ctns +/- mice. Staining with periodic acid Schiff and hematoxylin (A-F). (A) Proximal tubular atrophy and thickening of the basement membrane (arrow), (B) tubular dilatation and epithelial layer effacement (arrow), (C) cellular infiltration (arrow), tubular atrophy and thickening of basement membrane (arrowhead), (D) tubular cast (arrow). Magnification x40. (E) Collapsed (arrow) and sclerosed (arrowhead) glomeruli with severe tubulo-interstitial lesions. (F) wild-type C57BL/6. Magnification x20. Serial kidney sections (G-I). (G) Focal disappearance of proximal tubules (arrow; LT lectin labelling), (H) partial disappearance of TAL and DCT (arrow; Tamm-Horsfall labelling), (I) thickened basement membrane and mild interstitial fibrosis (arrow; Sirius red staining). Magnification x10. The age of the corresponding mouse is indicated on each photo. Scale bar represents 100 μm.

Nevo et al, NDT 2010