Cystinosis: an update

Elena Levtchenko

University Hospitals Leuven, Belgium

April 30, 2019
Disclosures

E. Levchenko performs consultancy for Orphan Europe, Chiesi, Kyowa Kirin, Advicenne and was supported by a research grant from Horizon Pharma
Overview of the lecture

• Introduction
  • biochemical and genetic basis of cystinosis

• Insights into pathogenesis of cystinosis

• Diagnosis of cystinosis

• Treatment of cystinosis
  • cysteamine treatment
  • novel therapies

• Take home messages
Cystinosis

- An autosomal recessive disease caused by lysosomal accumulation of cystine due to defective exodus of cystine out of the lysosomes

- Incidence ~1:100,000 - 200,000 newborns (clustering in some populations)

- Most common cause of inherited generalized proximal tubular dysfunction (renal Fanconi syndrome) progressing to end stage renal disease (ESRD)
Lysosomal cystinosin (*CTNS*, 17p13) is mutated in cystinosis

Most common mutation in North European population: 57 kb deletion

> 140 other mutations described (David et al. 2019)
  - Mutation detection rate > 95%:
    - Nonsense, missense, splice-site, promoter, micro-deletions, duplications
  - Genotype – phenotype correlation: severe mutations → severe phenotype

Town et al. Nat Genet 1998
Attard et al. Hum Mol Genet 1999
Kalatzis et al. Hum Mol Genet 2004
Levtchenko et al. Eur J Hum Genet 2014
Clinical forms

- **Infantile form (>90%)**:  
  - Fanconi syndrome ~ 3-6 months  
  - End stage renal disease (ESRD) ~ 10 years

- "**Late-onset** (juvenile) form (~5%)":  
  - Later onset (often during puberty)  
  - Mild tubulopathy, more pronounced proteinuria  
  - Later progression to ESRD

- **Ocular form**
Fanconi syndrome
Renal failure

Photophobia
Keratopathy
Retinopathy

Hypothyroidism

Diabetes
Exocrine pancreas deficiency

Cerebral atrophy
Neuro-cognitive deficits
Pyramidal symptoms
Stroke-like episodes

Muscular wasting

Delayed puberty
Male infertility

Liver enlargement,
fibrosis

Cystine
Kidney is the first organ affected by cystinosis
Pathogenesis of kidney disease in cystinosis

**Podocyte disease:**
glomerular proteinuria, FSGS

**Proximal tubule (PT) disease:**
renal Fanconi syndrome

**Renal interstitial inflammation and fibrosis:**
progressive CKD
Proximal tubule (PT) dysfunction

- Loss of PT cells into urine
  (Ivanova et al. 2016)

- PT cell apoptosis
  (Park et al. 2002, 2006; Gaide Chevronnay et al. 2014)

- Impaired mitochondrial function & oxidative stress & ↓ mit cAMP
  (Baum 1998, Wilmer et al. 2011, Bellomo et al. 2018)

- Impaired vesicle trafficking & autophagy

• Loss of PT mass

• Renal Fanconi syndrome
  • Dedifferentiation
  • Reduced expression of PT transporters
    → Renal Fanconi syndrome

• Oxidative stress
  → Inflammation, fibrosis

Mahoney et al. 2000
Podocyte dysfunction

- Loss of podocytes into urine
  (Ivanova et al. 2016)

- Increased podocyte motility and decreased adhesion in vitro
  (Ivanova et al. 2016)

- Morphologic podocytes changes
  - Podocyte foot process effacement
  - Multi-nucleated podocytes
  (Ivanova et al. 2016; Elmonem et al. 2017)

[Images of multi-nucleated podocytes, FSGS lesions, and global collapse]

- Glomerular proteinuria
- FSGS lesions
Renal interstitial inflammation and fibrosis

- Cystine crystals are mainly located in renal interstitium (free or in histiocytes), and rarely in PT cells or podocytes.

- Inflammasome activation by cystine crystals (increased expression of inflammasome-related genes Casp-1, Pycard, Il-18, Il18r1, Il1r1, Il1rl2):
  - Production of pro-inflammatory cytokines and chemokines
  - Renal interstitium inflammation and fibrosis

Prencipe et al. JASN 2014
Diagnosis of cystinosis

- **Suspected clinical presentation**
  - cystinosis - most common cause of Fanconi syndrome
  - unexplained eye complaints, photophobia
  - glucosuria & proteinuria (check for low molecular weight proteins)

- **Measurement of elevated cystine content in granulocytes:**
  - controls < 0.3 nmol ½ cystine/mg protein
  - heterozygotes < 1 nmol ½ cystine/mg protein
  - patients at diagnosis > 2 nmol ½ cystine/mg protein
  - patients on cysteamine therapy < 1 nmol ½ cystine/mg protein
  - values of your own laboratory!

- Cystine crystals in cornea (>1 year)

- Molecular analysis of cystinosis gene
Treatment of cystinosis
Management of renal Fanconi syndrome

• Free access to water and toilet, **avoid dehydration**

• Nutritional support 100-130% RDI

• Supplementation of electrolyte losses *(Veys et al. Curr Opin Pediatr 2017)*:
  - (Na) K citrate 2-10 mmol/kg/day QID
  - Na bicarbonate 2-15 mmol/kg/day QID
  - K chloride 2-10 mmol/kg/day QID
  - Salty food, Na chloride is rarely required

• Treatment & prevention of rickets:
  - (Na) K phosphate 0.2-2 mmol P/kg/day QID
  - Alphacalcidol 0.2-2 µg/day QD

• Copper deficiency: copper 1-10 mg/day BID

• Severe polyuria: indomethacin 0.5-3mg/kg/day TID

• In patients with adequate metabolic control, but persistent poor growth:
  - rhGH treatment 0.045 mg/kg/day QD
Indomethacin treatment reduces urinary losses due to renal Fanconi syndrome

- Rational: increased urinary PGE + successful use of indomethacin in one child (Bétend et al. 1979)
- 3 children with cystinosis
- Dose: 2 mg/kg/day, 9-18 months
- Increased sodium reabsorption, reduced free water clearance, improved plasma concentrations of Na, K, bicarbonate, P
- No acceleration of kidney function deterioration

Fig. 3 Effects of 2 weeks’ treatment with indomethacin on plasma electrolyte concentrations.

Haycock et al. Arch Dis Child 1982
Probability of CKD5 depending on indomethacin use
(data from EUNEFRON cohort)

Log Rank test: $p = 0.014$

Number at risk:

<table>
<thead>
<tr>
<th></th>
<th>INDO: No</th>
<th>INDO: Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>137</td>
<td>160</td>
</tr>
<tr>
<td>18</td>
<td>122</td>
<td>135</td>
</tr>
<tr>
<td>16</td>
<td>67</td>
<td>90</td>
</tr>
<tr>
<td>14</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>12</td>
<td>11</td>
<td>3</td>
</tr>
</tbody>
</table>
Anti-proteinuric treatment: use of ACE inhibitors

Wilmer et al et al. AJKD 2008

Greco et al. Pediatr Nephrol 2010: use of ACE inhibitors decreased risk of chronic renal failure in cystinosis (H.R. 0.15 (95% C.I. 0.03-0.68))
Probability of CKD5 depending on ACEi use
(data from EUNEFRON cohort)

No information on:
- dose
- duration
- anti-proteinuric effect

Avoid combination of indomethacin and ACEi!!

Slide Courtesy F. Emma
Cysteamine depletes intra-cellular cystine accumulation

Thoene JG, Oshima RG, Crawhall JC, Olson DL, Schneider JA
Cystinosis. Intracellular cystine depletion by aminothiols in vitro and in vivo.
J Clin Invest. 1976, 58: 180
Cysteamine treatment improves kidney function survival

Markello TC, Bernardini IM, Gahl WA.
Improved renal function in children with cystinosis treated with cysteamine

**Recommended dose:**
1.3 – 1.9 g/m²/day

Divided in:
- 4 daily doses (Cystagon®)
- 2 daily doses (Procysbi®)

**Side effects:**
- GI complaints
- Bad breath and body smell

→ limiting compliance
Probability of CKD5 depending on age at start of cysteamine (data from EUNEFRON cohort)
Renal replacement therapy in cystinosis

- ESPN/ERA-EDTA 2016: 255/14,366 1.8%
- NAPRTCS 2008: 104/7,037 1.5%
- ANZDATA 2009: 4/369 1.1%

- Both peritoneal dialysis and hemodialysis are suitable for cystinosis patients
- No evidence that cysteamine dose adjustment is required in patients on dialysis (Besouw et al. 2011)
- Metabolism of cysteamine might be impaired in ESKD (communication C. Langman 2018)
Kidney transplantation in cystinosis

• Graft survival is excellent
• Nephrectomy of the native kidneys because of persistent polyuria is rarely required (Sharbaf et al. 2012)
• Immunosuppressive treatment is similar to non-cystinosis patients:
  • preference for steroid-free regimen
  • CAVE! diabetes due to steroid and tacrolimus treatment
• Disease doesn’t recur in kidney graft
• Parents are accepted as kidney donors
• Cysteamine treatment has to be re-started when patient can take oral medications after transplantation and continues life long
ESPN/ERA-EDTA Registry Report of Transplantation in Childhood Cystinosis

Figure 2. Five-year graft survival of patients with nephropathic cystinosis (NC) and non-NC patients.
Fanconi syndrome
Renal failure

Photophobia
Keratopathy
Retinopathy

Hypothyroidism

Diabetes
Exocrine pancreas deficiency

Cerebral atrophy
Neuro-cognitive deficits
Pyramidal symptoms
Stroke-like episodes

Muscular wasting

Delayed puberty
Male infertility

Liver enlargement, fibrosis
Nephropathic Cystinosis in Adults: Natural History and Effects of Oral Cysteamine Therapy
Gahl et al., Ann Intern Med. 2007;147:242-250

Cysteamine treatment postpones or prevents extra-renal manifestations of cystinosis, prolongs life expectancy and should be continued after kidney transplantation.
Novel therapies

Cysteamine has no effect on renal Fanconi syndrome

Cysteamine

Altered exocytosis

New drugs

Improvement of renal Fanconi syndrome in vitro and in animal models

Enhanced apoptosis

Cysteamine has no effect on renal Fanconi syndrome

Altered vesicle trafficking

Altered lysosomal morphology and dynamics

Oxidative stress, inflammation

Degradation and recycling of intracellular substrates

Degradation and recycling of extracellular substrates

Enhanced apoptosis

Adapted from Settembre et al. Nat Rev Mol Cell Biol. 2013
**Hematopoietic stem cell (HSC) transplantation in cystinosis**

- HSC transplantation (HSC Tx) is efficient in cystinosis mouse model (Syres et al. 2009, Yeagy et al. 2011, Harisson et al. 2013)
  - Decrease of cystine accumulation in different tissues
  - Preservation of kidney function on short and long term
    - Effect is dependent on efficiency of engraftment
    - Improves extra-renal complications (thyroid) (Gaide Chevronnat et al. 2016)

- **Mechanism of action**
  - Engraftment of HSC in interstitium of organs → differentiation into tissue macrophages → clearance of cystine crystals
  - Lysosomal cross correction via tunneling nanotubes between macrophages derived from HSC and epithelial cells of recipient
Effect HSCTx due to formation of the tunneling nanotubes between donor cells and recipient epithelial cells

Naphade et al. Stem Cell 2015
A Phase I clinical trial on stem cell gene therapy for cystinosis

- Autologous HSC after lentiviral gene therapy to supplement CTNS
- Adults > 18 y.o.
- At least 1 year after kidney Tx
Male patient with cystinosis (het 57kb deletion & c.926dup exon 11):
  diagnosis at 2 years and 8 months
  severe renal Fanconi syndrome, deterioration of kidney function
  signs of cysteamine toxicity

16 years of age
- Pre-HSCTx myeloablative conditioning (treosulfan, fludarabine, thiotepa, ATG)
- 7,88 x 106 CD34+ HSC/kg; 166 x 106 CD3 + T-cells/kg from 10/10 HLA matched donor
- Post - HSTCx GvHD prophylaxis (tacrolimus, MMF, MTX)
- 22 days post-HSCTx: engraftment (Filgrastim D16, D17, D19)
- Full donor chimerism (>95%) in BM up to 184 days after Tx, and in blood up to 462 days after Tx

Elmonem et al. AJT 2018
Expression of WT CTNS in patient’s tissues after Allo-HSC Tx (24-30 months)

CTNS mRNA expression

**Pre-HSCT**
Healthy: 100% WT CTNS allele
Patient: 100% mutant CTNS allele

**Post-HSCT**
Healthy: 100% WT CTNS allele
Patient:
  - PTEC: 22% WT CTNS allele
  - Liver: 44% WT CTNS allele

Elmonem et al. AJT 2018
Allo-HSC Tx in cystinosis: clinical case (2)

- Acute Graft-versus-Host Disease (GvHD)
- Central nervous system complications (central pontine myelinolysis, pyramidal syndrome, recurrent epileptic seizures, neurologic toxicity of multiple drugs)
- Partial graft failure (parvovirus B19): second HSC Tx from the same donor
- Therapy resistant chronic GvHD
- Death due to multi-resistant Pseudomonas infection

Elmonem et al. AJT 2018
Take home messages

• Diagnosis of cystinosis: high level of suspicion in patients with renal Fanconi syndrome or unexplained proteinuria and glucosuria
  • Eye examination (cystine crystals)
  • Cystine measurements in WBC, DNA test

• Treatment with cysteamine remains the main therapy
  • Early administration improves kidney function prognosis
  • Treatment should be continued after kidney transplantation to protect extra-renal organs

• Novel therapies are underway to clinical trials

  risk – benefit balance should be carefully considered
Acknowledgements

Multi-disciplinary cystinosis clinics University Hospitals Leuven

Pediatric nephrologists: M. Van Dyck, K. Veys
Nephrologists: D. Kuypers, B. Bammens, K. Claes
Metabolic physician: D. Cassiman
Ophthalmologists: I. Casteels, C. Cassiman
Neurologist: L. De Walle
Psychologist: L. Willem
Youth worker: C. Cooreman
Compliance nurse: A. Van Hulle
Coming soon...

Beata Lipska-Zietkiewicz
Schimke immune-osseous dysplasia

May 7, 2019, 4 PM CET
University Hospitals Leuven

elena.levtchenko@uzleuven.be