Recent advances in cystinosis

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Clinical case (1)

- Female, born after 40 weeks normal pregnancy
- Birth weight 3200g
- No symptoms up to 6 months
- 6-9 months: **failure to thrive, vomiting, slowed development**
- 9 months:
  - urine test: glucosuria ++, proteinuria +
  - blood test: K 2.8 mmol/l, bicarbonate 16 mmol/l, phosphate 1.2 mmol/l
Clinical case (2)

- Female, born after 40 weeks normal pregnancy
- Birth weight 3500g
- No symptoms up to 10 months
- 10-24 months: **progressive development of rickets**
- 24 months:
  - blood test: K 3.2 mmol/l, bicarbonate 19 mmol/l, phosphate: 1.1 mmol/l, Ca 2.1 mmol/l, vitamin D (25 OH) 10 µg/l
Clinical case (3)

- Female 11 years old: **eye pain and photophobia**

- Blood test: normal electrolytes, bicarbonate, Ca, phosphate
  
  creatinine 0.8 mg/dl, GFR 77 ml/min/1.73 m²

- Urine test: protein/creatinine: 2.3 g/g
  
  glucose 1.2 g/l
Clinical case (4)

- Male 17 years old:

  - Severe headache and blurry vision
  - Blood pressure 200/120 mm Hg
  - New onset kidney failure, requiring dialysis

- Blood test:
  - Creatinine 9 mg/dl, BUN 180 mg/dl, K 6.8 mmol/l, bicarbonate 15 mmol/l, Hb 7.5 g/l
  - PTH 200 ng/l

- Urine test:
  - Glucose +, protein ++
Clinical case (5)

- Female 29 years old: 
  **incidental finding of proteinuria during routine examinations for inflammatory bowel disease**

- Blood test: creatinine 0.7 mg/dl, BUN 41 mg/dl, normal electrolytes, normal albumin

- Urine test: glucose -, protein 2.2 g/g creatinine, albumin: 560 mg/l, alpha-1 microglobulin: 104 mg/l

HD-OCT of the skin

Veys, Boone, 2016
HD-OCT: High definition optical coherence tomography
Cystinosis

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

- Orphan disease: incidence ~1:100,000-200,000 (clustering in some populations)

- most common cause of inherited generalized proximal tubular dysfunction (renal Fanconi syndrome)
Lysosomal cystinosin (*CTNS*, 17p13) is mutated in cystinosis (Town et al. 1998)
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
A novel gene encoding an integral membrane protein is mutated in nephropathic cystinosis

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Nephropathic cystinosis, an autosomal recessive disorder resulting from defective lysosomal transport of cystine, is the most common inherited cause of renal Fanconi syndrome. The cystinosis gene has been mapped to chromosome 17p13. We found that the locus D17S829 was homozygously deleted in 23 out of 70 patients, and identified a novel gene, CTNS, which mapped to the deletion interval. CTNS encodes an integral membrane protein, cystinosin, with features of a lysosomal membrane protein. Eleven different mutations, all predicted to cause loss of function of the protein, were found to segregate with the disorder.
CTNS gene structure (17p13, 23 kb)

Most common mutation in North European population: 57 kb deletion Town et al. 1998

100 other mutations described

CARKL encodes sedoheptulose kinase within pentose phosphate pathway, role in pathogenesis?

TRPV1 encodes transient receptor potential vanilloid 1Ca channel

Turkey: no 57 kb deletion 7/12 alleles: c.681 G>A Topaloglu et al. 2012
Reduced sensitivity of TRPV1 receptor in patients with hom57kb deletion

Pathogenesis of cystinosis

Adapted from Settembre et al. Nat Rev Mol Cell Biol. 2013

Regulation of apoptosis

Mito-chondrion

Enhanced apoptosis

Cystin

Altered exocytosis

Decreased expression of endocytotic receptors

Altered lysosomal dynamics

Degradation and recycling of intracellular substrates

Degradation and recycling of extracellular substrates

Plasma membrane

Autophagosome

\[ \uparrow \text{Cystine} \]

\[ \downarrow \text{ATP}, \uparrow \text{oxidative stress, inflammation} \]

\[ \uparrow \text{Enhanced apoptosis} \]

Adapted from Settembre et al. Nat Rev Mol Cell Biol. 2013
Cystinosin interacts with H⁺-ATPase-Ragulator-Rag

mTOR pathway is altered in cystinosis

Andrzejewska et al. JASN 2015

Ivanova et al. JIMD 2016
Transcription Factor EB (TFEB)

Phosphorylated by mTOR → inactivation (cytosolic localization)

Active TFEB (nuclear localization)

- Phosphorylated by mTOR → inactivation (cytosolic localization)
- Autophagy
- Lysosome biogenesis
- Lysosomal exocytosis
- Cellular clearance

Rega et al. Kidney Int 2016

Slide courtesy Francesco Emma

Sardiello et al, Science 2009
Sardiello and Ballabio, Cell Cycle 2009
Palmieri et al, Hum Mol Genet 2011
TFEB overexpression in CTNS -/- ciPTEC restored abnormal lysosomal morphology and decreased cellular cystine content.

Rega et al. Kidney Int 2016

Slide courtesy Francesco Emma
Glomerular damage in cystinosis

- Glomerular proteinuria (grams/day):
  - intermediate and high molecular weight proteins

- Renal histology: signs of podocyte damage

Podocyte hypertrophy, foot process effacement

Focal and segmental glomerulosclerosis

Glomerular collapse

The nephron
The podocyte

- **in vivo**
  - Smoyer (1998)
  - Foot process effacement

- **in vitro**
  - A podocyte in culture
  - Podocin DAPI

**Podocyte damage:**

- **in vivo**
  - Foot process effacement

- **in vitro**
  - Acquisition of motile phenotype
Urinary podocyte loss by cystinotic patients

Ivanova, Arcolino et al. Kidney Int 2016
Increased motility of cystinotic podocytes

Increased general motility (individual cell tracking assay):

- Control
- Cystinosis urine podocytes
- CTNS KD
Defective endocytosis
Defective Na\(^{+}\)-dependent transport
Altered lysosomal morphology
Altered mTOR signaling
Enhanced cell death
Increased oxidative stress

Inflammatory cell recruitment and fibrosis of renal cortex
Impaired glomerular permselectivity
Foot process effacement
Defective adhesion
Enhanced motility
Increased cell loss in urine

Courtesy Mohamed Elmonem
Clinical forms of cystinosis

- **Infantile form (>90%)**:  
  - Fanconi syndrome ~ 3-6 months  
  - End stage renal disease (ESRD) ~ 10 years
- **“Late-onset” (juvenile) form (~5%)**:  
  - Later onset (childhood, puberty, adulthood)  
  - Mild tubulopathy, more pronounced proteinuria, (even in nephrotic range)  
  - Later progression to ESRD
- **Ocular form**  
  - Overlap between ocular and juvenile forms  
  (Servais et al. 2008)
Diagnosis of cystinosis

- **Suspected clinical presentation**
  - cystinosis - most common cause of Fanconi syndrome
  - late onset forms can mimic common proteinuric disorders

- **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

• **Symptomatic:**
  – free access to water and toilet
  – replacement of urinary losses due to renal Fanconi syndrome
  – indomethacin
  – hormone replacement when required (thyroxin, insulin, testosterone)
  – growth hormone in children with poor growth

• **Specific treatment with cysteamine**
Cysteamine depletes lysosomal cystine accumulation in cystinosis

Adapted from Besouw et al. 2014
Efficacy of cysteamine treatment

• Postpones the deterioration of renal function \((\text{Markello et al. 1993})\)
• Improves growth \((\text{Wuhl et al. 2001})\)
• Postpones or prevents extra-renal complications \((\text{Nesterova & Gahl 2013})\)
Emma et al. Nephrol Dial Transplant 2015
Challenges with cysteamine therapy

• Delayed initiation due to delayed diagnosis of cystinosis

• Non-compliance with cysteamine therapy:
  – Difficult dose regimen (4 times daily):
    • < 25% of the patients follow the prescription (Levtchenko et al. 2006)
  – Gastro-intestinal complaints (Dohil et al. 2003)
  – Bad breath and sweat odor (Besouw et al. 2007)

Need for earlier diagnosis and better therapy
Delayed-release cysteamine (RP103)

Langman et al. CJASN 2012
Effectiveness of slow release cysteamine

**Short-term study** (Langman et al. 2012)

- WBC lowering effect: non-inferiority of RP103 compared to Cystagon
- Decreased use of PPI
- 80% of initial Cystagon® dose

**Long-term study: 24 months** (Langman et al. 2014)

- Sustained WBC lowering effect
- Stable eGFR
- Improvement in social function, school function, and in total function scores on the Pediatric Quality of Life

Delayed release cysteamine (Procysbi®) is approved by EMA
Natural history of patients with cystinosis

- Patients born before 30th of last century
  - death from dehydration and electrolyte disturbances
- Patients born before 70th of last century
  - death from ESPN ~10 of age
- Patients born after 80th of last century and treated with cysteamine
  - kidney function preservation until adolescence or young adult age (there are exceptions)
- 21st century: novel era
  - improving existing therapy
  - search for curative therapies (cell and gene therapy)

- Growing population of adult cystinosis patients (transition!)
- Issues of quality of life
- Issues of compliance
Take home messages

• Cystinosis is a **treatable** multi-organ lysosomal storage disorder:
  - Earlier diagnosis and treatment improve renal and extra-renal prognosis

• Diagnosis can be made at **any age**:
  - Think about cystinosis in patients with proteinuria *e causa ignota* (proximal tubulopathy can be mild!) and patients with ESRD of unknown etiology
  - Eye examination: corneal cystine crystals

• Cysteamine administration should be administered **life-long**, also after kidney transplantation

• Novel therapies (pharmacological, stem and gene therapy) are under investigation
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Per vivum ad verum

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