The Cystinosis Research Network Symposium

Tuesday, August 31, 2010
12:15—6:30 pm
Welcome to The Cystinosis Research Network Symposium!

We thank you all for joining us today for The Cystinosis Research Network Symposium as part of the 15th Congress of the International Pediatric Nephrology Association. We recognize time as one of the most valuable possessions we have and as such we are very grateful you have decided to commit a portion of your time to the rare disease of cystinosis.

We are deeply grateful to Dr. Frederick Kaskel and the entire IPNA 2010 Committee for including CRN and cystinosis as part of this important event. We would also like to thank Dr. William Gahl for chairing the meeting, as well as our speakers, poster presenters, and cystinosis families in attendance today.

It is our hope that the information shared from all over the world during this session regarding diagnosis and management, as well as strategies for integration of a worldwide cystinosis registry, will go far towards advancing our understanding and treatment of this disease. In the end, we hope it will increase the potential for proactively identifying and ultimately curing individuals with this debilitating disease.

Once again, thank you for joining us.

Best Regards,

Christy Greeley
President and Executive Director
Cystinosis Research Network
302 Whytegate Court
Lake Forest, IL 60045 USA
greeleycd@aol.com
Toll Free: 866-276-3669
Fax: 847-235-2773

www.cystinosis.org
Mission

The Cystinosis Research Network (CRN) is a volunteer, non-profit organization dedicated to supporting and advocating research, providing family assistance and educating the public and medical communities about cystinosis.

Vision

The Cystinosis Research Network’s vision is the discovery of improved treatments and ultimately a cure for cystinosis.
Key Areas of Focus

Research
- Established CRN Clinical Cystinosis Fellowship at the NIH under the direction of Dr. William Gahl. Dr. Galina Nesterova named to the Fellowship in 2010
- Funded nearly $2 million in cystinosis research

Development
- Funding received through individual donations, grassroots fundraisers, CRN sponsored programs, and corporate and government grants

Family Support
- Identification and support of newly diagnosed families
- Communication with existing families
- Provision of information via website www.cystinosis.org, newsletter, online resource library
- Support groups online
- Biennial Family Conference

Education and Awareness
- Sponsorship of IPNA Cystinosis Symposium
- Exhibition at professional conferences (IPNA, ASN, ASPN, etc.)
- Active member organization of NORD. CRN Board of Directors member Marybeth Krummenacker serves on the NORD Board of Directors as Advocacy Committee Chair
- Grants educational scholarships to individuals with cystinosis and their siblings
- Provision of professional information via brochures and website
The Cystinosis Research Network Symposium

**Poster Presentations***

12:15 – 1:00 PM        Cystinosis Poster Presentations with Presenters in Attendance

*Lunch provided by Cystinosis Research Network

**Plenary Lecture**

1:00 -1:30 PM   Cystinosis Introduction  
William A. Gahl, MD, PhD  
National Institutes of Health  
Bethesda, MD, USA

**Platform Talks**

*Chairs: Jerry A. Schneider, MD, University of California, San Diego, CA, USA and Robert Kleta, MD, PhD, University College London, London, UK*

1:30 – 1:45 PM       Growth Hormone in Cystinosis  
Elke Wuhl, MD  
University Children’s Hospital  
Heidelberg, Germany

1:45 – 2:00 PM       Cell Death in Cystinosis  
Elena Levtchenko, MD  
University Hospitals Leuven  
Leuven, Belgium

2:00 – 2:15 PM       Electrophysiological Properties of CTNS  
Bruno Gasnier, PhD  
Institut de Biologie Physico-Chimique  
Paris, France

2:15 – 2:30 PM       Newborn Screening for Cystinosis  
Silhoun Hahn, MD, PhD  
Seattle Children’s Hospital  
Seattle, WA, USA/T. Vilboux, Bethesda

**Panel Discussion: Availability of Cysteamine and Leucocyte Cystine Assays in Less Well Developed Countries**

*Chair: William G. Van’t Hoff, MD, Great Ormond Street Hospital, London, UK and Nilzete Bresolin, MD, Children's Hospital Joana de Gusmo, Florianopolis, Brazil*

2:30 – 2:35 PM       Vera H. Koch, MD  
Hospital das Clinicas of the Sao Paulo Medical School  
Sao Paulo, Brazil
2:35 – 2:40 PM  Neveen A. Soliman, MD  
Cairo University  
Cairo, Egypt

2:40 – 2:45 PM  Rezan Topaloglu, MD  
Hacettepe University School of Medicine  
Ankara, Turkey

2:45 – 2:50 PM  Leticia Belmont, MD  
Cystinosis Mexico  
Mexico City, Mexico

2:50 – 2:55 PM  Velibor Tasic, MD, PhD  
University Children’s Hospital  
Skopje, Macedonia

3:00 – 3:30 PM  Break

**Controversies**  
*Chairs: Craig B. Langman, Children’s Memorial Hospital, Chicago, IL, USA and Chebl Mourani, MD, Hotel Dieu De France, Beirut, Lebanon*

3:30 – 3:45 PM  Early Management of Cystinosis and Fanconi Syndrome  
Paul Goodyer, MD  
Montreal Children’s Hospital  
Montreal, Canada

3:45 – 4:00 PM  Dosing of Cysteamine in Children and Adults; Treatment of Newborns and Pregnant Women  
Jess G. Thoene, MD  
University of Michigan  
Ann Arbor, MI, USA

**Plenary Lecture**  
*Introduction: Francesco Emma, MD, Ospedale Pediatrics Bambino, Rome, Italy*

4:00 – 4:30 PM  Mouse Models for Studying Cystinosis  
Corinne Antignac, MD, PhD  
Necker Hospital  
Paris, France

4:30 – 4:45 PM  Break
International Cystinosis Registries Presentation & Discussion

Chairs: William Gahl, MD, PhD, National Institutes of Health, Bethesda, MD, USA, Patrick Niaudet, MD, Hopital Necker-Enfants Malades, Paris, France, and Galina Nesterova, MD, National Institutes of Health, Bethesda, MD, USA

4:45 – 5:00 PM  The European Cystinosis Registry Project  
Patrick Niaudet, MD  
Hopital Necker-Enfants Malades  
Paris, France

5:00 – 5:10 PM  The North American Cystinosis Research Network Registry  
Paul Goodyer, MD  
Montreal Children’s Hospital  
Montreal, Canada

5:10 – 5:20 PM  Cystinosis Registry  
Kyle Brown  
Innolyst, Inc.  
San Mateo, CA, USA

5:20 – 5:30 PM  National Organization for Rare Disorders  
Mary Beth Krummenacker  
Hicksville, NY, USA

5:30 – 5:40 PM  NIH Presentation  
Yaffa Rubinstein, PhD  
National Institutes of Health – Office of Rare Diseases Research  
Bethesda, MD, USA

5:40 – 5:50 PM  OxalEurope Registry  
Bernd Hoppe, MD  
Universität Köln  
Köln, Germany

5:50 – 6:00 PM  Oxalosis Registry  
Dawn Milliner, MD  
Mayo Clinic Rochester  
Minneapolis, MN, USA

6:00 – 6:10 PM  Alport Syndrome Registry  
Clifford Kashton, MD  
University of Minnesota  
Minneapolis, MN, USA

6:10 – 6:20 PM  Autosomal Recessive Polycystic Kidney Disease Registry  
Lisa M. Guay-Woodford, MD  
University of Alabama at Birmingham  
Birmingham, AL, USA

6:20 – 6:30 PM  Open Discussion and Summary
Speaker Presentations
Presentation #1

Overview of Cystinosis: Natural History and Treatment

William A. Gahl and Galina Nesterova
Section on Human Biochemical Genetics, NHGRI, NIH, Bethesda, Maryland, USA

The devastating renal tubular and glomerular effects of intracellular cystine storage in nephropathic cystinosis have been known for a century. The lysosomal location of the stored cystine and the feasibility of renal transplantation were recognized four decades ago. The basic transport defect was discovered almost 30 years ago, and the CTNS gene was identified a decade ago. In the 1980s, diligent use of oral cysteamine therapy (60-90 mg/kg/d dosed every 6h) was shown to lower cellular cystine content by >90% and to prevent or attenuate the growth retardation and glomerular deterioration of pre-transplant patients. Oral cysteamine was approved by the FDA in 1994. We now know that the ability of chronic oral cysteamine therapy to stave off renal failure has practical limitations; cystinosis patients, treated from early childhood, generally require dialysis or renal replacement therapy by early adulthood. We also recognize that nephropathic cystinosis destroys non-renal organs in the third through fifth decades and carries enormous morbidity and mortality. Of 100 patients seen as adults at the NIH between 1986 and 2006, 33 had died; their mean age at death was 29 ± 1 (SEM) years. Most of the late pathology of cystinosis (e.g., swallowing difficulty, coronary artery calcifications, posterior segment ophthalmic complications, myopathy, and diabetes), however, were prevented by long-term oral cysteamine therapy. Cysteamine eyedrops dissolve the corneal cystine crystals of cystinosis patients.

The future looks strong for basic research into cystinosis. Cellular models hold promise for revealing the precise cause of the tissue destruction in cystinosis. Animal models are revealing the potential benefits of stem cell transplantation. In the clinical arena, better delivery systems for oral cysteamine are being developed. Cysteamine eyedrops are moving toward approval. Spurred by an effective therapy, physicians across the globe are diagnosing cystinosis earlier and applying treatments more diligently. There may be potential for newborn screening for cystinosis, and for stem cell therapy. Finally, patient advocacy groups such as the Cystinosis Research Network are inspiring clinical and basic research into cystinosis and promoting improved care for affected individuals.
Overview of Cystinosis: 
Natural History and Treatment

Cystinosis Research Network

IPNA Cystinosis Symposium
August 31, 2010
New York, NY

William A. Gahl, MD, PhD
National Human Genome Research Institute
National Institutes of Health, DHHS

Cystinosis

- Natural History Without Treatment
- Treatment
- Effects of Cysteamine Therapy

CYSTINOSIS

- AR; 1 in 200,000 births
- Lysosomal storage disease due to impaired transport of cystine out of lysosomes.
  - High intracellular cystine content
  - Crystals in many tissues
- Therapy: Replacement of renal losses; cystine depletion with cysteamine.
The Cystinosis Gene (CTNS)

• 1998: CTNS isolated
  – 12 exons spanning 23 kb of genomic DNA
  – 367 amino acids
  – 7 transmembrane domains

• 2000: Common deletion includes first 10 exons of CTNS and 57,257 bp
  - ~50% of N. European patients have this deletion

• 2010: Over 80 different mutations
**CYSTINOSIS NATURAL HISTORY**

<table>
<thead>
<tr>
<th>Age</th>
<th>Clinical Manifestation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth</td>
<td>None</td>
</tr>
<tr>
<td>Infancy</td>
<td>Renal tubular Fanconi syndrome, Growth retardation</td>
</tr>
<tr>
<td>Early childhood</td>
<td>Photophobia</td>
</tr>
<tr>
<td>Late childhood</td>
<td>Renal failure</td>
</tr>
<tr>
<td>Adults</td>
<td>Cerebral calcifications, diabetes, retinal blindness, swallowing, difficulty, myopathy, pseudohypoparathyroidism, cerebellar, liver involvement, coronary artery calcifications</td>
</tr>
</tbody>
</table>

**Transmission EM of conjunctival cell**
(Dr. T. Kuwabara)

**Scanning EM of liver Kupfer cell**
(Dr. Kamal Ishak)
Nephrocalcinosis

Renal Allografts in Cystinosis

- Patients do well.
- Disease does not recur in graft, although crystals may appear because of invading host cells.
- Cystine accumulation continues in other organs, causing post-transplant complications.

Non-renal Organ Involvement (Post-transplant Complications)

- CNS: Calcifications, atrophy, pseudotumor cerebri
- Retina: Blindness
- Cornea: Crystals, band keratopathy
- Muscle: Distal vacuolar myopathy
- Liver: Nodular regenerating hyperplasia
- Coronary arteries: Calcification
- Pancreas: Diabetes; exocrine insufficiency
- Testes: Low testosterone; azospermia
Cerebral atrophy in a 24-year old man

Cerebral calcifications in an adult with cystinosis

Pseudotumor cerebri; increased intracranial pressure

Peripheral Retinal Pigmentary Changes in Cystinosis

Visual Acuity in logmar units

<table>
<thead>
<tr>
<th>Age at last visit</th>
</tr>
</thead>
<tbody>
<tr>
<td>No light perception</td>
</tr>
<tr>
<td>Light perception</td>
</tr>
<tr>
<td>Count fingers</td>
</tr>
</tbody>
</table>
Band keratopathy in a 14-year old girl

Distal Vacuolar Myopathy of Cystinosis

- Muscle atrophy, weakness
- Begins in hands, then central parts of body, swallowing muscles
- Progressive with age
- Related to muscle cystine content
- Occurs in patients who did not receive cysteamine therapy
- Carnitine is not deficient in post-transplant patients.

22-year old with cystinosis, myopathy, and swallowing difficulty. Died of aspiration.

Cystinosis patient with atrophy of tongue muscles.
Swallowing Difficulty

Pooling in valleculae and pyriform sinuses

Rare double bolus

Coronary Artery Calcification

Coronary angiogram of 25-year old man

Liver Disease in Cystinosis

Mild portal fibrosis

Increased reticulin staining with nodularity; Nodular Regenerative Hyperplasia
Diagnosis

- Postnatal
  - Family history
  - Fanconi syndrome (poor growth, polyuria, dehydration, acidosis)
  - Corneal crystals (>1 year old)
  - Elevated leucocyte (pmn) cystine level
- Prenatal
  - Elevated cystine level in chorionic villi or amniotic fluid cells

Cystinosis

- Natural History Without Treatment
- Treatment
- Effects of Cysteamine Therapy

Cystinosis Therapy

- Symptomatic
  - Replacement of renal losses (citrate, phosphate, potassium, water, calcium)
  - L-thyroxine, testosterone
  - Growth hormone
- Cystine Depletion
  - Oral cysteamine (Cystagon®)
  - Cysteamine eyedrops
HS-CH$_2$-CH$_2$-NH$_2$

CYSTEAMINE
Cystinosis

- Natural History Without Treatment
- Treatment
- Effects of Cysteamine Therapy

Pre-Transplant, Early, Adequate Oral Cysteamine Therapy:

1. Allows for a normal growth rate.
2. Preserves thyroid function.
3. Helps maintain renal function.
4. Still, most patients begun on cysteamine therapy early (1-2 years of age) require a renal transplant in their late teens or early twenties.
Post-Transplant Cystinosis

<table>
<thead>
<tr>
<th>Complication</th>
<th>Cysteamine helps?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swallowing difficulty</td>
<td>Yes</td>
</tr>
<tr>
<td>Vascular calcifications</td>
<td>Yes</td>
</tr>
<tr>
<td>Retinopathy</td>
<td>Yes</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>Yes</td>
</tr>
<tr>
<td>Myopathy</td>
<td>Yes</td>
</tr>
<tr>
<td>Pulmonary dysfunction</td>
<td>Yes</td>
</tr>
<tr>
<td>Death</td>
<td>Yes</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>Yes</td>
</tr>
<tr>
<td>Liver damage</td>
<td>?</td>
</tr>
<tr>
<td>Pseudotumor cerebri</td>
<td>?</td>
</tr>
<tr>
<td>Male hypogonadism</td>
<td>?</td>
</tr>
</tbody>
</table>
41 Post-Transplant Cystinosis Patients had Chest CT Scans:
- 28 Normal (mean 22 y)
- 13 Coronary Artery Calcification (mean 36 y)
Cystinosis - Outcomes

- 1955 - Death in infancy/childhood
- 1965 - Death or transplant, complications
- 1975 - Death or transplant, complications
- 1985 - Delay until age 15-25 in transplant
  - Expect no late complications

Early diagnosis is critical!

### CYSTEAMINE EYEDROPS

<table>
<thead>
<tr>
<th></th>
<th>Untreated</th>
<th>Treated</th>
</tr>
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<tbody>
<tr>
<td>3-year old</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>20-year old</td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
</tbody>
</table>

### Cysteamine Eyedrop Therapy

<table>
<thead>
<tr>
<th>Time</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>69 mo</td>
<td>3.00</td>
</tr>
<tr>
<td>85 mo</td>
<td>0.00</td>
</tr>
<tr>
<td>133 mo</td>
<td>3.00</td>
</tr>
<tr>
<td>157 mo</td>
<td>0.00</td>
</tr>
<tr>
<td>86 mo</td>
<td>3.00</td>
</tr>
<tr>
<td>109 mo</td>
<td>0.00</td>
</tr>
<tr>
<td>237 mo</td>
<td>3.00</td>
</tr>
<tr>
<td>249 mo</td>
<td>0.25</td>
</tr>
</tbody>
</table>
Cysteamine - Summary

- Oral and topical cysteamine help preserve function of all organs studied in cystinosis.
- Early and diligent therapy is essential.
- Cysteamine should be given to patients of all ages.
- Leucocyte cystine depletion below 1 nmol/mg protein may not be needed for post-transplant complications.

Cystinosis – Basic Research

- Many investigators are studying the mechanism of cell death/dysfunction in cystinosis.
- Cell models will soon include induced pluripotent stem cells made into kidney cells.
- Mouse models are available for studying stem cell transplantation, with good early results.

Cystinosis – Clinical Advances

- Cysteamine eyedrops nearing approval
- Microencapsulated enteric release cysteamine in clinical trial.
- Newborn screening based upon molecular mutations is a possibility.
- Gene therapy via stem cell transplantation is on the horizon.
- Organizations provide registries and foster access to cysteamine.
Growth Hormone in Cystinosis

Elke Wühl, MD
Center for Pediatrics and Adolescent Medicine, University of Heidelberg, Germany

Growth retardation is a characteristic symptom in patients with nephropathic cystinosis. Various factors are contributing to early growth impairment, e.g. metabolic acidosis and electrolyte disturbances due to Fanconi syndrome, decreased renal function, poor nutritional status, and hypothyroidism - all in consequence of cystine accumulation in the tissue. In the ‘pre-cysteamine’ era mean adult height in patients with cystinosis was extremely poor: 146 cm (-4.6 SDS) and 137 cm (-4.7 SDS) in North American male and female, and 136 cm (-6.1 SDS) and 124.0 cm (-6.9 SDS) in French male and female patients, respectively. According to EDTA registry data published in 1991, only a few patients reached 150 cm of height. Nowadays treatment with cystine depleting agents may prevent growth retardation when administered from early infancy onward but does not induce catch-up growth in children who are already growth-retarded at the start of treatment. Thus in children with persisting growth failure despite adequate cysteamine treatment and nutrition early growth hormone (GH) treatment should be considered.

In the ‘European Study on GH Treatment in Children with Nephropathic Cystinosis’, 72 children aged 3 to 18 years where treated with GH over a mean period of 3.1 (1 to 10) years. Mean height SDS at baseline was –4.0 in patients with CKD stage I-IV, –4.4 in patients on dialysis, and –4.9 in patients after renal transplantation. During the first treatment year, height velocity doubled in the CKD group, increased by 80% in the dialysis group, and increased by 45% in the renal transplant group. Within 3 years the height SD score increased by +1.6 in prepubertal CKD patients, and percentile parallel growth was maintained thereafter without major side effects. By now final height data is available from 42 patients. Mean duration of GH treatment was 3.4 (1-12) years resulting in a mean final height of 154.7 cm (-2.5 SDS) in boys and 146.7 cm (-2.7 SDS) in girls. The effect of GH was best in younger CKD children, resulting in a normal final height in almost 50% of patients; effects were less distinct in peripubertal patients receiving renal replacement therapy.

In conclusion, long-term GH treatment is safe and effective in young children with nephropathic cystinosis. GH treatment should be started early in the course of the disease if adequate nutrition and cysteamine treatment do not prevent growth retardation.
Phenotype of Nephropathic Cystinosis

- Fanconi syndrome: hypophosphatemic rickets, metabolic acidosis, aminoaciduria, Ca-, K-, Mg-loss, polyuria, polydipsia, dehydration, vomiting, failure to thrive
- Growth retardation
- Progressive chronic renal failure
- Photophobia, corneal cystine deposits
- Hypogonadisms, hypothyreoidisms
- Exocrine and endocrine pancreatic insufficiency
- Liver- und bone marrow fibrosis, myopathy
- CNS: cerebral atrophy, swallowing disorders

Growth Impairment in Nephropathic Cystinosis

Final Height

**US**

- Males: 146 cm (-4.6 SDS)
- Females: 137 cm (-4.7 SDS)

**France**

- Males: 136.5 cm (-6.1 SDS)
- Females: 124.0 cm (-6.9 SDS)
Factors influencing Growth in Cystinosis

Cystine Accumulation

- Endocrine glands
- Bone
- Kidney
- Intestine

Chronic renal failure / Uremia
Fanconi Syndrome
Hypothyroidism
Osteodystrophy
Metabolic acidosis
Electrolyte disturbances
Poor nutrition

Growth retardation

Factors influencing Growth in Cystinosis

Cysteamine

- Endocrine glands
- Bone
- Kidney
- Intestine

Thyroxin
Vitamin D
Bicarbonate / Electrolyte Substitution
Tube feeding

Growth Hormone

Cysteamine Treatment and Growth

Kleta et al., J Pediatr 2004: 145:555-60
**Inclusion Criteria**

- Age > 2 years
- Height < -2 SDS (3rd pct) and/or height velocity < 0 SDS (50th pct)
- Prepubertal or start of puberty within last 12 months
- Cysteamine treatment, if any, > 1 year
- Normal thyroid function or on stable thyroid hormone substitution
- No diabetes mellitus
- Stable renal function in patients after renal transplantation (> 1 year)

**rhGH Dose**

1 IE (0.33mg/kg/week) = 0.05 mg/kg/d s.c. (Genotropin, Pfizer)

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**Baseline Clinical Characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Conservative treatment (n = 52)</th>
<th>Dialysis (n = 7)</th>
<th>Transplant (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>28 M/24 F</td>
<td>5 M/2 F</td>
<td>7 M/8 F</td>
</tr>
<tr>
<td>Prepubertal/prepuberal</td>
<td>56/2</td>
<td>7/1</td>
<td>9/6</td>
</tr>
<tr>
<td>Age (y)</td>
<td>7.1 ± 2.6</td>
<td>12.5 ± 2.7</td>
<td>14.8 ± 1.7</td>
</tr>
<tr>
<td>Bone age (y)</td>
<td>6.3 ± 2.5</td>
<td>9.0 ± 2.6</td>
<td>9.9 ± 2.2</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>123.3 ± 8.6</td>
<td>122.5 ± 8.6</td>
<td>129.6 ± 6.7</td>
</tr>
<tr>
<td>Height (SDS)</td>
<td>-4.6 ± 1.2</td>
<td>-4.4 ± 1.2</td>
<td>-4.9 ± 1.1</td>
</tr>
<tr>
<td>Height velocity (cm/y)</td>
<td>3.5 ± 1.6</td>
<td>2.8 ± 2.1</td>
<td>3.7 ± 2.4</td>
</tr>
<tr>
<td>Height velocity (SDS)</td>
<td>-2.2 ± 1.6</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>25.9 ± 6.2</td>
<td>23.6 ± 5.2</td>
<td>32.6 ± 8.6</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>17.1 ± 1.5</td>
<td>17.1 ± 1.5</td>
<td>19.1 ± 3.3</td>
</tr>
<tr>
<td>GFR (ml/min/1.73 m²)</td>
<td>94 ± 24</td>
<td></td>
<td>94 ± 24</td>
</tr>
</tbody>
</table>

Data are given as mean ± SD.
**Effect of GH in Nephropathic Cystinosis**

<table>
<thead>
<tr>
<th>Height SDS</th>
<th>Height velocity [cm/year]</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
</tr>
</tbody>
</table>

- - -

**Effect of GH Treatment on Serum Creatinine**

- without Cysteamine
- with Cysteamine

<table>
<thead>
<tr>
<th>Serum Creatinine [mg/dL]</th>
<th>Chronological Age [years]</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>B</td>
</tr>
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</table>

- - -

**Summary**

- Increase of height SDS by +1.6 in prepubertal patients on conservative treatment (CKD I-IV)
- Effect less expressed in peripubertal children on RRT
- No deterioration of renal disease progression
- No major side effects.
Final Height?

Questionnaire sent to participants in 2008:
- Final height? Age at attainment of FH?
- Pubertal stage? Duration of GH treatment?
- Renal function? RRT? Duration of CKD?

Summary – Analysis of Final Height Data

- Long-term GH treatment increases final height significantly
- Mean final height 154.7 cm (-2.5 SDS) in males,
  146.7 cm (-2.7 SDS) in females,
  significant difference compared to historical controls.
- Best growth response in young patients with early initiation
  of GH treatment, long-time GH treatment prior to RRT

Conclusions

Long-term GH treatment in children with nephropathic cystinosis is safe and effective.

GH treatment should be started early in the course of the disease if adequate nutrition and cysteamine treatment do not prevent growth retardation
### European Study Group on rhGH Treatment in Nephropathic Cystinosis

- Angers - Dr. Champion, Berlin - Dr. Gellermann,
- Birmingham - Dr. Hulton, Essen - Dr. Bonzel, Giessen - Dr. Kreuder,
- Hamburg - Dr. Dietz, Hannover - Drs. Offner, Albers,
- Helsinki - Dr. Holmberg, Heidelberg - Drs. Wühl, Haffner, Mehls,
- London - Drs. van't Hoff, Rigden, Lüdenscheid - Dr. Weber,
- Lyon - Dr. Cochaf, Milano - Dr. Ardissino, Moers - Dr. Firnhaber,
- Nancy - Drs. Krier, André, Nijmegen - Dr. de Jong,
- Paris - Drs. Broyer, Loirat, Bensman, Reuilingen - Dr. Trefz,
- Rostock - Dr. Wigger, Rouen - Dr. Landthaler,
- Strasbourg - Dr. Fischbach, Toulouse - Dr. Bouissou,
- Tours - Dr. Nivel, Utrecht - Dr. Lilien
Cell death in cystinosis

Elena N. Levtchenko, University Hospitals Leuven, KU Leuven, Belgium

Cystinosis belongs to a large group of lysosomal storage disorders (LSD), that are mostly caused by the deficiency of soluble lysosomal hydrolases, but may also result from defective non-enzymatic lysosomal or non-lysosomal proteins with impact on lysosomal function. Despite distinctive mechanisms leading to storage of lysosomal material, many of these diseases share common clinical, biochemical and cellular features. Among common pathogenetic pathways, altered cell death mechanisms such as autophagy and apoptosis have been demonstrated.

In cystinosis a defect in the lysosomal membrane transporter cystinosin leads to lysosomal accumulation of the amino acid cystine, causing a multi-organ disorder. Kidney disease is far most severe compared to other LSD with renal impairment. Contrasting many other LSD, neurodegeneration occurs late in cystinosis, generally during the 3rd decade of life. In respect to pathogenesis, cystinosis shares at least some mechanisms with other LSD. Enhanced apoptosis was first demonstrated in cystinotic fibroblasts by the group of J. Thoene in 2002. Lowering cystine content with cysteamine reduced the rate of apoptosis, whereas loading the cells with cystine dimethyl ester (CDME) dramatically increased sensitivity to apoptotic stimuli (Park et al. 2002). We observed that CDME loading inhibited mitochondrial function and provoked oxidative stress leading to cell death, a phenomenon that was not found in cystinotic cells with equal degree of cystine accumulation, thus, warranting a cautious interpretation of the results obtained in the CDME-loading model of cystinosis (Wilmer et al. 2007). Lysosomal involvement in apoptosis has been observed over a decade (Trump et al. 1997) and is accompanied by a permeabilization of the lysosomal membrane and concomitant translocation of lysosomal cathepsins to the cytosol. Park et al. demonstrated increased translocation of cathepsin B in cystinotic cells exposed to apoptotic stimuli (2006). The proposed causative mechanisms leading to apoptosis in cystinosis encompasses lysosomal cystine leakage with subsequent cysteinylation of proapoptotic protein kinase C delta, initiating protein activation (Park et al. 2006).

Mitochondrial abnormalities have been shown in many LSD (reviewed in Bellettato et al. 2010). The autophagic/mitochondrial pathway has been recognized as the major route of removal of damaged mitochondria, which are sources of reactive oxygen species with potential cell-damaging properties. Sansanwal et al. demonstrated abnormal patterns of mitochondrial autophagy in cystinotic fibroblasts and renal proximal epithelial cells. Cystinotic cells demonstrated an enhanced apoptosis rate, decreased ATP generation and an increase in ROS. The autophagy inhibitor 3-methyl adenine rescued cell death in cystinotic cells (Sansanwal et al. 2010).

Despite significant progress made during the last decade, the question remains whether the complexity of the cystinotic phenotype can be solely explained by aberrations in cell death mechanisms. The results obtained from in vitro models do not necessarily reflect the in vivo processes and require confirmation by animal models of cystinosis and human verification.

Unraveling the pathogenesis of cystinosis will allow for interference with the disease mechanisms downstream of the cystinosin defect and will enhance our understanding of the lysosomal function in general.

References:
Cell death in cystinosis

Elena Levtchenko, MD, PhD

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August 31, 2010

Lysosomal cystinosin (CTNS, 17p13) is mutated in cystinosis (Town et al. 1998)
Underlying pathogenetic mechanisms of cystinosis

Cystinosin defect → Cell damage

Decreased intracellular ATP → Altered glutathione → Enhanced oxidative stress → Increased cell death

Cell death

process that counteracts cell division:

- **Necrosis**: rupture of plasma membrane, release of cell content → inflammation
- **Apoptosis** (Greeks “falling of”): programmed cell death 1
- **Autophagy** (“self eating”): programmed cell death 2

Indication of enhanced cell death in cystinosis

- **Proximal tubular atrophy in cystinosis**
- **Cortical atrophy in cystinosis**
- **Muscular atrophy in cystinosis**

Mahoney et al. 2000
Chevalier et al. 2008
Novo et al. 2010
Lysosomal – mitochondrial axis:
lysosomes as “suicide bags”

Control + CDME
Control
Control + CDME
Control
Cystinosis + MEA
Cystinosis
Cystinosis + MEA
Cystinosis

Cystine dimethyl ester (CDME) induces cell death unrelated to cystine accumulation

Park et al. 2002

Increased apoptosis in cystinotic fibroblasts

Wilmer et al. 2007

Cystinotic fibroblasts with equal cystine content:
- normal mitochondrial ATP generation
- preserved cell viability

Wilmer et al. 2007

Toxic effect of CDME on control cells
Increased apoptosis in cystinotic proximal tubular cells

![Bar chart showing increased apoptosis in cystinotic cells compared to controls](image)

- Control (HK2)
- Cystinosis (RPTE, transformed with SV40)

Proposed mechanism of increased apoptosis in cystinosis

- Lysosomal membrane permeabilization
- Increased cytosolic cysteine
- Cysteinylation of protein kinase C delta
- Increased activity of protein kinase C delta
- Increased apoptosis rate

Lessons from other lysosomal storage disorders

- Plasma membrane
- Early endosome
- Recycling endosome
- Late endosome
- Lysosome
- Autophagosome

![Diagram showing lysosomal storage disorders](image)
Activated autophagy in cystinosis

Can the complex cystinotic phenotype be solely explained by enhanced cell death?

Apoptosis and autophagy assays strongly depend on:
- origin of cells
- availability of adequate controls
- passage number / viral transformation
- cautious interpretation of in vitro results (in vivo confirmation of in vitro data is required)

Other mechanisms involved?

Discussion

ATP depletion
Disturbed glutathione metabolism
Enhanced apoptosis
Enhanced autophagy
Cystinosin defect
Cystine accumulation
Missing piece

Inflammation?
Electrophysiological Dissection of Cystinosin

Raquel Ruivo¹, Gian Carlo Bellenchi², Giovanni Zifarelli³, Corinne Sagné¹, Xiong Chen¹, Cécile Debacker¹, Michael Pusch³, Stéphane Supplisson⁴, and Bruno Gasnier¹

¹Université Paris Descartes and Centre National de la Recherche Scientifique, UMR 8192, Institut de Biologie Physico-Chimique, 13 rue Pierre et Marie Curie, F-75005 Paris. ²Istituto di Biofisica e Genetica, CNR, Napoli, Italy. ³Istituto di Biofisica, CNR, Genova, Italy. ⁴Ecole Normale Supérieure, CNRS UMR 8544, Paris, France.

Lysosomal catabolites are exported to the cytosol by H⁺-driven transporters. In nephropathic cystinosis, cystine export is impaired by mutations affecting cystinosin, a lysosomal transporter harbouring two PQ-loop motifs and distantly related to archaeal rhodopsins. To characterize human cystinosin, we performed voltage clamp analysis of a sorting mutant misrouted to the plasma membrane. Application of cystine in acidic extracellular medium elicited an inward current and extracellular alkalinization consistent with a 1:1 H⁺/cystine symport. At mildly acidic pH, the $K_m$ for cystine increased exponentially with voltage, a property suggesting that cystine binding is coupled to protonation from the extracellular (lysosomal) compartment of a residue buried in the membrane. Mutational analysis unveiled three aspartates essential for transport. Voltage jumps applied to cystine-bound cystinosin triggered capacitive currents which required D305, but not D161 or D205, and were carried by extracellular protons. We conclude that cystine binding is coupled with protonation of D305 through a high-field access channel open to the lysosomal lumen. This residue, which is mutated in some cystinosis patients and conserved in 46% of PQ-loop proteins, may act as a relay site for H⁺ translocation.
Electrophysiological dissection of cystinosin

Whole-cell approach of cystinosin


Cystinosin activity induces an inward current

- (zwitterionic) cystine is co-transported with ions
- Transport is selective for cystine

Combined pH-out current and cystine uptake measurements

Cystinosin catalyzes a 1:1 H⁺/cystine symport
Summary of steady-state kinetic study

• $K_M$ for cystine depends on pH$_{in}$ and membrane potential in a complex manner

• Hypothesis: cystine binding is coupled to protonation of a residue buried in the membrane

• 3 conserved Asp are essential for transport

Voltage jumps reveal charge movements in cystine-bound cystinosin

Transient current analysis of the 3 essential Aspartates

Charge movements requires D305
Charge movements are carried by extracellular protons

1) Dependence of ‘titration curve’ on extracellular (lysosomal) pH

‘Titration curve’ of the charge movements

\[
Q_{\text{max}} \rightarrow V_{1/2} \left( \text{mV} \right) = -96.0 \text{ mV}
\]

\[
\begin{align*}
\text{Observed: } & \Delta V_{1/2} = -96.0 \text{ mV} \\
\text{Expected (Nernst, } \delta = 0.64): & \Delta V_{1/2} = -90.9 \text{ mV}
\end{align*}
\]

Charge movements are carried by extracellular protons

2) Transient currents are slowed down in D_2O (isotopic effect)

Conclusions

- cystinosin is a 1:1 H⁺/cystine symporter
- cystine binding is coupled to protonation at D305
- D305 is open to the lysosomal lumen through a ‘proton well’
- the well spans 65% of voltage gradient \( \rightarrow \) D305 may act as a relay site for H⁺ translocation
Implications, perspectives

- the position and role of D305 suggest that 2nd PQ-loop motif is implicated in transport mechanism
- TMs 2 and 3, and 1st PQ loop, may interact with TM6
- Adult, non-nephropathic mutation G197R lies within region affecting voltage dependence

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Cécile Debacker
Corinne Sagné
CNR Genova, Italy
Giovanni Zifarelli
Michael Pusch
Newborn Screening for Cystinosis

Sihoun Hahn, MD, PhD, Department of Pediatrics, University of Washington School of Medicine, Seattle Children’s Hospital, Seattle, WA, USA

Early diagnosis allows physicians to provide a proper treatment that, in majority, prevents patients from developing permanent organ damage, and at the very least encourages preventative measures which increase the patient’s quality of life significantly. Newborn screening is a prototype which has proven to be effective at reducing healthcare costs, improving outcomes, and avoiding long-term disability in affected children. Current newborn screening methods are able to identify only disorders in which abundant specific amino acids or small molecule metabolites accumulate in the serum. Cystinosis does not fall into this category because the accumulation of cystine is not measurable in blood spot samples. There is no method available yet for newborn screening for cystinosis.

MS/MS is currently used in most newborn screening laboratories as the platform to detect the amino acids and small molecule metabolites associated with inborn errors of metabolism. We hypothesized that we can extract low abundance proteins, such as cystinosin in dried blood spots. This central hypothesis has been formulated based on our preliminary data focused on ceruloplasmin, relatively abundant protein in serum and therefore, easily extractable from dried blood spots (deWilde et al., 2008). Our preliminary results indicate that peptides from cystinosin can be effectively screened by the method we developed. Cystinosin is localized in the lysosomal membrane; therefore it is present at a much lower concentration than many plasma proteins such as ceruloplasmin. Purified cystinosin is not readily available, so performing the actual trypsin digest to identify key signature peptides is not trivial. As a result, we have explored the use of in silico tryptic digestion as an approach to predict key signature peptides. The signature peptides candidates for cystinosin were further explored and confirmed that the sequences were unique within the human genome through BLAST search. Actin was investigated for use as an internal marker. We first investigated the candidate peptides on white blood cells. Three samples from patients with cystinosis were analyzed for signature peptide and compared with control samples. The cystinosin peptide was not detectable in two patients and one patient showed low cystinosin/actin ratio compared to control. Our result indicates the peptide analysis for cystinosis is feasible for diagnostic and screening test. A larger scale study is required to determine the detection rate with this methodology. In addition, the depletion of abundant proteins should be optimized for dried blood samples. Nevertheless, these tools have the potential to increase our capacity for early and rapid identification of many other genetic conditions advancing the use of proteomic methodologies, an area that holds great promise but is largely untapped.
Newborn Screening for Cystinosis

Sihoun Hahn, MD, PhD
Professor, Department of Pediatrics
University of Washington School of Medicine
Seattle Children’s Hospital
Seattle, WA

NEWBORN SCREENING

A Public Health Program
• Aimed at identification of conditions for which early intervention can prevent mortality, morbidity, and disabilities
• Performed by analysis of diagnostic markers in blood spots collected on filter paper at birth
• MS/MS is a standard method

WHO Criteria for Population Screening
• Latent stage
• Suitable test
• Test acceptable to population
• Agreement on whom to treat
• Natural history understood
• Cost effective
**IDENTIFICATION OF SIGNATURE PEPTIDES**

**Trypsin Digestion of Purified Ceruloplasmin**

Use Ion Trap and Q-TOF Mass Spectrometry to Identify Precursor Ions with High Homology to Ceruloplasmin

Identify the Precursor Ions with LC/MS/MS Using Purified Ceruloplasmin

Identify the Precursor Ions in a Complex Mixture of Peptides

Identify the Precursor Ions in the Dried Blood Spots of Newborns

**In Silico Trypsin Digestion of Cystinosin**

Select Signature Peptides Unique Within Human Genome

Choose Appropriate Daughter Ion for MRM experiment

Confirm Signature Peptides by Synthetic, isotopically labeled peptide

Optimization
**SELECTION OF TARGET PEPTIDES**

- FLVIR
- EQFLK
- LAVTLVK
- RPGYDQLN

**BRIEF METHOD**

- WBC pellets are dissolved in detergent (0.1% ProteaseMAX, Promega) to liberate cytoplasmic and membrane bound proteins
- Total protein concentration of an aliquot is measured using the Bradford Assay.
- Proteins are reduced with dithiothreitol
- Sample is digested at 37 °C, overnight, with trypsin
- Sample is dried under N₂ at 40 °C
- Dried peptides are re-suspended in a solution with 50 nM labeled peptides
Cystinosin 115-119 in WBC

Cystinosin 115-119

Patient Samples

<table>
<thead>
<tr>
<th>Compound</th>
<th>Patient 041</th>
<th>57kb del</th>
<th>Normal Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>heterozygote 57kb del and c.971-12G&gt;A</td>
<td>ND</td>
<td>0.2 pmol/mg</td>
<td>ND</td>
</tr>
<tr>
<td>and c.971-12G&gt;A</td>
<td>ND</td>
<td>0.2 pmol/mg</td>
<td>ND</td>
</tr>
</tbody>
</table>

Actin Peptides

Patient Samples

<table>
<thead>
<tr>
<th>Compound</th>
<th>Patient 041</th>
<th>57kb del</th>
<th>Normal Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>heterozygote 57kb del and c.971-12G&gt;A</td>
<td>0.2 nmol/mg</td>
<td>0.8</td>
<td>2.1</td>
</tr>
<tr>
<td>and c.971-12G&gt;A</td>
<td>0.2 nmol/mg</td>
<td>0.8</td>
<td>2.1</td>
</tr>
</tbody>
</table>
**Concentration of Cystinosin Peptide**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cystinosin pmole/mg protein</th>
<th>Actin nmole/mg protein</th>
<th>C/A ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control &gt;5 y/o</td>
<td>0.3 ± 0.18</td>
<td>0.4 ± 0.21</td>
<td>8.3 E±2 2.8 E±</td>
</tr>
<tr>
<td>Patient 041</td>
<td>Not Detected</td>
<td>0.2</td>
<td>N/A</td>
</tr>
<tr>
<td>Patient 042</td>
<td>Not Detected</td>
<td>0.8</td>
<td>N/A</td>
</tr>
<tr>
<td>Patient 045</td>
<td>0.8</td>
<td>2.1</td>
<td>3.8 E±</td>
</tr>
</tbody>
</table>

**BTK and WASP in lymphocyte cell line U937**

**SUMMARY**

- Cystinosin can be detected by peptide analysis in WBC
- Actin can be an useful internal marker for quantitation of cystinosin
- Cystinosin or Cystinosin/Actin ratio is altered in patients with cystinosis
- Our method can be applied to other genetic conditions such as primary immune deficiency
- A larger scale study is required to determine the detection rate with this method
- Further optimization on blood spot samples with depletion of abundant protein is necessary
ACKNOWLEDGMENT

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  – Bill Gahl, MD, PhD
Presentation #6

Availability of Cysteamine and Leucocyte Cystine Assays in Less Well Developed Countries – Brazil

Vera H. Koch, MD, Hospital das Clinicas of the Sao Paulo Medical School, Sao Paulo, Brazil

Presentation Unavailable at time of printing.
Presentation #7

Cystinosis in Egypt: Availability of Cysteamine and Leucocyte Cystine Assay

Neveen A. Soliman, MD, Cairo University, Cairo, Egypt

In developing countries overwhelmed by common and endemic diseases, the care for rare disorders is considered a luxury which the country can not afford. Nevertheless, patients and families living with cystinosis in Egypt have the right to medical care and access to treatment just as equal to those afflicted with more common illnesses. Egyptian Group for Orphan Renal Diseases (EGORD), as a rare renal disease advocate, believes that these hurdles should not be viewed as insurmountable because there are compelling social and ethical reasons to address the needs of patients with rare diseases. Sponsoring cystagon therapy is still an unsolved issue in our country. Efforts are building up to establish Leucocyte cystine assay in Egypt. Pediatric nephrologists, geneticists, chemical pathologists, patients & families, health policy making authorities, and society are partners in the ongoing challenge of caring for cystinosis in Egypt, noting that the impact of rare disease should not be measured by numbers alone.
Cystinosis in Egypt:
Availability of Cysteamine and Leucocyte Cystine Assay

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15th Congress of IPNA, New York 2010

Cystinosis
How did we manage to start caring for it?

Egyptian Group for Orphan Renal Diseases (EGORD):

- National health group
- An adoptive parent for patients with rare renal diseases
- Established as a group of the Egyptian Society of Pediatric Nephrology & Transplantation (ESPNT)

EGORD is committed to the identification, and treatment of rare renal disorders through programs of:

- Awareness
- Support
- Education
- Research
- Advocacy.
Nephropathic Cystinosis

Nephropathic cystinosis is a rare genetic disease characterized by defective lysosomal cystine transport and increased lysosomal cystine. Corneal Cystine Crystal Scoring (CCCS) for diagnosis of nephropathic cystinosis was studied in all suspected children with renal Fanconi syndrome and siblings of diagnosed cases over a two year period. In addition to oral cysteamine, cysteamine eye drops were provided to all diagnosed patients and CCCS was followed up on a quarterly basis. Of 33 screened cases, 14 had corneal cystine crystals. Crystals were absent in two cystinotic patients under the age of 20 months. The mean age at diagnosis was 52.7 months and five patients had ERSD. After six months of treatment, the mean CCCS did not increase from the initial value of 1.81; associated with a decrease of 0.5 in two cases and a similar increase in two others. Scores decreased in two other patients after 12 months. Compliance was generally inadequate due to the high frequency of administration and the need for multi-drug regimen. CCCS is a simple and reasonably sensitive method for diagnosis of nephropathic cystinosis above two years of age. Topical treatment with cysteamine eye drops prevents progression of deposits and may decrease it with adequate compliance. Further follow up is still recommended to monitor long term effects of both systemic and topical cysteamine therapy.

PMID: 19414947


Soliman NA, El-Baroudy R, Rizk A, Bazaraa H, Younan A.

Center of Pediatric Nephrology and Transplantation, Cairo University, Egypt.

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Challenges?

- Time is our enemy:
  - Promotion of awareness for early diagnosis
  - WBC Cystine assay
  - Identification of affected families

- Lifetime treatment
  - Finance:
    - Sponsoring body
    - Orphan Drug Act
    - Cysteamine eye drops
    - Cystagon
  - Drug compliance & counselling:
    - Education & support
• Difficult cases:

• Limited number of patients:
  Multicenter research studies

... out of the darkness into the light...
• Pediatric nephrologists
• Geneticists
• Patients
• Health policy making authorities
• Society are partners in the ongoing challenge of caring for rare disorders, noting that the impact of such diseases should not be measured by numbers alone!
EGORD Team

Thanks

Cystinosis Research Network

NIH, Bethesda
W. Gahl
I. Bernardini

The Cystinosis Foundation

H. Bazaraa, M. Nabhan, H. Aziz, R. Aly, R. Hassan

Bambino Gesù Children's Hospital, Rome
F. Emma
A. Taranta

Thank you
Presentation #8

Availability of Cysteamine and Leucocyte Cystine Assays in Turkey

Dr. Rezan Topaloglu, Hacettepe University, Faculty of Medicine, Department of Pediatric Nephrology, Ankara, Turkey

Cystinosis is an autosomal recessive disease characterized by intracellular cystine accumulation due to impaired cystine transport across the lysosomal membrane. It is a rare disease with the estimated incidence of 1 case in 100,000-200,000 live births. Three clinical forms of cystinosis have been described based on the severity of symptoms and the age of onset. Patients with the most common and the most severe infantile form develop renal Fanconi syndrome in infancy and renal failure in the first decade. Other organs frequently affected are cornea and thyroid causing painful photophobia and hypothyroidism respectively. Furthermore, myopathy, swallowing dysfunction, pulmonary dysfunction, diabetes, male hypogonadism, and central nervous system involvement seen in adolescence and young adults with nephropathic cystinosis. Accurate measurement of intracellular cystine content is necessary for the diagnosis and monitoring of treatment with cysteamine. Different methods have been studied and developed to measure cystine content in leukocytes. Lately, cystine binding assay liquid chromatography-tandem mass spectrometry (LC-MS/MS) has been used in reference labs.

In terms of cystinosis and Turkey although expected number of patients may be more than this figure, we were able to reach 85 patients from all over Turkey for the 1st International Cystinosis Conference in East Mediterranean held in Turkey in 2009. As in other parts of the world in general we noticed that there is substantial delay between onset of symptoms and confirmation of the diagnosis of cystinosis and also insufficient monitoring of the treatment. The main reasons for these observations are: cystinosis is being a rare disease, lack of knowledge among the patients, parents and primary care physicians. Furthermore, leucocyte cystine measurement is not routinely performed in Turkey even the presence of the knowledge and technology.

In our center we are able to perform the test time to time but not the steady state basis. The major factor for this drawback is; financial problems to obtain consumables, maintain of the equipment and the short of technical assistance. We aim to overcome the challenges and be able to run the assay on the steady state regular basis.

Treatment and availability of the drug which is not problem anymore. Although Orphan Europe does not have headquarters in Turkey, Orphan Europe is supplying Cystagon through a distributor company “Med Supplies”. Med Supplies Company has all the necessary permission from Turkish Ministry of Health and the Turkish Union of Pharmacists. On the other hand, supplying and reaching the eye drops is being always problem.

All of above we strongly believe that continuous training and cooperation with patients, the families and primary care physicians is a great help for the patients suffering from Cystinosis.
Availability of Cysteamine and Leucocyte Cystine Assays in Turkey

Rezan Topaloglu, MD
Hacettepe University Faculty of Medicine
Department of Pediatric Nephrology
Ankara Turkey

Cystinosis in Turkey

Although expected number of patients may be more than this figure, we were able to reach 85 patients from all over Turkey for the 1st International Cystinosis Conference in East Mediterranean

Problems and difficulties
Diagnostic difficulties

- Substantial delay between onset of symptoms and confirmation of the diagnosis of cystinosis
- rare disease
- lack of knowledge, awareness
- leukocyte cystine measurement

Leukocyte cystine measurement

- Leucocyte cystine measurement is not routinely available at medical centers in Turkey
- Some private labs collaborating with the international labs but very expensive for the patients

Hacettepe Experience

- Leucocyte cystine level could measured at our metabolic diseases lab by cystine binding assay with the use of ion exchange high performance liquid chromatography

High cystine in platelets from patients with nephropathic cystinosis: a chemical, ultrastructural, and functional evaluation

Hacettepe Experience
The test could not routinely performed due to
- Rare disease
- Costly test
- Time consuming
- Lack of consumables due to financial reasons
- Short of technical assistance
- Near Future aim to be able to measure by LC-MS/MS

Problems in monitoring
- Monitoring cystinosis therapy: regular measurement of leucocyte cystine level every 3 month is required
- In our experience not being able to monitoring at regular basis is causing poor management and poor outcome

Treatment
- Availability of the drug which is not problem anymore Orphan Europe and the distributor company Med Supplies
- Social Security problems
- Long Procedures to get the drug (Turkish Pharmacist Union)
  Usually lack of eye drop
Near Future aim to be able to measure Leukocyte cystine level by LC-MS/MS for both diagnosis and monitoring

What we can do more

- Training and cooperation
  - Patient and the family
  - Primary care physicians, Pediatricians
  - High index of suspicion in infants presenting with dehydration, RTA, rickets, failure to thrive
  - Adult doctors
- Achieve better compliance
  - Diminish side effects of the drug
- Transition and psychological support
- Practical approach to diagnose earlier
- Further investigations
Thank you
Presentation #9

Tacules Pitfalls and Problems Founded in the Cysteamine Availability and in the Implementation of a Methodology for Cystine Determination in Leukocytes by High Performance Liquid Cromatography in Mexico

Laboratorio de Errores Innatos del Metabolismo y Tamiz
Instituto de Investigaciones Biomédicas UNAM-Instituto Nacional de Pediatría SS

INTRODUCTION:

Mexico is a developing Country and epidemiological priorities of infant care are mainly focused on infectious diseases and cancer. Inherited metabolic disorders (IMD) are considered not important by the authorities, so they are almost neglected diseases and as a consequence the knowledge and research on this area is poor. Since 2002 a group of physicians heading by the author got interested in establishing a research line for the study of cystinosis with the main objective of give a better quality of life for the patients with cystinosis creating an integral model of care for this disease which includes the consultation with specialists like nefrologists, endocrinologists, gastroenterologists, ophthalmologists, genetists and others as well as the inclusion of mental health evaluation and social issues. Evidently one of the first concerns in the establishment of the model was to count with an intraleukocitary cystine determination methodology for the diagnosis monitor treatment. On December 2008, the CRN generously gave a financial grant in order to implement a methodology for cystine determination.

OBJECTIVE: To present the advances in the model of attention of patients with cystinosis, the cysteamine availability and obstacles and pitfalls in the implementation of the methodology for cystine determination in Mexico.

RESULTS: 1) Advances in the model of attention, after an intensive work of diffusion and medical training we have increased the clinical awareness on physicians, especially pediatricians, nephrologists, ophthalmologists and geneticists, as a result we have observed that physicians are suspecting the disease early. On the other hand we have established a network with physicians from other health institutions for information, counseling, detection, evaluation of the patients. One of the obstacles for the adequate operation of the model has been that as the model was created in a pediatric institution it has been difficult to get the attention of the adult patients. 2) Thanks to the efforts made by the Asociación Mexicana de Cistinosis we have got that all our patients receive the Cysteamine by donor program. However, the drug is not available neither registered in our Country. Actually social security systems neither the governmental institutions do not pay the treatment. 3) With the CRN donation we got the reagents for the cystine determination in leukocytes by HPLC but the reduction and carboxilation reactions carried for the analysis were unstable which caused imprecision of the analytical methodology and the detection limit obtained was not low enough to diagnose patients according with the reference values reported on the literature. This situation coincided with moving to new installations of our laboratory in which we have a tandem mass spectrometry (MSMS) equipment so we are considering migrating from HPLC to MSMS for this determination as last reports on the literature indicates.

CONCLUSIONS. We have created The cystinosis model of attention. We require that the cysteamine be introduced on the COFEPRIS registry in order to get it available in the Country. All the Mexican federal institutions are governed by a very bureaucratic administrative system which makes all the processes very low including the input purchase. On the other hand the dollar exchange rate affects directly the Mexican peso value and the costs elevate. On the other hand the lows for importation of chemical substances is very strict, this causes delays in the availability of reagents in the research field.
Presentation #10

Availability of Cysteamine and Leucocyte Cystine Assays in Less Well Developed Countries – Macedonia

Velibor Tasic, MD, PhD, University Children’s Hospital, Skopje, Macedonia

Presentation Unavailable at time of printing.
Presentation #11

Early Management of Cystinosis and Fanconi Syndrome

Paul Goodyer, MD, Montreal Children's Hospital, Montreal, Canada

Presentation Unavailable at time of printing.
Dosing Cysteamine

Jess G. Thoene, M.D.
University of Michigan
Pediatric Genetics

Cysteamine Structure

\[ \text{H}_2\text{N} - \text{SH} \]

Dose schedule

- Initial Dose:
- 10 mg/kg/day for 2 weeks
- Increase by 10 mg/kg/day every 2 weeks
- Obtain WBC cystine level after patient stable on 60 mg/kg/day for at least 2 weeks
Why?

• Initial patients started on 60 mg/kg/day developed lethargy, rash, hyperthermia, and in one case, a Stevens-Johnson-like syndrome
• By analogy to what was then in use in penicillamine treatment, the tapered dose was adapted with many fewer acute side effects

Frequent side effects:

• Nausea
• Vomiting, particularly in early am
• Lethargy
• Allergic rash
• Increased gastric acidity
• Halitosis

Infrequent side effects

• Seizures (rare)
• Neutropenia (rare)
Effect of cysteamine in preserving renal function

Treatment of Newborns and Adults

- Newborns: No adjustment of dose from that described

- Adults: Dose calculation is changed to 1.3-1.9 g/m² at above 40 kg
- Maximum recommended dose is 2.0 g/day
Fetopathy in the rat

- Cysteamine produced dose dependent adverse effects on the fetus during organogenesis and histogenesis. Specific malformations can be associated with this effect (cleft palate, kyphosis, vertebral anomalies), as well as IUGR and fetal death.
- Neither 37.5 nor 75 mg/kg/day produced a significant increase in adverse effects on the fetus. While adverse effects were seen in the 100 mg/kg/day group, the most severe effects on fetal outcome were seen in the 150 mg/kg/day group, in which there was no apparent maternal toxicity.

Treatment during pregnancy

- Listed as Class C by US FDA
- No controlled trials to determine safety during pregnancy
- Most practitioners cease treatment during pregnancy
- Given long course of disease, potential risk to mother is outweighed by risk of dysmorphology in fetus

Dose change during renal failure

- What does one do when patient is in renal failure?
- Decrease dose?
- When?
- How much?
- Continue during dialysis
- D/C peri-transplant
Possible answers

- Monitor plasma cysteamine
- Monitor WBC cystine and titer dose to maintain reasonable cystine depletion, anticipating that as renal failure proceeds, half life of cysteamine may increase, magnifying drug effect and permitting a decrement in dose
- Other ideas?
Dosing of Cysteamine in Children and Adults; Treatment of Newborns and Pregnant Women

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The practice of cysteamine administration to infants and children is well established: Begin at a dose of 10 mg/kg/d po divided every 6 hours, and increase the dose by 10 mg/kg/d every two weeks until a dose of 60 mg/kg/d is reached. At that time measure the WBC cystine. If is is < 1.0 nmol/mg protein measured 6 h after the last dose, maintain at that level and follow WBC cystine every three months, making adjustments in dose as required by weight gain.

When patients approach 40 kg, the dose is recalculated using an initial dose of 1.3 g/m2/day because maintaining the dose of 60mg/kg/ per day at body weights greater than approximately 40 kg leads to increased side effects. As the disease has permitted growth to adulthood, the number of patients taking the maximum recommended dose (2.0 g/day) has increased.

Use in pregnancy has not been studied systematically. Most physicians caring for pregnant cystinosis patients interrupt treatment during pregnancy because Cystagon is classified as a Category ‘C’ drug by the USFDA, and dysmorphology has been seen in the offspring of experimental animals given high doses of cysteamine during gestation.

A difficult question to answer in the absence of data is what to do when the patient reaches ESRD. At low GFR the blood cysteamine content is expected to increase and seizures have been observed. When to diminish the dose, and by how much, and when to discontinue the drug are open questions. If dialysis is initiated, then if near normal clearance is maintained, cysteamine may be continued, using either plasma cysteamine concentration (if available) or WBC cystine, as a surrogate marker to assess the necessity for dose adjustment. Many centers discontinue the drug peri-renal transplant to avoid interference with engraftment.

After successful renal transplantation, cysteamine must be re-initiated to avert the long-term side effects of the disease: diabetes, pulmonopathy, peripheral neuropathy, esophageal dysmotility.
Presentation #13

Mouse Models for Studying Cystinosis

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Presentation Unavailable at time of printing.