Pediatr Nephrol
DOI 10.1007/s00467-008-0943-6
© IPNA 2008

ABSTRACTS

First International Cystinosis Research Symposium

Jerry A. Schneider* and Ranjan Dohil*, Symposium Co-Chairs

Cystinosis is an autosomal recessive disorder with an estimated incidence of 1 in 100,000 to 200,000 live births. In nephropathic cystinosis, free cystine accumulates in lysosomes, eventually causing damage to most body tissues. Major advances in our understanding of cystinosis in the past quarter-century include finding that cystinosis is caused by a defect in cystine egress from lysosomes in 1982, the approval of Cystagon™ by the FDA in 1994 and mapping the defective gene, *CTNS,* to chromosome 17p13 in 1995 and cloning this gene in 1998. In the last ten years it has become increasingly difficult to obtain funding from government agencies to study rare diseases. This was especially true for a disease like cystinosis where so much is already known. Thus, support from private groups has been essential for continuing studies of this disorder.

The Cystinosis Research Foundation was formed in 2003 by parents of a cystinosis patient and since then this foundation has provided over $5 million for cystinosis research. On April 3 and 4, 2008 the Cystinosis Research Foundation sponsored an International Cystinosis Research Symposium at the Arnold and Mabel Beckman Center of the National Academies of Sciences and Engineering in Irvine, California. Most of the researchers who presented at the Symposium have received significant financial support from the Cystinosis Research Foundation.

Over 40 investigators from the United States, France, Germany, England, Italy, Belgium and the Netherlands attended. Some of these investigators have been studying cystinosis for many years; others just received funds to study cystinosis and presented their plans, rather than their results. A major goal of the Symposium was to promote collaborations between investigators who are studying cystinosis in order to hasten progress in understanding and treating this disease. Future research advances will determine the value of this approach.

*Department of Pediatrics, University of California, San Diego. Both JAS and RD are consultants to Raptor/Bennu Pharmaceuticals, Inc.

SCIENTIFIC PROGRAM

HISTORY (HI)

1. History of Cystinosis in the United States	Jerry A. Schneider, MD
2. History of Cystinosis in Europe. Experience at .Necker Hospital: Outcome after age 20y	Michel Broyer, MD
3. Cystinosis: Insights into an Adult Lysosomal Storage Disorder	Robert Kleta, MD, PhD

METHODOLOGY (ME)

1. Tandem Mass Spectrometry Cystine Determination	Jon Gangoiti, PhD Bruce Barshop, MD
2. Methodology in Clinical Cystinosis Studies	Meredith Fidler, PhD

CYSTEAMINE (RX)

1. History of Cysteamine in Cystinosis	Jess Thoene, MD
2. Enteric-Coated Cysteamine in Cystinosis	Ranjan Dohil, MD
3. In Vivo Confocal Microscopy to Detect Cystine Crystals in the *Cnts -/-* Mouse.	James V. Jester

GENETIC THERAPY (GT)

1. Treatment of Cystinosis Nephropathy with Adult Stem Cells in Murine Cystinosis Model	Stephanie Cherqui, PhD Daniel Salomon, MD
2. Gene Transfer Studies for Cystinosis	Claire Hippert (Trainee) Vasiliki Kalatzis, PhD (mentor) Eric J. Kremer, PhD (mentor)
3. Parthenogenetic Embryonic Stem Cells as a Source of Immunocompatible Renal Progenitor Cells for Therapy of Nephropathic Cystinosis	Holger Willenbring, MD
4. Targeted Cell Fusion for the Correction of Tubulopathy Due to Cystinosis	Silvia Espejel Carbajal, PhD (trainee) Holger Willenbring, MD (mentor)

NEUROLOGICAL (NE)

1. Cognitive Domain of Executive Functioning . in Cystinosis	Angela Ballantyne, PhD
2. Auditory and Visual Processing in Cystinosis	Rita Ceponiene, MD, PhD

MITOCHONDRIA (MI)

Project	Investigator(s)
1. The Biology and Genetics of Mitochondrial Disease and Their Relationship to Cystinosis	Douglas Wallace
2. Mitochondrial Dysfunction in Cystinosis Myopathy	Doris Trauner, MD
3. Lysosomal Cystine Enhanced Apoptosis in Cultured Human Cells	Jess Thoene, MD
4. Molecular and Pathogenic Study of Cystinosis	Sha Tang, PhD (trainee) Taosheng Huang, MD, PhD (mentor)

TRANSPORT (TR)

Project	Investigator(s)
1. Characterization of cystinosin intracellular trafficking	Corinne Antignac, MD, PhD
2. Identification and Characterization of the Lysosomal Transporter in Cysteamine Mediated Cystine Efflux	Bruno Gasnier, PhD Ellen Closs, PhD

PATHOLOGY (PA)

Project	Investigator(s)
1. The Pathogenesis of Renal Disease in Cystinosis	Elena Levtchenko, MD, PhD
2. Cysteamine Effects on Extracellular Matrix Accumulation in Chronic Kidney Disease	Allison Eddy, MD

GENES (GN)

Project	Investigator(s)
1. Complex Genetic Approaches to Monogenic Disease: Genomic and Transcriptomic Dissection of Normal Expression of *CTNS*	Katy Freed, PhD (trainee) Eric Moses, PhD (mentor)
2. Transcriptional and Post-transcriptional Regulation of the *CTNS* Gene	Francesco Emma,MD Anna Taranta, PhD Elena Levtchenko, MD, PhD

DERM (DE)

Project	Investigator(s)
1. Molecular Mechanisms of Hypopigmentation in Cystinosis	Christine Chiaverini, MD (trainee) Robert Ballotti, PhD (mentor)

YEAST (YE)

Project	Investigator(s)
1. Yeast Model for Cystinosis	Seasson Phillips, PhD (trainee) David Pearce, PhD (mentor)

HI 1

Cystinosis: crystals to genes

Jerry A. Schneider
Department of Pediatrics, University of California,
San Diego, CA

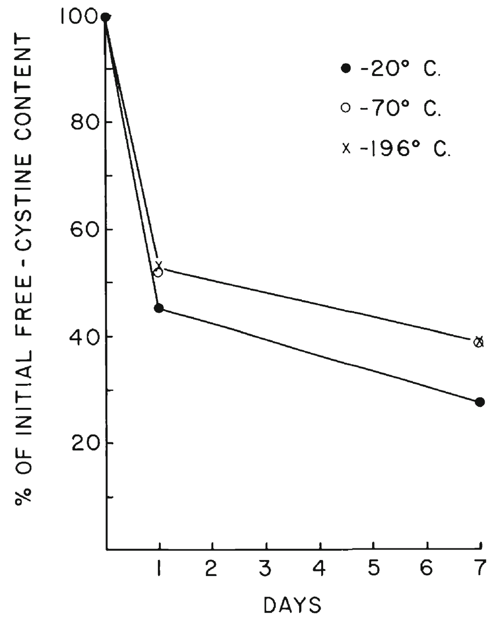
In my opening address I will review advances in cystinosis research over the past 25 years. In this abstract I will discuss the importance of accurate cystine measurement, including sample preparation, for the diagnosis, treatment and study of cystinosis.

In the past, biochemical studies of cystinosis were handicapped by scientists' inability to measure cystine. This changed in the 1960s with the development of ion-exchange chromatography. In 1974, Oshima described a cystine assay based on a specific cystine-binding protein. This became the method of choice for measuring cystine. In the 1990s, many laboratories described high-performance methods to measure cystine, and in 2004 Dalton and Turner described an double-tandom mass spectrometry assay that was as sensitive and accurate as the binding assay. Dalton summed up the situation at the 3rd International Cystinosis Conference in 2004, "There are many ways to accurately assay cystine, the critical step is how you prepare the sample."

When planning our first clinical trial of cysteamine, we knew we would have to enroll patients from many centers and accurately measure their leukocyte cystine levels in response to therapy. We attempted to develop a method to have heparinized blood shipped to us for leukocyte preparation and assay. We were unsuccessful. After 24 h the results were very erratic regardless of temperature. Thus, we required the collaborating centers to prepare the leukocytes shortly after the blood was drawn, acidify the cells to stabilize the cystine and then ship the sample to us on dry ice. We sent the referring laboratories a kit with the necessary reagents and instructions for preparing the cells. Our Cystine Determination Laboratory continues to require this procedure.

I am concerned that some laboratories in the USA are now accepting heparinized blood 24–48 h after it is drawn and reporting the leukocyte cystine value. I fear that mistakes will be made. At this Symposium Dr. Fidler will report her recent experience in storing whole blood for 24 h before leukocyte isolation.

Another concern I have is that some cystine determination methods consist of immediately freezing the sample in liquid nitrogen before acidifying it, and then preparing the sample for assay at a later date. Many years ago we reported that this procedure led to significant loss of cystine in cystinotic fibroblasts.



(from Biochem Med 10:368, 1974)

HI 2

Outcome of cystinosis after 20 years of age. A study of the Enfants-Malades series

Michel Broyer, MD, Marie-Jo Tête
Geneviève Guest, and Patrick Niaudet, Paris, France

Records from 56 patients born before 1988 and alive at 20 years of age were retrieved for this series. All patients had infantile nephropathic cystinosis and a documented mutation in the *CTNS* gene. The aim of this study was to report the latest status of these patients. Considering the fact that those born after 1980 started

cysteamine before the age of 3 years, which was not the case of those born before 1980, the patients were classified according to their year of birth into two cohorts: those born before 1980 (39) and those born thereafter (17). On December 31, 2007, 38 patients aged 20–39 years were alive, and 18 had died between 21 and 40 years of age. The cause of death was: encephalopathy (nine), cerebral hemorrhage (one), infection (four), Acquired Immunodeficiency Syndrome (AIDS, one), car accident (one), porto-caval encephalopathy (one) and unknown (one). No deaths had occurred in those patients born after1980.
Results.

1) The *evolution of renal function* was different according to the year of birth. For the 39 patients born prior to 1980, the mean age at end-stage renal disease (ESRD) was 9.6 years (range 6–14 years) versus 17.4 years (7–21 years) for the 12 patients born after 1980 who reached ESRD, while five additional patients of the latter cohort were still on conservative treatment at a mean age of 23.5 years (20–27 years) with a plasma creatinine of 80–290 µmol/l (=0.9–3.3 mg/dl).
2) *The stature* was also different in the two cohorts: the males born before 1980 had a mean height of 144.5 cm (123–158 cm) versus 167 cm (157–175 cm) for those born thereafter; the females born before 1980 had a mean height of 132 cm (111–153 cm) versus 153.5 (147–158 cm) for those born after.
3) *Eye involvement*: the last visual acuity was obviously better in those born after 1980, with a mean score of 8.5 (6–10), versus those born before 1980, with a mean score of 5 (0–10). Photophobia was noted in both cohorts but corneal grafts were performed in only 3 patients, all born < 1980.
4) *Thyroxine* was prescribed at the mean age of 11.6 and 12.6 years in those born before and after1980, respectively,, which is not significantly different, but the number of patients not requiring thyroxine was four of 34 in the first group and nine of 14 in the second group.
5) *Gonadal functions* were not assessed in a systematic way, but four males in the cohort born before 1980 received testosterone versus none in the post-1980 cohort. Two female patients became pregnant: one born in 1978 stopped cysteamine and gave birth to a normal baby now 5 years old; the other one, born in 1982, decided not to stop cysteamine and voluntarily aborted.
6) *Glucose intolerance* (GI) was investigated in 33 of the patients born before 1980 and was found in 29 of these patients at a mean age of 15.7 years (8–29 years); however, four of the patients of this cohort had no GI at last examination at ages 21–40 years. Of 11 patients born post-1980 who were evaluated, only two had GI at 11 and 17 years, respectively, while the nine others did not have GI at the last examination (age 20–27). Diabetes requiring insulin was observed in nine patients, all in the cohort born prior to 1980, with a mean time delay of 6.9 years (1–15 years) after the discovery of the GI.
7) *Encephalopathy*, as defined by at least one of the following symptoms, cerebellar s., pyramidal s., or mental deterioration, occurred in 14 patients, all born prior to1980.
8) *Myopathy*, as defined by oropharyngeal dysfunction and/or distal muscle atrophy, was observed in 15 patients, all born prior to1980.
9) *Hepatosplenic involvement*: of the 36 documented patients born prior to 1980, 45% had portal hypertension and 54% were splenectomized. None of the patients born after 1980 had such problems.

In conclusion, in this series the year 1980 appears to be a crucial one for children born with cystinosis, with a clear impact of starting cysteamine treatment before the age of 3 years on renal impairment and the development of extra renal complications.

HI 3

Cystinosis: insights into an adult lysosomal storage disorder

Robert Kleta[1] and William A Gahl[2]
[1]University College London, London, UK,
and [2]National Institutes of Health, Maryland, MD

Cystinosis is an autosomal recessive lysosomal storage disorder affecting children and adults all over the world. In cystinosis, cystine is trapped in the lysosomal compartment due to a defect in its egress transport protein cystinosin encoded by the gene *CTNS*. By mechanisms still not fully understood, this leads to tubular and glomerular kidney failure and deterioration of other organs (e.g., thyroid, muscles) if left untreated.

Kidney involvement is prominent from shortly after birth as renal tubular Fanconi syndrome, with the clinical consequences of failure to thrive and rickets. Cystinosis is the single most common cause of renal Fanconi syndrome in childhood. Therefore, any recognition of renal glucosuria, generalized aminoaciduria, phosphaturia, small molecular weight proteinuria, polyuria and metabolic acidosis (due to renal bicarbonate loss) should lead to prompt consideration of cystinosis as a possible cause. This can be done utilizing biochemical analytical methods measuring the cystine content of polymorphonuclear leucocytes. Corneal cystine crystals, seen on slit lamp examination, are pathognomic as well, but may not be visible before 16 months of age. Left undiagnosed and untreated, patients will develop, in addition to the existing tubular insufficiencies, pronounced impairment of glomerular function. Such kidney damage is often present at diagnosis and inevitably leads to end-stage renal failure, typically at the end of the first decade of life. Kidney failure in cystinosis presents differently from other forms of renal insufficiency because of the overlap of tubular and glomerular involvement. Kidney transplantation will be curative with respect to both renal tubular and glomerular function.

Cysteamine treatment, which reduces intracellular cystine stores in cystinosis patients, was introduced in the 1970s and approved in the 1990s.

The full burden of nephropathic cystinosis in adulthood and the effects of long-term oral cysteamine therapy on its nonrenal complications have not been systematically investigated. Therefore, we assessed the severity of cystinosis in adults receiving and not receiving oral cysteamine therapy. One hundred persons (58 men and 42 women) age 18 to 45 years with nephropathic cystinosis were examined between January 1985 and May 2006 at the National Institutes of Health. Historical data were collected on renal transplantation, administration of oral cysteamine and time and cause of death. Patients were evaluated for: height and weight; thyroid, pulmonary, and swallowing function; muscle atrophy; hypogonadism (in men); retinopathy; vascular and cerebral calcifications; diabetes mellitus; homozygosity for the common 57-kb deletion in *CTNS*. Laboratory studies were also performed.

Of the 100 adults with nephropathic cystinosis, 92 had received a renal allograft and 33 had died. At least half of the patients had hypothyroidism, hypergonadotropic hypogonadism (in men), pulmonary insufficiency, swallowing abnormalities or myopathy. One third of the patients had retinopathy or vascular calcifications, and 24% had diabetes. Homozygosity for the 57-kb *CTNS* deletion was associated with an increased risk for death and morbidity. The 39 patients who received long-term (>8 years) oral cysteamine therapy were taller and heavier, had a renal allograft later in life, had lower cholesterol levels, and experienced fewer complications and deaths than patients who

received cysteamine for fewer than 8 years. The frequency of diabetes mellitus, myopathy, pulmonary dysfunction, hypothyroidism, and death increased as time off cysteamine treatment increased, and it decreased as time on cysteamine therapy increased.

Untreated nephropathic cystinosis causes extensive morbidity and death in adulthood. Long-term oral cysteamine therapy mitigates these effects; early diagnosis and diligent treatment can prevent or ameliorate major organ complications. We conclude that diligent treatment with oral cysteamine to lower the intralysosomal cystine content of cells is as important after kidney transplantation as it is before transplantation to prevent or attenuate organ failure in cystinosis patients.

ME 1

Mass spectrometric analysis of cystine dynamics

Jon A. Gangoiti and Bruce A. Barshop
Department of Pediatrics, University of California San Diego, San Diego, CA

We have been developing tandem mass spectrometric applications for the analysis of cystine metabolism in cystinosis. The specific applications have been in the pharmacodynamics of cysteamine and cystamine and leukocyte cystine determination, and we are moving on to study intracellular dynamics of related intermediates.

We developed a method for the quantitation of cysteamine by liquid chromatography–tandom mass spectrometry (LC–MS/MS), using 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB) to generate adducts of endogenous cysteamine and 2,2-dimethyl-ethane aminothiol added as an internal standard and then monitoring the loss of the resulting 230 amu moiety in the tandem mass spectrometer collision cell [i.e. multiple reaction monitoring (MRM) experiments with the transitions *m/z* 274.8→229.8 for cysteamine and *m/z* 302.8→229.8 for the internal standard]. This method was applied to define pharmacokinetics and pharmacodynamics in patients given: (1) cysteamine delivered into different sites along the alimentary tract (compare Fidler et al., Br J Clin Pharmacol, 2006, and Dohil et al., J Pediatr, 2006), and (2) different formulations of cysteamine, including the conventional pharmaceutical product and an encapsulated time-release formulation. Further development, in order to be able to detect and distinguish cystamine as well as cysteamine, required an alternate strategy which did not involve disulfide tagging. That method utilized $^{2}H_4$-ethanolamine as an internal standard and isocratic hydrophilic interaction liquid chromatography (HILIC). The MRM allows quantitation of cysteamine (*m/z* 78.2→61) and cystamine (*m/z* 153.1→108) relative to $^{2}H_4$-ethanolamine (*m/z* 66.2→48.1). Plasma analyzed with and without the addition of the reducing agent tris(2-carboxyethyl) phosphine (TCEP) indicated that a very high proportion of plasma cysteamine is acid-precipitable. We observed an extremely rapid and quantitative conversion of cystamine to cysteamine in vivo. Though this conversion limits the applicability of this method to measure cystamine, the measurement of plasma cysteamine by HILIC–high-performance liquid chromatography–MS/MS was straightforward, accurate, and suitable for pharmacokinetic studies.

We adapted the methodology of Dalton and Turner (Abstracts of the 3rd International Cystinosis Conference 2004, J. Inher. Metab. Dis. 2005) to analyze leukocyte cystine, using stable-isotope dilution with deuterated $^{2}H_4$-cystine as the internal standard and a Teicoplanin (Chirobiotic T) column to prevent ion suppression. Cystine and $^{2}H_4$-cystine are measured using MRM experiments for the transitions *m/z* 241.1→152.1 and *m/z* 245.1→156.1 respectively. Precision and reproducibility were established (average intra-assay repeatability 4.70%, average inter-assay repeatability 6.23%; for three concentrations ranging from 0.12 to 3.3 μ*M*). Accuracy was assessed by the analytical recovery, which was 104 ± 0.07% for nine concentrations ranging from 0.02 to 10 μ*M*. Linearity was demonstrated over the range 0.02 to at least 4 μ*M*. The lower limit of quantitation (LLOQ, determined by variation <15%) was found to be 0.005 μ*M*. The agreement of results using this method and the standard cystine binding assay was excellent ($r^2 = 0.9706$) with no variance bias at low or high concentration levels. The method was validated according to the guidelines of the College of American Pathologists. The run time is 3 min, and injection-to-injection time is 3.5 min.

The LC–MS/MS method has allowed a much higher throughput and many more assays to be performed, permitting us to expedite several cystinosis research projects. One such application is verification of the simplified methods of leukocyte isolation to reduce the demands at the point-of-care. We have demonstrated that sample shipment may be facilitated from remote locations where dry ice and express courier services are not affordable, by placing isolated leukocyte extracts onto bloodspot cards, such as those used in newborn screening worldwide. In addition to the straightforward studies with various anticoagulants, we have plans to study various agents which may stabilize lysosomes. We also can use isotopically-labeled cystine, cysteamine, and other related compounds to follow the dynamics of lysosomal release. Preliminary work is underway with the dimethyl ester of 3,3'-$^{13}C_2$-cystine to load lysosomes of cystinotic cells and then examine the flux of the carbon label through the glutathione cycle. Conversely, we can use $^{2}H_4$-cysteamine to examine the intracellular behavior of cysteamine, which could reflect its stoichiometry in the process of lysosomal egress and could indicate whether cysteamine re-enters the lysosome.

Further application of mass spectrometry to cystinosis research should come from a more comprehensive approach to analysis, including not only sulfhydryl compounds, but all those whose metabolism is perturbed by cystinosis. A global analysis of a myriad of compounds is feasible with mass spectrometry; we propose that an untargeted metabolomic approach could find new biomarkers and give new insights into metabolic ramifications of cystinosis, unravel the complexities of the disease, and explain the complex clinical phenotypes.

ME 2

Methodology in clinical cystinosis studies

Meredith Fidler, PhD
Department of Pediatrics, University of California San Diego, San Diego, CA

Leukocyte cystine levels are used to diagnose cystinosis and monitor patients' Cystagon therapy and for multiple purposes in research studies. The determination of cystine in leukocytes is carried out only in a small number of laboratories worldwide; the UCSD Cystine Determination Laboratory is one of these. Currently, the UCSD Cystine Determination Laboratory requests all leukocyte samples submitted for measurement of cystine content be prepared within 1 h of drawing the heparinized blood. A number of laboratories in the USA and Europe, however, accept whole blood samples and prepare leukocytes approx-

imately 24–48 h after the blood sample is drawn, using ethylenediaminetetraacetic acid (EDTA) or heparin as anticoagulant.

A systematic evaluation of the effect of delayed leukocyte preparation and anticoagulant on leukocyte cystine content has, to our knowledge, not been published. To date, the tedious method used for determining cystine content has made an evaluation of storage time and condition too labor intensive to study. The purchase of a new tandem mass spectrometer has reduced sample analyses time drastically, enabling us to study this important issue.

The aims of our study were to evaluate whether leukocyte separation from whole blood can be delayed for up to 48 h without affecting cystine levels and to evaluate the effect of anticoagulants on cystine content and storage.

To date, four cystinotic patients have been enrolled into the study. Patients on Cystagon therapy were asked to discontinue therapy 24 h before participating in the study. Depending on age and hemoglobin concentration, a total of 20–150 mL of blood was drawn from each subject, with aliquots anticoagulated with EDTA, acid citrate dextrose (ACD), Li heparin or Na heparin. Blood was divided into 3-mL samples, and leukocytes were prepared in triplicate by the ACD-dextran method immediately after collection (0 h), or after being stored for 24 ±1 or 48 ±1 h at either room temperature or 4°C. Leukocyte cystine content was calculated as nmol ½ cystine per milligram protein.

Samples stored for 48 h at room temperature and collected in Li heparin, Na heparin, or EDTA did not separate when mixed with ACD-dextran, and leukocytes could not be isolated from these samples. Leukocytes could be prepared from all other samples.

Preliminary results indicate that leukocytes need to be prepared immediately when blood is anticoagulated with Li or Na heparin. The leukocyte cystine content determined from blood samples collected in Li or Na heparin and stored at room temperature or 4°C for 24 h did not correspond well with the levels determined in heparinized samples prepared immediately after collection. After 48 h, regardless of the anticoagulant and storage temperature, cystine levels did not compare well to samples prepared at 0 h. However, when blood collected in ACD was stored for 24 h at room temperature or 4°C, the leukocyte cystine content was comparable to that determined in ACD samples prepared immediately. Similar results were seen for EDTA samples stored at room temperature for 24 h. Results from EDTA samples stored for 24 h at 4°C are pending.

Surprisingly, the protein content in leukocyte samples prepared from equal amounts of whole blood varied between anticoagulants, even at 0 h. Protein content was higher in samples prepared from heparinized blood (Na or Li) than blood anticoagulated with ACD or EDTA. The total cystine per leukocyte sample did not vary greatly between anticoagulants. Thus, the leukocyte cystine content, calculated as nmol ½ cystine per mg protein, differed vastly between Na or Li heparin and both EDTA and ACD. This unexpected finding is now leading our research in a new direction, and we are currently investigating the reason for the difference in the observed protein content.

RX 1

History of cysteamine in cystinosis

Jess Thoene, MD
Pediatric Genetics, University of Michigan, Ann Arbor, MI

Discovery of the lysosomal location of cellular cystine storage in cultured cystinotic fibroblasts led to a search for the means of reducing this content. Early studies of cystine-depleting compounds included penicillamine and dithiothreitol. Ascorbic acid, a benign endogenous compound, caused approximately 50% cystine depletion in cultured cystinotic fibroblasts but failed to demonstrate improved renal function in a double blind clinical trial. Cysteamine (2-amino-ethanethiol) was found to produce rapid (50% in 30 min) and complete (approx. 90%) cystine depletion in cultured cystinotic fibroblasts incubated in cystine-deficient media. This depletion was superior to that produced by other analogues and resulted in little tissue toxicity at the doses required for cystine depletion. Cystamine caused equivalent cystine depletion in vitro, but at that time cysteamine had been used in the UK to treat paracetemol (Tylenol) overdose, hence a emergency Investigational New Drug Exemption (IND) for cysteamine was requested from the U.S. Federal Drug Administration (FDA) to treat one child who was nearing renal failure and whose parents were opposed to renal transplantation. Cysteamine, both intravenously and orally, caused a dramatic decrease in her circulating leukocyte cystine content. On this foundation, a group consisting of Dr. Schneider at UCSD, Dr. Thoene at the University of Michigan, and Drs. Schulman and Gahl at NIH sought funding to launch a clinical trial of cysteamine in nephropathic cystinosis. The initial one-patient trial at UCSD occurred in 1976. After receiving funding from several sources, including NIH, Ministry of Defense, and both the generic and brand name pharmaceutical industry, and having the product adopted temporarily by three separate drug companies, cysteamine was approved as Cystagon (Mylan Laboratories) on Aug 15,1994. Data presented to the FDA in the New Drug Application (NDA) showed that renal failure was precluded in patients with nephropathic cystinosis if the drug were instituted early in childhood (ideally less than age 2 years) and if compliance, as determined by the measurement of leukocyte cystine content, was maintained. It initially appeared that renal failure could be avoided throughout the lifespan of the patient. It now appears that renal failure is averted, but that it still occurs, though more a decade later than in untreated patients. Our studies demonstrated that cysteamine reacts intra-lysosomally with cystine to form a mixed disulfide, which is recognized by the intact lysine transporter. Cysteamine enters the lysosomes via its own transporter, thus allowing a cycle of lysosomal cystine depletion to occur. Study of other analogues of the parent molecule have included phosphocysteamine and pantethine. The side effects of cysteamine are primarily limited to nausea and vomiting associated with the noxious thiol odor and taste, although a Stevens–Johnson-like rash and seizures have occurred, usually when the drug was instituted at a high dose. Current practice is to begin at 10 mg/kg per day and increase the dose by 10 mg/kg per week at weekly intervals until a dose of 60 mg/kg per day is reached. Further dose adjustments are based on maintaining leukocyte cystine at less than 1.0 nmol/mg protein. As children reach adolescence, the dose is converted to surface area instead of bodyweight, usually 1.3g/m^2 per day, with a maximum dose of 2.0 g/day. Cysteamine eye drops are required to treat the accumulation of cystine crystals in the cornea. The FDA approval of that formulation has been pending for over 15 years.

RX 2

Enteric-coated cysteamine in cystinosis

Ranjan Dohil*
Department of Pediatrics, University of California,
San Diego, CA

Longitudinal data have shown regular cysteamine therapy to be effective in reducing the rate of deterioration of renal and thyroid

function as well as improving growth in patients with cystinosis. Unfortunately, however, cysteamine may cause gastrointestinal symptoms, such as abdominal pain, nausea, and vomiting, and this, along with the requirement to take the drug four times daily, may lead to poor compliance to therapy and the rapid onset of renal failure and the need for kidney transplantation.

At this point in time there are no effective alternatives to chronic cysteamine therapy for the treatment of cystinosis. We are therefore reliant upon cysteamine therapy for the foreseeable future. Results from our earlier studies suggest that it may be possible to improve the efficacy of the cysteamine simply by altering the way that the drug is formulated and, therefore, its site of intestinal uptake. Through a custom-made naso-enteric tube we were able to selectively deliver cysteamine bitartrate solution into the stomach, small intestine (SI), and colon. The direct delivery of the drug to the SI resulted in higher plasma cysteamine levels and prolonged depletion of white cell cystine. The uptake of cysteamine from the stomach and colon was not as good as from the SI. The results of this study prompted the ongoing study reported here in which cysteamine capsules have been altered in an attempt to target drug delivery to the SI; this will hopefully increase the systemic uptake of cysteamine and allow more drug to reach the lysosome. This, in turn, will hopefully prolong the drug's effect, and fewer daily doses of cysteamine will be required. In order to prove our hypothesis, we have taken commercially available cysteamine bitartrate (Cystagon) and coated the capsules so that they will now rapidly disintegrate in the SI rather than the stomach. Patients with cystinosis are receiving the enteric-coated Cystagon (EC-Cystagon), and serial plasma cysteamine and white cell cystine levels are measured. Subjects will then continue to receive EC-Cystagon twice daily for at least 1 month, initially at the same total daily dose of cysteamine that they received in the form of Cystagon before the study commenced. During this time 12-h trough white cell cystine levels will be measured each week, and these levels will be compared to 6 -h trough white cell cystine levels measured previously (while patient was taking regular Cystagon therapy). Two patients were treated for an extended period of time (11–16 months) and underwent further pharmacokinetic and serial white cell cystine studies. Patients' gastrointestinal symptoms will be monitored.

The results of our study so far suggest that white cell cystine levels can be maintained within the expected target range (<1) when cysteamine bitartrate is delivered through a SI-targeted system. In fact, our study patients receiving EC-Cystagon therapy appear to need less of this formulation in order to maintain adequate 12-h trough cystine levels; subjects required only 60–70% of their previous total daily dose of cysteamine bitartrate (when given as Cystagon, four times daily). The gastrointestinal symptoms were minimal on EC-Cystagon when given at the lower dose.

*RD is a consultant to Raptor/Bennu Pharmaceuticals, Inc.

RX 3

In vivo confocal microscopy to detect cystine crystals in the *Cnts -/-* mouse

James V. Jester, Ron Kurtz, and Jennifer Simpson
The Eye Institute, University of California, Irvine, Irvine, CA

In order to develop a screening system to evaluate novel therapeutic interventions for the treatment of corneal cystinosis, we have evaluated the ability of in vivo confocal microscopy to detect cystine crystal formation in the cornea of the *Cnts -/-* mouse. Eyes from 7-month-old *Cnts -/-* ($n = 9$) and wild-type ($n = 3$) mice, kindly provided by Dr. Cherqui of The Scripps Research Institute, were initially fixed in 2% paraformaldehyde in phosphate buffered saline (PBS) and shipped to University of California, Irvine Medical Center (UCIMC). Whole, intact eyes were scanned using a tandem scanning confocal microscope to obtain reflected light images from the peripheral and central cornea. A series of images extending from the surface epithelium into the cornea were obtained from all eyes and then digitally reconstructed to identify the location of cystine crystals within the eye.

As shown in Fig. 1 A, three-dimensional reconstruction of an in vivo confocal microscopic scan at the peripheral cornea shows a cross section through the sclera, cornea, and iris. Evaluation of single images from the scan detected the presence of thin, needle-like crystals in the anterior (Fig. 1 a, arrow-B) and posterior (Fig. 1 a, arrow-C) peripheral cornea. Correspondingly, high-resolution images of the crystals at these locations are shown in Fig. 1 b and c (arrows). Overall, crystals were detected in the peripheral cornea in all *Cnts -/-* mice, and none were detected in the wild-type control mice. No crystals were detected in the central cornea at this age, which was to be expected based on previous mouse studies and the pathogenesis of human corneal cystinosis, which progresses from the peripheral to central and anterior to posterior cornea.

Future studies will focus on characterizing the development and progression of corneal cystinosis to identify optimal times for therapeutic intervention using novel drug delivery modalities, stem cell transplantation, and corneal electrophoresis.

GT 1

Treatment of cystinosis nephropathy using genetically modified adult stem cells in the murine cystinosis model

Kimberly Syres, Frank Harrison, Daniel R. Salomon, and Stephanie Cherqui
Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA

Cystinosis patients typically present with proximal renal tubular dysfunction (Fanconi syndrome) before the age of 1 year and without specific treatment progress to end-stage renal failure by the end of their first decade of life. Other tissues are also damaged by cystine accumulation. Unfortunately, the proximal renal tubulopathy is not sensitive to treatment with cysteamine. Patients with renal failure require dialysis or renal transplantation, both of which have significant negative health effects, particularly for young adults.

Our premise is that adult stem cells are a potential treatment for the nephropathy of cystinosis. Indeed, it has been proven that bone marrow-derived stem cells can give rise to renal cells after migrating to the kidney, particularly in the setting of a progressive renal injury. Because bone marrow-derived stem cells also have the capacity to colonize essentially any organ, this approach may also ameliorate the multi-systemic defects seen in children with cystinosis.

The clinical strategy would be to isolate adult stem cells from the bone marrow of patients, genetically modify them ex vivo to introduce the defective gene (*CTNS*), and inject them back into the patients. As a proof of concept, we are using the $Ctns^{-/-}$ murine model for cystinosis.

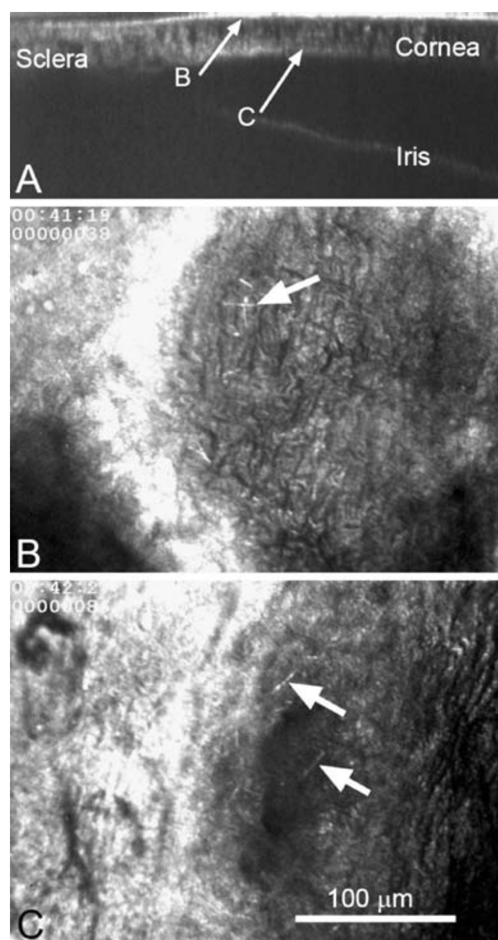


Fig. 1 In vivo confocal microscopy scan

These animals accumulate cystine and cystine crystals in all organs tested. *Ctns*$^{-/-}$ mice develop ocular changes similar to those observed in affected humans as well as bone and muscular defects and behavioral anomalies. However, the *Ctns*$^{-/-}$ mice do not develop proximal tubulopathy or renal failure despite the fact that they accumulate a significant quantity of cystine in the kidney and exhibit histological anomalies (i.e., giant mitochondria).

By isolating bone marrow stem cells from strain-matched mice constitutively expressing a Green Fluorescent Protein (*GFP*) gene, we can follow and measure the engraftment of the bone marrow cells in the transplanted mice. A key question is what is the best way to introduce these cells—systemic injection versus directly into the kidney via the ureter. We are also testing the efficacy of using whole bone marrow stem cells or mesenchymal stem cells. Outcome measures for the potential repair of renal injury are kidney tissue cystine content, histology by confocal microscopy, plasma and urine testing, and renal function. The potential that *CTNS*-expressing stem cells will also engraft in other tissues and reduce cystine levels is also being tested.

In parallel, we are developing a complementary gene therapy strategy to directly prevent the kidney injuries in cystinosis using an Adeno-Associated Virus (AAV2) vector expressing *CTNS*. As a proof of concept for renal specific delivery, we are injecting the vector by a retrograde ureteral delivery.

Finally, we propose a strategy to optimize the murine model for the renal defects in cystinosis in order to better represent the nature of the human disorder for testing new treatments. We are generating double knock-out mice that are deficient for the *Ctns* and superoxide dismutase (*SOD*) genes, *Ctns*$^{-/--/-}$ and *Ctns*$^{-/-+\ /-}$, based on the principle that the proximal tubulopathy in cystinosis is due to an oxidative stress leading to the decrease of ATP production, which in turn results in proximal renal cell dysfunction.

GT 2

Gene transfer studies for cystinosis

Claire Hippert[1], Grégor Dubois[1], Carole Morin[1],
Olivier Disson[1], Sandy Ibanes[1], Chantal Jacquet[1],
Corinne Antignac[2], Eric J. Kremer[1] and Vasiliki Kalatzis[1] [1]Institut de Génétique Moléculaire de Montpellier, CNRS-Universités Montpellier I & II, Montpellier, France,
and [2]Inserm U574, Hôpital Necker-Enfants Malade,
Paris, France

We report the first viral-mediated *CTNS* gene transfer studies and evaluate the feasibility of this strategy as an alternative or complementary treatment for cystinosis. We initially transduced human *CTNS*$^{-/-}$ fibroblast cell lines and primary murine *Ctns*$^{-/-}$ hepatocyte cultures with *CTNS*-expressing adenovirus vectors in vitro and demonstrated that gene transfer can reduce cystine storage. However, in vitro transduction of hepatocytes from young (≤3 month olds) and older (≥5 months old) mice suggested that the efficiency of correction was age-dependent. We validated these observations in vivo by performing short- (1 week) and long- (1 month) term *CTNS*-transduction studies. Short-term *CTNS* gene transfer in young *Ctns*$^{-/-}$ mice resulted in a significant decrease in cystine levels as compared to levels in non-transduced mice. In contrast, *CTNS* gene transfer in older mice did not significantly reduce cystine levels. A possible explanation for the age-dependent efficiency of cystine clearance is that a longer post-transduction period is required to reduce the higher cystine levels in older mice. Thus, a mild immunosuppression protocol was used to extend the post-transduction period from 7 to 28 days. Interestingly—and consistent with our short-term in vivo transduction data—we significantly reduced cystine levels in young *Ctns*$^{-/-}$ mice but not in older mice. Our data suggest that the age-dependent phenomena may be due to a factor other than the duration of cystinosin expression. Taken together, our data provide the proof-of-concept that gene transfer is feasible for correcting defective lysosomal transport but suggest that, in the case of cystinosis, it is likely to be preventive but not curative.

GT 3

Parthenogenetic embryonic stem cells as a source of immunocompatible renal progenitor cells for therapy of nephropathic cystinosis

Holger Willenbring, MD
Institute for Regeneration Medicine,
Department of Surgery, University of California San Francisco, San Francisco, CA

Early onset cysteamine therapy can preserve life-long kidney function in nephropathic cystinosis. However, several circumstances exist

under which cysteamine therapy is not sufficiently applied. In addition, renal proximal tubular injury frequently occurs despite diligent cysteamine therapy. This leads to Fanconi syndrome, which can have severe complications and impairs the quality of life. Kidney transplantation is the only therapy currently effective at restoring failing kidney function, but donor organs are sparse and organ transplantation necessitates life-long immunosuppression. Unlike other organs or kidney structures, the renal proximal tubular compartment is a promising target for cell therapy. Intriguingly, significant progress has recently been made in differentiating embryonic stem cells into progenitors of proximal tubular cells. What remains to be achieved is the compatibility of the transplanted cells with the recipient's immune system. Several methods exist that establish pluripotency in recipient-derived cells, but none of these is currently efficient or safe enough for human application. By contrast, parthenogenetic embryonic stem cells can be efficiently derived from unfertilized human oocytes, and these have a high likelihood of matching between relatives or even within the general population. In this proposal, we outline the critical experimental steps that will establish the principal feasibility of using parthenogenetic embryonic stem cells for the therapy of Fanconi syndrome due to nephropathic cystinosis.

GT 4

Targeted cell fusion for the correction of tubulopathy due to cystinosis

Silvia Espejel Carbajal, PhD
Institute for Regeneration Medicine, Department of Surgery, University of California San Francisco, San Fancisco, CA

Recent findings show that cell fusion between transplanted bone marrow cells and host renal proximal tubular cells produces cells that regenerate the tubular compartment and cure renal Fanconi syndrome. Studies from our laboratory suggest that bone marrow-derived macrophages (BMM) represent the hematopoietic cells that fuse with and genetically repair proximal tubular cells. These findings highlight an opportunity for using readily available BMM for the correction of genetically encoded tubulopathies. However, the application of cell fusion for therapy is currently hampered by its low spontaneous frequency.

The objective of my research project is to significantly induce fusion between donor macrophages and host renal proximal tubular cells. For this purpose, I will utilize the fusogenicity of the measles virus. Measles virus fusion is initiated by an attachment protein which, upon binding to its specific cellular receptor CD46, signals to a fusion protein to execute the fusion process. Importantly, both the attachment and fusion protein sequences can be dissociated from the virus and are therefore safe for human application. To establish induced cell fusion, I will transiently express these two sequences in BMM, which I will then transplant into mice transgenic for human CD46. Similar to humans, these mice express CD46 in most cells. Hence, this model allows me to investigate essential aspects of therapeutic cell fusion, including efficiency and functional reprogramming. Moreover, this model shows that inducing cell fusion with measles virus proteins will require retargeting in order to be efficient and safe. To target the fusion of the adoptively transferred BMM specifically to proximal tubular cells, I will either genetically alter the tropism of the measles virus attachment protein or use a bispecific antibody. Both approaches are based on the recognition of a surface antigen specific for proximal tubular cells by a monoclonal antibody. Isolating the genetic sequences that encode the specificity of this antibody or simply conjugating the antibody with a BMM-specific antibody are approaches that can be used to direct the nonspecific fusion to proximal tubular cells. Provided that targeted cell fusion is efficient and safe, this procedure would have therapeutic potential for nephropathic cystinosis.

NE 1

A Study of the cognitive domain of executive functioning in individuals with cystinosis.

Amy Spilkin, PhD, Angela Ballantyne, PhD,
and Doris Trauner, MD
Department of Neurosciences, University of California,
San Diego, CA

Executive functions underlie some of the most important aspects of daily life, including problem solving, planning, organizing, and the monitoring of behavior. These functions, however, are often not assessed in more structured situations, such as cognitive or school testing. Executive dysfunction has been observed in a number of genetic, neurologic, and/or metabolic disorders. Cystinosis is a genetic metabolic disease that affects multiple organs, including the brain. A specific cognitive profile of visuospatial deficits and intact language and general cognition has been found, yet there are no data on executive functions in these individuals. Here, I present test data from a comprehensive and standardized measure of executive functioning as well as questionnaire data from parents on the daily executive functioning of their children with cystinosis. The prevalence and nature of executive functioning difficulties found in individuals with cystinosis will be discussed, as will the implications for everyday functioning, intervention, and brain-behavior relationships in cystinosis.

NE 2

Perception, attention, and target detection in auditory and visual modalities in cystinosis

R. Ceponiene, D. Trauner, M. Kinnear, A. Spilkin, K. Jong, H. Girard, and J. Townsend
University of California, San Diego, CA

Background and rationale. Previous research has shown that individuals with cystinosis have subtle cognitive deficits, specifically in the visuo-spatial, attentional, and academic achievement domains (Scarvie et al., Percept Mot Skills 82:67–77,1996; Ballantyne and Trauner, Nwuropsychol Behav Neurol 12:254–263, 2000; Ballantyne et al., Am J Med Genet 74:157–161, 1997). Drawing from these data, our study aimed at elucidating the specific neuro-functional underpinnings of these behavioral difficulties. Three information processing "levels" were targeted. The *first* one was sensory, which denotes cortical representation of physical stimulus attributes, e.g., pitch or loudness for sounds, color or shape for visual stimuli. Sensory processing provides the foundation for higher cognitive functions by supplying information for building verbal and abstract mental representations in the brain. The *second* processing "level" was

attention. This included spatial attention as well as orienting to novel, unexpected events. Spatial attention denotes one's ability to focus on a certain location in space. Both the strength and breadth of attention were examined. These two attentional capacities—focusing and orienting—are critical balancing scales of our ability to perform goal-oriented behavior. Individuals with cystinosis have been shown to have significantly higher rates of attentional problems; however, the specific attentional profile has not been established. The *third* processing aspect was target detection. This ability includes maintaining mnemonic representation of target stimulus, detecting the target among non-target stimuli, and producing a motor response (button press) to target stimuli. This ability models active interaction with environment by representing a stimulus-assessment–decision-response cycle. Two main sensory modalities, attentional and visual, were studied. While behavioral data is available in visual modality, data from auditory modality are lacking. However, audition is also critical for cognitive functioning

Methods. The research method consisted of recording event-related brain potentials (ERPs), which are electrophysiological markers of the brain's responses to stimuli or internally generated mental events (e.g., decision making). The major advantage of the ERP method is a millisecond-scale temporal resolution. The three above-delineated processing "levels" to a large degree build one onto another, and the ERP method allows the researcher to identify the processing stage where information breakdown occurs and, often, identify its nature.

Subjects. To date, 14 individuals with cystinosis and 14 age- and gender-matched controls have been studied in two (auditory and visual) experiments. The age of the participants ranged from 6 to 52 years (mean 27 years).

Procedure. Three types of stimuli were presented randomly at four different locations. In the visual experiment, these were four boxes drawn on the computer screen; in the auditory experiment, they were four speakers evenly spread out in front of a participant. The three stimulus types were frequent "standard" stimuli, infrequent target stimuli, and varied novel stimuli. The auditory stimuli were complex tones, and the visual stimuli were shapes. The stimuli were presented in blocks of 400 stimuli. The participants had to attend to one pre-determined location during the duration of a block and to press a button in response to the target stimulus. They had to look directly in front of them at the fixation mark at all times. Attended location changed from block to block, for a total of four blocks per location. The electroencephalogram was cleaned from blink, motion, and electrical artifacts, segmented, and averaged by stimulus type. Behavioral response times to target stimuli and accuracy were also determined.

Results. *Behavior*: No group differences in target detection accuracy were found in the visual task. However, in the auditory task, the cystinosis group performed significantly poorer than their controls. Almost half of participants with cystinosis had severe difficulty localizing sounds. Interestingly, in both modalities, reaction times were longer for the central than peripheral targets, and there was a strong tendency for this to be more pronounced in the cystinosis group. *Visual ERP*. Unexpectedly, ERP amplitudes were overall larger in the cystinosis group than in the controls. At the level of visual sensory processing, we found an enhanced P2 peak in the cystinosis group. This finding is typical in neuro-developmental populations; however, its significance is as yet unclear. Nonetheless, the cystinosis group showed appropriate enhancement of sensory response upon spatial attention. At the level of target detection, the cystinosis group showed a broader spread of visuo-spatial attentional preference. While the controls showed a clear right-hemi-field facilitation, as indexed by P300 latency and amplitude, in the cystinosis group this included right-sided as well as a left middle location. Finally, their novelty response was strongly diminished and, importantly, showed no spatial selectivity.

Auditory ERP. As in the visual modality, in the auditory modality the cystinosis group showed a strongly enhanced sensory P2 peak. Spatial attention effects differed substantially between the groups. The processing of negativity, which indexes attentional enhancement of attended, as compared with unattended, stimuli was either delayed, diminished in amplitude, or absent in the cystinosis group. These differences were more pronounced with attention to peripheral speakers. Target detection appeared to be more efficient in the middle than in peripheral locations in the cystinosis group. No such differences were found in the control group. Finally, spatial selectivity of auditory novelty detection was less consistent in the cystinosis group than in the controls.

Significance. Behavioral data indicate that, overall, individuals with cystinosis have difficulties with perceptual localization. This is more severe in the auditory modality. The electrophysiological finding of overall larger ERP amplitudes in the cystinosis group may be caused by function non-specific factors, such as increased water retention and/or thinner scalp bones due to renal osteodystrophy. In addition, function-specific electrophysiological differences between the cystinosis and control groups were found at all levels of processing, with those at the attentional level predominating. The sensory differences were similar across the auditory and visual modalities and represented a finding frequent in neuro-developmental populations that has been associated with increased stimulus feature-non-specific sensory activation, potentially interfering with feature-specific encoding. Spatial attention effects were largely intact in the visual modality but deviant in the auditory modality. Auditory localization is a more challenging ability in general and, as evidenced by behavioral and ERP data, also for the individuals with cystinosis. Voluntary stimulus parsing appears to be least affected in this disorder; however, subtle spatial attention differences are a possibility. Finally, dampened response to visual novelty is striking. Although the neural generators of the Nc response are not known, its frontal predominance strongly suggests frontal lobe involvement.

Summary. Our findings indicate that individuals with cystinosis have subtle electrophysiological deficits, with more challenging, higher-level functioning being affected the most. This includes auditory localization and visual novelty detection.

MI 1

Cystinosis and redox biology: a mitochondrial connection?

Douglas C. Wallace, PhD
Center for Molecular and Mitochondrial Medicine
and Genetics (MAMMAG), University of California,
Irvine, CA

There are marked similarities between the symptoms observed in cystinosis patients and those of mitochondrial disease patients. These include renal dysfunction, such as Fanconi syndrome, growth retardation, ophtalmological problems, myopathy that preferentially affects oxidative Type I fibers, neurological lesions, among others. Both conditions also

affect the production of reactive oxygen species (ROS), glutathione redox status, and apoptosis. Cytinosis is due to a defect in the lysosome transport protein, cystinosin, which transports cystine across the lysosomal membrane. Excessive cystine trapped in the lysosome ultimately precipitates in crystals. In the cytosol and other subcellular compartments, cystine and cyteine, together with reduced and oxidized glutathione and the thioredoxins, form a network of thiol (-SH)-disulfide (-S-S-) oxidation-reduction (redox) couples that regulate enzymes and transcription factors. Target transcription factors include Oct4 for development, p53 for apoptosis, Fos/Jun for cell growth, NFkB and Nrf1 for oxidative stress response, and many others. All of these subcellular redox compartments are interconnected through the mitochondria, which regulate the cellular redox status via oxidative phosphorylation (OXPHOS) and ROS production in addition to regulating apoptosis. The coupling efficiency of OXPHOS, which determines cellular redox status, is modulated by mitochondrial DNA (mtDNA) variants, which in turn correlates highly with the indigenous ethnic origins of the individuals. Ethnicity is a major cystinosis risk factor, with cystinosis being common in persons of European descent, less common in African-Americans, and virtually absent in Asians. Assuming that the correlations between mitochondrial disease and cystinosis signify a common etiological basis, than emerging mitochondrial disease therapies may prove beneficial for treating cystinosis patients.

MI 2

Mitochondrial dysfunction in cystinosis myopathy

Doris A. Trauner, MD
Departments of Neurosciences and Pediatrics,
University of California, San Diego, CA

Background. Progressive muscle weakness and wasting are common complications in adults with nephropathic cystinosis. The cause of the myopathy is not well defined. This study was performed to determine whether the myopathy associated with nephropathic cystinosis is the result of mitochondrial dysfunction with resultant deficiency in respiratory transport chain function.

Methods. Eleven adolescents and adults (age range 18–43 years) underwent exercise testing, electromyography (EMG), nerve conduction studies, and muscle biopsies. One was excluded because of participation in a conflicting research study. In the second phase of the study, five patients received a combination of carnitine, coenzyme Q10, biotin, riboflavin, and thiamine daily for 3 months, and five received a placebo. After 3 months, strength was assessed by a neurologist who was blind to the treatment status, and the control group was offered the same treatment as the experimental group. All ten individuals were re-tested at the end of 6 months and 1 year.

Results. Of the ten participants, five demonstrated weakness on the initial testing, primarily in the upper extremities. Over the 12-month follow-up, three additional subjects displayed mild muscle weakness. The results of the EMG studies were abnormal in six of the eight patients tested: four showed myopathic changes, one showed only neuropathic changes, and one showed a combination of myopathic and neuropathic changes. Only one muscle biopsy was normal; all of the others demonstrated changes consistent with myopathy. None of the participants had significant cystine crystal deposition. Biochemical studies revealed evidence of mitochondrial dysfunction with lower than normal coenzyme Q levels, and significantly reduced complex IV/CS activity. There was no evidence of improvement during the clinical trial. However, biochemical testing indicated that the subjects were not compliant with treatment.

Conclusions. The results of this study support the hypothesis that there is mitochondrial dysfunction in nephropathic cystinosis.

MI 3

Lysosomal cystine enhanced apoptosis in cultured human cells

Jess Thoene, MD
Pediatric Genetics, University of Michigan, Ann Arbor, MI

The role of lysosomal cystine in the development of the phenotype in cystinosis is problematic in that the cystine is effectively isolated from the rest of cellular metabolism. Several models have been proposed, but most do not provide a mechanism for such an interaction. During early apoptosis the lysosomes are permeabilized, providing such access. We have shown that lysosomal cystine enhances apoptosis two- to 3.5-fold in cultured normal and cystinotic fibroblasts, cultured renal proximal tubule epithelial cells, and cultured human mesenchymal stem cells. The induction of apoptosis in such cells yields cysteinylation of protein kinase C-theta isoform (PKC δ), and the increased apoptotic rate is reduced by pretreatment of cells with siRNA to PKC δ. PKC δ is a pro-apoptotic mediator whose activity increases 300% after treatment with cystine. Enhanced apoptosis could provide an explanation for the cystinosis phenotype, via increased and inappropriate apoptosis during the first decade of life. The “swan neck” deformity of proximal renal tubules, long a hallmark of cystinosis, is explicable via this model, as is the renal failure that results from progression of tubule cell loss to atubular glomeruli. Modification of this process by other genes may explain the milder forms of the disease.

Additional cysteinylation targets were sought after apoptotic stimulus in normal and cystinotic fibroblasts utilizing ICAT and matrix-assisted laser desorption/ionization (MALDI) tandem mass spectrometry (MS/MS) to target Cys-containing peptides. Cells were treated for 16 h with tumor necrosis factor-alpha (TNFα), washed, and harvested by trypsin and the protein pellets frozen. Extant thiols were blocked with iodoacetamide, the proteins were then reduced with tributylphosphine, and the newly exposed thiols reacted with ICAT, an isotope-tagged, cleavable, thiol-reactive reagent containing a biotin affinity moiety. The mixture was passed through an avidin affinity column, and the effluent analyzed by MS/MS. Cysteinylated proteins appear only in cystinotic cells after an apoptotic stimulus, and not in normal cells. We have identified three candidate proteins that meet the listed criteria in at least one trial. One of the three, MFGE8, has been identified in two independent experiments. Gratifyingly, all three contain cyste(i)ne residues and are thought to have some role in apoptosis. Two of these, calmegin and calreticulin, may work together in the apoptotic cascade. An improved harvest procedure has been instituted to increase the yield of cysteinylated proteins recovered by this protocol.

MI 4

Molecular and pathogenesis study of cystinosis

Taosheng Huang, MD, PhD, and Sha Tang, PhD
University of California, Irvine, CA

We hypothesized that a cystine transporter defect in the lysosome of cystinotic patients results in a cysteine shortage in the cytosol, where the three major thio/disulfites, glutathione (GSH), thioredoxin, and cysteine, are coupled. Cysteine is also one of the precursors for GSH synthesis. Therefore, the GSH and thioredoxin levels are decreased in the cytosol and mitochondria in patients with cystinosis. This affects mitochondrial function and other nuclear and cytoplasmic redox processes.

We observed a slower growth rate of cystinotic cells when compared to normal cells. Accordingly, augmented programmed cell death in cystinotic cells was recorded even without apoptotic stimuli, suggesting that apoptosis does play an important role in the pathogenesis of cystinosis. Cystinotic cells generally displayed reduced ATP content and total GSH level as well as an increase in the glutathione disulfide (GSSG)/total GSH ratio, indicating a perturbed redox balance due to cystine being trapped in the lysosomes and consequent defective energy production capability of the mitochondria. However, the differences in GSH or ATP contents between cystinotic and normal cells were not definite; this further demonstrated the complexity of the disease and could partially explain the inconsistencies of similar data in previous reports. Meanwhile, we performed the first comprehensive gene expression analysis of human cystinotic cells and identified four differentially expressed genes (FN1, BMP4, HDAC1 and JUNB) in cystinotic cells that are involved in cell proliferation and development.

We are now analyzing the expression data in more details. We will obtain more control cell lines in the near future and will use quantitative RT-PCR to validate the differentially expressed genes. Apoptosis rate and the GSH and ATP levels of additional control cell lines will also be investigated to further increase our understanding of the differences in redox status and energy metabolism of cystinotic versus normal cells. Based on our preliminary data, mitochondrial malfunction could be a major factor in cystinosis. We will analyze mitochondrial respiration rate and complex I–IV activities to see if mitochondrial respiratory chain activities are compromised in cystinotic cells. The production of reactive oxygen species (ROS) will also be studied by measuring either aconitase activity or MitoSox fluorescence. We expect to see elevated ROS levels in cystinotic cells, since ROS can damage the mitochondria, reduce ATP production, and initiate apoptosis. In addition, the effects of antiapoptotic reagents (broad-spectrum caspase inhibitors) on cystinotic fibroblasts will be investigated to see if they can reverse the increased apoptotic rates in cystinotic cells we observed.

TR 1

Characterization of cystinosin intracellular trafficking

Anne Bailleux, Nathalie Nevo, and Corinne Antignac Inserm U574, Université Paris-Descartes, Paris, France

Cystinosis is an inherited lysosomal storage disorder characterized by a defective lysosomal efflux of cystine. The causative gene, *CTNS*, encodes a lysosomal membrane protein, cystinosin, that contains seven transmembrane domains and is targeted to the lysosome by two lysosomal sorting signals, a classical tyrosine-based GYDQL lysosomal sorting motif in its C-terminal tail and a novel conformational lysosomal sorting motif localized to the fifth inter-transmembrane (TM) domain loop, both of which are oriented toward the cytoplasm. We have shown that cells transiently overexpressing a cystinosin–GFP fusion protein display a striking aggregation of lysosomes into a few large juxtanuclear structures as well as a diminution of the usual pattern of small discrete intracytoplasmic vesicles characteristic of lysosomes. These large juxtanuclear structures are reminiscent of what is observed in cells overexpressing hVam6p, a protein of the vesicle-associated membrane protein (Vamp) family, which has been identified as a mammalian tethering/docking factor with an intrinsic ability to promote lysosome fusion in vivo or the lysosomal proteins ocular albinism type 1 (Oa1) and LGP85. When the fifth inter-TM loop of cystinosin was altered, the expression of cystinosin was divided between the plasma membrane and small intracellular vesicles, but the large lysosomal structures were no longer observed. The same results have been found in MDCK cells stably overexpressing cystinosin–green fluorescent protein (GFP) as either the wild-type cystinosin or cystinosin deleted from one or both lysosomal targeting signals. These data suggest that this region of the fifth inter-TM loop might play a role in lysosomal fusion.

The global aim of the research project is to characterize intracellular trafficking of cystinosin and determine its potential role in lysosomal fusion.

To address these questions, we searched for cystinosin-interacting proteins using a proteomics approach. By immunoprecipitating cystinosin in cells overexpressing cystinosin–GFP, followed by electrophoretic separation of co-immunoprecipitated proteins and mass spectrometry, we were able to show that cystinosin consistently interacts with several subunits of the proton-pumping vacuolar ATPase (V-ATPase) as well as with galectin 3, a beta-galactoside binding lectin that has recently been shown to be required for apical sorting. We confirmed the interaction with galectin 3 by immunoprecipitation of endogenous galectin in MDCK cells stably transfected with cystinosin–GFP, which showed that the interaction is abolished when the cells were incubated with lactose or thiogalactoside, proving that the interaction proceeds through a carbohydrate-dependent mechanism.

We have also mapped the region of interaction of cystinosin with the V-ATPase to the fifth inter-TM loop, which is the putative region involved in lysosome biogenesis/fusion, and we are now testing whether overexpression or inactivation of cystinosin modifies the pH of lysosomes and endosomes; a defect in the acidification of endosomes has recently been shown to interfere with endocytic functions, which could in turn explain several features of the Fanconi syndrome in cystinosis.

TR 2

Identification and characterization of the lysomal transporter involved in cysteamine-mediated cystine efflux

Bruno Gasnier[1] and Ellen I. Closs[2]
[1]Institut de Biologie Physico-Chimique, Paris, France,
and [2]Johannes Gutenberg University, Mainz, Germany

The treatment of cystinotic patients by cysteamine is based on the presence of non-affected amino acid transporters in the lysosomal

membrane that remain unknown at molecular level. After entering patients' cells, cysteamine accumulates in the lysosomes through the mediation of cysteine/cysteamine transporters. In the lysosomal lumen, this cysteamine reacts with cystine to form a cysteine–cysteamine conjugate and because of its structural similarity to lysine, this mixed disulfide is able to leave the lysosomes through another transporter selective for cationic amino acids. Following the reduction of the mixed disulfide to cysteamine and cysteine in the cytosol, the cysteamine reenters the lysosomes to remove more cystine. The lysosomal cationic amino acid transporter, known biochemically as lysosomal 'system *c*', thus represents a 'salvage pathway' which by-passes the need for a functional cystine transporter.

We have identified members of a family of cationic amino acid transporters (CAT) that localize to lysosomes and thus represent a candidate system for the *c* proteins. In the first part of the talk, given by EIC, the CAT proteins will be introduced and compared to what is known about the function of system c. The second part of the talk, given by BG, will provide an overview on the approaches to study lysosomal transporters and current attempts to develop an in situ lysosomal efflux assay. In addition, preliminary data on cysteamine–cysteine interactions with CATs will be provided.

PA 1

The pathogenesis of renal disease in cystinosis

M. Wilmer[1]; L. van den Heuvel[1]; R. Masereeuw[1],
H. Blom[2], L. Monnens[1]; E. Levtchenko[3]
[1]Radboud University Nijmegen Medical Centre,
The Netherlands, [2]VU University, Amsterdam;
The Netherlands, and [3]University Hospital, Leuven, Belgium

Renal disease in cystinosis is characterized by various degrees of proximal tubular dysfunction, proteinuria, and progressive deterioration of glomerular filtration rate (GFR) due to interstitial renal damage. Lysosomal cystine accumulation is the hallmark of cystinosis and leads to renal damage by pathways that are still elusive. The *goal of our project* is to unravel the pathogenesis of renal dysfunction in cystinosis. Because proteinuria, a known factor of renal disease progression in diverse nephropathies, is invariably present in all patients with cystinosis, we examined the pathogenesis of proteinuria in cystinosis.

To this end, we established conditionally immortalized cloned proximal tubular cell lines (ci-PTEC) from patients with cystinosis carrying known genetic defects in the *CTNS* gene ($n = 3$) and from age-matched healthy controls ($n=2$). The proximal tubular origin of these cells was confirmed by demonstrating the presence of diverse proximal tubular cell markers (dipeptidyl-peptidase IV, aquaporin-1, aminopeptidase N, p-glycoprotein, organic cation transporter 2, and multidrug resistance-protein 4) and by the functional activity of alkaline phosphatase. The expression of zona occludens 1 tight junction protein confirms the epithelial origin of these cells.

We found normal protein levels of multi-ligand receptors megalin and cubilin in cystinotic renal tissue, which is in contrast to findings in other proximal tubular disorders with slower renal disease progression rate (such as Dent's disease and Lowe syndrome). These receptors are responsible for proximal tubular uptake of albumin and low-molecular-weight proteins. Functional endocytosis of fluorescein isothiocyanate (FITC)-labeled albumin was demonstrated in both cystinotic and control ci-PTEC and could be inhibited by receptor-associated protein (RAP), a known inhibitor of megalin-mediated absorption.

Increased urinary excretion of high-molecular-weight protein immunoglobulin (Ig)G in cystinotic patients starting from an early age suggests that glomerular damage in cystinosis is already present before any measurable deterioration of the GFR has occurred and points to the potential therapeutic benefit of early administration of angiotensin-converting enzyme inhibitors. Studies on cytokine and chemokine secretion in cystinotic renal tissue and control ci-PTEC after the application of albumin and cysteamine as well as measurements of oxidative stress markers are ongoing.

PA 2

Cysteamine effects on extracellular matrix accumulation in chronic kidney disease

Allison A. Eddy, MD
Children's Hospital & Regional Medical,
University of Washington, Seattle, WA

Cysteamine treatment has had an incredible impact on the severity and progression of nephropathic cystinosis. Although its use is associated with a significant reduction in lysosomal cysteine levels, it is still unclear if this is its primary mechanism of renoprotection. Based of the absence of significant renal disease in the cystinosin knockout (*Ctns-/-)* mice, it may be that a second "hit" in additional to cysteine loading is required for renal disease to develop. Like all chronic and progressive kidney diseases, the nephropathy of cystinosis is characterized by interstitial fibrosis. In recent years it has been recognized that cysteamine has several biological effects that prevent collagen accumulation, and it has been shown to reduce fibrosis in an experimental model of liver disease. Our laboratory will soon begin studies with the support of a grant from the Cystinosis Research Foundation to determine if cysteamine prevents fibrosis in another experimental kidney disease model and to determine if endogenous cysteamine depletion or superimposed kidney injury represent a second hit that will exacerbate nephropathy in the *Ctns-/-* mice.

It is anticipated that cysteamine will alter one or more of the following fibrosis-promoting pathways in the kidney and other organs. (1) Cysteamine may modify interstitial collagens, making them more susceptible to protease-dependent degradation. Tissue transglutaminase 2 (Tgase2) catalyzes collagen cross-linking through the formation of bonds between glutamine and lysine residues. Cysteamine is a potent Tgase2 inhibitor; this effect has been associated with the neuoorotective effects of cysteamine. (2) Lowering lysosomal cysteine levels may enhance the activity of the lysosomal cathepsins that have been implicated in intracellular pathways of collagen degradation. (3) As a consequence of interstitial fibrosis, renal tubules are destroyed via apoptosis-dependent pathways. Through its anti-apoptotic actions as an inhibitor of caspase-3, cysteamine may help to preserve intact and functional nephrons. (4) A state of oxidative stress develops in chronically damaged kidneys which is thought to contribute to ongoing injury by mechanisms that have not been fully elucidated. Glutathione is an important endogenous defense pathway against oxidative stress. Cysteamine increases glutathione synthesis by

increasing γ-glutamyl cysteine synthetase activity. In addition, cystine/cysteine levels also influence tissue redox potential.

We speculate that the great efficacy of cysteamine as therapy for nephropathic cystinosis extends well beyond its ability to reduce lysosomal cystine levels. If this hypothesis is correct, there will be opportunities to develop new therapies that should benefit children with cystinosis as well as patients with chronic kidney disease due to other etiologies.

GN 1

Complex genetic approaches to monogenic disease: genomic and transcriptomic dissection of normal expression of *CTNS*

K.A. Freed, E.K. Moses, J.E. Curran, M.P. Johnson, M. Carless, T.D. Dyer, J.Charlesworth, S.A. Cole, J.W. MacCluer, H.H.H. Göring, J. Blangero
Department of Genetics,
Southwest Foundation for Biomedical Research,
San Antonio, TX

While rare human genetic diseases like cystinosis are caused by mutations in a single gene, there is a growing realization that genes rarely work alone but, rather, are positioned within complex global regulatory networks in which they may potentially interact with many other genes. While *CTNS* mutations represent the known causes of cystinosis, there are likely to be other genes involved in the pathogenesis of cystinosis, acting either upstream as regulators of *CTNS* gene expression or downstream having a more direct involvement in disease pathology.

In this study, we describe a novel approach for elucidating the biological functions of a monogenic disease locus via the application of complex genetic analysis to normal variation in gene expression. To examine the potential functions of the *CTNS* gene, we utilized a unique data set of genome-wide lymphocyte transcriptional profiles from 1240 individuals in large extended Mexican American families. To identify potentially functional regulatory variants, we resequenced the entire *CTNS* gene in 189 founder individuals. Over 200 variants were detected, including 105 novel single nucleotide polymorphisms (SNPs). Association analysis in the sequenced subset revealed strong evidence for *cis*-regulation (with p-values as low as 1.0×10^{-10}). When these variants were genotyped in all 1240 individuals, evidence for *cis*-regulation increased (with p values as low as 2.4×10^{-39}), thus providing convincing support for *cis*-acting regulatory variants within (or near) the *CTNS* gene.

Using the most highly associated SNPs, we then performed association analysis on the genome-wide lymphocyte transcriptional profiles to identify genes that are causally downstream of *CTNS*. Multiple genes were identified, including COPS8, which functions as an important regulator in multiple signaling pathways and acts as a positive regulator of E3 ubiquitin ligases.

In order to identify *trans*-acting upstream genes that may influence *CTNS* expression levels, we performed a linkage-based genome scan. Using this approach, we identified a potential linkage region at chromosomal region 9q21 (p=0.0025). Examination of the genetic correlations of expression levels of genes located within this linkage region with *CTNS* expression levels identified a positional candidate gene, *VPS13A* (vacuolar protein sorting 13A). *VPS13A* expression levels were inversely genetically correlated with *CTNS* expression levels (ρ_G=−0.551, p =9×10^{-6}). Thus, *VPS13A* may be a potential inhibitor of *CTNS* expression. In order to identify those variants most likely to be functionally involved in the *trans*-regulation of *CTNS* expression, we have genotyped over 143 *VPS13A* SNPs in 1240 individuals. Association analysis of 143 variants revealed strong evidence for *cis* effects on *VPS13A* expression levels (p=1.9×10^{-7}). Association analysis using six best “*cis*-acting” *VPS13A* SNPs on *CTNS* expression level was performed. All six SNPs were significantly associated with *CTNS* expression (p=0.009–0.05). These genetic results strongly indicate that *VPS13A* is an upstream *trans*-acting regulator of *CTNS*.

We also performed a genome-wide association study on 900 individuals using Illumina’s HumanHap550 Genotyping BeadChip. With a false discoverby rate (FDR) of 0.1, 5 *trans* loci for *CTNS* were identified, including RAB12. RAB12 is a member of the RAS oncogene family and is involved in vesicular trafficking.

Another gene of interest is *STUB1*. In many biological systems redundancy has been observed in gene function. This has been observed for the yeast homologue (*ERS1*) of human *CTNS*, with the recent identification of the functionally related *MEH1* gene. Using a combinatorial strategy involving database sequence interrogation and genetically correlated expression data from our transcriptome data set, we have identified *STUB1* to be a plausible candidate for the human homologue of yeast *MEH1*. This raises the possibility that Stub1 may work in parallel with Ctns by having an overlapping or complementary function. We have exhaustively enumerated all genetic variation in *STUB1* and genotyped these SNPs in 1240 individuals. Association analysis is ongoing, and results will be presented.

In order to verify and extend these studies, the next phase of our research program involves the collection of genetic material from cystinosis families. In July 2007, the Cystinosis Research Network Family Conference took place in San Antonio, Texas. Blood was collected from consenting individuals affected by cystinosis and their first degree relatives. The blood samples (147) were transported to the Southwest Foundation for Biomedical Research, San Antonio, Texas where lymphocytes were isolated, transferred to vials, and then snap frozen in liquid nitrogen. In the future, tissue culture techniques will be used to generate stable, long-term viable cell lines from these lymphocytes. Each cell line will represent one cystinosis family member; as such, we will have the full spectrum of disease severity, from early onset severe cystinosis to unaffected individuals. This will provide us with a unique biological repository for future genetic studies. An update on the progress of this project will be presented.

GN 2

Transcriptional and post-transcriptional regulation of the cystinosin gene

Anna Taranta[1], Serena Corallini[1], Francesco Bellomo[1], Francesco Emma[1], Martijn J. Wilmer[2],
Lambert P. van den Heuvel[2] and Elena N. Levtchenko[2] [1]Department of Nephrology and Urology,
Division of Nephrology, Bambino Gesù Children’s Hospital
and Research Institute, Rome, Italy,
and [2]Department of Pediatrics, Pediatric Nephrology,
Radboud University Medical Center,
Nijmegen, Netherlands.

The main goal of this project is to study the transcriptional and post-transcriptional regulation of the *CTNS* gene.

Our results to date demonstrate that the *CTNS* gene can be upregulated in vitro by cystine deprivation: HK2 cell lines cultured in cystine-deprived medium showed a 2.5-fold increase in mRNA transcription.

Based on this result, we have sub-cloned several segments of the *CTNS* promotor in the luciferase pGL4 reporter vector and have begun testing the activity of the promotor under cystine depletion. Using a similar approach, we have studied mRNA stability after actinomycin D inhibition of transcription. Our initial data show that there is no significant enhancement of mRNA stability after cystine deprivation.

In another set of experiments, we have identified a CTNS isoform, termed cystinosin-LKG. This CTNS isoform is characterized by a different carboxy-terminal tail, caused by an alternative splicing of exon 12, which results in the removal of the lysosome-targeting signal (GYDQL). By transfecting green fluorescent protein (GFP)–fusion plasmids, followed by immunocytochemistry and transmission electron microscopy after immunogold-labelling, we were able to show that in transfected cells cystinosin-LKG is expressed in the plasma membrane, lysosomes, and other cytosolic vesicles. The localization of cystinosin-LKG in the plasma membrane was confirmed by the demonstration of a pH-dependant ^{35}S-cystine uptake in transfected COS-1 cells. The differential expression of the two known cystinosin isoforms was then analyzed in different human tissues and in different cell lines under different culture conditions. Our preliminary results show that in most tissues, the lysosomal cystinosin isoform accounts for more then 90% of the transcripts. In the testis, however, cystinosin-LKG corresponds to approximately 50% of the transcripts. To date, we have not observed a differential regulation of these two cystinosin isoforms under different conditions, in particular after cystine deprivation.

Finally, our project includes “rescue” experiments, in which the two isoforms are transfected in cultured tubular cell lines obtained from patients with cystinosis carrying homozygous deletion of the *CTNS* gene. We have now identified the experimental conditions that enable successful transient transfection of the *CTNS* gene in these cells and are beginning to test the metabolic effects of each of these isoforms.

DE 1

Molecular mechanisms of hypopigmentation in cyctinosis

Christine Chiaverini, Stefania Briganti, Enrica Flori,
Mauro Picardo, Jean-Paul Ortonne, and Robert Ballotti INSERM U895, Biologie et Pathologies des Cellules Mélanocytaires: de la Pigmentation Cutanée au Mélanome, Université de Nice Sophia-Antipolis, Nice

Cystinosis is a rare autosomic recessive disease with multi-systemic involvement caused by *CTNS* mutations. Kidney involvement is clearly the foremost clinical characteristic of cystinosis. Several other symptoms are frequently associated with cystinosis, including hypothyroidism, photophobia, hypogonadism, and pulmonary dysfunction. Several other symptoms have been observed in patients with cystinosis, including fair skin complexion, blond hair, and blue eyes. Obviously, pigmentation alterations are not the most crippling symptom of cystinosis. However, we sought to investigate the role of *CTNS* in melanocyte differentiation and melanin pigment production because such studies in other multi-systemic diseases with pigmentation involvement have contributed important clues to a better understanding of the other symptoms of the pathologies.

The cells responsible for skin hair and eyes pigmentation are melanocytes. These cells, derived from the neural crest, produce melanin within specialized intracellular vesicles called melanosomes, which belong to the lysosome-related organelle family. In skin, melanosomes, which contain all of the enzymatic machinery (tyrosinase, Tyrp1, Dct) required for melanin synthesis, are transported to the dendrite tips of the melanocyte and transferred to surrounding keratinocytes to allow an uniform skin pigmentation and an efficient photo-protection against the carcinogenic effect of UV radiation of the solar light. Two types of pigments are produced by melanocytes, eumelanins, which are black/brown pigments, and phaeomelanins, which are yellow/red ones. Eumelanins play a key photoprotective role against UV-induced DNA damage and skin cancer. Conversely, pheomelanins, which are red/yellow sulfur-containing pigments, appear to be deleterious and could favor skin cancer development. Although it has been shown that the level of tyrosinase, tyrp1, and Dct play a key role in pigment production, cystine content may also be very important parameters in melanogenesis. Indeed, the sulfur in the pheomelanins may be provided by cystine readily available in the melanosome.

Taking into account that cystinosin transports cystine out of the lysosome and that melanosomes are lysosome-related vesicles, it is tempting to propose that cystinosin is involved in the active melanosomal efflux of cystine and therebyregulates melanogenesis. In cystinosis, hypopigmentation could result from an intramelanosomal cystine accumulation, which could favor pheomelanin synthesis. Additionally, the H+ cotransporter activity ascribed to cystinosin could participate in the regulation of intramelanosomal pH. Since melanosome pH is a key parameter of melanogenesis, mutation in the *CTNS* could affect H+ efflux and, consequently, pigment synthesis. Another hypothesis is that cystine accumulation may be toxic to melanocytes, leading to a decreased cell growth or increased apoptosis sensitivity. To verify this hypothesis, we have initiated a study to elucidate the role of cystinosin in melanogenesis as well as in melanocyte growth and apoptosis.

YE 1

Yeast as a model system for cystinosis

Seasson Phillips Vitiello and David A Pearce
Center for Neural Development and Disease,
Department of Biochemistry and Biophysics,
University of Rochester School of Medicine and Dentistry, Rochester, NY

The lesser complexity of model organisms enables researchers to obtain information about a certain protein and the processes it is involved in, and to extrapolate that knowledge with the aim of unraveling the more complex systems in higher organisms. The budding yeast *Saccharomyces*

cerevisiae has been used in scientific research for over half a century, resulting in a highly annotated genome and many optimized approaches and tools. We have successfully used yeast to study Batten disease, a fatal childhood neurodegenerative disease. Batten disease, or juvenile neuronal ceroid lipofuscinosis, is caused by mutations in CLN3. The yeast homolog to CLN3 is denoted Btn1p. To date, we have identified many pathways that are altered upon deletion of *BTN1* and, importantly, have applied these observations to mammalian cells. Likewise, we aim to use a yeast model of cystinosis to gain a better understanding of cystinosin function and the disease pathologies, ultimately resulting in the identification of novel therapeutic targets. The amino acid sequence of the yeast homolog Ers1p is 31% identical and 47% similar to cystinosin. Moreover, it was previously reported that deletion of *ERS1* (*ers1-Δ*) causes yeast to become sensitive to the drug hygromycin B. This sensitivity can be rescued by human CTNS expression, suggesting that these two proteins are functional homologs. Ers1p localizes to the vacuole, the analogous organelle to the lysosome (Gao et al., FEBS J 272:2497–2511, 2005). However, several questions about the mechanism of Ers1p and defects found in *ers1-Δ* remain unanswered. By using yeast genetic techniques, such as yeast two-hybrid analysis, synthetic genetic array, and phenotypic screens, we propose to study Ers1p in order to gain insight into cystinosin.

SPEAKER INDEX

Speaker	Session
Antignac, C	TR 1
Ballantyne, A	NE 1
Ballotti, R	DE 1
Barshop, B	ME 1
Broyer, M	HI 2
Ceponiene, R	NE 2
Cherqui, S	GT 1
Chiaverini, C	DE 1
Closs, E	TR 2
Dohil, R	RX 2
Eddy, A	PA 2
Emma, F	GN 2
Espejel Carbajal, S	GT 4
Fidler, M	ME 2
Freed, K	GN 1
Gangoiti, J	ME 1
Gasnier, B	TR 2
Hippert, C	GT 2
Huang, T	MI 4
Jester, JV	RX 3
Kalatzis, V	GT 2
Kleta, R	HI 3
Kremer, EJ	GT 2
Levtchenko, E	PA 1, GN 2
Moses, E	GN 1
Pearce, D	YE 1
Phillips, S	YE 1
Salomon, D	GT 1
Schneider, JA	HI 1
Tang, S	MI 4
Taranta, A	GN 2
Thoene, J	RX 1, MI 3
Trauner, D	MI 2
Wallace, D	MI 1
Willenbring, H	GT 3, GT 4