



# **Cystinosis Research Symposium 2011**

*Ground-breaking cutting-edge research  
in an ultra-rare disease presented in layperson's terms*

**Where: Theatre, Conway Institute, UCD**

**When: Thursday, 13th October  
2pm (sharp) to 6pm**

**Presentations and Posters from Researchers at  
University College Dublin, University College Cork  
and Stanford University California**

**ALL WELCOME!  
WTF METRONIA!**

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## **The Cystinosis Research Symposium 2011**

The Cystinosis Research Symposium 2011 presents research results from national and international projects funded by Cystinosis Foundation Ireland and other cystinosis research in Ireland. It attempts to bring different research groups together and make the results of their work accessible to patients, to donors, to the public and to others in the scientific and medical field.

We are grateful to Orphan Europe and to the Health Research Board for supporting this Symposium.

Special thanks to Dr Thomas McDonald and Professor Achim Treumann, members of the Research Group of Cystinosis Foundation Ireland.

## **Cystinosis Foundation Ireland**

Cystinosis Foundation Ireland is an Irish registered charity, registered number CHY15517. It is an all volunteer, non-profit organisation dedicated to providing services for those affected by cystinosis.

Cystinosis Foundation Ireland was founded in 2003 by patients' families and friends with the following four aims:

- ⤴ **Research Support:** The Foundation raises funds to promote research into the causes and improved treatments of cystinosis and to hopefully one day find a cure.
- ⤴ **Patient/Parental support**
- ⤴ **Providing an Internet website** with information on the disease and any updated information or news as it becomes available.
- ⤴ **Education & Awareness** about the condition to the medical profession and the public. Almost nothing is known about the condition in the general public and indeed the medical profession. Many patients have experienced misdiagnosis, sometimes taking months and on occasion, years to be correctly diagnosed. Early diagnosis is crucial so that cysteamine therapy (Cystagon™) is started as soon as possible.

Cystinosis Foundation Ireland operates an open-call system. Applicants can contact the Foundation, if they are interested in carrying out research into the disease. Please refer to our website (<http://www.cystinosis.ie>) for our particular areas of interest.

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## Programme

Time	Presenter	Title
14:00-14:10	Anne Marie O'Dowd, Cystinosis Foundation Ireland	Welcome
14:10-14:40	Patrick Harrison, Dept. Physiology, University College Cork	100 years of cystinosis – one drug, no cure, and a lot of unanswered questions
14:40-15:00	Ciaran Lee, Dept. Physiology, University College Cork	Cystinosis Gene Repair I: Generation of new tools to correct defects in the CTNS gene
15:00-15:20	Katrin Kaschig, Dept. Physiology, University College Cork	Cystinosis Gene Repair II: How to check if the function of the CTNS gene has been restored
15:20-16:00	<b>Poster Session and Refreshments</b>	
16:00-16:20	Rodolfo Sumayao, University College Dublin	How can laboratory cell line studies help us to understand cellular dysfunction in Fanconi syndrome in cystinosis?
16:20-16:40	Bernadette McEvoy, University College Dublin	Cystinosis affects pancreatic beta cells, the cells that make insulin in the body
16:40-17:00	Poonam Sansanwal, Stanford University	A new discovery to understand kidney damage in cystinosis
17:00-17:10	Mick Swift, Chairman Cystinosis Foundation Ireland	Chairman Summary
17:10-18:00	<b>Poster Session and Discussions</b>	

## **Presentation Abstracts.**

### **100 years of cystinosis – one drug, no cure, and a lot of unanswered questions**

*Patrick T Harrison, University College Cork, Ireland*

Cystinosis is a rare inherited disorder which affects all organs of the body. The disease is triggered by mutations in a gene called CTNS. The normal version of the gene makes a protein called cystinosin which ensures that the regular recycling of the amino acid cystine in all cells in the body. In cystinosis patients, both copies of the gene are defective in every cell, which results in the accumulation of cystine in a region of cell called lysosomes. This accumulation causes cells throughout the body to malfunction.

The best available drug, cysteamine, can prevent the build up of cystine, and significantly improve the overall health of children and teenagers with cystinosis. However, kidney transplantation followed by a life-long dependence on immunosuppressive drugs is almost inevitable for virtually all patients before they reach the age of 20.

Nearly 1200 research papers have been published on cystinosis, and an international cystinosis registry is well under way, yet several substantial questions remain unanswered.

- (i) exactly how does cystine accumulation result in the symptoms of cystinosis?
- (ii) ~5% of patients with mutations in both copies of the CTNS gene do not have any kidney problems; a similar observation has been made in the mouse model of cystinosis – is there a second gene which determines the severity of the disease?
- (iii) cysteamine has to be administered every six hours and many patients report side effects of significant halitosis – can new formulations address these problems?
- (iv) an allogenic bone marrow transplant model of cystinosis results in a significant reduction in cystine in all tissues – what is the mechanism by which this occurs?
- v) a gene-modified autologous bone marrow transplant model of cystinosis could potentially be used to treat cystinosis without the need for life-long dependence on immunosuppressive drugs; a similar approach has recently been successful in a human patient with a rare blood disorder – could this approach eventually become a cure for cystinosis?

Cystinosis research offers patients a potentially brighter future. There is still much basic research to be done to understand how the gene mutation causes the disease, but there are many opportunities for immediate improvements to existing treatments, and even the longer term possibility of a cure for this disease.

A child's most persistent question on any journey is, "Are we there yet?". Cystinosis is a challenging journey for many children; without continued research, the answer to their question will remain, "no".

## **Cystinosis Gene Repair II: Generation of new tools to correct defects in the CTNS gene**

(Generation of CTNS-specific Zinc Finger Nucleases using oligomerised pool engineering (OPEN))

Ciaran M. Lee<sup>1,2</sup>, Martina F. Scallan<sup>2</sup>, Patrick T. Harrison<sup>1</sup>

<sup>1</sup>Department of Physiology, <sup>2</sup>Department of Microbiology, BioSciences Institute, University College Cork, Ireland

Cystinosis is an autosomal recessive disease caused by mutations in the CTNS gene. CTNS encodes for the lysosomal cystine transporter, cystinosin. The most profound effects are seen in the kidneys with end stage renal failure occurring in teenage years even when treated with cystagon. However, transplanted kidneys do not accumulate cystine in the lysosome. This opens up the possibility that correction of the CTNS gene could restore normal kidney function.

In 2005, a method for correcting genes in up to 20% of cells was described. This gene correcting system uses designer enzymes called Zinc Finger Nucleases (ZFNs) to target a mutant gene and initiate its repair (HDR). Subsequent studies have confirmed the potential of ZFN-HDR technology to revolutionise gene and stem cell therapy. At present, there are no set guidelines on how best to design ZFNs. The work currently underway in our lab is to assess three different methods to design ZFNs to target the CTNS gene. Two simple methods have already yielded five independent ZFN pairs, however none have successfully targeted the CTNS gene in cells, even though some of them could recognize the CTNS target *in vitro*.

The third, a more complicated and thorough design method, oligomerised pool engineering (OPEN) is currently under investigation. This involves the construction of a library of a variety of different ZFNs for testing, and then screening this library for ZFNs that can recognise the CTNS gene. The library will contain a total of  $1.3 \times 10^7$  ZFNs, and will be screened for nucleases that can recognise eight different sites in CTNS. The ability to efficiently target these different sites should allow the repair of about half of the known mutations in the CTNS gene. Some of these ZFNs will also be used to generate novel cell lines to further investigate the mechanism by which mutations in the CTNS gene cause cystinosis.

## **Cystinosis Gene Repair I: How to check if the function of the CTNS gene has been restored**

(A functional assay to detect restoration of *CTNS* via homology-directed repair using Zinc finger nucleases)

Katrin Kaschig<sup>1,2</sup>, Ciaran M. Lee<sup>1,2</sup>, Jennifer A. Hollywood<sup>1,2</sup>, Martina F. Scallan<sup>2</sup>, Patrick T. Harrison<sup>1</sup>

<sup>1</sup>Department of Physiology, <sup>2</sup>Department of Microbiology, BioSciences Institute, University College Cork, Ireland

Cystinosis is a multi-system autosomal recessive disorder caused by mutations and/or deletions in both alleles of the *ctns* gene, encoding for the acid-dependent lysosomal cystine exporter cystinosin. The lack of cystine export from the lysosome disrupts a number of cellular processes, including the normal regulation of autophagy, a natural process, which degrades and recycles components of damaged organelles, membranes and proteins of the cells to ensure survival. This dysregulation of autophagy, especially in the mitochondria of the cells, has been shown to lead to an increase in apoptosis [1].

Our lab is developing a method to repair the mutation(s) in the *ctns* gene which disrupt the cystine transporter. We have developed specific proteins, so called Zinc Finger Nucleases (ZFNs), which should be able cleave *CTNS* near to the mutated region. By adding corrected *CTNS* DNA sequences, the site-specific cleavage by the ZFNs should trigger a naturally occurring repair process in cells called homology-directed repair (HDR). We have already shown that mutations in a different gene (*CFTR*) can be corrected in cells using the ZFN repair strategy [2]. We have already made specific ZFNs that can target the mutated region in the *ctns* gene *in vitro* [3] and now plan to evaluate them in cells. Gene repair can be measured by PCR-based assays.

In order to determine if gene repair results in a reversal of the cystinotic state of the cell, we will use an assay that can measure the level of autophagy. The level of autophagy can be determined using a fluorescently labeled monoclonal antibody specific for a protein called LC3. The level of LC3 can then be visualised by confocal microscopy.

The sensitivity of the assay will be established in cystinotic proximal tubular epithelial cells, and also in healthy cells that have been treated with cystine dimethylester (CDME), which mimics the cystine accumulation seen in cystinotic cells, and thus provide a positive control for the autophagy process.

Once verified, the assay will be used to test how effective ZFN-mediated repair of mutations in the *CTNS* gene is at reversing autophagy and cell death.

### **References**

- [1] Sansanwal, P *et al.* 2010 *J Am Soc Nephrol* 21: 272-83.
- [2] Hollywood JA *et al.* 2011 *ESGCT Congress* (submitted)
- [3] Kaschig K *et al.* 2009 *Irish Journal of Medical Science* **178** (S12)S478-9

**How can laboratory cell line studies help us to understand cellular dysfunction in Fanconi syndrome in cystinosis?**

(*CTNS* gene inhibition alters redox status in a human kidney epithelial cell line: implications for cell dysfunction associated with Fanconi syndrome in cystinosis)

*Rodolfo Sumayao, Bernadette McEvoy, Tara McMorrow, Philip Newsholme*

UCD School of Biomolecular and Biomedical Sciences and UCD Conway Institute

Cystinosis is a lysosomal storage disorder caused by a defect in the gene for a lysosomal cystine transporter. This leads to widespread accumulation of the amino acid cystine in different tissues resulting in multi-organ damage but the loss of kidney function remains the foremost clinical characteristic of this disease. This is manifested by increased urinary losses of essential nutrients, minerals and electrolytes, known as *Fanconi syndrome*.

We investigated the effects of mimicking the basic genetic defect in cystinosis by silencing the causative gene responsible for this disease, in human-derived kidney cells. Specifically, we examined the levels of harmful reactive molecules and different parameters relating to cell stress and cell death.

Mimicking the genetic defect using molecular techniques in kidney cells resulted in a significant increase in the levels of reactive molecules, decreased antioxidant capacity, decreased cell energy and enhanced cell death. These alterations are likely to contribute at various levels to the chain of events that lead to cell damage and, ultimately, cell death associated with Fanconi syndrome in cystinosis.

**Cystinosis affects pancreatic beta cells, the cells that make insulin in the body**  
(Molecular mechanisms of pancreatic beta cell dysfunction associated with cystinosis: Determination of optimal approaches for cell protection)

*Bernadette McEvoy, Rodolfo Sumayao, Tara McMorrow and Philip Newsholme*

UCD School of Biomolecular and Biomedical Science, University College Dublin, Belfield, Dublin 4

Cystinosis is a genetic, lysosomal storage disease. The main characteristic of this disease is the accumulation of the amino acid, cystine, in one compartment of the cell as a result of a genetic defect in the protein transporter that removes cystine from this compartment. The cells of the kidney are the first to be affected by this accumulation resulting in kidney failure at a young age which requires a kidney transplant. Other organs and systems in the body are also affected by the cystine accumulation including the pancreas which can lead to diabetes.

In this study, cystine dimethyl ester (CDME) was used to generate cystine accumulation in pancreatic beta cells. In the body these cells produce and secrete insulin in response to blood glucose levels. Increased levels of cystine and reduced glucose and amino acid stimulated insulin secretion were observed in pancreatic beta cells following CDME treatment. This indicated that these cells are under stress and therefore could not perform their normal function. The level of stress the cells may be under was assessed by measuring the antioxidant levels within these cells which displayed a significant increase in these levels.

These results suggest that CDME treatment, which mimics cystinosis, has a negative impact on the pancreatic beta cells causing cell stress however the main stressors have yet to be identified. Our studies have suggested a higher death rate of cells perhaps due to less energy being available to the cell to carry out its normal functions. Further analysis could lead to identifying the main stressors involved with the aim of reducing them, to prevent further damage to the pancreatic cells.

**A new discovery to understand kidney damage in cystinosis (Identification and characterization of novel cellular injury molecules and pathways in cystinosis)**

Poonam Sansanwal

Stanford University, Sarwal Laboratory

The main goal of this study is to investigate mechanisms leading to kidney injury in nephropathic cystinosis patients.

Using whole-genome gene expression profiling of blood, skin and kidney cells obtained from nephropathic cystinosis patients, several novel molecules and pathways are identified that provide critical insights into cellular injury mechanisms in cystinosis. Various experiments were conducted to assess the expression and function of one such molecule, an apolipoprotein. This molecule has two different forms, one resides in the cell and the other one is secreted out of the cell. The intracellular form of this protein has been shown to be associated with cell death.

Experiments were conducted to modulate the level of this protein and bring it down and investigate if the cell death can be rescued. Our data shows that the cell death can be significantly alleviated by knocking down the level of this protein. This novel molecule potentially could be a new therapeutic target, and a marker of renal injury in human nephropathic cystinosis.

**Posters**

<b>University</b>	<b>Presenter</b>	<b>Poster title</b>
UCC	Ciaran Lee	Cystinosis Gene Repair I: Generation of CTNS-specific Zinc Finger Nucleases using Oligomerised Pool ENgineering (OPEN)
UCC	Katrin Kaschig	Cystinosis Gene Repair II: A functional assay to detect restoration of <i>CTNS</i> gene via homology-directed repair using Zinc Finger Nucleases
UCC	Jennifer Hollywood	Cystinosis gene repair III: use of donor sequences containing AAV-2 inverted terminal repeat sequences to enhance zinc finger nuclease homology directed repair efficiency
UCD	Bernadette McEvoy	Molecular mechanisms of pancreatic beta cell dysfunction associated with cystinosis: Determination of optimal approaches for cell protection
UCD	Rodolfo Sumayao	CTNS gene inhibition alters redox status in a human kidney epithelial cell line: implications for cell dysfunction associated with Fanconi syndrome in cystinosis.