Lowe Syndrome Trust Supporters' Press Release

LST-Funded Medical Research Update

Professor Robert L Nussbaum
Chief, Division of Genomic Medicine
Department of Medicine and
Institute of Human Genetics
UCSF

'Building on current research funded by the Lowe Syndrome Trust, this project presents the next small but significant step in a very long journey – hopefully leading to understanding the basic underlying defect of the disease'

LST Grant: £163,000

UCSF
University of California
San Francisco

The defective gene in Lowe Syndrome is *OCRL*, which serves as the blueprint for the Ocrl enzyme. This enzyme's job is to remove a molecule of phosphate from a natural substance called PI(4,5)P_2 ("substrate").

What is PI(4,5)P_2? The PI part stands for PhosphatidylInositol. Looking at the molecular structure in the figure, you can see that PI contains a ring with 6 numbered places. PI can have a molecule of phosphate attached at the 3,4, or 5 positions as well as in combinations of two or even all three positions. PI(4,5)P_2 is one form of PI with two phosphates attached, one at the 4 and the other at the 5 position.

Where do you find PI(4,5)P_2 and why does it matter if the Ocrl enzyme is defective? The cell brings materials in, sends materials out, and dispatches materials to various destinations inside the cell in little packages called "vesicles". Vesicles are bubbles of lipid ("fat") that enclose cargo and separate them from the rest of the watery inside of the cell. Different vesicles are given a name that usually ends with the syllable "-sōme" such as endosome, lysosome, etc.

How do cells know which vesicles go where? By "barcoding" the vesicle surfaces. Vesicles have PI on their outer surfaces. The various PIs with phosphates attached at the 3,4 and/or 5 positions in the ring are the Barcode Address Labels for vesicles that identify what kind of vesicle it is and allow the cell to direct the vesicles to the correct locations in the cell. Ocrl removes the phosphate at the "5" Position from PI(4,5)P_2 and therefore changes a PI(4,5)P_2 label to a PI(4)P label. Loss of this enzyme causes vesicles to remain mislabeled with a PI(4,5)P_2 barcode long after they were supposed to have the barcode changed to PI(4)P. The vesicles containing the incorrect PI hang up in the cell and interfere with the normal machinery of uptake, delivery, and release of material from the cells. Furthermore, Ocrl does more than just convert PI(4,5P_2) to PI(4)P . Ocrl also partners with other proteins and brings them to the vesicle surface. These proteins are needed to direct and process the vesicle. Many of the problems in Lowe Syndrome can be traced to this "traffic jam "in the vesicle trafficking system.

We created an authentic animal model lacking Ocrl function and showed the mouse has the abnormal kidney function that mimics what occurs in Lowe syndrome. This mouse gives us a system from which scientists can remove kidney cells and brain cells to study how a deficiency in this enzyme results in Lowe syndrome, particularly vesicle trafficking. It also gives us an animal in which to test potential therapies in a whole animal, an important step when developing a drug therapy.

Dr R. Claudio Aguilar
Department of Biological Sciences
Purdue University
And
Professor Philip Beales
Institute of Child Health
UCL

'Toward a greater understanding of the cellular biology underlying Lowe syndrome through integration with Cerebro-Renal diseases'

LST Grant: £180,000

The Lowe Syndrome Trust (LST) performs an outstanding job divulging knowledge, providing funds for research and organizing symposia where scientist can discuss ideas and findings concerning the Lowe syndrome.

Thanks to funds provided by the LST, our lab just discovered that Lowe syndrome patient's cells display cellular abnormalities shared with other developmental diseases known as *ciliopathies*. In addition to shed light on the cellular causes of this disease, these findings change the status of Lowe syndrome from an isolated disease to be associated with a broad and better-studied pathology group.

This innovative view will have a major impact in the field and ultimately on the patients' well-being. Specifically, we believe that breakthroughs in terms of mechanistic insights or novel therapeutic approaches, arising in the field of ciliopathies could be immediately capitalized upon by Lowe researchers (and vice versa).

We speculate that drugs capable of counteracting cellular defects in ciliopathies (or their derivatives) will be beneficial for LS patients as well. Indeed, thanks to funds from the LST, we are already testing agents proven to suppress cellular deficiencies in certain ciliopathies on LS patient's cells with very promising results. This exciting new direction is the object of intense investigations in our lab.

No cure or health improvement for the patients will arise without fundamental research, and the LST plays a crucial and unique role in supporting these efforts.

Professor Pietro De Camilli and
Dr Laura Swan
The De Camilli Laboratory
Yale University

'Novel interactors of the Lowe syndrome protein OCRL'

LST Grant: £80.000

Despite the genetic cause of Lowe Syndrome (mutations in the enzyme OCRL) being well known for the past several years, it has been difficult to understand how the loss of OCRL function impinges on the function of specific cells and tissues, and thus to find the correct approach for how to treat or cure patients with this syndrome. In our laboratory, a series of experiments, funded in large part by the activity of the Lowe Syndrome Trust UK, helped define the precise mechanisms through which the protein OCRL interact with other proteins. This work, in turn, helped pinpoint sites of actions of this enzyme in cells and thus brought us closer to understand how OCRL dysfunction causes disease in Lowe Syndrome patients.

A significant subgroup of Lowe syndrome patients has genetic lesions which affect one part of the OCRL protein, called the ASH-RhoGAP domain. This region is important for targeting OCRL to its appropriate cellular location(s) so that it can function properly. Currently, we believe that OCRL functions to help direct membrane "traffic", i.e. the shuttling of proteins and materials from the surface of the cell into interior compartments and back. This process is critical for the cell, much like how a city relies upon properly functioning highways. For this reason, we have concentrated heavily on identifying the proteins which bring OCRL to specific interior compartments, and which do not function in Lowe syndrome patients.

We have shown that defective OCRL proteins found in some patients cannot bind two other proteins: APPL1 and Ses1/2 (also called IPIP27A and B). Both proteins bind OCRL via a similar sequence (which we called an F&H peptide). We identified in turn a specialized surface on OCRL that binds F&H peptide. This surface is conserved in OCRL-like proteins throughout evolution, underscoring the importance of these interactors and suggesting additional interactors. We are now investigating what is the most ancient function of this portion of OCRL. We thank the Lowe Syndrome Trust UK for its efforts, both in fundraising and providing international symposia on this problem, without which, the field of OCRL biology would be far less advanced.

Dr Tim Levine
Department of Cell Biology
UCL Institute of Ophthalmology

'Identifying Interactors of OCRL 1 at intercellular junctions in renal tubular cells as potential drug targets of Lowe Syndrome'

LST Grant: £130,000

Here at the UCL Institute of Ophthalmology, this will be our second grant from the charity, and enables us to continue in our quest to understand and treat Lowe Syndrome. We were fortunate in that our first project made the unexpected finding, that in Lowe Syndrome, cells that form sheets and tubules do not grow together normally. This might explain why complex cellular structures, such as the tubules that concentrate urine in the kidney, fail to function in Lowe Syndrome. Our new project will develop this important new line of research to show how we can rescue growth of cells in tubules, a necessary first step on the road to developing drugs that reverse the effects of Lowe Syndrome, restoring vital functionality.

Dr Martin Lowe
Faculty of Life Sciences
University of Manchester

'Investigating renal dysfunction in a zebrafish model for Lowe syndrome'

LST Grant: £253.000

My laboratory at the University of Manchester has been fortunate enough to receive 3 Lowe Syndrome Trust (LST)-funded research grants, each for ~£80,000. This has funded 3 projects, each of 3 years duration and carried out by a different PhD student. One project has finished, one is at the end of its second year, and the other is in its first year.

We have made a number of important advances through our funding from the LST charity. A major breakthrough was the generation of a zebrafish model for Lowe syndrome. Having an animal model that faithfully recapitulates the symptoms seen in Lowe syndrome patients will allow researchers, ourselves included, to perform experiments to work out how the symptoms of Lowe syndrome occur i.e. to decipher the mechanisms at play. Once we know the mechanisms involved, then we can target them to devise specific drugs to correct the processes that are defective in the disease state. Importantly, by using the zebrafish model we have managed to discover defects that occur in the development of the nervous system, as well reveal for the first time how the kidney problems arise in Lowe syndrome. The latter discoveries on the kidney have led to a current LST-funded project to devise a drug screen in zebrafish. This will necessitate genetically engineering a zebrafish strain in which we can monitor kidney function in living animals. The advantage of zebrafish is that they are amenable to high throughput drug screening. We hope to identify compounds that can restore kidney function in the Lowe syndrome model. These lead compounds can then be used in additional tests in other model systems prior to testing in human patients.

We have distributed our zebrafish Lowe syndrome model to other labs around the world, so they can focus on other specific aspects of the disorder. This includes the lab of Scott Baraban at the University of California San Francisco, who is an expert in studying seizures and epilepsy, and Dr Yang Sun at the Indian University School of Medicine, who is investigating how loss of OCRL1 causes cataracts and glaucoma. We will continue to distribute the fish to any labs that request them.

For further information on Martin's work see a BBC article from 2008 entitled 'Zebrafish used in Syndrome Study'

Dr Anthony Norden
Addenbrooke's Hospital, Cambridge
University of Cambridge

'The role of the megalin-cubilin system in the proteinuria of Lowe Syndrome'

LST Grant: £20,000

Awarded in 2005 by Jonathan Ross and Oscar, this grant extension has enabled Dr Anthony Norden to continue his exploration in partnership with Professor Robert Unwin. Their vital research is an important part of learning more about Lowe Syndrome and its various causes and effects.

UNIVERSITY OF CAMBRIDGE

Dr Van't Hoff, Professor Robert Unwin, and Dr Guido Laube

'An investigation of intracellular metabolism in renal proximal tubular cells from patients with Lowe Syndrome'

LST Grant: £9,000

During 2002 a grant of £9000 was made to support a Kidney Research Project at Gt Ormond Street Hospital/ICH London, headed by Dr Van't Hoff, Robert Unwin and Guido Laube, to whom Lowe Syndrome patients have donated urine samples so that OCRL kidney cells can be cultured and also made available for other research projects. The research is entitled "An investigation of intracellular metabolism in renal proximal tubular cells from patients with LOWE-Syndrome". The latest paper has been published in May 2008 Renal Phenotype in Lowe Syndrome. This concludes "Patients with Lowe syndrome do not have renal Fanconi syndrome but a selective proximal tubulopathy, variable in extent and dominated by low molecular weight proteinuria and hypercalciuria, the classical features of Dent disease"

Professor Shamshad Cockcroft
Lipid Signalling Group
UCL

'Assessment of Golgi structure and membrane traffic in OCRL Cells'

LST Grant: £50.000

UCL

The work funded by The Lowe Syndrome Trust has produced the following findings:

The X-linked oculocerebrorenal syndrome (Lowe Syndrome) is caused by a mutation of the OCRL1 protein, an enzyme that breaks down a unique phospholipid by removing a phosphate. The phospholipid, phosphatidylinositol (4, 5) bisphosphate (PIP2) is special because it has a multiplicity of functions in cell signalling, modulation of the actin cytoskeleton and membrane traffic.

Two major outcomes of our work are: [1] we have shown that endocytosis and Cell Signalling are intricately linked and that stimulation of signalling negatively regulates endocytosis. The possibility that a build-up of PIP2 in OCRL patients can lead to aberrant calcium signalling needs to be considered and this should be examined in a polarized epithelial cell model. [2] The activation of OCRL by a protein, Rab6 which functions as a molecular switch. Rab6 can cycle between the OFF position to ON position. In the ON position, it can bind to OCRL and increase the activity of OCRL. Future work should focus on identifying the upstream regulator of the Rab6 protein.

Professor Kleta, Professor Unwin, and Dr Bockenhauer
UCL, Royal Free Hospital, and Great Ormond Street

'Molecular Studies into familial renal Fanconi syndromes'

LST Grant: £84,000

Funded by a grant of £84,000 from the Lowe Syndrome Trust we have recently discovered a new disease mechanism that sheds light onto how the kidney works. Our competitive team comprised of renal doctors, geneticists, bioinformaticians, computer scientists, cell and molecular biologists and physiologists focused on the study of rare renal disease, discovered a new genetic cause of renal Fanconi syndrome presenting in children and adults - renal Fanconi syndrome is also the kidney manifestation in Lowe syndrome. Our research showed that incorrect placement of one particular enzyme within kidney cells can cause disease, not because it is absent from the place it was meant to be, but because it disturbs the function of the organelle it was wrongly re-routed to. We discovered that a genetic mutation in a protein usually sent to structures called peroxisomes caused misrouting to other structures called mitochondria, which produce the cell's energy. This misplaced enzyme significantly interfered with mitochondrial energy production thereby causing renal Fanconi syndrome.

This work has now been published in the world's leading medical journal, the New England Journal of Medicine (Klootwijk et al., NEJM, 2014, 370, 129-138).

This provides new insights into how one part of the kidney maintains its normal function and therefore provides ideas about how kidney problems, such as those present in Lowe syndrome, can potentially be better diagnosed and treated. We are now continuing with additional studies into other forms of renal Fanconi syndrome to better understand what goes wrong with kidneys in patients with Lowe Syndrome.

Dr John Lucocq
College of Life Sciences
University of Dundee

'OCLR1 and its lipid products'

LST Grant: £60,000

In April 2007 a £10,000 extension was awarded to Dr John Lucocq and his team at the University of Dundee to further their investigations into Lowe Syndrome. This was in addition to a grant of £50,000 awarded in 2003 to undertake research on the protein, Ocrl1, which is produced by the faculty gene and is thought to reside in the Golgi apparatus. The Golgi apparatus is responsible for the processing, sorting, packaging and distribution of proteins in cells to the right destinations within cells. Dr Lucocq believes that defects in kidney and nerve cell function are linked to problems of protein transport from Golgi to other parts of the cells. The research continues to move forward and funding from the Lowe Syndrome Trust remains absolutely vital in the perpetuation of this vital research.

Lowe Syndrome Association

LST Grant: £30,000

The UK charity initially supported the USA Lowe Syndrome Assocation (LSA) with an award of $15,000 towards a total of $60,000 funding for two research projects. The first grant was to Prof. Jeremy W Thorner, Division of Biochemistry, Molecular Biology University of California, Berkeley, USA entitled "Phosphatidylinositol 4-Kinase, Pik1, Shuttles Between the Nucleus and the Cytosol Exploring the Physiological function of PtdIns (4) Generation in the Nucleus". The other research project entitled "Genetic Suppressors of 5-Phosphotase Mutants in C.Elegans" was carried out by Erik M.Jorgensen, Ph.D and Kimberley R. Schuske Ph.D in the department of Biology at the University of Utah in Salt Lake City USA. LSA reviewers commented on the projects as "well conceived and outstanding", "promising" and "have the potential to reveal significant insights about Lowe Syndrome and lead to new treatments".

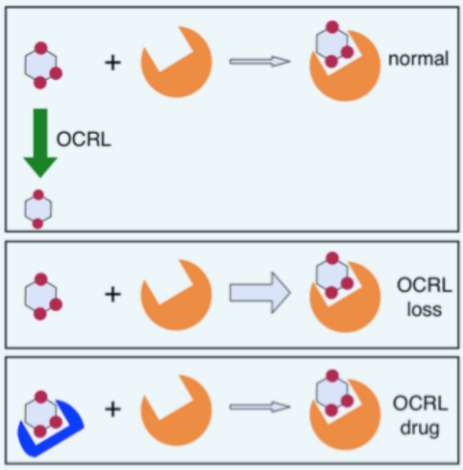
Dr Rudiger Woscholski, Department of Chemistry
Imperial College London

"Chemical intervention of PIP2 dependent pathways'

LST Grant: £330,000

Imperial College London

The loss of the OCRL gene is responsible for the symptoms experienced by Lowe syndrome patients. Any therapeutic intervention would have to either restore the healthy genes in every affected organ (eyes, brain, kidney, skin, etc) using gene therapy, a method that has yet to be made safe for human application, or rely on chemical intervention to alleviate the symptoms. The latter approach is used with many diseases such as cystic fibrosis or even cancer that are caused by genetic mutations. The loss of OCRL will thus increase the level of a particular lipid called PIP2 abnormally and as a consequence alter signalling cascades and cellular behaviour and function. As the OCRL gene product is either absent or "dead" in the patient's organs, the raised PIP2 levels are the only remaining targets. As illustrated in figure 1, PIP2 levels are recognised by other proteins (shown in orange), which convey signals or alter cellular function. When OCRL is lost these signals increase abnormally (thicker arrow indicating this transition), but if a chemical (shown in blue) was able to selectively bind the PIP2 a balance of power could be restored.



We have been funded by the Lowe Syndrome Trust to deliver this type of chemical intervention tool. Our data show that we can use our compound to selectively bind PIP2 in the test-tube and in the living cells. Current funding is directed to support experimental work that will facilitate the translation of this discovery into a viable diagnostic tool and ultimately a future drug programme. While the latter is not yet in our reach, we made considerable progress with the former goal. Attaching a fluorescent reporter to the PIP2 binding molecule we validated earlier, allowed us to visualize the lipids in cells. This is an important step towards a future diagnostic kit development, which will provide a substantial boost in filling the current void in diagnostic capabilities for this disease. Current methods for the determination of Lowe Syndrome in potential patients are cumbersome and laborious. The research underpinned by the funding of the LST has greatly enhanced the possibility to create a diagnostic kit within the next couple of years. The progress achieved would have not been possible without the continuous and unwavering support of the LST. It is the only organization in the world funding research to deliver medical and pharmaceutical solutions for the young boys affected by this disease. Since the launch of the LST nearly a decade ago, money raised through numerous events (i) had changed public and scientific awareness towards this disease on a global level, created a critical mass of research expertise networked through the LST, supported the necessary science to provide a cure for future generations of sufferers of Lowe Syndrome.

To support the Lowe Syndrome Trust please visit the website: www.lowetrust.com or email: lowetrust@gmail.com