
# The Lowe Syndrome Trust International Symposium

# Molecular and Clinical Advances in Lowe Syndrome

## The Royal Society, London
## December 14th 2012

*"Care today … cure tomorrow"*
**LOWE SYNDROME TRUST**

Thank you for attending today's Lowe Syndrome Trust UK symposium. The programme and a brief synopsis of each presentation is attached.

Lowe syndrome is a genetic disorder caused by a missing enzyme and this can affect many organ systems, particularly the brain (with seizures, mental retardation, impaired speech and developmental delay), the kidneys (with loss of important salts and nutrients, and eventual kidney failure), the eyes (with cataracts), the bones (with deformity and arthritis), and the muscles (with weakness). Unfortunately, few children survive beyond their late teens or early adulthood.

The Lowe Syndrome Trust was founded in June 2000 with an aim to raise funds to support research into Lowe Syndrome. All research projects funded by the charity can be found on www.lowetrust.com.

Yours sincerely

Lorraine Thomas
Chair & Trustee
The Lowe Syndrome Trust

**The Fourth International Symposium of the Lowe Syndrome Trust**
**Clinical and Molecular Advances in Lowe Syndrome**
**To be held at The Royal Society, Pall Mall, London, December 14th 2012**

**09.00h – Registration: tea and coffee**

09.30h: Welcome - **Lorraine Thomas**, Founder and Chair of the Lowe Syndrome Trust, London, UK

**MORNING SESSION**

**Chair: *Dr Richard Sandford***

09.40h: **Professor Joris A. Veltman** (Guest speaker) **- "Novel genetic insights into learning disability"**
10.10h: **Professor Helen Cross - *"Cysts and seizures and Lowe syndrome"***

**Chair: *Prof. Robert Unwin***

10.40h: **Professor Robert Kleta - *"The renal Fanconi syndrome"***
11.10h: **Professor Daniel Bichet** (Guest speaker) **– *"The physiology and pathophysiology of water balance"***

***11.45h - Coffee break***

**Chair: *Dr Detlef Bockenhauer***

12.00h: **Steven Scheinman, President and Dean,The Commonwealth Medical College,Pennsylvania, USA - " *Mechanisms of hypercalciuria in Lowe Syndrome and Dent Disease ".***
12.30h**: Dr John Lieske** (Guest speaker) **- *"The Rare Renal Stone Disease Consortium: Dents 1 and 2"***
13.00h: **Debbie Jacobs - "LSA overview of Lowe syndrome 2008: A comprehensive survey"**

***13.20h - Lunch***

**AFTERNOON SESSION**

**Chair: *Prof. Philip Beales***

14.15h: **Dr Claudio Aguilar** - "***Lowe Syndrome and Ciliopathies: Role of Ocrl1 in primary cilia assembly"***
14.45h: **Dr Martin Lowe** - ***"OCRL1 and endocytic membrane traffic"***
15.15h: **Dr Tim Levine** - "***Correct polarisation of renal tubular cells requires OCRL1"***

***15.45h - Tea and Coffee***

**Chair: *Prof. Shamshad Cockcroft***

16.00h: **Dr Rudiger Woscholski** -***"PH-domain mimetics: potential drugs for Lowe syndrome?"***
16:30h: **LauraSwan "*Molecular Interactions of OCRL in the endocytic pathway*"**

**Chair: *Prof. Philippe Jaeger*** *(Guest Chair)*

17.15h: **Professor Roland Baron** (Guest speaker) - "**Bone remodeling: Cellular basis and clinical implications"**
18.00h: **END of meeting**

## ***Talk Abstracts***

**"Novel genetic insights into learning disability"**

*Professor Joris A. Veltman* Department of Human Genetics, Radboud University Nijmegen Medical Centre, PO Box 9101, 6500 HB Nijmegen, The Netherlands.

Germline coding *de novo* mutations (SNVs, indels as well as CNVs) are an important cause of moderate to severe forms of intellectual disability (ID) and associated syndromes. Exome sequencing now allows us to reliably identify these mutations using a single genomic test and we have recently implemented exome sequencing in the diagnostic follow-up of these patients. In this presentation, I will first discuss the role of *de novo* mutations in genetic disease and the associated risk factors such as the local genomic structure and paternal age. Next I will describe our recent work on using a diagnostic family-based exome sequencing approach to test this *de novo* mutation hypothesis in 100 patients with unexplained ID, as well as targeted follow-up studies of several candidate ID genes in 750 additional patients. A total of 79 unique coding *de novo* mutations were identified and validated in 52 patients. Damaging *de novo* (n=10) as well as X-linked maternally-inherited (n=3) mutations were detected in known ID genes, resulting in a minimal diagnostic yield of 13% in this cohort. In addition, potentially causative *de novo* mutations in novel candidate ID genes were detected in 22 patients. For three of these candidate genes, recurrent *de novo* mutations were identified in patients with similar phenotypes, confirming that they are true ID genes. To further expand the possibilities of exome sequencing for mutation detection we have recently validated automatic CNV detection on exome data, and compared its performance to that of high resolution genomic microarrays. This analysis shows that exome sequencing can reliably detect the large majority of pathogenic *de novo* CNVs, responsible for and additional ~15% of ID. In conclusion, *de novo* mutations represent an important cause of ID and exome sequencing is an effective diagnostic strategy for their detection.

**References:**

1. de Ligt J, Willemsen MH, van Bon BWM, Kleefstra T, Yntema HG, Kroes T, Vulto-van Silfhout A, Koolen DA, de Vries P, Gilissen C, del Rosario M, Hoischen A, Scheffer H, de Vries BBA, Brunner HG, Veltman JA, Vissers LELM. Diagnostic Exome sequencing in Patients with Severe Intellectual Disability. New England Journal of Medicine, available online (2012).
2. Vissers LELM, de Ligt J, Gilissen C, Janssen I, Steehouwer M, de Vries P, van Lier B, Arts P, Wieskamp N, del Rosario M, van Bon BWM, Hoischen A, de Vries BBA, Brunner HG, Veltman JA. A *de novo* paradigm for mental retardation. Nat Genet 42: 1109-12 (2010).
3. Hehir-Kwa JY, Rodríguez-Santiago B, Vissers LE, de Leeuw N, Pfundt R, Pérez-Jurado LA, Veltman JA. *De novo* copy number variants associated to intellectual disability have a paternal origin and age bias. J Med Genet 48: 776-8 (2011).
4. Veltman JA & Brunner HG. *De novo* mutations in human genetic disease. Nat Rev Genet 13: 565-575 (2012).

**"Cysts and seizures and Lowe syndrome Cysts, Seizures and Lowe syndrome"**

J Helen Cross.
The Prince of Wales's Chair of Childhood Epilepsy, UCL-institute of Child Health & Great Ormond Street Hospital for Children, London.

Seizures occur in 30-50% of children with Lowe syndrome. Children present with a variety of seizure types including myoclonic seizures, generalised tonic clonic seizures, spasms and atonic seizures. There is also a higher prevalence of febrile seizures than the general population. The epilepsy usually presents before the age of 6 years, and responds variably to antiepileptic drugs. Magnetic resonance imaging has been reported in isolated cases, when signal change in the white matter is seen, with occasional white matter cysts. There is no evidence to date of progressive white or grey matter change. Magnetic resonance spectroscopy of the white matter has been reported to show a peak of myo-inositol, suggestive only of gliosis consistent with the structural MRI findings. A recent Zebrafish model deficient in OCRL1 showed increased susceptibility to heat induced seizures and cystic brain lesions as reported in those with Lowe Syndrome. Presentation of seizures appears likely to be the result of the underlying impaired brain development rather than a marker of further difficulty; MRI structural changes may or may not be related and show little evidence of progression. Clinical chronology of the seizure disorder in those where it occurs appears scant and further study, with EEG and MRI correlation is warranted.

**"The Renal Fanconi syndrome"**

*Professor Robert Kleta,* Potter Chair of Nephrology, University College London, UK

Lowe syndrome is also called oculo-cerebro-renal syndrome, due to the cardinal manifestations of the syndrome in these three organs. Clinical problems (e.g., rickets) can start early in infancy due to renal Fanconi syndrome with urinary losses of vital nutrients. Renal Fanconi syndromes accompany a variety of medical important syndromes including Lowe syndrome. Renal Fanconi syndromes occur due to malfunctioning of the proximal tubule, the part within the renal tubule and nephron, which reabsorbs most of all freely filtered substances (including glucose, phosphate and amino acids) from the urine back to the blood. In order to better understand the pathophysiology of this particular kidney failure we investigate clinically and by use of modern genetic tools rare familial forms of isolated renal Fanconi syndromes in children and adults.

Work presented is a collaboration with Professor Robert Unwin's group (at Centre for Nephrology, Royal Free Hospital, UCL, London), Dr Detlef Bockenhauer's group (at Great Ormond Street Hospital and the Institute of Child Health, UCL, London), and Professor Richard Warth's group (Medical Cell Biology, University Regensburg, Germany).

**"The physiology and pathophysiology of water balance"**

*Professor Daniel Bichet,* Department of Medicine, Hopital du Sacre-Coeur de Montreal

The physiology and pathophysiology of water balance has been enlightened by the identification of genes responsible for key components of water conservation and water excretion. These genes are mutated in rare hereditary disorders characterized by dehydration or, extremely rarely, water excess. With genes mutations leading to defects in renal water reabsorption, affected children have early dehydration episodes since these very young patients are unable to drink enough to satisfy thirst and replace urinary losses. Mental retardation could be a consequence of repeated dehydration episodes in

infancy. Early recognition and treatment of X-linked NDI with an abundant intake of water allows a normal lifespan with normal physical and mental development.

In a recent editorial Richard Lifton [1] indicated that mutations causing more than 2000 Mendelian diseases have been identified, and this has led to rewriting of pathophysiology textbooks on every organ system and the identification of rational targets for therapeutic interventions . I will use the new information provided by the *AVP*, the gene responsible for the antidiuretic hormone production (Arginine Vasopressin) at the hypothalamic level, *AVPR2*, the gene coding for the renal vasopressin V2 receptor, and *AQP2*, the gene coding for the renal collecting duct water channels expressed at the luminal membrane in response to vasopressin, to describe a molecular view of water conservation.

The kidney is responsible for numerous homeostatic functions and, as summarized recently by Sands, Layton and Fenton [2] body fluid tonicity is tightly controlled by regulation of renal water excretion and extracellular fluid volume is controlled by regulated NaCl excretion. There is independent regulation of water and solute excretion, that is, in the absence of changes of solute intake or of changes in metabolic production of waste solutes, the kidney is able to excrete different volumes of water in response to changes in water intake. This ability to excrete the appropriate amount of water without marked perturbations in solute excretion is dependent on renal concentrating and diluting mechanisms. Renal water excretion is tightly regulated by the peptide hormone arginine-vasopressin, the antidiuretic hormone. Circulating vasopressin levels perceived by vasopressin$V_2$ receptors on the basolateral side of principal cells of renal collecting ducts, are determined by tonoreceptors inputs in the hypothalamus and periphery [3] and modulated by other non-osmotic stimuli including Angiotensin II levels [4].The term "tonoreceptors" is used here to distinguish receptors perceiving only "effective" osmoles, from osmoreceptors perceiving both effective osmoles and relatively ineffective osmoles like urea.

**Renal tubular sites of urine concentration and dilution**

The results of micropuncture studies of the mammalian nephron are clear: proximal tubule fluid is always isosmotic with plasma, regardless of whether the kidney is diluting or concentrating urine. In contrast, the fluid in early distal convoluted tubule is always hypotonic regardless of the final osmolality of the urine: the loop of Henle is the major site of dilution of tubule fluid and that dilution processes in the loop occur regardless of whether the final urine is dilute or concentrated. In contrast, the principal site of urine concentration is beyond the distal tubule, in the collecting duct system .

**Math: in the adult kidney 18 liters are reabsorbed under the regulation of vasopressin.**

In adult humans, the glomerular filtration can filter 180 L/day of fluid from the plasma, of which 90% (about 162 L) is returned to the circulation by reabsorption in the proximal tubule and in the descending limb of the loop of Henle. The ascending loop of Henle is completely impermeable to water, that is, does not express any water channel, but rather reabsorbs close to 30% of the filtered NaCl thereby diluting the urine and generating accumulation of NaCl in the medulla. This process, in conjunction with urea sequestering, will increase medullary tonicity, a key component of vasopressin induced water reabsorption through permeabilization of principal cells .

There are three actions of vasopressin aimed to increase urine concentration [5]:

1) By activating the vasopressin V2 receptor, vasopressin will increase the water permeability of principal cells by increasing the luminal membrane accumulation of AQP2 water channels.

2) Vasopressin acts on thick ascending limbs of Henle to stimulate NaCl reabsorption, which increases the osmolality of the medullary interstitium.

**3)** Vasopressin facilitates the transepithelial movement of urea along its concentration gradient in terminal portions of the collecting duct, which allows high levels of urea to be excreted without reducing urinary concentrating ability.

**4) Vasopressin V2 receptors and aquaporin 2 expression at the luminal membrane of principal cells of the collecting ducts.**

AVP is bound to the $V_2$ receptor (a G-protein-linked receptor) on the basolateral membrane. The basic process of G-protein-coupled receptor signaling consists of three steps: a hepta-helical receptor that detects a ligand (in this case, AVP) in the extracellular milieu, a G-protein ($G_{\propto s}$) that dissociates into a subunits bound to GTP and beta-gamma subunits after interaction with the ligand-bound receptor, and an effector (in this case, adenylyl cyclase). AVP activates adenylyl cyclase, increasing the intracellular concentration of cyclic AMP. Protein kinase A (PKA) is the target of the generated cAMP. The binding of cAMP to the regulatory subunits of PKA induces a conformational change, causing these subunits to dissociate from the catalytic subunits. These activated subunits are anchored to an aquaporin-2 (AQP2)-containing endocytic vesicle via an A-kinase anchoring protein. Cytoplasmic vesicles carrying the water channels are fused to the luminal membrane in response to AVP. When AVP is not available, AQP2 water channels are retrieved by an endocytic process, and water permeability returns to its original low rate. Aquaporin-3 (AQP3) and aquaporin-4 (AQP4) water channels are expressed constitutively at the basolateral membrane.

**Hereditary central and nephrogenic diabetes insipidus**

Hereditary diabetes insipidus, a disorder characterised by the excretion of abnormally large volumes (greater than 30 ml/kg body weight per day for an adult patient) of dilute urine (less than 250 mmol/kg) could be hypothalamic, also called central diabetes insipidus, secondary to mutations in the gene coding for AVP, or nephrogenic secondary to mutations affecting either the vasopressin V2 receptor gene or the aquaporin 2 gene [6].

Complex nephrogenic polyuric cases are characterized by loss of water and electrolytes including sodium, potassium, calcium, and chloride. They are secondary to the loss of function of sodium, potassium or chloride transporters/channels that are key to the construction of the counter-current concentration system or to physical alterations of the renal medulla induced by other hereditary renal diseases [7.]

We are recommending the sequencing of the nephrogenic diabetes insipidus genes in all the affected patients. The genes involved are, with a few exceptions, relatively small and easy to sequence. This genomic information is key to the routine care of patients with congenital polyuria and, as in other genetic diseases, reduces health costs and provides psychological benefits to patients and families.

**Related papers**


**1.** Lifton RP. Individual genomes on the horizon. N Engl J Med. 2010;362:1235–6

2. Sands JM, Layton, H.E., Fenton, R.A. Urinary Concentration and Dilution. In: Taal MW, Chertow GM, Marsden PA, Skorecki K, Yu ASL, Brenner BM, eds. *Brenner & Rector's The Kidney*. 9th ed. Philadelphia: Elsevier Saunders; 2012:326-352.

**3.** Lechner SG, Markworth S, Poole K, et al. The molecular and cellular identity of peripheral osmoreceptors. *Neuron.* Jan 27 2011;69(2):332-344.

**4.** Prager-Khoutorsky M, Bourque CW. Osmosensation in vasopressin neurons: changing actin density to optimize function. *Trends Neurosci.* Feb 2010;33(2):76-83.

**5.** Brown D, Fenton, R.A. The Cell Biology of Vasopressin Action. In: Taal MW, Chertow GM, Marsden PA, Skorecki K, Yu ASL, Brenner BM, eds. *Brenner & Rector's The Kidney*. 9th ed. Philadelphia: Elsevier Saunders; 2012:353-383.

**6.** Bichet DG. Clinical manifestations and causes of nephrogenic diabetes insipidus[www.uptodate.com:](http://www.uptodate.com:) UpToDate; 2012.

**7.** Bockenhauer D, van't Hoff W, Dattani M, Lehnhardt A, Subtirelu M, Hildebrandt F, and Bichet DG. Secondary nephrogenic diabetes insipidus as a complication of inherited renal diseases. *Nephron Physiology* 116: p23-29, 2010.

**"Mechanisms of hypercalciuria in Lowe Syndrome and Dent Disease"**

*Steven J. Scheinman, M.D.*
President and DeanThe Commonwealth Medical College

Patients with the Lowe syndrome and Dent disease typically have recurrent kidney stones and develop nephrocalcinosis. The major contributing factor for stones in these patients is hypercalciuria. A fuller understanding of the mechanisms of hypercalciuria is now emerging. In growing boys with Dent disease and in CLC-5 knockout mice there is a component of fasting hypercalciuria that most likely reflects abnormal bone turnover. However, the largest component of the hypercalciuria in patients and KO mice is diet-dependent. It has been known that the normal rise in urinary calcium following oral calcium loading is exaggerated in patients with Dent disease, with relatively suppressed PTH levels and elevated levels of 1,25-dihydroxyvitamin D. Thus, it has been speculated that excessive production of 1,25(OH)D, either in response to hypophosphatemia or as a manifestation of proximal tubular dysfunction, drives excessive intestinal absorption. Recent observations point to a direct effect of mutations in OCRL1 and CLC-5 on intestinal calcium transport mechanisms. The TRPV6 calcium channel is expressed at the apical surface of intestinal epithelial cells and mediates absorptive calcium transport. Expression of TRPV6 is increased in response to 1,25(OH)D. In *Xenopus* oocytes, OCRL suppresses the activity of the TRPV6 intestinal calcium channel through modulation of $PI(4,5)P_2$ levels that affect activity of the calcium channel. Mutations in OCRL that cause Dent 2 disease reduce this suppression and thus increase TRPV6-mediated calcium transport. In CLC-5 knockout mice, TRPV6 expression is increased, and preliminary data indicate that, as with OCRL, expression of CLC-5 in *Xenopus* suppresses TRPV6 abundance. These observations indicate that mutations in these genes causing the Lowe syndrome and Dent-1 and Dent-2 disease may produce hypercalciuria through a direct increase in TRPV6-mediated intestinal calcium absorption.

References:

1. Wu G, Zhang W, Na T, Jing H, Wu H, Peng JB. Suppression of intestinal calcium entry channel TRPV6 by OCRL, a lipid phosphatase associated with Lowe syndrome and Dent disease. *Am J Physiol Cell Physiol*. 302(10):C1479-91, 2012.
2. Scheinman, S.J.: Dent's disease. Chapter 12, pp. 213-226 in *Genetic Diseases of the Kidney*, eds. R. Lifton, G. Giebisch, S. Somlo, D. Seldin, Elsevier, 2009

**"The Rare Renal Stone Disease Consortium: Dents 1 and 2"**

*Dr John Lieske M.D.*, Mayo Clinic

The Rare Kidney Stone Consortium (RKSC; see www.rarekidneystones.org) is increasing awareness and improving the management of 4 genetic causes of kidney stones: Dent Disease, Primary Hyperoxaluria, APRT deficiency, and cystinuria. Each is characterized by frequent kidney stone attacks, a risk for renal damage and even kidney failure. Although nephrolithiasis is common, affecting up to 10% of Americans, these rare genetic causes make up only a small percent of the total and are easily missed. Even when the diseases are recognized, they are often not managed optimally due to

physicians' lack of experience.

Registries are now active for all 4 diseases. The Dent Disease registry accumulates information about the clinical manifestations of patients with Dent disease in order to increase knowledge about this rare disorder, in order to develop better treatments in the future. Increasing evidence suggests that there is a relationship between Dent Disease and Lowe Syndrome. At least some patients with Lowe syndrome have kidney stones and other kidney problems like persons with Dent disease. Indeed, some of the Dent disease patients have the same gene mutation as patients with Lowe syndrome. Furthermore, the other gene that can cause Dent disease acts along similar pathways within cells as the Lowe Syndrome gene. Therefore patients with Lowe Syndrome genetic mutations and kidney manifestations can also be included in the Dent disease registry. We encourage all patients and affected families to participate in order to advance progress towards a cure for these diseases.

Data from 71 patents in the registry as of June 2012 were recently presented at the American Society of Nephrology (San Diego, CA; 11/2/12). Patients are currently enrolled from North America (n=43), Europe (n=9), Asia (n=9), and other locations (n=10). Disease type was confirmed as Dent 1 (*CLCN5* mutation, n=44), Dent 2 (*OCRL1* mutation, n=1), neither Dent 1 nor Dent 2 (n=4), or unknown (n=22). Age at diagnosis was 13.6 (±11.7) yrs. (range 0-44; 9 patients (13%) diagnosed after age 30). Mean followup was 3.8 yrs. Fourteen patients had kidney stones at an age of 17.2 (±10.2) years, most as the presenting symptom but 3 substantially (6.8 yrs) later. For those with data, low molecular weight proteinuria (95%), hypercalciuria (87%), and hematuria (44%) were common, while kidney stones (27%) and bone disease (11%) were less common. Total protein excretion was 1.8 (±1.6) g/24-hr. Random urinary calcium:creatinine ratio was 0.61 (±0.60) mg/mg. Among those with normal kidney function and greater than 16 yrs old (n=8), 24-hr urine calcium was 361 (±174) mg. First available serum creatinine was 1.1 (±1.9) mg/dl at an age of 14.1 (±11.6) yrs. 6 patients progressed to ESRD at a mean age of 30.5 (±13.2) yrs; 4 currently have functioning renal allografts.

Therefore, our data suggest patients with Dent disease are most often diagnosed in their early teens and only a minority ever experience clinical kidney stones. Moderate proteinuria, hypercalciuria, and hematuria are more commonly present. Clinicians should maintain a high index of suspicion for Dent disease in younger males with unexplained proteinuria and CKD.
Please join us on the new Rare Kidney Stone Consortium Dent Disease Facebook page for further updates:

http://www.facebook.com/home.php#!/pages/Dent-Disease-Mayo-Clinic/218213651553992

**"LSA overview of Lowe syndrome 2008: A comprehensive survey"**

*Debbie Jacobs* President, Lowe Syndrome Association (LSA), an international non-profit, all-volunteer organization made up of parents, friends, professionals, and others who are interested in this rare condition.

A brief overview of the Report on the Lowe Syndrome Comprehensive Survey - 2008 Study strategies including survey software, content of survey, confidentiality, taking the survey, analysis of results, presentation of results, dissemination of results and updating the 2000, 3th edition of the Living with Lowe Syndrome book, A Guide for Families and Professional.

**"Lowe Syndrome and Ciliopathies: Role of Ocrl1 in primary cilia assembly"**

*Dr. Claudio Aguilar,* Department of Biological Sciences, Perdue University, USA

I will present evidence indicating that Lowe patient cells have defects in the assembly of primary cilia and this phenotype was reproduced in cell lines by knock-down of Ocrl1. Importantly, this defect could be rescued by re-introduction of WT Ocrl1 in both patient and Ocrl1 knock-down cells. In addition, a zebrafish animal model of LS exhibited cilia defects and multiple morphological and anatomical abnormalities typically seen in ciliopathies. Mechanistically, we show that Ocrl1 is involved in protein trafficking to the primary cilia in a Rab8-and IPIP27/Ses-dependent manner. Taking into consideration the relevance of the signaling pathways hosted by the primary cilium, our results suggest hitherto unrecognized mechanisms by which Ocrl1 deficiency may contribute to the phenotypic characteristics of Lowe syndrome. This conceptual change in our understanding of the disease etiology may provide an alternative avenue for the development of therapies.

**"OCRL1 and Endocytic Membrane Traffic"**

*Martin Lowe, Faculty of Life Sciences, University of Manchester, UK*

Mutation of OCRL1 causes two disorders in humans, namely oculocerebrorenal syndrome of Lowe, or Lowe syndrome, and Dent-2 disease, which are characterized by renal tubulopathy accompanied by eye and neurological defects. Recent studies have indicated that a number of cellular processes are affected by loss of OCRL1, including the trafficking of cargo proteins within the endocytic pathway. To better understand the cellular role of OCRL1 we have sought to identify novel interaction partners for this protein, resulting in the discovery of IPIP27 (inositol phosphatase interacting protein of 27 kDa) A and B, also referred to as Ses1 and 2. Previously we reported that IPIP27A and B are regulators of cargo recycling in the endocytic pathway. In this presentation I will describe our most recent progress on the IPIP27 proteins, and propose a model for how they cooperate with OCRL1 to regulate cargo trafficking in the endocytic pathway. I will also discuss progress made in a zebrafish model for Lowe syndrome and Dent-2 disease, which faithfully recapitulates many of the symptoms seen in human Lowe and Dent-2 patients. Our findings support the hypothesis that defects in endocytic trafficking are responsible for the manifestations of Lowe syndrome and Dent-2 disease.

Work completed in collaboration with Chris Noakes, Zenobia Mehta, Guanhua Yan and Grzegorz Pietka.

**" Correct polarisation of renal tubular cells requires OCRL1"**

*Timothy Levine,* Department of Cell Biology, Institute of Ophthalmology, UCL, UK

Mutations in the phosphoinositide 5-phosphatase OCRL1 cause Lowe syndrome and Dents-2 disease, both of which can be classified as renal Fanconi syndromes. The epithelial cells lining the renal proximal tubules are highly polarised with distinct apical and basolateral surfaces separated by intercellular junctions. Using Madin–Darby canine kidney

(MDCK) cells as a model of renal epithelia we found that a pool of OCRL1 targets intercellular junctions and is required for the correct organisation of apical and basolateral surfaces. MDCK cells depleted of >90% OCRL1 polarise along a horizontal rather than vertical axis, with eventual formation of the apical domain in the lateral membrane and not at the cell apex. One of the first events to occur upon polarisation is recycling of plasma membrane components with specific targeting to the newly forming apical domain. In the absence of OCRL1, this apical recycling is blocked and apical cargo accumulates in Rab11 positive compartments that have unusually elevated levels of PI(4,5)P2, the OCRL1 substrate. This apical cargo is subsequently mis-targeted to the lateral membrane. These results suggest that OCRL1 is required for exit of apical cargo from recycling apical endosomes, and that without OCRL1 polarising cells fail to orient an axis of polarity in the vertical plane. An initial problem in polarisation is likely to affect downstream events such as primary cilium formation. Since cell types affected by Lowe Syndrome such as neurons are highly polarised, polarisation defects may contribute to the eventual disease.

Work completed in collaboration with Rachel Daniels, Adam Grieve, Karl Matter, Rob Bacallao, George Ojakian and Martin Lowe.

**"PH-domain mimetics: potential drugs for Lowe syndrome? "**

*Dr Rudiger Woscholski,* Division of Cell and Molecular biology, Imperial College London, UK

Lowe syndrome is caused by the loss of function of the OCRL phosphatase, which in turn results in elevated levels of the inositol lipid PI(4,5)P2. Our hypothesis is that lowering the levels of this lipid will mimic the action of the missing phosphatase. We provide evidence that a chemical receptor designed to bind PI(4,5)P2 mimics the action of the endogenous cellular PI(4,5)P2 binding proteins, such as PH domains. This PH domain mimetic (PHDM) is capable of penetrating cells, where it will seek out the PI(4,5)P2, making it inaccessible for the endogenous signalling molecules. Treatment with PHDM would thus mask cellular PI(4,5)P2 levels and in doing so mimic the missing OCRL phosphatase. This proof of principle shows that there are promising chemical lead compounds that could be the basis for future drug discovery programmes for Lowe Syndrome.

**"Molecular Interactions of OCRL in the endocytic pathway"**

*Laura Swan*, Department of Cell Biology and Howard Hughes Medical Institute, Yale University School of Medicine, USA

Evolutionary conservation of the F&H motif binding site of OCRL

Laura Swan[abc], Alexandre Luscher[d], Florian Fröhlich[a], Florence Leuba[d], Michelle Pirruccello[abc], Tobias Walther[a], Thierry Soldati[d] and Pietro De Camilli[abc]

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We will present an update of our studies on the molecular properties of OCRL and of its interactors. Our lab identified the endocytic proteins APPL1 and Ses1/2 as binding partners for the C-terminal region of OCRL through a common short peptide motif (F&H motif) present in these proteins. Such interaction is disrupted by several patient mutations. The crystal structure of the ASH-RhoGAP-like domain of OCRL in a complex with the F&H peptide of Ses1 shows that the F&H peptide binds to an evolutionary conserved patch on the surface of the RhoGAP-like domain. Curiously, while we have identified three interaction partners for the F&H binding surface of OCRL (APPL1, Ses1 and Ses2) in humans, lower organisms preserve a F&H binding surface on OCRL in the absence of any clear homologue of any of these 3 interactors. Thus, we investigated the ancient F&H binding surface of the single OCRL homologue Dd5p4 in the social amoeba *Dictyostelium discoideum*. Previous work has shown that expression of human OCRL in the *Dd5P4* background partially rescues survival defects, suggesting that amoeboid OCRL and human OCRL have similar function.
Differential label free Mass Spectrometry identified three proteins whose interaction with Dd5P4 is abolished by mutation of the F&H surface. Remarkably, despite there being no identifiable sequence homology, the domain structure of the dictyostelium interactors (BAR and PH) are remarkably similar to that of known human F&H partners APPL1 and Ses1/2. This suggests that there may well be an evolutionarily conserved triad of interaction partners for the F&H domain, which can variably direct OCRL activity to needed sites of OCRL phosphatase activity in the cell.

Related Papers:
Swan LE*, Pirruccello M*, Folta-Stogniew E, De Camilli P. 2011. Recognition of the F&H motif by the Lowe Syndrome protein OCRL, *Nat Struct Mol Bio.* 18:789-95 PMID:21666675

Swan LE, Tomasini L, Pirruccello M, Lunardi J, and De Camilli P. 2010. Two closely related endocytic proteins that share a common OCRL binding motif with APPL1. *Proc. Nat. Acad. Sci.* 107: 3511- 1516. PMID: PMC2840126

**"Bone remodeling: Cellular basis and clinical implications"**

*Professor Roland Baron,* Department of Medicine, Harvard Medical School

The objective of Dr Baron's lecture is to provide an up-to-date encompassing view of bone biology, its relationship with other organs and its relevance to our current understanding of skeletal diseases and to new drug development. After describing the cellular and extracellular organization of bone as an organ and as a tissue, the lecture will go in a detailed description of the molecular mechanisms that govern the differentiation and function of the three cell types that are present in bone: the osteoblast, the osteocyte and the osteoclast and their coordinated activities in the context of bone growth and bone remodeling. Particular emphasis will be placed on the crosstalk between these cells and cells of the hematopoietic bone marrow environment and the resulting reciprocal regulation of bone remodeling and hematopoiesis. The lecture will then present and discuss the role of bone as an endocrine organ and an integrated player in the overall regulation of mineral metabolism, its relationship with other organs such as the kidney and the brain, and its role in the regulation of fat, glucose and energy homeostasis, in addition to skeletal homeostasis. The novel therapeutic options to treat skeletal diseases that have been generated as a result of this recent progress in our understanding of bone biology will also be presented and discussed.