Lowe Syndrome Research Meeting 2023

Lay Abstracts

R. Claudio Aguilar: Studying different genetic mutations in Lowe syndrome
His research team is looking at different genetic mutations in Lowe syndrome patients. They found that not all mutations have the same effects on the OCRL1 protein. In future this could help us understand the different symptoms in Lowe syndrome patients.

Antonija Jurak Begonja: Bleeding problems in Lowe Syndrome
Some Lowe syndrome patients have bleeding problems during surgery. She and her team found that the cells have abnormal blood clotting. They are studying how this is related to the genetic changes in Lowe syndrome.

Antonella de Matteis

TBC

Arnaud Echard: Studying how cells control certain processes
His research team found that cells use certain proteins to control the way cells move substances around inside them. This knowledge can help us understand how Lowe syndrome affects cells.

Francesco Emma: Kidney problems in Lowe Syndrome
Kidney problems in Lowe syndrome are called Fanconi syndrome. He is an expert in how to measure and monitor this condition and is establishing how to use this information in clinical trials.

Kai Erdmann: Using tiny organ models to study Lowe Syndrome
His research team is developing and using tiny organ models to study Lowe syndrome. They found that these models can mimic the disease and help test new treatments.

Jenny Gallop: Studying how fatty chemicals called phosphoinositide lipids affect kidney and brain cells
Her research team are finding out how changes in phosphoinositides can affect cells in the kidney and brain. In cells they tested a medicine that might improve kidney

function in Lowe syndrome patients. They are now studying how this medicine affects brain cells.

Paul James: Showing there can be Positives
I will be talking about my work and the opportunities I have as a person living with Lowe syndrome. I hope to continue to educate the general public and give hope to those families with someone with Lowe syndrome.
www.livingwithlowes.com Twitter: @livingwithlowes

Jeri Kubicki: Strength in Unity: the LSA's Commitment to community, family voices, and research advancement
In my presentation I will share LSA's priorities and desires, and a bit more about our broad community and how we support it. I'll also share perspectives from our families about research desires, and challenges living with Lowe syndrome.

Herb Lachman: Making a new model for studying Lowe syndrome
His research team has made a model using stem cells to study the brain and mental aspects of Lowe syndrome. They used cells from patients with certain gene mutations and found that some mutations were worse than having no gene at all. This suggests that different treatments will be needed for the different parts of the body affected in Lowe syndrome.

Martin Lowe: Using fish to study Lowe syndrome
His research team are using genetically altered fish to study Lowe syndrome. They found similar problems in the fish's nervous system and kidneys to patients. These fish models can help us understand and treat Lowe syndrome.

Raghu Padinjat: Studying the brain development in Lowe syndrome
His team are using stem cells to study brain development in Lowe syndrome patients. They found changes in how brain cells become specialized and how they function.

Leopoldo Staiano: Studying kidney function decline in Lowe syndrome
He and his team are studying how the *OCRL1* gene and changes in fatty chemicals in cells affect kidney function in Lowe syndrome. They found that this can lead to kidney damage and cell death. They are using tiny organ kidney models to study the disease and find new treatments.

Yang "Young" Sun: Eye problems in Lowe syndrome
Children with Lowe syndrome often have eye problems, like cataracts and vision loss. His research team is studying these eye problems to better understand and treat them.

Vidhu Thaker: Finding the role of the *OCRL* gene in the brain for hormones and growth
Her research team are studying how a specific gene variant affects the brain. This may help us understand how Lowe syndrome affects growth.

Andrew Thomas: Lowe Syndrome Trust
TBC

Scientific Abstracts

Prof R. Claudio Aguilar
Purdue University, USA

Phenotypic and Biochemical Abnormalities Displayed by Conformationally Affected OCRL1 Patient's Variants

Abstract:
Lowe Syndrome (LS) is a condition due to mutations in the *OCRL1* gene, characterized by congenital cataracts, intellectual disability, and kidney malfunction. Unfortunately, patients succumb to renal failure after adolescence. This study is centered in investigating the biochemical and phenotypic impact of patient's OCRL1 variants (OCRL1^{VAR}). Specifically, we tested the hypothesis that some OCRL1^{VAR} are stabilized in a non-functional conformation by focusing on missense mutations affecting the phosphatase domain but not changing residues involved in binding/catalysis. The pathogenic and conformational characteristics of the selected variants were evaluated *in silico* and our results revealed some OCRL1^{VAR} to be benign while others pathogenic. Then we proceeded to monitor the enzymatic activity and function in kidney cells of the different OCRL1^{VAR}. Based on their enzymatic activity and presence/absence of phenotypes, the variants segregated in 2 categories that also correlated with the severity of the condition they induce. Overall, these two groups mapped to opposite sides of the phosphatase domain. In summary, our findings highlight that not every mutation affecting the catalytic domain impairs OCRL1's enzymatic activity. Importantly, data support the inactive-conformation hypothesis. Finally, our results contribute to establish the molecular and structural basis for the observed heterogeneity in severity/symptomatology displayed by patients.

Dr Antonija Jurak Begonja
University of Rijeka, Croatia

Bleeding tendency in Lowe syndrome: OCRL controls cytoskeletal rearrangements during platelet adhesion

Bleeding events during surgery have been reported in patients with Lowe syndrome (LS). The main function of platelets is to activate when the vessel wall is injured and to stop bleeding by clot formation. Recent studies indicate impaired primary hemostasis and abnormal platelet function in the presence of OCRL deficiency or inhibition, although normal or sometimes low platelet counts are found in LS patients. In our work, we demonstrate altered reorganization of the cytoskeleton of human platelets with pharmacologically inhibited OCRL: platelets do not fully spread, form mainly filopodia, accumulate actin nodules, and retain microtubular coils.

Prof Antonella de Matteis
TBC

Dr Arnaud Echard
Institut Pasteur, France

Rab35/OCRL control PI(4,5)P_2 and F-actin both during endocytosis and cytokinesis

I will present an overview of our findings showing that the Rab35 GTPase directly interacts with and recruits OCRL to the plasma membrane and on newly formed endosomes to limit PI(4,5)P2 and F-actin accumulation. This is key for cytokinetic abscission and endocytic recycling back to the plasma membrane, the latter being likely relevant for understanding the cellular basis of the Lowe syndrome.

Dr Francesco Emma
Osperdale Bambino Gesu, Italy

Renal Fanconi syndrome and its measurement in clinical trials

The kidney problems in Lowe syndrome are in a particular clinical pattern called Fanconi syndrome. I will explain and discuss the clinical aspects and how we monitor Fanconi syndrome. I will show how clinically we can measure it and the utility of nuclear medicine scans for use as an endpoint in clinical trials.

Dr Kai Erdmann
University of Sheffield, UK

Organ on a chip technology to model Lowe syndrome

Organs on chips are microfluidic devices, which allow the incorporation of fluid flow in in vitro tissue models. We have applied this technology to successfully mimic disease phenotypes of the gut and kidney, i.e., for inflammatory bowel disease and Lowe syndrome. There is a need for physiological human in vitro models for Lowe syndrome/Dent II disease to study the underpinning disease mechanisms and to identify and characterise potential drugs and drug targets. We have established a proximal tubule organ on chip model combining a 3D tubule architecture with fluid flow shear stress that phenocopies hallmarks of Lowe syndrome/Dent II disease. We demonstrate the suitability of our in vitro model for drug target validation. Furthermore, using this model, we demonstrate that proximal tubule cells lacking OCRL expression upregulate markers typical for epithelial-mesenchymal transition (EMT), including the transcription factor SNAI2/Slug, and show increased collagen expression and deposition, which potentially contributes to interstitial fibrosis and disease progression as observed in Lowe syndrome and Dent II disease. Furthermore, our work on inflammatory bowel disease also demonstrates as a proof of principle the potential of our organ on chip platform to use induced pluripotent stem cells as starting material to build patient specific in vitro models as well as to

expand the complexity of the model by including additional cell types such as macrophages into the model. Similar strategies are currently followed to further improve our existing organ on a chip model for Lowe syndrome.

Dr Jenny Gallop
University of Cambridge, UK

Understanding how the changes in phosphatidylinositol (4,5)-bisphosphate metabolism alter kidney and brain cells and how to rebalance it

We have been investigating the molecular mechanisms by which elevated $PI(4,5)P_2$ levels affect the actin cytoskeleton within cells. We generated a set of preclinical data in kidney proximal tubule cells, demonstrating that the approved drug alpelisib, a class 1 PI3K inhibitor, improves proximal tubule cell function in a mouse model of Lowe syndrome and Dent disease 2. Building on this finding, we are now examining how altered phosphoinositide metabolism in neuronal growth cone membranes influences actin-rich protrusions and axon guidance. Our goal is to make basic science-based predictions on the potential effects of alpelisib on these processes in Lowe syndrome cells and ultimately in patients.

Prof Herb Lachman
Albert Einstein College of Medicine, USA

Modeling Lowe Syndrome using induced pluripotent stem cells (iPSC)
A mouse model has been developed for Lowe syndrome (LS) that recapitulates the renal pathology. However, the cognitive and neuropsychiatric manifestations of LS are not seen. Consequently, we developed an iPSC model system in which molecular and cellular phenomena that could potentially underlie the cognitive and neuropsychiatric features could be analyzed in patient-specific neurons and neural progenitor cells (NPCs). To this end, we generated iPSC lines from three LS cases who have mutations in the 3' end of OCRL in which the catalytic domain is preserved, albeit is non-functional. In addition, we generated true *OCRL* KO lines using CRISPR-Cas9 gene editing on iPSCs from derived from typically developing controls. No OCRL protein is produced from the KO lines. We found that certain phenotypes, in particular, F-actin polymerization and WAVE1 formation in NPCs, were dramatically affected by patient mutations. However, the KO lines did not show any defects in F-actin or WAVE-1, suggesting that the hypomorphic patient mutations are worse than having no OCRL at all, indicative of a type of dominant-negative effect. Indeed, when iPSCs containing a patient specific hypomorphic variant was knocked out using CRISPR, the F-actin and WAVE1 abnormalities were rescued. On the other hand, other functions associated with LS mutations, such as endosome recycling, were equally impaired in both the patient and KO lines, suggesting that this phenotype is not subjected to a dominant negative effect. This dichotomy is likely due to substrate specific differences in OCRL binding. The finding has therapeutic implications in that treatment directed at a particular organ system or pathway might work in one but not the other. In addition, proteomics data will be presented that point to several potential novel targets for therapeutic intervention.

Prof Martin Lowe
University of Manchester, UK

Using zebrafish to model Lowe syndrome

To investigate the underlying mechanisms of Lowe syndrome we have generated a number of genetically altered zebrafish lines and characterised their phenotypes. These include a gene-trap line that has residual Ocrl expression, a Sanger ENU mutant that has complete loss of Ocrl, and a TALEN-generated mutant in the Ocrl-binding partner Ipip27A. We have used these lines to investigate phenotypes in the nervous system and kidney, two of the three major affected tissues in Lowe syndrome. Our findings indicate perturbed neurodevelopment, with recent analysis showing defective endocytic traffic in the neuroepithelial tissue during early embryonic stages. We have also observed defective renal tubular function in both the Ocrl and Ipip27A models, which suggest defective endocytic traffic and lysosomal homeostasis in the proximal tubular region, which could help account for the renal manifestations seen in human Lowe syndrome patients. In the longer term, these zebrafish models may be used for drug screening as well as further phenotypic characterization, including exploration of genetic adaptation that can occur upon loss of Ocrl or its paralogue Inpp5b.

Prof Raghu Padinjat
National Centre for Biological Sciences-TIFR, India

Cellular mechanisms underlying the neurodevelopmental phenotype of Lowe syndrome.

A neurodevelopmental delay is one of the key clinical features of Lowe syndrome. However, the cellular and physiological mechanisms that lead to altered brain development and function remain unknown. I will present our recent work using iPSC derived culture of brain tissue from Lowe syndrome patients that provide insights into the mechanisms that include altered differentiation and physiology in the developing brain of patients with Lowe syndrome.

Prof Yang “Young” Sun
Stanford University, USA

Ocular presentations of Lowe syndrome— challenges and advances

Children with Lowe syndrome typically develop bilateral congenital cataracts, which can lead to early vision loss. The causes of vision loss may include cataract-related procedures, congenital glaucoma, microphthalmia, nystagmus, corneal scarring/keloid formation, and amblyopia. Bilateral dense congenital cataracts are often the first signs of Lowe syndrome. Early identification and removal of cataracts can promote visual development. To gain a better understanding of the ophthalmic findings in Lowe syndrome patients, we conducted a comprehensive analysis of their clinical data, which included a review of their ocular conditions and clinical management.

Dr Leopoldo Staiano
TIGEM and IRGB-CNR, ITA

Kidney organoids as a model to study novel roles of OCRL associated with the progressive decline of kidney function in Lowe Syndrome.

Fatty Acids (FAs) are transported from Lipid Droplets (LDs) to peroxisomes and mitochondria where they are used as a source of energy. Kidney proximal tubule epithelial cells mostly rely on FAs metabolism for energy production and its impairment results in severe cell damage and cell death. Phosphatidylinositol 4,5bisphosphate ($PI4,5P_2$) favours the contacts between LDs and peroxisomes, allowing FAs metabolism and transport to mitochondria. $PI4,5P_2$ level is increased in Lowe Syndrome (LS), a rare genetic disease caused by mutations in OCRL (a $PI4,5P_2$ 5-phosphatase) characterized by congenital cataracts, intellectual disabilities, and Renal Fanconi Syndrome. The decline of kidney function towards chronic kidney disease (CKD) has been observed in several LS patients, although its pathogenesis is understudied. To discover new molecular mechanisms of proximal tubule dysfunction and CKD in LS we investigated the role of OCRL and $PI4,5P_2$ in the regulation of LDs-peroxisomes-mitochondria interplay and its effect on FAs metabolism. In kidney proximal tubule cells depleted of OCRL, $PI4,5P_2$ accumulates on LDs and peroxisomes reducing the transport of fuelling lipids to mitochondria that display lower mitochondrial membrane potential, increased ROS and structural abnormalities. Cells and kidney organoids lacking OCRL display an increased number of $PI4,5P_2$-rich LDs because of the combined impairment of LDs contact with peroxisomes. Finally, kidney organoids lacking OCRL display increased deposition of extracellular matrix and thus increased fibrosis, which is a hallmark of CKD. In summary, this study is paving the way to a wider understanding of kidney dysfunction in LS and aims at the discovery of new fundamental cell biology mechanisms controlled by $PI4,5P_2$ and OCRL.

Prof Vidhu Thaker
Colombia University, USA

Establishing the role of *OCRL* in the hypothalamus based on transcript specific expression.

Variable splicing of *OCRL* resulting from a 24-bp exon was identified with the physical mapping of the gene and subsequently confirmed in two independent studies. We identified an individual with frameshift variant limited to the 24-bp region with loss of expression of the long isoform while preserving the short isoform. The tissue specific loss of this isoform may help define the role of OCRL in the brain, specifically the neuroendocrine pathways. This presentation will discuss our current findings on the potential neuroendocrine function of *OCRL* and hypotheses based on tissue expression and patient specific induced pluripotent stem cell lines.

Glossary

1. Alpelisib: A drug used to treat certain types of breast cancer, and has potential therapeutic effects on Lowe Syndrome.
2. Chronic kidney disease (CKD): A condition in which kidney function gradually declines over time, potentially leading to kidney failure.
3. Congenital cataracts: Clouding of the eye lens present at birth, which can cause vision problems.
4. CRISPR-Cas9: A tool used to edit genes by adding, removing, or changing specific DNA sequences.
5. Cytokinesis: The process by which a cell divides into two daughter cells during cell division.
6. Cytoskeleton: A network of proteins within a cell that provides structural support and plays a role in various cellular processes.
7. Endocytosis: The process by which cells take in molecules from the environment by engulfing them in their membrane.
8. Epithelial-mesenchymal transition (EMT): A process by which epithelial cells, which line organs and blood vessels, transform into more mobile and invasive mesenchymal cells. This process is involved in tissue repair, but can also contribute to disease progression.
9. F-actin: A protein that forms the structure of cells and is involved in various cellular processes.
10. Fanconi syndrome: A kidney disorder causing excessive loss of nutrients and electrolytes in the urine.
11. Hemostasis: The process by which blood flow is stopped after an injury.
12. Induced pluripotent stem cells (iPSC): Adult cells that are reprogrammed into a state similar to embryonic stem cells, capable of developing into different cell types.
13. Lipid droplets (LDs): Storage compartments within cells that hold lipids, or fat molecules.
14. Lowe Syndrome (LS): A rare genetic disorder affecting the eyes, brain, and kidneys, causing congenital cataracts, intellectual disabilities, and kidney problems.
15. Neuroendocrine pathways: Communication networks between the nervous system and the endocrine system, responsible for regulating hormone release.
16. Neural progenitor cells (NPCs): A type of precursor cell that can give rise to different types of cells in the nervous system.
17. Nuclear medicine scans: Imaging tests that use small amounts of radioactive materials to diagnose and treat various diseases.
18. OCRL1 gene: A gene responsible for encoding the enzyme OCRL1, mutations in which can lead to Lowe Syndrome.
19. Organ on a chip: A microfluidic device that simulates the functions of an organ, providing a more realistic environment for studying diseases and testing drugs.
20. Organoid: A three-dimensional, miniaturized, and simplified version of an organ, grown in vitro from stem cells. Organoids mimic the structure and

function of their corresponding organ, providing a valuable tool for studying organ development, disease modeling, and testing potential treatments.

21. Peroxisomes: Small cellular structures involved in the breakdown of fatty acids and the elimination of toxic substances.
22. Phosphatidylinositol (4,5) bisphosphate (PI(4,5)P2): A type of lipid molecule found in cell membranes that plays a crucial role in cell signaling and membrane trafficking.
23. Platelets: Blood cells that help form clots to stop bleeding.
24. WAVE1: A protein involved in the regulation of actin polymerization, which is essential for cell shape and movement.
25. Zebrafish: A small tropical fish used as a model organism for studying human diseases.