

Report on 2nd Asia-Pacific Lysosomal Conference, Auckland, NZ, February 14-16, 2019

The 2nd Asia-Pacific Lysosomal Conference was held at the Holiday Inn Auckland Airport with the purpose to bring together experts in the field of Lysosomal Diseases. Our theme, “***Lysosomes and disease: Today and tomorrow***” was chosen to reflect the state of knowledge now and the future prospects in these exciting times as longed-for treatment options become realisable. 165 people attended, to hear presentations on the science, diagnosis, clinical care and personal stories of the impacts of these diseases on patients and families, and their often difficult journey through the health system.

Summary

1 – Scientific presentations.

On days 1 and 2 of the conference, 30 speakers on the science of these diseases gave us a good insight into the history of research into lysosomal diseases, from the early studies that led to the first tentative developments of therapies for them, and the advances made over subsequent decades to build on those developments to improve on therapeutic options, refine delivery methods for enzyme replacement therapies, and to explore the application of promising new technologies such as gene therapy, chaperone therapies, stem cell therapies, and substrate reduction therapies.

Of note in these presentations were themes of:

- International collaborations being essential to making good progress, with NZ researchers playing a significant part in discoveries across several of these diseases.
- Good animal models providing significant research advantages, with NZ making a big contribution with several large naturally occurring models.
- Collaboration with patients and their families being an important part of motivation, fundraising and provision of samples and natural history to enable research.
- The progressive improvement in knowledge over time, which needs support to continue the momentum, rather than assuming the first advance is the whole and complete story.
- Regional variations in diagnosis, care, and treatment of patients which are subject to political priorities, healthcare investment, social expectations, and economic power.

See the appendix to this report for a more detailed summary of the scientific sessions, prepared by Prof David Palmer, who was the Scientific Chair for the conference.

2 – Diagnosis, clinical care, screening and patient/family experiences.

Day 3 of the conference saw 19 presentations on topics that covered the whole range of experiences that families have with these diseases, and how the areas of diagnosis and clinical care have changed over time.

The early days saw these diseases often missed altogether, leaving the family with missed opportunities for therapeutic intervention, or accusations that the family was the problem, and frequent struggles to find coherent medical care and social support when clinicians or officials did not understand what the disorder was, or even when they did, inadequately responded to because of its rarity, or complexities in the health bureaucracy. These problems, along with challenges in getting access to therapies, to a greater or lesser extent, were a reality in both Australia and New Zealand, where all but one of our speakers on day 3 were from.

Personal testimony from parents and patients talked about the challenges they faced, some of them harrowing to hear, and how just occasionally things did line up for them for better care. We had

some presentations that day from health professionals to describe how services are changing to respond to these needs, and how new possibilities in screening and diagnosis can change future management, and how improved medical interventions in pain management, and cardiac and skeletal surgeries, can improve health and quality of life for Lysosomal patients.

LDNZ and other patient advocacy groups in the region have worked for many years to improve health and disability sector response to our diseases, and the personal stories highlighted issues we have frequently directed here in NZ to politicians, Ministry officials and District Health Boards. Topics have included delayed diagnosis, problematic transitions to adult services, problems with access to tertiary services across DHB boundaries, improvements needed to palliative care for young patients, problems with carer support and respite care, and access to funding for treatments. Special thanks are given to Ra Timms, Kirsty Taylor, Samantha Prior, Dan Peach, and Vanessa Ede-Scott who gave personal accounts of their experiences as lysosomal disease parents or patients. So much of what has been achieved, or will be achieved in the future, was encapsulated in the experiences they talked about so eloquently, and which identified problems and possible solutions.

These problems have been the core of the work plan for LDNZ, the Australian MPS society, and other Australian Lysosomal support groups for decades now. We know this set of issues is a core problem for other advocacy groups in the region too, but unfortunately we did not have talks from outside Australia and NZ on them.

Talks from Ross Drake from the Paediatric Palliative Care service, Bryony Ryder from the Metabolic service, and Rosie Marks, a Paediatrician at Starship hospital, identified service improvements that we had worked to achieve as a direct result of learning from families what the needs and issues were. The PPC service and the metabolic service exist in part because of direct advocacy by LDNZ for them to be funded, and services in Australia have received similar support for their establishment from the Australia patient groups. But there is still a long way to go before healthcare for lysosomal diseases in both countries meets an ideal standard, and certainly much work is needed in the rest of the region.

Talks on an Australian carrier screening research project, and a pilot of newborn screening for Pompe disease in Taiwan, gave us a taste of emerging applications of technologies that may help in future to improve treatment by early detection, and possibly to prevent the disease altogether, but these talks also highlighted challenges in making such screening reliable and effective tools that would deliver real benefits with minimal harms. Discussions around this topic highlighted different viewpoints and the need for more research before a consensus is likely about the way forward for a range of possible applications of screening technologies for our diseases.

An important part of the day's program was an opportunity to present problems of access to medicines in NZ to the Chair and CEO of Pharmac who attended. This gave the chance to outline a decade and a half of problems with Pharmac's approach to funding novel treatments, and a series of dishonest and manipulative processes they had gone through to make very little sound like a lot. Given the new Minister's commitment that progress needed to be made in funding treatments for rare diseases, and that he saw this as fundamentally being about equity, we had a unique opportunity to challenge Pharmac about their approach, knowing they would be reviewing their policy on rare diseases and equity at a meeting a few weeks later. While the response from Pharmac was predictably non-committal in advance of their meeting, we have subsequently received confirmation from them that their policy on equity will change in line with our requests and the Minister's expectations, demonstrating the significant value of having them there at the meeting.

3 – Appreciation to conference funders and supporters.

LDNZ is very grateful for the many companies and institutions that provided grants, travel subsidies, and other assistance to make this meeting possible.

Bringing experts from around the world was so important, as the rarity and complexity of these diseases needs the best brains internationally, in cooperation, to make progress. And that progress is happening. Even though it is slow progress, decades of relentless pursuit of knowledge by teams of international researchers and clinicians, supported by patient advocacy groups, has shifted these diseases to a point when we really are on the cusp of major breakthroughs in therapies for them.

Attendance at the meeting also enabled a number of invited speakers to attend other research meetings in Christchurch and Auckland in the following week, to build their collaborations with NZ researchers and explore new avenues in their research projects.

Your support helped make this meeting a very successful event in many ways.

John Forman
Chair of Lysosomal Diseases NZ
For the LDNZ board

Appendix – Conference scientific report, by Prof David Palmer

Day one.

The conference opened with a very powerful science session “*Lysosomal biology and pathophysiology of lysosomal storage diseases*”. First up was a Keynote presentation from Prof Hans Aerts, from Leiden University, Netherlands. “*LSD's and their challenges, as illustrated by Gaucher disease – Looking back and looking forward*” in which he outlined the progress made in understanding Gaucher disease and treating it along with Fabry disease and Pompe disease. Hans stressed how much progress has been made since the first enzyme replacement therapy (ERT) for GD, which required extraction of glucocerebrosidase, coded for by the GBA gene, from 3,000 placentas and lamented at how the cost for ERT had not come down, even though recombinant enzyme is now readily available. He highlighted the poor correlation of GBA genotype and GD phenotype, even among monozygotic twins, and the poorly understood increased risk for Parkinson's disease in individuals carrying a mutant GBA gene, suggesting that glucocerebrosidase may have substrates additional to glucosylceramide. He also stressed that all genetically diagnosed Pompe patients will benefit more from being treated as soon as possible, regardless of their clinical status.

That was followed by a seminal talk “*Membrane lipids and storage compounds regulate lysosomal sphingolipid catabolism and trigger a secondary accumulation of lipids in lysosomal disease*” from Konrad Sandhoff, from Bonn University, Germany. Konrad told us how he discovered the basis of what became Sandhoff disease in the first 18 months of his Ph D, and his subsequent experiences and insights into the world of sphingolipid catabolism and the sphingoliposes. This included an exposition of the complexity and specificity of membrane lipids, the roles of activator proteins and the interaction of substrate inhibition of enzyme activities and ionic lipids leading to complicated phenotype genotype correlations of which Konrad and colleagues have developed an enzyme kinetics understating. He also emphasized the importance of natural, rather than synthetic, substrates in the analysis of the actual hydrolytic activities of the enzymes degrading sphingolipids and to understand the primary and secondary accumulations in the LSDs.

“*Lysosomal proteins, proteomics and disease*” presented by David Sleat from Rutgers University, USA outlined an exquisite suite of biochemical experiments he conducted with Peter Lobel that led to the discovery of the enzyme and gene activities missing in CLN2, classical late infantile Batten disease. Central to this was the innovative use of mannose-6-phosphate receptors isolated from 300 litres of cow serum for affinity blotting of mannose-6-phosphate tagged proteins in tissues from normal controls and patients of CLN2 disease. Partial protein sequencing, degenerate primer design and PCR was used to identify the gene (TPP1 for tripeptidyl protease 1), then causative mutations were identified by DNA sequencing. The information gained has been used for the first ERT for this disease. In recent developments this strategy, coupled with protein mass spectroscopy, has been used to define the soluble lysosomal proteome leading to the discovery of some more soluble lysosomal proteins.

One of the hard lessons I have learnt at these meetings is that the funders and regulators want all our works to be the final word and no further development should be considered. To me this is as daft as stopping aeroplane development after the Wright brothers managed 230 metres at an altitude of 7 metres in a minute, and is against the theme of our meeting *Lysosomes and Disease, Today & Tomorrow*. Thus the next talk “*Carbohydrate-mediated lysosomal protein trafficking, and modifications to improve therapies*” was very timely. In this Antony Fairbanks, University of Canterbury, described some exciting possibilities to improve the uptake of ERT currently under investigation.

Fernanda Copeland, Avobrio, USA, continued this theme with a discussion of the current advances in gene therapy, particularly *ex vivo* lentiviral based gene therapy in which stem cells are loaded outside the body prior to delivery. "*Identifying therapeutic targets to treat Niemann–Pick type C disease*" by Andrew Munkacsi, Victoria University of Wellington (VUW), continued the theme of ongoing development, this time using yeast models to understand the roles of various histone deacetylases in the development and regulation of Niemann–Pick type C.

Sometimes things deemed to be old hat suddenly become very important as neuroanatomy has in this case. Lots of our increased understanding of the LSDs comes from classical style neuroanatomy studies of small and large animal models of LSDs, both natural and "man-made." In "*Neuropathological assessments of animal LSD therapy trials*" the world leading specialist, Jon Cooper from Washington University, St Louis, USA, told us about his collaborative work with a number of us and the advances in our understanding of the course and causes of neurodegeneration, and the effects of therapies, from careful studies of small animals (mainly mice) large animals (sheep and some dogs) and carefully comparing the neuroanatomical changes.

Day two.

Day two opened with "*Disease models and therapy studies*". Nadia Mitchell, Lincoln/Otago presented long-term successful gene therapy of CLN5 affected sheep treated by a single pair of intraventricular injections of AAV9 derived vectors in her talk "*Sheep as a pre-clinical model for human gene therapy*." This therapy blocked the disease development and neurodegeneration when administered preclinically and blocked or dramatically slowed further neurodegeneration when administered to clinically affected sheep, meaning that it will be useful to children after clinical diagnosis and thus particularly relevant to the human conditions. Nadia outlined progress in treating the retinal degradation by gene therapy and highlighted the value of sheep as models for human disease and a lot of work on in vivo testing of disease progression in them, which enhances their value.

This presentation was followed by two others on the subject of sheep as models of lysosomal disease. Samantha Eaton, Edinburgh/Roslin Institute, Scotland, described a successful "*Novel CRISPR generated ovine model of CLN1 disease*." Three CLN1 affected lambs had been created by this method and the next crop were due for further studies. Imke Tammen, University of Sydney described the "*Generation of CRISPR/Cas9 genome edited sheep embryos in preparation to create an ovine CLN7 Batten disease research flock*" using more advanced methods to increase the proportion of successful CRISPR gene transfers as well as a helpful "*Survey of LSDs in large animals including cattle with Niemann-Pick disease*."

Next Miguel Sena-Esteves, University of Massachusetts, Boston, USA, told us of "*Therapeutic efficacy studies of a bicistronic AAV9-Hexa/b vector delivered systemically or to CSF in Sandhoff mice*" in which separate AAV vectors containing the α and β hexaminidase (HexA and HexB) subunits (α or β gangliosidosis; Tay Sachs and Sandhoff diseases) were intracranially injected into mice together, followed by intracerebroventricular injections of AAV9 constructs coding for both, each with its own promoter, reading either from the ends or from the middle. These worked really well in Sandhoff mice. In "*Gene therapy for the gangliosidoses - from the bench to the bedside*" Heather Gray-Edwards, also University of Massachusetts, told us more of sheep, with an α GM2 gangliosidosis (Tay Sachs disease) and of cats with a β GM2 gangliosidosis (Sandhoff disease) in which disease was significantly delayed by AAV gene therapy delivered to the CNS, intraventricularly, intrathecally and by lumbar injection. Following the success of these trials an application has been made to start clinical trials next year.

In "*Pathogenesis and treatment of skeletal disease in MPS children and Stem cells to understand MPS pathogenesis*" Sharon Byers, University of Adelaide, described how neither ERT nor bone marrow

transplants (BMT) clear glycosaminoglycans (GAGs) from chondrocytes and osteocytes in mucopolysaccharidoses (MPS's) and that the skeletal system remains a significant site of pathology in MPS children who continue to need orthopaedic intervention. Trabecular bone mass, (the very porous highly vascularized type of bone that contains red bone marrow) is reduced in cats with MPS VI (Maroteaux-Lamy syndrome) caused by deficiency of arylsulfatase B (ASB) and storage of dermatan sulphate. Growth plates in mice with MPS VII (Sly syndrome, caused by a deficiency of the β -glucuronidase) display all the hallmark characteristics of slowly growing bones. These animal studies point to two interventions to improve skeletal health; therapies that increase or maintain bone mass and therapies that promote growth plate chondrocyte division and/or hypertrophic maturation to normalise bone length, both of which can be tested in animal models.

Next, in a teleconference presentation "*Gag reduction in MPS VI – Odiparcil a potential GAG clearance therapy*" Mireille Tallandier, Inventiva Pharma, France, outlined the effectiveness of this drug in diverting cellular GAGs to become odiparcil bound and soluble, allowing urine excretion. Firstly it bound GAGs in affected fibroblasts leading to inhibition of storage. Next oral administration to affected mice led to urinary excretion, less GAG storage in cartilage, better joint movement and improved corneal thickness. These data support the clinical investigation of odiparcil in a clinical trial which is now underway.

Phillip Rendle, Ferrier Institute, VUW then gave a short presentation "*The development of analytical reagents and standards to aid in the diagnosis of and monitoring treatment of Morquio A syndrome (MPS IVA)*" caused by an N-acetylgalactosamine 6-sulfatase deficiency. This talk highlighted the increasing importance of carbohydrate chemistry and carbohydrate chemists to improving our understanding and developing our tool-kits for evaluation and therapies.

A group of four talks on MPS IIIA (Sanfilippo syndrome) caused by deficiencies of heparan N-sulfatase followed starting with three from Sandra Orgeig's group at the University of Adelaide focusing on mouse model studies of airway obstruction, restrictive lung disease and respiratory infections that can lead to death in children. Tamara Paget talked about "*Heparan sulphate and lipid analysis of lung tissue and alveolar surfactant in the Mucopolysaccharidosis IIIA (MPS IIIA, Sanfilippo syndrome) mouse.*" Sandra herself told us that "*Pulmonary surfactant activity is reduced in mucopolysaccharidosis IIIA mice*" and Emma Parkinson-Lawrence that "*The mucopolysaccharidosis (MPS) IIIA mouse demonstrates increased airways resistance.*" These talks were admirable for their clarity and interesting in that these systemic physiological complications of LSDs are important and life threatening but putative treatments tend to be pushed aside in favour of things like high throughput cell culture studies. I am also aware that mucus build-up and lung infections can be fatal contributors to CLN2 Batten disease. Relieving these symptoms may be invaluable, especially as it has been deemed that in relation to gene therapy for Sanfilippo "for any future (gene or enzyme) treatment to be successful, it must be administered as early as possible" (Wikipedia). Currently MPS-III A is mainly diagnosed clinically, by which stage it is probably too late for any treatment to be very effective. Neonatal screening programs would provide the earliest possible diagnosis. Adeline Lau, also from Adelaide then gave a talk about "*The evaluation of a novel substrate reduction therapy for Sanfilippo syndrome.*"

The afternoon started with session 3; *Lysosomal diseases in other parts of the world* started with a very interesting and sobering presentation from Sheela Nampoothiri, Kerala, India "*Spectrum of lysosomal storage disorders: 13 years' experience from a single tertiary care centre in Kerala*". Sheela works in the single care centre for all of the 37 million people of this state which has extremely limited resources. In spite of this enormous load they have dealt with 241 patients with LSDs, MPS's being the most common, followed by mucopolysaccharidoses II and III. Consanguinity was positive in 74/241 (30.7%) which explains the high incidence of homozygous mutations. Since 2008 some patients with Pompe, Gaucher, Fabry and MPS have been treated successfully with ERT. Particularly germane to

LDNZ was the successful ERT treatment of patients with Pompe disease, as Hans Aerts indicated most successful when began based on preclinical genetic diagnosis. However this treatment, dependent on the “largesse” of a drug company, was stopped when the Indian government indicated it would take responsibility but could not afford the bill. For me this was a chilling indication of the way LSD treatment development and delivery is handled currently, through the interactions of public sector research, triaging agencies and proprietorial profit driven drug companies. My growing unease centres on how this outcome is backward looking, stymies progress, is unfair and does not deliver to the majority of patients.

In *“Improving the efficacy of enzyme replacement therapy for infantile neuronal ceroid lipofuscinosis”* Ivanhoe Leung, University of Auckland told us of work towards improving ERT for infantile NCL (CLN1) by inhibiting (deactivating) palmitoylation of the enzyme through inhibition of the palmitoylating activities of DHHC 3 and 7, thus increasing the stability of the enzyme.

Yin-Hsiu Chien, National Taiwan University Hospital, Taipei followed. In *“Newborn screening for lysosomal storage diseases by tandem mass spectrometry: update in Taiwan’s experience.”* She told us of the power of tandem mass spectroscopy newborn screening in diagnosing Pompe, Fabry, Gaucher, iduronidase (MPS-1), iduronate 2-sulfatase (IDS, MPS II), N- α -acetylglucosaminidase (NAGLU, MPS IIIB), galactosamine-6-sulfate sulfatase (GALNS, MPS IVA), and arylsulfatase B (ARSB, MPSVI) patients from newborn blood spots. She emphasised the power of beginning ERT as soon as possible after diagnosis rather than waiting for the development of clinical symptoms and irreversible manifestations, highlighting Pompe disease as her example.

Next Ines Noher de Halac, Children’s Hospital, Cordoba, Argentina. Outlined the South American situation in *“Understanding the role of neurogenetics in translational research of neuronal ceroid lipofuscinoses in Argentina.”* Two things struck me; the sheer enormity of being responsible for half a continent, and the genetic differences between and European Amero-Indian derived late infantile NCL2 patients. Both populations show the range of disease subtypes on presentation, but the mutations underlying them are unique to each. This indicates that the disease causing mutations are rather recent and that treatments and genetic diagnoses based on common mutations may not transfer automatically to distant ethnic groups.

Kazunori Tanizawa, JCR Pharmaceuticals, Japan told us of *“A novel therapeutic approach for treatment of CNS manifestations in patients with mucopolysaccharidosis”* using an antibody based “J-Brain Cargo” to deliver ERT across the blood brain barrier for MPS II, now in phase II and phase III therapy trials.

Session 4, *Existing and Emerging Therapies* opened with Steve Gray, UT Southwestern Medical Center, Dallas, USA and *“Intrathecal administration of AAV9: A platform-based gene transfer approach to treat lysosomal storage diseases”* in which he outlined the translational potential of AAV9 vectors for treating inherited CNS disorders. Intravenous injection has been the route used in at least 3 clinical trials, for spinal muscular atrophy (SMA), MPS IIIA, and MPS IIIB. However this route of administration can be hampered by the presence of natural anti-AAV neutralizing antibodies, high peripheral organ gene transfer causing gene-dependent toxicity and the high doses required to achieve sensible CNS transfer. These factors can be ameliorated by direct CNS administration, intrathecal administration of AAV9 leading to broad CNS gene transfer in mice, pigs, and non-human primates. This approach has been used to treat giant axonal neuropathy (GAN), Batten disease (CLN6), and SMA. Nine additional clinical trials are being initiated, including aspartylglucosaminuria, CLN1 Batten disease, CLN5 Batten disease, CLN7 Batten disease, multiple sulfatase deficiency, Tay-Sachs and Sandhoff diseases.

In the next talk *“Pathogenesis, enzyme replacement and gene therapies for glycoprotein and glycolipid storage diseases”* Alessandra d’Azzo, St. Jude Children’s Research Hospital, Memphis, TN,

USA talked of the roles of lysosomal enzymes and their substrates in basic cellular processes by applying these concepts to studies of the lysosomal diseases sialidosis, galactosialidosis (GS) and GM1-gangliosidosis caused by single or combined deficiency of the lysosomal enzymes neuraminidase 1 (NEU1), protective protein/ cathepsin A (PPCA) and β -galactosidase (β -GAL). She indicated that NEU1 is a negative regulator of lysosomal exocytosis, a ubiquitous calcium-dependent pathway that affects the way cells communicate with each other and with the extracellular environment. The excessive exocytosis of lysosomal contents that ensues as a consequence of NEU1 deficiency and impaired sialic acid processing of one of its substrates, LAMP1, is at the basis of many of the phenotypic alterations characteristic of sialidosis and GS in both mouse models and in patients.

In *"The challenges of developing therapies for mucopolysaccharide diseases in the 21st century"* Brian Bigger, University of Manchester, England alluded to the ERT, substrate reduction and haematopoietic stem cell therapies for MPS then focused on exciting advances in gene therapies, including recent work from his own lab for three of the different Sanfilippo (mucopolysaccharidosis types IIIA, IIIB and IIIC) diseases and Hunter disease (MPSII) and options for gene therapy likely to be tested in the clinic over the next few years. Brian highlighted the use of cell type specific promoters, inducing tolerance to avoid antibody responses, exploring different vector capsids and the difficulties in moving from mouse studies to translation. He spoke of the value of larger animals to bridge this gap, including sheep studies to determine the effectiveness of different brain parenchymal injection procedures, one being carefully targeted convection enhanced delivery for human brain surgery.

Another approach to treatments for LSDs is the use of molecular chaperones, discussed by Katsumi Higaki, Tottori University, Yanago, Japan, in the *"Development of pharmacological chaperone therapy for lysosomal storage diseases."* Chaperones are small molecules that bind to proteins ensuring that they are locked into their biologically active conformation. Some mutations lead to a loss of the relevant conformation (and thus enzyme activity) without otherwise affecting the enzyme. In these cases binding a chaperone can restore the required conformation and thus the enzyme activity. In this regard Migalastat has been approved for treating Fabry disease in Japan. Katsumi spoke the characterisation of other chaperone candidates to move this strategy forward to other LSDs, including of Gm₁-gangliosidosis.

Mark Thomas, Royal Perth Hospital, Perth, returned to gene therapy in his presentation *"Stem cell gene replacement therapy (GRT) for Fabry disease"* He concentrated on a more sophisticated delivery of gene therapy, using adult stem cells loaded with codon optimised genes of interest using lentiviral vectors to deliver the cargo ex vivo. In this, cells from the patient's blood or bone marrow are removed and grown in the laboratory, then transduced with the vector. In particular he concentrated on ex vivo autologous stem cell (CD34+) self-inactivating third-generation lentiviral GRT, containing the human codon-optimised α -galactosidase A gene. So far there has been a good outcome in three out of four Fabry disease patients treated this way and other trials are planned. This method of treatment is a "one off" avoiding the potential antibody problems from ERT and the need for lifelong treatment required for that and for chaperone therapy.

To conclude: I think the meeting provided an excellent forum reflecting the state of knowledge and the future prospects in these exciting times as longed-for treatment options become realisable. The scientific progress has been remarkable as has the transformation to "can do" from the "too rare, too hard, too bad" diagnoses of 20 years ago when LDNZ was formed.