CDKL5 FORUM

MEETING REPORT

WELLCOME TRUST CONFERENCE CENTRE, LONDON
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Hosted and organised by

loulou FOUNDATION

In collaboration with

International Foundation For CDKL5 RESEARCH
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CDKL5 INSIEME VERSO LA CURA

EXECUTIVE SUMMARY

The Loulou Foundation, established in 2015, is a private non-profit UK Foundation dedicated to advancing the scientific understanding of CDKL5 deficiency while actively pursuing multiple approaches towards effective treatments For the disorder.

On October 10-11th 2016, the Loulou Foundation hosted the second CDKL5 Forum meeting which showcased the latest research into CDKL5 deficiency with investigator presentations and posters of original data. It also facilitated brain-storming sessions exploring existing and future therapeutic approaches. The outcomes and conclusions of the meeting will also guide the Loulou Foundation's research funding priorities for the coming year.

The Forum chairman, **Sir Colin Blakemore**, noted the extraordinary progress in the past year since last year's meeting, with 14 new research grants into CDKL5 deficiency awarded, supporting the work of 50 scientists at 24 labs in 13 leading institutions around the world.

The Forum also welcomed two special guest speakers at the meeting. The first day keynote address from **George Freeman MP**, the first UK Minister for Life Sciences, said the CDKL5 Forum was to be congratulated for bringing together academics, industry and patient advocates, "What's so important about your community is that the patient voice sits right at the heart of everything you do".

The second day keynote address was a very moving presentation by **John Crowley**, Chairman & CEO of Amicus Therapeutics. He provided insights 'learnt along the way' in his quest to find a cure for Pompe Disease, the life threatening condition affecting two of his three children. His story formed the basis of the 2010 film 'Extraordinary Measures' with Harrison Ford and Brendan Fraser. The two essential elements for successful drug development, Crowley explained, are vision and the ability to take risks. With vision, you need to think where they want to be in five or ten years, and then work backwards from there. With risk taking, people need to appreciate that it is acceptable to make mistakes. "Failure is ok as long as you are transparent and honest, and think, what can we learn, and where should we go next".

Amicus Therapeutics, said Crowley, has recently started a CDKL5 pre-clinical programme, based on an enzyme replacement approach. "We are going to move heaven and earth to do everything we can," he said.

The main CDKL5 Forum programme was divided into 5 sections with workshop sessions supporting four of the topics to allow for more wide ranging discussion.

The **First Session, 'Improving Biological Understanding To Enable Translational Research'**, addressed key questions – how is CDKL5 regulated? - what are the upstream and downstream pathways controlling function? and – how do mutations lead to disease? The answers are really important said moderator **Dario Alessi**, because they will provide us with the framework of knowledge that will enable us to play the engineer and work with clinicians to improve therapies for this devastating condition."

The main function of CDKL5 is as a kinase that needs to be activated to have biological effects, with the terminal domain involved in upstream and downstream regulation. Pathogenic mutations are known to occur throughout the protein, including the kinase domain, so discovering how the kinase domain binds and how the process can be disrupted will be important for greater understanding of the biology.

In neurons, CDKL5 expression has been shown in both the cytoplasm and dendrites. It is found at the synapse, both pre- and post-synaptically. CDKL5 has the ability to traffic between the cytoplasm and nucleus in response to glutamatergic stimulation. **Giles Hardingham** reported preliminary data probing presynaptic function using a pH-sensitive probe which revealed CDKL5 over-expression slowed endocytosis, while knock-down and neurons from knock-out mice showed a trend toward the acceleration of endocytosis. He also reported promising data centred on characterisation of a new anti-human CDKL5 antibody with preliminary data showing the presence of CDKL5 in synaptic fractions of the mouse brain.

CDKL5 is expressed in multiple brain regions and different types of neurons, with the highest levels found in forebrain neurons. **Joe Zhou** and colleagues removed CDKL5 expression only from forebrain excitatory neurons. They found that these mice show learning and memory deficits which mimick intellectual disability-like features, but not other phenotypes related to CDKL5 disorder. "We now think the intellectual disability features of CDKL5 may be due to a dysfunction in the excitatory neurons," said Zhou.

Knowledge of gene transcript isoforms and their developmental expression profiles, **Ralph Hector** explained, is essential for understanding the mechanistic roles of CDKL5 in brain development. He proposed a new nomenclature system for naming CDKL5 gene products that encompasses both the range of transcript isoforms and predicted protein isoforms.

"In order to understand the function of the CDKL5 protein and resulting pathology in the brain when it is lost, we need to discover the biological substrates, and understand how they are regulated and what the downstream effects are," explained **Sila Ultanir.** "It's only when we understand in more depth what CDKL5 is doing that we will be able to come up with intelligent therapeutics." To date her laboratory has revealed three substrates of CDKL5.

In **Session Two, 'Clinical Research and Study Design', Laura Mamounas** (NINDS/NIH) explained, in neurology over the past decade, the vast majority of drug trials have failed despite promising preclinical findings. Companies and sponsors often have little understanding of why their trials failed. This has led to recognition of the need to improve the quality and reproducibility of animal research, and to bring the 'rigor and quality' of study design expected in clinical trials to preclinical animal studies.

NINDS has recently set up the NeuroNext Network to support early-stage and exploratory clinical trials in neurological disorders. The idea is to conduct a series of progressive, exploratory trials each building upon what we learned from the previous trial before launching the pivotal Phase III trial.

Concluding the session, moderator **Helen Cross** said: "The key thing that has come across is the importance of sharing of information, not just in CDKL5 but with other rare diseases. It is vital in order to not make the same mistakes all over again. It is also important to remember that negative results are just as important as positive results, but time and again they never make it to publication."

In the accompanying workshop session the need to create better open databases and registries of CDKL5 patients was identified as critical in order to provide a worldwide research resource. Despite the area being highlighted for action at last year's CDKL5 Forum, there had been few advances. It was agreed the next step should be a 'scoping exercise' exploring issues raised in the session and considering the technology to be used. Organisations already running established databases should be consulted.

In **Session Three, 'Disease Modelling', Peter Kind** provided insights into new rat models for CDKL5. The models, he said, have been made possible by the new DNA editing technology Crispr/ Cas 9, which introduces a cut into exon 8 resulting in a 10 base pair deletion and premature stop codon in exon 9.

There are now opportunities to move beyond mouse models of conditions like CDKL5 to rats, pigs and monkeys. Having more models will allow us to determine whether key phenotypes are conserved across mammalian species and hence are relevant to humans with CDKL5; "ultimately this will help us move into trials earlier," he said.

Advantages of mouse models, **Joe Zhou** explained, are that mice are readily amenable to genetic manipulations which reflect human genetic mutations, and represent the 'lowest species' that can be used to assess higher cognitive functions with brain disorders. Despite current CDKL5 mouse models lacking the manifestation of spontaneous seizures, we are starting to see robust phenotypes emerge in these models that are reproducible in different mutation models and in different labs.

Steven Sheridan (Massachusetts General Hospital) outlined patient-specific in-vitro cellular modelling of CDKL5 using human induced pluripotent stem cells (iPSCs). Human iPSCs are a genetically accurate

model allowing for the study of different cell types specific to neurological disease which provides the opportunity to grow large numbers of cells facilitating chemical and genetic screening. “In addition we can study various steps in early development, later development and even neurodegeneration,” said Sheridan. “Possibilities, exist to model individual patient variation and targeted treatments”, he added.

Ashley Winslow emphasized the valuable role that could be played by the CDKL5 Portal in communicating information. “There are potential tools that the community could benefit from, which hopefully they can share. The CDKL5 Forum Portal is one way to make that knowledge public. We want to link people up collaboratively,” she said.

John Crowley moderated a panel discussion in **Session Four** on the **Industry Perspective** that considered where industry should be focusing their research efforts to best enable clinical development in rare diseases.

Companies apply different strategies to identify which rare diseases to focus on. For example, GSK looks for clinical data showing treatment efficacy as well as strategic platforms that could be applied across other therapies, where Roche is focussed on unmet medical need and providing innovative medicines.

Small patient numbers related to rare diseases were not regarded as a deterrent. It is understood that more patients are likely to be identified once drugs became available. It was recommended that pharma companies should engage with patients through social media and advocacy groups in the discovery phase rather waiting for Phase 3 clinical trials.

In **Session Five** on **‘Novel Therapeutic Approaches to Treat CDKL5 Deficiency’, Michael Green** described the potential for pharmacological reactivation of the X-linked CDKL5 gene as a treatment.

The typical CDKL5 patient is a female who is heterozygous for CDKL5 deficiency. In female cells the normal biological process is for one of the two X-chromosomes to become inactive (Xi) and the other to remain active (Xa), with the result that approximately 50% of cells express the mutant CDKL5 gene and 50% wild-type. Green’s hypothesis is that reactivation of Xi would lead to cells containing both wild-type and mutant CDKL5. “Since CDKL5 is an enzyme even small amounts of reactivation might be sufficient to bring clinical benefits,” said Green.

Green and colleagues are in the process of identifying X-chromosome inactivation factors (XCIFs), which may provide desirable targets for the development of drugs that function by reactivating the silent Xi-linked CDKL5 gene. Already, the approach has resulted in the identification of several dozen new epigenetic regulators and protein kinases involved in X-chromosome inactivation.

David Cavalla described Healx’s repurposing approach that matches the genetic profiles of patients with rare diseases to drugs already on the market. A recent project using pluripotent stem cells derived from CDKL5 patients provided a ‘match’ with the marketed antidepressant tianeptine. Preliminary studies with tianeptine are encouraging. Other possible matches for CDKL5 found in the repurposing initiative, added Cavalla, included anisomycin, harmol, monastrol, resveratrol, and chlorpromazine. These drugs however, have yet to be validated experimentally.

The delivery of potential drugs was recognized as a particular challenge as they must cross the blood-brain barrier. Direct systems for targeted delivery, may be required, including use of additional agents to enhance brain delivery. Protein replacement approaches may have challenges including short half-lives and immunogenicity.

Rare diseases, said **Tuyen Ong**, represents a huge unmet need with approved treatments making up only 5% of the 7,000 different rare diseases existing today. Of the 2,000 rare diseases known to be monogenic, 5 to 15% are due to nonsense mutations which could be addressed with nonsense mutation read-through.

“Typically in the disease state, you have a ribosome terminating when it reaches the stop codon resulting in the absence of a full length functional protein,” explained Ong.

Ataluren (Translarna TM) has a novel mechanism of action that facilitates read through beyond the premature stop codon allowing for the production of the full length functional protein that is lost in the disease state.

Ataluren could be suited to CDKL5, Ong added, since it is a small molecule that can penetrate the blood brain barrier, and seizure frequency offers a well-defined study endpoint.

In a collaboration with **Orrin Devinsky** at NYU, PTC Therapeutics is about to embark on a prospective placebo cross over study with Ataluren in CDKL5 patients.

In Conclusion

The mix between clinicians, academic researchers, patient groups and the corporate sector is the magic of the CDKL5 Forum. Last year the Loulou Foundation set the goal of treatments within five years and cures within 10 years. Following the progress made in just one year, these ambitious targets could well be achievable. A huge thank you to everyone helping us to achieve our goal.

INTRODUCTION

On October 10-11th 2016, The Loulou Foundation hosted the second CDKL5 Forum meeting in collaboration with CDKL5UK, The International Foundation for CDKL5 Research IFCR, and CDKL5 Insieme verso La Cura.

The meeting, held at the Wellcome Trust, London, was attended by over 120 delegates, including academic and clinical researchers from over 40 institutions, Leadership of the patient advocacy groups and representatives from 14 pharmaceutical and biotech companies in the field of rare diseases. [See appendix for list of attendees]. The forum show-cased the latest research into CDKL5 disorder with investigator presentations and posters of original data as well as facilitated brain storming sessions exploring existing and future therapeutic approaches.

Welcoming delegates, Sir Colin Blakemore, the meeting chair, said: "The Foundation has achieved extraordinary progress in the short time that it has been active and this is to a very large extent due to the energy and intelligence of its founders."

Following feedback from the 2015 CDKL5 Forum, a more interactive programme has been devised, Sir Colin explained, with increased scientific presentations and opportunities for discussion in moderated panel sessions and break-out workshop sessions, addressing four separate topics rather than just one as in 2015. [See appendix for programme].

The outcomes and conclusions of the meeting will be used to guide the Loulou Foundation's research funding priorities for the coming year. Since last year's meeting, the Foundation has awarded 14 new research grants into CDKL5 deficiency, supporting the work of 50 scientists at 24 labs in 13 leading institutions. Eleven of these grants are funded through a new partnership with the Orphan Disease Center at the University of Pennsylvania and the establishment of a Program of Excellence in CDKL5 Deficiency.

CDKL5 deficiency is a rare monogenic condition for which there are approximately 1,200 patientsdiagnosed worldwide. CDKL5 is a gene that provides instructions for making the cyclin-dependent kinase-like 5 protein, also known as serine/threonine kinase 9 (STK9), whose deficiency causes a neurological disorder that was first described clinically in 2004 as an early-onset variant of Rett Syndrome. Symptoms of CDKL5 deficiency include severe cognitive deficits, seizures, sleep disturbance, and decreased visual acuity.

The Loulou Foundation, established in 2015, is a non-profit UK foundation committed to further the development of research to better understand CDKL5 and actively pursue multiple approaches to achieving rapid treatments and cures.

KEYNOTE ADDRESS

GEORGE FREEMAN MP

In the first day keynote address, George Freeman MP described the revolution in genomics and informatics currently 'rebooting' drug development as the 'Apollo 11' mission of our time. "I want to signal a very strong support on behalf of the (UK) government for that mission," said Freeman, who currently chairs the UK Prime Minister's Policy Board.

Freeman, who was Minister for Life Sciences 2014-16 (with responsibilities for NICE, Genomics England and the UK biotech and pharma industries), had three key messages for the CDKL5 community:

- Genomics and informatics should drive treatment and diagnosis
- A good understanding of the true cost of poorly diagnosed/ treated conditions supports budgetary discussions about the value of new medicines. Patient groups, he added, can be more effective than the health service at striking innovative deals with pharma.
- Both direct and indirect costs of living with a condition need to be articulated to governments.

Freeman called on the NHS, with its genomics and informatics infrastructure, to form partnerships with the pharmaceutical industry and become the place where new drugs are tested. Advantages of such an alliance, he explained, would include quicker access to innovative treatments for patients whilst driving and supporting the life science industry. "If really bold the NHS could even end up getting royalties for the drugs," said Freeman.

In terms of Brexit, Mr Freeman pointed out that there could be advantages in science being liberated from some of the EU regulations on clinical trials, data, genomics and stem cells. "It's vital that we seize that freedom and put in place a regulatory environment that should be the envy of the world," he said. There would be opportunities for the UK, he added, to develop leadership in accelerated drug development and rare diseases.

The CDKL5 Forum was to be congratulated for bringing together academics, industry and patient advocates. "What's so powerful about your community (CDKL5) is that the patient voice sits right at the heart of everything you do," said Mr Freeman.

SESSION 1:

IMPROVING BIOLOGICAL UNDERSTANDING TO ENABLE TRANSLATIONAL RESEARCH

Moderator: Prof Dario Alessi, University of Dundee

The key questions to be addressed in the session, said moderator Dario Alessi, included how CDKL5 was regulated, the upstream pathways controlling function, the downstream pathways substrates, and how mutations led to disease. "The answers are really important because they'll provide the framework of knowledge that will enable us to play the engineer and work with clinicians to improve therapies for this devastating condition," said Alessi. Additionally, understanding these pathways and substrates may uncover potential biomarkers important to clinical development.

Probing Physiological Consequences of Acute and Chronic Changes in CDKL5 Expression

Prof Giles Hardingham, University of Edinburgh, reported on studies using Joe Zhou's knock-out CDKL5 mice to look at key developmental physiological milestones in the forebrain. With the mice arriving in Edinburgh in April 2016 (following a collaboration that was initiated at last year's CDKL5 Forum), the studies are still at a preliminary stage.

Hardingham explained, synaptic transmission relies on the efficient fusion of neurotransmitter synaptic vesicles with the presynaptic membrane and their recycling back into the presynaptic terminal. Even small perturbations can result in the formation of defective synaptic vesicles and altered neurotransmitter release. CDKL5 has been proposed to phosphorylate the presynaptic protein amphiphysin, which has been implicated in synaptic vesicle clathrin-mediated endocytosis.

Hardingham reported preliminary data probing presynaptic function using a pH-sensitive probe which revealed CDKL5 over-expression slowed endocytosis, while knock-down and neurons from knock-out mice showed a trend toward the acceleration of endocytosis. Such findings require further work to firmly establish a definitive influence of CDKL5 on synaptic vesicle cycling.

Hardingham also reported promising data centred on characterisation of a new anti-human CDKL5 antibody, generated at the University of Dundee. Currently, he explained, the field lacks reliable CDKL5 antibodies which are essential to establish where in the cell CDKL5 is located and whether it redistributes during development or in response to stimulation. A reliable antibody is also necessary to identify binding partners. The new CDKL5 antibody's specificity was validated against knock-out tissue, and preliminary data showed that it could identify the presence of CDKL5 in synaptic fractions of the mouse brain, as well as being suitable for immunolocalisation studies at the subcellular level.

New Characterisation of the CDKL5 Gene: Implications for Research and Diagnostics

Dr Ralph Hector, University of Glasgow, provided an overview of studies combining bioinformatics analyses and molecular methods to characterise the CDKL5 gene in human, rats and mice, and to predict the protein isoforms translated from each transcript. The approach, said Hector, may aid the development of isoform-specific antibodies.

While a number of studies have investigated CDKL5 structure and transcript expression in different tissues, the suite of isoforms and resulting proteins have been incompletely understood. Knowledge of gene transcript isoforms and their developmental expression profiles, Ralph explained, is essential for understanding the mechanistic roles of CDKL5 in brain development. An ongoing analysis of CDKL5 variants also has the potential to reveal insights into regions of the gene important for CDKL5 function, and how this relates to the updated characterisation of protein isoforms.

In the literature, the number of alternative designations used to describe CDKL5 gene products has been an obstacle to consistent CDKL5 terminology that takes into account the range of isoforms. In a recent

study Hector proposed a new nomenclature system for naming CDKL5 gene products that encompasses both the range of transcript isoforms and predicted protein isoforms. The system, said Ralph, will also enable naming of novel CDKL5 transcripts identified in other species.

For further information. Hector R. Characterization of CDKL5 transcripts in human and mouse. PLOS One 2016. Published: June 17, 2016.

Genetic Dissection of CDKL5 Function in Mice

Taking a genetic approach to dissection of the spatiotemporal requirement for CDKL5, said **Dr Zhaolan (Joe) Zhou, University of Pennsylvania**, could help provide key information for targeted therapy. He noted, the three major questions that need to be addressed are:

- Where is CDKL5 expression required for brain function?
- When is CDKL5 expression required for brain function?
- When and where could CDKL5 expression be sufficient for brain function?

Mouse models of CDKL5 show phenotypes including poor performance on various maze tests (indicating impaired learning and memory), and reduced sociability and nesting activities (representing autistic-like behaviours). Notably, CDKL5 knockout mice (including hemizygous males and heterozygous females) do not exhibit spontaneous seizures. "Mouse models of CDKL5, however, still hold potential to understand pathophysiology, to identify translatable mechanisms, and to support therapeutic development," said Zhou.

A number of previous studies have shown that CDKL5 is expressed in multiple tissues, including brain, liver and lungs. To determine if CDKL5 in the brain is responsible for phenotypes related to CDKL5 disorder, the Zhou laboratory removed CDKL5 expression selectively from the brain (known as conditional knock-out studies), they found that these mice develop similar behavioural phenotypes to whole-body knockout mice. Thus, CDKL5 expression in the brain is essential.

CDKL5 is expressed in multiple brain regions and different types of neurons, with the highest levels found in forebrain neurons. To determine where in the brain CDKL5 expression is required, Zhou and colleagues removed CDKL5 expression only from forebrain excitatory neurons. They found that these mice show learning and memory deficits, mimicking intellectual disability-like features, but not other phenotypes related to CDKL5 disorder.

Further, they found neural circuit functions are selectively disrupted in these mice.

"We now think the intellectual disability features of CDKL5 may be due to a dysfunction in the excitatory neurons," said Zhou.

He added that questions remain which include the discrepancies found between conditional knockout studies of in vivo mice and knock-down studies in cultured cells in vitro, the cellular origins of other CDKL5 related phenotypes, the timing of when CDKL5 expression is required, and whether there is a potential for phenotypic reversal.

Identification of Direct Substrates of CDKL5

Last year's CDKL5 Forum said **Dr Sila Ultanir, The Francis Crick Institute, London**, provided the inspiration for her group to explore CDKL5 point mutations. Her team has found that the majority of human missense mutations occur in the kinase domain of the gene rather than the C-terminal tail. "This indicated to us that the kinase domain plays an important role in the disease," said Ultanir.

This finding led the lab to focus attention on kinase substrates and direct their efforts towards

understanding how they are regulated by CDKL5. “In order to understand the function of the CDKL5 protein and resulting pathology in the brain when it is lost, we need to discover the biological substrates, and understand how they are regulated and what the downstream effects are,” explained Ultanir. “It’s only when we understand in more depth what CDKL5 is doing that we will be able to come up with intelligent therapeutics.”

Ultanir reported that their kinase substrate identification method, has revealed three substrates of CDKL5 including the proteins GEF-H1, EB2 and MAP1S.Her methoduses analog specific mutants of CDKL5 which can use bulky ATP analogs to label CDKL5 substrates, followed by trypsin digestion, covalent capture of phosphorylated substrate peptides and then mass spectrometry to identify substrates and phosphorylation sites.

Other noteworthy results include the finding that CDKL5 phosphorylates EB2 and MAP1S in vivo and affects their microtubule binding.

The next goal, said Ultanir, will be to investigate the significance of CDKL5 and its substrate in neuronal development.

SESSION 2:

CLINICAL RESEARCH AND STUDY DESIGN

Moderator: Prof. Helen Cross, University College London

NINDS Perspectives on Translational and Clinical Research for Neurodevelopmental Disorders: New Directions and Opportunities

Prof Laura Mamounas, NINDS Neurogenetics Cluster, NIH, outlined new NINDS and NIH programmes to support the translational pipeline and enhance 'rigor and reproducibility' of research.

In neurology over the past decade, the vast majority of drug trials have failed despite promising preclinical findings. This has led to recognition of the need to improve the quality and reproducibility of animal research, and to bring the 'rigor and quality' of study design expected in clinical trials to preclinical animal studies. NINDS/ NIH recommendations for improvement include:

- Rigorous experimental design (e.g., blinding, randomization, appropriate controls and statistics) to ensure robust and unbiased results in animal studies
- Animal models should possess good construct validity and correspond to the clinical disease condition (genetic/molecular mechanisms, sex, age, etc.)
- Identifying robust and reproducible phenotypes (e.g., conserved across models and species) to increase confidence that preclinical results translate to humans
- Using human relevant doses in animal models and incorporating PK/PD measures
- Replicating promising preclinical treatment findings in more than one model and in independent laboratories
- Publishing all results (both positive and negative outcomes).

There is also an urgent need to improve 'clinical trial readiness' before launching human trials. For example, we need a better understanding of natural history and clinical disease mechanisms, and to develop better clinical outcome measures (more sensitive, dynamic measures tailored to the disease population) along with clinical biomarkers to inform trials.

Lessons, said Mamounas, can be learnt from the Fragile X story, where despite 31 papers from 17 different labs showing that mouse phenotypes could be reversed with mGluR5 blockers, three large phase 2b trials by Roche and Novartis failed to show benefits. "The outcome was that both Novartis and Roche abandoned their programmes and got out of the Fragile X field altogether," said Mamounas. But Fragile X experts questioned whether the trials were testing what the mouse models had told them and whether it was advisable to discard this target based on these trials.

Questions raised from Fragile X studies included:

- Whether the endpoints used in the human studies (parent reported behaviour) are related to the most robust and reproducible outcomes from the mouse studies (i.e., improvements in synaptic function and plasticity; learning and memory).
- Whether much younger children would be needed to see improvements in synaptic plasticity and learning/cognition as predicted by the animal studies; the Novartis and Roche trials enrolled adults and adolescents, and it is not known whether older subjects have sufficient plasticity in their more mature brains or have passed critical developmental windows.
- Whether human trial durations should have been appreciably longer to see changes due to improved synaptic plasticity, or whether trials will require combining the drug intervention with a training/learning paradigm to see an effect.
- Whether adaptive dosing in humans should be used to take into account individual drug responses.

The reality, said Mamounas, is that despite spending billions of dollars on drug development, companies or sponsors often have little idea about why their trials fail.

NINDS has recently set up the NeuroNext Network to support early-stage and exploratory clinical trials in

neurological disorders. The idea, explained Mamounas, is to conduct a series of progressive, exploratory trials each building upon what we learned from the previous trial before launching the pivotal Phase III trial.

For example, NINDS recently funded a new NeuroNext trial to look at the effects of AFQ056 (the same mGluR5 blocker used in the 'failed' Novartis trial) on language and learning in young children with Fragile X (clinicalTrials.gov: NCT02920892). "This is an exploratory trial to test out a new paradigm for mechanism-targeted drug development in neurodevelopmental disorders: can AFQ056 enhance neural plasticity in the form of improved language learning during an intensive language learning intervention in very young children? We hope to learn something about the biomarker or the language intervention that will enable the next trial," said Mamounas.

NINDS also just awarded another exploratory clinical trial in a neurodevelopmental disorder. Using the antiepileptic drug vigabatrin before seizures begin, the PREVeNT trial (NCT02849457) will assess whether preventing or delaying the onset of epilepsy in infants with tuberous sclerosis complex can improve developmental and cognitive outcomes in these children.

Novel Mechanism of Action Facilitating Read Through in Non - sense Mutations

Rare diseases, said **Dr Tuyen Ong, PTC Therapeutics**, represents a huge unmet need with approved treatments making up only 5% of the 7,000 different rare diseases existing today. Of the 2,000 rare diseases known to be monogenic, 5 to 15% are due to nonsense mutations which could be addressed with non-sense mutation read-through.

"Typically in the disease state, you have a ribosome terminating when it reaches the stop codon resulting in the absence of a full length functional protein," explained Ong.

Ataluren (Translarna TM) has a novel mechanism of action that facilitates read through beyond the premature stop codon allowing for the production of the full length functional protein that is lost in the disease state.
This has potential as a treatment in a number of conditions including ion channel disorders, genetically defined epilepsy, metabolic disorders, muscle disorders, eye disorders, skin disorders, neurological disorders and pulmonary disorders. Ataluren could be suited to CDKL5 deficiency, Ong added, since it is a small molecule that can penetrate the blood brain barrier, and seizure frequency offers a well-defined study endpoint.

In a collaboration with Prof Orrin Devinsky at NYU School of Medicine, PTC Therapeutics is about to embark on a prospective placebo cross over study where CDKL5 patients aged between two and 12 years, will be randomised to ataluren or placebo. The primary outcome will be safety, with secondary outcomes including change in total number of monthly motor seizures, number of episodes of status epilepticus, use of rescue visits, ER visits/ hospitalisations and effect on myoclonic seizures. "We look forward to sharing results in due course," said Ong.

DISCUSSION

Helen Cross moderated the discussion of clinical research and study design, with panellists consisting of **Sam Amin** (Bristol Children's Hospital), **Orrin Devinsky** (New York University), **Laura Mamounas** (NIH/ NINDS), **Tuyen Ong** (PTC Therapeutics) and **Albena Patroneva** (Marinus Pharmaceuticals).

Endpoints: Use of behavioural change as an endpoint should be viewed with caution as it was a subjective measure affected by years of developmental influence. The ideal timing of the measurement of different endpoints needed to be taken into consideration. For example, three months was sufficient for seizures, but inadequate for long term plasticity at the synaptic level, which needed eight to 18 months. While

seizures may appear to be an 'easy' endpoint, it should be remembered that there are complexities, such as subjects experiencing non-epileptic seizures. Improvements in quality of life and behavioural function need to be distinguished from improvements in epilepsy.

Questions surround how translatable animal models are to clinical endpoints. The CDKL5 phenotype needs to be fully understood to test new agents. One recent questionnaire of parents suggested that in addition to epilepsy and developmental delay around 40% of CDKL5 children experience cardiac arrhythmias. There was a need to create a scientific group that could educate the FDA about feasible endpoints and adaptive trial design for use in studies relevant to developmental disorders. While the FDA talk about use of biomarkers in early phase trials, they will not approve a drug with such data and still expect clinically meaningful endpoints.

Age in trials: The discussion debated whether clinical trials in CDKL5 should be started in adults and move to children or undertaken first in children. One approach was to start at the youngest possible age, identify the 'critical window' where efficacy signals occur and then move into older age groups. In a disorder like CDKL5, with a high burden of seizures and behavioural problems, a risk-benefit analysis favoured giving children the drug. It should also be considered that any drug preventing or delaying seizures administered at an early age might allow more normal development to occur.

Recruitment to clinical trials: A major issue facing rare diseases, such as CDKL5 deficiency, is having enough subjects available to suitably power trials. Patients cannot be recruited to more than one trial at the same time, leading to concerns that subjects may abandon one trial in favour of another. Registries can provide a valuable resource to identify suitable patients.

Requirements for starting clinical trials: While some delegates felt there was a need for preclinical and clinical research to have 'rock solid' foundations, the counter point was that such stringent requirements would prevent chance investigations that might allow the field to advance more rapidly.

Summarising the debate, **Cross** said: "The key thing that has come across is the importance of sharing of information, not just in CDKL5 but with other rare diseases. It is vital in order to not make the same mistakes all over again. It is also important to remember that negative results are just as important as positive results, but time and again they never make it to publication."

SESSION 3:

DISEASE MODELLING IN VITRO AND IN VIVO

Moderator: Prof. Jim Wilson, University of Pennsylvania

Rat Models for Studying Neurodevelopmental Disorders

Prof Peter Kind, Edinburgh University provided insights into new rat models for CDKL5. The models, he said, have been made possible by the new DNA editing technology Crispr/ Cas 9, which introduces a cut into exon 8 resulting in a 10 base pair deletion and premature stop codon in exon 9.

There are now opportunities, Kind said, to move beyond mouse models of conditions like CDKL5 to rats, pigs and monkeys. "It's really exciting times – having more models will allow us to determine whether key phenotypes are conserved across mammalian species and hence are relevant to humans with CDKL5; ultimately this will help us move into trials earlier," he said.

Rats are not big mice, Kind stressed, but separated in evolutionary terms by 12 to 25 million years. "Rats have greater cognitive flexibility, and bigger brains making it possible to perform functional MRI," adding that rats also produce bigger litters resulting in the need for fewer breeding pairs.

In addition to CDKL5, current rat models at Edinburgh include Fmr1, Syngap, Grin2A, Mecp2, Nlgn3, Pten, Cntnap and Shank3. Cellular phenotypes, Kind and his team have found, are well conserved between the mouse and rat models, but behavioural phenotypes are less conserved. "This is very likely because the evolutionary pressures in terms of behaviour are very different between the two. Mice evolved on dry plains while rats evolved on wet swamplands," he said.

Addressing the major question of whether CDKL5 rats experience seizures, Kind said that it was currently too early to say. But it was noteworthy, he added, that in rat models of Fragile X, audiogenic seizures occur throughout the lifespan, whereas in mouse models of the condition they are restricted to a narrow time window around the end of the third postnatal week. "So it may be that rat models have a greater seizure susceptibility," he said. The team have also demonstrated (using western blot and antibody tests) that female rats heterozygous for CDKL5 show reductions in CDKL5 protein levels.

Overview of Mouse Models and Phenotyping

Dr Joe Zhou, University of Pennsylvania, provided an overview of currently available mouse models of CDKL5 and additional mouse models his laboratory is in the process of developing. The models, he said, include the knockout and knock-in (recapitulating a nonsense mutation) models. Both models are distributed by the Jackson Laboratory (**https://www.jax.org/strain/029642** and **https://www.jax.org/strain/029643**).

Advantages of mouse models, Zhou explained, are that mice are readily amenable to genetic manipulations reflecting human genetic mutations, and represent the 'lowest species' that can be used to assess higher cognitive functions with brain disorders. "But the cons remain that a mouse is a mouse," said Zhou.

Highlighting his new knock-in model with Nonsense R59X mutation, , Zhou said that that this model provided good construct validity and face validity (referring to behavioural phenotypes mimicking symptoms in CDKL5 patients) and could 'jump-start' drug testing in animal models.

Despite current CDKL5 mouse models lacking the manifestation of spontaneous seizures, we are starting to see robust phenotypes emerge in these models that are reproducible in different mutation models and in different labs. Looking across two different models and phenotyping conducted across three different labs, Joe Zhoe, Cornelius Gross, and Psychogenics, hind limb clasping, cued and contextual

fear conditioning, and sociability are emerging as reproducible endpoints, while new behaviours should continue to be explored. Visual evoked potential (known as VEP) and auditory evoked potential (known as auditory ERP), suggested Zhou, might be the sought-after biomarkers as outcome measures in future preclinical and clinical studies.

“As a community we need a set of reliable phenotypes and biomarkers that have clinical relevance, with outcome measures that have high predictive validity,” said Zhou. The next step, he added, will be to see which phenotypes are recoverable and reversible and at what age.

Patient-Specific In-Vitro Cellular Modelling of CDKL5-Associated Neurodevelopmental Disease: Derivation of Matched Isogenic iPSCs, Neural Stem Cells and Neurons by Stable X-Inactivation

Dr Steven Sheridan, Massachusetts General Hospital, outlined patient-specific in-vitro cellular modelling of CDKL5 using human induced pluripotent stem cells (iPSCs).

The rationale for developing human iPSCs, Sheridan explained, includes producing a genetically accurate model allowing for the study of different cell types specific to neurological disease, with the opportunity to grow large numbers of cells facilitating chemical and genetic screening. “In addition we can study various steps in early development, later development and even neurodegeneration,” said Sheridan. Possibilities, he added, exist to model individual patient variation and targeted treatments.

In monogenic neurological disorders the pathways and players often overlap. “So trying to understand as many of these diseases as possible helps us understand not only individual diseases, but also to tackle those where we don’t know the genetic cause,” explained Sheridan.

Modified Synthetic RNA-Based iPSC Reprogramming, he said, was the technology used to create iPSC lines from patient that, due to high efficiency of reprogramming, results in a large amount of pooled bankable cell samples that retain the ability to further isolate clones from. So far Sheridan and colleagues have derived and banked more than 250 patient iPSC lines, with the ultimate goal of achieving 400 lines including polygenic, monogenic and control patients.

One trick the investigators have utilized to model X-linked diseases (e.g. Rett Syndrome and CDKL5), where females are predominantly affected, is to use X-inactivation to produce isogenic clonal pairs from the iPSC pool from a patient that either expresses the wild type or affected allele. To date, they have made 48 iPCS CDKL5 clones, of which 26 are wild type and 22 express CDKL5 mutations.

The CDKL5 clonal iPSC lines are enabling phenotypic assay development and explorations looking at gene expression, CDKL5 sub-cellular localization, synaptic function, oxidative stress/ mitochondrial function and real time neuron differentiation (including neurite outgrowth and dendritic arborization).

One of the goals of the biobank being developed, said Sheridan, will be to offer the cell lines to the research community.

PANEL DISCUSSION

Prof Jim Wilson (University of Pennsylvania) moderated the panel discussion around what current models can tell us about disease development in the absence of seizures. The panellists were **Prof Peter Kind** (University of Edinburgh), **Dr Robin Kleiman** (Boston Children's Hospital), **Dr Tommaso Pizzorusso** (University of Florence), **Dr Steve Sheridan** (Massachusetts General Hospital) and **Dr Joe Zhou** (University of Pennsylvania).

The following points were made:

- Biomarkers should be studied from a developmental perspective since wild type animals and mutants are likely to diverge from each other at different stages in development. Investigators should consider early on in the research process which is the most appropriate biomarker to use to test their hypothesis.
- Evoked potentials represent a quantitative biomarker with the potential for longitudinal studies and the possibility to record before and after treatment. Additionally, management of evoked potentials reduces the variability associated with individual and performance like behavioural tasks. Conducting evoked potential studies in a wide range of phenotypes, it was felt, would provide greater confidence in trials of novel therapeutic approaches.
- New technical developments should help investigators to conduct cellular and molecular studies at the same time as behavioural readouts, such as combining auditory potential recordings with memory studies. This approach might allow correlations to be made between changes in sensory processing and memory deficits.
- With 95% of clinical trials failing despite sufficient animal model data to be accepted by the FDA, questions were raised about the value of animal models. Many behavioural endpoints, such as mouse social behaviour, it was felt do not translate to human endpoints. The Pharma industry, needs to ensure that animal model studies are conducted with greater 'rigor; and not just viewed as a means- to-an-end for starting a clinical trial.
- Some feel that one advantage of iPSC models is that theyt mimic human time frames opposed to those cultured from the mouse and they could also account for the time taken for human neurones to differentiate.
- Open access resources are needed relating patient clinical information to different mutations. Such an approach would help in understanding gene functionality and whether there are distinct types of CDKL5. DECIPHER, a Sanger initiative that allows the clinical community to share and compare phenotypic and genotypic data, has information on 14,000 patients with neurological disordersAt this time only three patients included in DECIPHER haveCDKL5 disease.
- CDKL5 patients with different mutations may respond in different ways to various drugs while it might be beneficial to 'turn on' the inactivated copy in some patients this might represent a problem in other patients
- To interpret pharmacology of in-vivo brain models, investigators need to be more proactive in measuring exact drug exposure levels.

KEYNOTE ADDRESS

JOHN F. COWLEY, Chairman and CEO of Amicus Therapeutics

In the second day keynote presentation, John Crowley provided insights into 'lessons learnt along the way' in his quest to find a cure for Pompe Disease, the life threatening condition affecting two of his three children. Crowley, now Chairman and CEO of Amicus Therapeutics, identified the two essential elements for successful drug development and the four traits he has observed in people who are leaders in the field.

Crowley's journey has been documented in the book "The Cure" by Geeta Anand, and the 2010 film 'Extraordinary Measures' starring Harrison Ford and Brendan Fraser. Crowley described how the family received the diagnosis that his daughter Megan had Pompe disease, a failure of glycogen metabolism, just seven days after his son Patrick, who also has Pompe disease, was born. "After going through the shock, the grief and the denial we finally settled on determination," said Crowley.

That determination involved leaving his secure job, and co-founding the biotech company Novazyme Pharmaceuticals. In the space of one and half years he grew the company to 100 people and raised $25 million in venture capital money. Ultimately the company was acquired by Genzyme Corporation who started a clinical trial for Pompe disease with the enzyme replacement therapy Moozyme. Finally in January 2002 Megan and Patrick became the 27th and 28th children to be treated in the clinical trial. "It saved their lives and helped them to achieve a quality of life that they would not have had without it," said Crowley.

Moozyme, he recognises, would eventually have been developed without the efforts of his family, "but I like to think we were an accelerator," he said.

The two essential elements for successful drug development, Crowley explained, are vision and the ability to take risks. With vision, biotech entrepreneurs need to think where they want to be in five or ten years, and then work backwards from there. With risk taking, people need to appreciate that it is acceptable to make mistakes. "Failure is ok as long as you are transparent and honest. There's a need to think what can we learn, and where should we go next," said Crowley. Penicillin, he added, would not have been discovered if Alexander Fleming had not left a petri dish out!

Crowley shared the four traits he has observed in great entrepreneurs:

- **Persistence.** Exemplified by Jonas Salk who refused to give up on developing the vaccine for polio
- **Optimism.** Exemplified by his daughter Megan, who despite three surgeries for scoliosis and seven weeks in ICU continued to be optimistic that she would be able to sit up straight.
- **Hope.** Exemplified by a quadriplegic speaker at a biotech meeting, who recognised that although research might be too late to directly benefit him, it could help future generations of patients.
- **Time.** Achieving a balance between the 'sprint' and 'marathon' of drug development and developing the ability to 'cherish' each precious moment.

Amicus Therapeutics, said Crowley, has this year announced a CDKL5 pre-clinical programme around enzyme replacement therapy approach. "We are going to move heaven and earth to do everything we can," he said.

SESSION 4:

THE INDUSTRY PERSPECTIVE

Moderator: John Crowley, Amicus Therapeutics

John Crowley moderated a panel discussion that considered where industry should be focusing their research efforts to best enable clinical development in rare diseases. The panellists were **Jonathan Appleby** (GSK Rare Diseases), **Quintus Ngumah** (PTC Therapeutics), **Omar Khwaja** (Roche Pharmaceuticals), **Tauhid Ali** (Takeda) and **Yael Weiss** (Ultragenyx).

Identifying Which Rare Diseases to Focus on

Appleby said that the two main drives for GSK were clinical data suggesting the treatment was likely to show efficacy and strategic 'platforms' that could be applied across other therapies. For Roche, said Khwaja, the focus was on identifying unmet needs and providing innovative medicines. One advantage of studying genetically defined rare diseases, he added, was that targets (unlike in heart disease and dementia) are often well defined. In selecting diseases to focus on companies need to identify their individual strengths and to consider the expertise and capabilities they already have in place. Ultragenyx, said Weiss, are 'agnostic' about modality and disease, with targets often arising from 'random' meetings. What was felt to be most important, she added, was for potential therapeutics to have understandable biology.

For PTC Therapeutics, said **Ngumah**, it was identifying conditions with unmet medical need, with the company actively soliciting proposals from physicians and patients. Indeed, they made the decision to work on CDKL5 after hearing a presentation by **Devinsky**.

Ali described Takeda's new in-house 'Accelerator Programme' designed to bridge the gap between academia and pharmaceutical R & D. The intention, he explained, was to get away from pharma models focused on risk analysis, and create a team of entrepreneurial MDs/PhDs willing to take risks. Through multiple models of collaboration, Takeda plans to invest in potential agents, then once efficacy signals are shown find other companies to take programmes forward.

Addressing the Issue of Patient Numbers in Rare Diseases

Panellists did not feel having small numbers of patients would deter pharmaceutical companies. It was felt to be the commercial team's role to find the patients. The Ultragenyx view, said **Weiss**, was that more patients were likely to be identified once drugs became available. Resources for identifying patients for trials included social media and patient advocacy groups providing access to their registries.

While being first in class represented the ideal situation, said **Khwaja**, developing second and third generation drugs was important when there was an unmet medical need and drugs could transform standards of care. Treatments did not have to be curative, if they could prolong life or deliver significant improvements in quality of life.

Rare diseases pharmaceutical companies should engage with patient groups in the discovery phase rather than waiting for phase 3 clinical trials. Patient groups can offer a valuable repository of the science, provide access to the patient population and give insights into the issues important to patients/families as well as clinically relevant trial endpoints. Patient advocates can help to record the natural history which may serve asvaluable comparative data for use in open label trials. At GSK, **Appleby** said, webcasts of interviews with patients and their families were used to help investigators understand the value of their work and create a sense of urgency.

Ali suggested, sharing data on the natural history of rare diseases, clinical endpoints and quality of life across pharmaceutical companies would be valuable. The approach would be achievable, he said, since such data had no bearing on intellectual property. In spinal muscular atrophy, **Khwaja** said, the patient group has already encouraged development of a precompetitive consortium of pharmaceutical companies to develop clinically meaningful endpoints and consider health related quality of life. The consortium he added, help to prevent patients and caregivers from getting repeated requests from different companies for the same data.

Crowley commented that the three things that had struck him about FDA Commissioner Robert Califf's report on the drug eteplirsen for muscular dystrophy were:

- The need for regulatory flexibility in rare diseases drug development
- The need for data to be considered in totality rather than single endpoints
- The need to consider the patient perspective throughout the drug development process.

SESSION 5:

NOVEL THERAPEUTIC APPROACHES TO TREAT CDKL5 DEFICIENCY

Moderator: Prof. John Christodoulou, University of Melbourne

Pharmacological Reactivation of the Xi-Linked CDKL5 Gene as a Potential Treatment for CDKL5 Deficiency

Prof Michael Green, University of Massachusetts Medical School, described the potential for pharmacological reactivation of the X-linked CDKL5 gene as a treatment.

The typical CDKL5 patient, explained Green, is a female who is heterozygous for CDKL5 deficiency. In female cells the normal biological process is for one of the two X-chromosomes to become inactive (Xi) and the other to remain active (Xa), with the result that approximately 50% of cells express the mutant CDKL5 gene and 50% wild-type. Green's hypothesis is that reactivation of Xi would lead to cells containing both wild-type and mutant CDKL5. "Since CDKL5 is an enzyme even small amounts of reactivation might be sufficient to bring clinical benefits," said Green.

Green and colleagues first undertook a genome-wide RNA interference screen that resulted in identification of 13 factors required for X-chromosome inactivation (XCIFs), none of which were encoded on the X-chromosome. (Bhatnagar et al 2014, PNAS 111(35):12591-12598). Short-hairpin RNA (shRNA)-mediated knockdown of any one of the XCIFs resulted in reactivation of X-linked genes including CDKL5. "Our results indicated that X-chromosomal inactivation is more reversible than previously thought," said Green.

An initial concern was that the approach might lead to elevated levels of gene expression, which may be deleterious. "Even though both chromosomes were expressed we found levels of the protein were normal, suggesting there are compensatory mechanisms," said Green.

Currently, Green and colleagues are in the process of identifying additional XCIFs, which may provide more desirable targets for the development of drugs that function by reactivating the Xi-linked CDKL5 gene. They are screening two focused mouse shRNA libraries, one directed against a comprehensive panel of repressive epigenetic regulators, and the other directed against a comprehensive panel of protein kinases.

Already, the approach has resulted in the identification of several dozen new epigenetic regulators and protein kinases involved in X-chromosome inactivation. Future steps, said Green, will include testing chemical inhibitors of XCIFs for efficient reactivation of the X-linked CDKL5 gene. The most efficacious, least cytotoxic candidates will then be tested for restoration of normal neuronal morphology using iPS cells derived from patients with CDKL5 mutations.

Defects in AMPA-R Expression in CDKL5 Deficient Neurons can be Restored by Tianeptine Treatment

Dr David Cavalla, Healx, Cambridge, described Healx's repurposing approach that matches the genetic profiles of patients with rare diseases to drugs already on the market. A recent project using pluripotent stem cells derived from CDKL5 patients provided a 'match' with the marketed antidepressant tianeptine.

The Healx methodology involves undertaking a transcriptomic analysis identifying which genes are different in the disease state from the healthy state, and therefore need to be either expressed more strongly or suppressed. It then looks for a match to available drugs, using a mathematical algorithm.

Tianeptine, first synthesized in 1968, has established effects on cognition in addition to antidepressant properties. A lack of understanding of the mechanism of action, however, has impeded wide use with

the drug currently only marketed in France, South America and parts of Asia. At the molecular level tianeptine (among other effects) stimulates glutamate currents via AMPA subtypes.

While AMPAkines have been proposed for use in cognitive dysfunction (schizophrenia, depression, Alzheimer's) concerns have been raised about neuroexcitation. Tianeptine, said Cavalla, works in an altogether different way, and does not provoke seizures in preclinical models or human studies.

In an animal model of Rett syndrome (female MECP2 mouse), tianeptine has been shown to rescue phenotypes associated with MeCP2 knockout. The drug improved respiratory dysfunction, gait features, motor coordination in the rotarod test, reduced clasping, and normalized pre-pulse inhibition of startle.

Cavalla suggested other possible matches for CDKL5 found in the repurposing initiative, included anisomycin, harmol, monastrol, resveratrol, and chlorpromazine. These drugs however, have yet to be validated experimentally.

Dr Charlotte Kilstrup-Nielsen, University of Insubria, described her lab's efforts to explore how defects in AMPA-receptor expression in CDKL5 mice are restored by tianeptine in collaboration with Healx's discovery efforts.

The team focused their attention on the AMPA-receptor, Kilstrup-Nielsen explained, since it is known that defects in the receptor occur in neurologic disorders characterized by cognitive defects.

The AMPA-receptor, mediating fast excitatory neurotransmission in dendritic spines, is composed of four subunits (designated GluR1, GluR2, GluR3 and GluR4) which combine to form tetramers.

Using hippocampal neurons from wild type mice with CDKL5 expression silenced by shRNAs, Kilstrup-Nielsen and colleagues demonstrated that in the absence of CDKL5, GluR2 levels were down regulated, but GLuR1 levels were unaffected.

Focusing attention on GluR2, the team went on to show when CDKL5 was missing, the GluR2 subunit was hyper-phosphorylated. Using immune florescence investigators showed that when CDKL5 expression was silenced, the amount of GluR2 inserted into the neuronal membrane was reduced. "We believe loss of CDKL5 impacts on neuronal functions through deregulation of GluR2,"said Kilstrup-Nielsen.

From the functional point of view such findings are significant since it is widely accepted that AMPA receptors containing the GluR2 subunit do not allow the passage of calcium ions; while those not containing GluR2 allow the passage of calcium ions.

Knowing that tianeptine is capable of enhancing synaptic activity, Kilstrup-Nielsen and colleagues performed in vitro studies exploring whether addition of tianeptine might restore GluR2 expression in neurons where CDKL5 expression was silenced. Results showed that addition of tianeptine resulted in both GluR2 levels and hyper-phosphorylation returning to normal. "Next we want to try to understand how tianeptine is having this effect," said Kilstrup-Nielsen.

The Pathophysiology of CDKL5 Disorder: Exploiting Visual Cortex Circuitry to Disclose the Synaptic Determinants of the Disease in a Mouse Model

Dr Maurizio Giustetto, University of Turin, described the work of his lab defining CDKL5 pathophysiology using visual cortex circuitry in CDKL5 mutant mice. "We decided to focus on the primary visual cortex because of the well-known visual defects in CDKL5 patients and mouse models of the condition," explained Giustetto.

In a previous study using 2-photon microscopy, Giustetto and colleagues showed that in the absence of CDKL5, the number and the stability of the dendritic spines was reduced. This effect, said Giustetto,

is most likely due to disassembly of postsynaptic machinery caused by altered interactions with PSD-95. Furthermore, in mice lacking CDKL5 the team showed severe defects in synaptic transmission and plasticity, and that treatment with IGF-1 was able to restore such deficits. Recent findings from the primary visual cortex (V1) studies of mice lacking CDKL5 using immunofluorescence and confocal microscopy have found:

- Levels of the immediate early gene c-fos (a marker of neuronal activity) are reduced.
- A severe disruption of both excitatory and inhibitory connectivity in the cortex together with alterations of key molecular constituents of the glutamatergic PSD.
- Excessive glutamatergic connectivity onto paravalbumin (PV) in V1 associated with more PV-positive synapses with corticofungal layer V pyramidal neurons.
- Profound alterations in the establishment of PV-positive interneurons assembly, together with defective formation of perineuronal nets at the end of critical period of plasticity.

"These data reveal overall disruption of V1 cellular and synaptic organization that may cause a shift in the excitation/inhibition balance which is likely to underlie the visual deficits characteristic of CDKL5 disorder," said Giustetto. "Moreover, they show for the first time that crucial steps underlying experience-dependent refinements of cortical circuits are defective in CDKL5 mutant animals, defects that may also be at the basis of the cognitive/sensorial impairments of CDKL5 patients."

The team have also considered the role of CDKL5 in-vivo assembly of glutamatergic synapses, with data suggesting that pathogenic CDKL5 mutations disrupt CDKL5-Shank 1 interactions and alter the expression of metabotropic class of glutamate receptors (mGlurs) at synapses

"These exciting new data opens up to completely new avenues of therapeutic intervention, exploiting the already available molecules to modulate mGlurs function in the brain," concluded Giustetto.

Forniceal Deep Brain Stimulation Rescues Hippocampal Memory in Rett Syndrome Mice

Dr Jianrong Tang, Baylor College of Medicine, provided an overview of his lab's studies exploring whether forniceal deep brain stimulation (DBS) rescues hippocampal memory in a mouse model of Rett syndrome (RTT).

RTT girls with mutations in the X-linked MECP2 gene, Tang explained, initially show normal development, then around six to 18 months of age develop RTT symptoms including stereotypic behaviours, dystonia, intellectual disability and autistic features.

Using a RTT mouse model (female Mecp2+/- mice) showing learning and memory deficits, hypoactivity, tremors, stereotypies, seizures and altered sociability), Tang and colleagues investigated the hypothesis that forniceal DBS improves hippocampus-dependent memory by modulating hippocampal synaptic plasticity and hippocampal neurogenesis.

At six to eight weeks, the mice had surgical implantation of the electrodes, and received two weeks of chronic DBS (consisting of 130 Hz, 60 μs pulses, one hour per day for 14 days) or sham treatment. Three weeks later, the mice were exposed to the fear conditioning or water maze tests.

Results in the RTT mouse model showed forniceal DBS improved hippocampal learning and memory, normalized hippocampal synaptic plasticity, restored synchrony patterns and increased adult neurogenesis. "This showed that the RTT brain, in mice at least, is responsive to neuromodulation." said Tang.

Questions currently being addressed include the duration of the of DBS effect, whether DBS restores learning and memory in older RTT mice, the percentage of new neurons induced by forniceal DBS that are MeCP2-positive, and the influence of forniceal DBS on other brain structures. Next, the team plan to test the effects of forniceal DBS on the cognitive deficits in CDKL5 mice.

(These results were recently published: Hao et al., Nature, 526: 430-434, 2015; Lu et al., Neuron, 91: 739-747, 2016)

ACCELERATOR WORKSHOPS

Four 'Accelerator Workshop' took place during the afternoon of day one. Each workshop covered a different topic and were designed to build upon the discussions of the earlier Sessions. **Dr Ashley Winslow, CSO, Loulou Foundation and Director of Neurogenetics at University of Pennyslvania Orphan Disease Center,** explained, the Workshops were intended to define where the CDKL5 field currently stood and to identify gaps in knowledge. "We want you to think about the obstacles that we're going to come up against in both the short and long-term start and how we overcome them as early as possible," said Winslow. Bringing the community of academics, pharma executives and patient advocates together, she added, provided opportunities to 'leverage' all resources. "We want to push the boundaries on traditional drug development timelines and move things faster."

WORKSHOP A: IMPROVING BIOLOGICAL UNDERSTANDING

Moderator: Prof Peter Kind, University of Edinburgh

Current Understanding of CDKL5 Biology: The main function of CDKL5 is as a kinase that needs to be activated to have biological effect, with the terminal domain involved in upstream and downstream regulation. Pathogenic mutations are know to occur throughout the protein including the kinase domain. Discovering how the kinase domain binds and how the process can be disrupted will be important for greater understanding of the biology. Non-pathogenic point mutations of CDKL5 are also likely to occur. Ability to predict pathogenicity is imperfect. Most mutations identified as pathogenic are analysed using computer algorithms, and relatively few have been confirmed through clinical studies. The clinical method of confirming whether mutations are pathogenic is to compare mutations between siblings and see whether they share genetic mutations and whether disease occurs in both cases.

Neuronal Understanding: In neurons, CDKL5 expression has been shown in both the cytoplasm and dendrites. It is found at the synapse, both pre- and post-synaptically, and also in the nucleus, moving between the cytoplasm and nucleus in response to glutamatergic stimulation. While the exact role in the nucleus is unknown, it appears to be involved in DNA damage, and may co-localise with proteins that deal with splicing. In non-neuronal cell types, there is evidence that localisation is dependent on cell-cycle. In the absence of CDKL5, synapses go through rearrangement and compartments appear

immature. Its influence is probably pre-synaptic, but no work has been done on synaptic localisation except for functional clinical studies. Looking at potential functions for CDKL5 on the circuit level may help to bridge the gap between molecular and behavioural, and provide a sensitive assay for potential drugs. In developmental studies, there is evidence CDKL5 might influence trajectories of interneurons and lack of CDKL5 delay maturation of the system. Looking at excitation and inhibition balance of neural circuits could be one way to understand its role, and CDKL5 inhibition might be linked to susceptibility to seizures. It is interesting there are no seizures in CDKL5 knockout mice, indicating differences in function between mice and humans. There is a need for good antibodies to study CDKL5. While there is investment in polyclonal and monoclonal antibodies, there are concerns that high levels of expression indicated by antibodies might just be descriptive and may not necessarily inform us about what is happening.

Pathologic Disease Events: CDKL5 deficiency is a very complex pathology with the scientific community still working out how well models relate to clinical disease. Even inactivated proteins can have biological effects, making it difficult to know whether behaviours seen in mice translate to the clinical condition and how predictive models are. Symptoms seen in CDKL5 deficiency can be caused by a range of mutations, and it is unclear whether mutations seen in the clinic relate to the loss of a protein or to a specific altered protein function. To understand the disease, more models are needed with improved understanding of how they relate to the human condition. It should not be assumed that high levels of CDKL5 expression correlate with high levels of function of the protein, and a much greater understanding of both molecular and circuit-level aspects of the mutations is needed if behavioural effects are to be understood.

Stem Cells: An exploration of stem cell data will be crucial in allowing understanding of how the proteins function, and what types of altered function relate to clinical conditions. However, data from human stem cells has not yet provided concrete answers to key questions about the role of CDKL5 in development. Both top down and bottom up approaches will be needed to fully understand pathology associated with CDKL5.

Short and Long Term Goals for Understanding CDKL5: Developing a battery of antibodies will be vital to understanding the role of the protein in both normal function and pathological conditions.

Understanding of upstream regulators of CDKL5 as well as downstream targets is needed to monitor not only expression, but also activity of CDKL5. Already it is known some activity only takes place when CDKL5 is incubated with ATP, and further elucidation of the auto-phosphorylation sites will be crucial. Understanding of the specific phosphates that phosphorylate CDKL5 could provide potential clinical targets. Robust phenotypes are needed in animal and stem cell models. The lack of seizures in mouse models can be read as either a problem (showing a lack of conservation of mechanisms responsible for the clinical disease), or as a benefit (requiring us to look more deeply at underlying mechanisms). Since it is known that treatment of seizures does not improve patient clinical outcomes, the mouse model may allow exploration of pathologies otherwise masked by compensatory physiology associated with seizures. In the long term, understanding pathophysiology from cells to circuits and to behaviours will develop a clearer understanding of the disease and lead to therapeutic advances.

Collaborations: There was recognised to be a need for greater understanding of key aspects of pathology to bridge gaps between research and the clinic. Questions include whether clinical cases can be stratified according to mutations, how seizures are manifested clinically, and whether the locus is consistent across cases? It was noteworthy that only one clinician was represented on the workshop panel. Stratifying patients into groups (such as those who are kinase defective and kinase overactive) could help theoretical understanding of the disease and provide a better appreciation of differences between mutations. The CDKL5 Forum is essential for facilitating discussion between researchers and clinicians, allowing the development of organic collaborations.

Best Therapeutic Avenues: It is too early to tell which is the best therapeutic approach, making it important for all avenues: (gene therapy, protein replacement, pharmacological interventions targeting translation (read-through), cellular or circuit deficits) to be explored provided they are based on solid scientific evidence.

In Conclusion, Ashley Winslow said, "The tools are starting to develop and catch up with the field so we can test hypotheses more critically in the models that we have." It is critical, she added, to develop good antibodies. "Additionally, studies at the circuit level might bridge the gap between molecular and behavioural knowledge where for neurological disorders there is a disconnect," she said.

WORKSHOP B: CLINICAL DATA AND TRIAL DESIGN

Moderator: Prof Orrin Devinsky, New York University

The workshop focussed entirely on how to create better databases and registries of CDKL5 patients in order to provide a worldwide research resource. The moderator and participants agreed that the need was 'critical'. Despite the area being highlighted for action at last year's CDKL5 Forum, there had been few advances. This was in stark contrast to 'massive' changes that have occurred in preclinical research. Currently there are several CDKL5 databases, with the largest containing details of 276 patients, mainly from the US, UK and Australia, though questionnaires have been prepared in other languages. The chair called for a new vision of how a patient database could be organised. Ideas considered included:

Governance: Databases, it was strongly felt, should not be owned by any one research institute or disease foundation, but should be collaborative, if centralised, ventures. Parental support groups will be critical to establishing such ventures, and there should be parent representation on governance bodies. A charter on governance was essential, in particular for setting out how data would be accessed. There could be no compromise on guaranteeing 'free and transparent' access for the research community. However, there were recognised to be considerable obstacles, including administrative issues around negotiating demands of US Institutional Review Boards (IRBs – ethics committees) that will need to be carefully thought through. The database must be international, but sharing information between countries was likely to raise further challenges.

Enrollment and data input: Ideally there should be both clinician and parent input to databases. Parents are often highly motivated and provide an 'incredible human resource' for accessing their children's medical records from health centres and uploading them. But both groups, however, are busy and could be facilitated by paid research assistants. Many parents have already devoted effort to supplying data to one of the current CDKL5 databases – ideally any new, over-arching database would merge existing data, rather than asking parents to start afresh. Some databases distribute apps enabling parents to capture what is happening in real time.

Access to information: People involved with database projects may have career and organisational interests in restricting access to data, making it critical to maintain free access for all. One participant felt projects would fail unless free access was guaranteed.

Funding: A database needs adequate funding to ensure input data is good quality, and that the database is curated and updated over the long term.

Content: New angles are likely to emerge on disease, yielding new research directions that in turn may require the collection of new categories of data. Careful thought, therefore, needs to be given to 'future-proofing' the data that is collected.

Next Steps: The workshop agreed the next step should be a 'scoping exercise' exploring issues raised in the session and considering the technology to be used. Organisations already running successful databases (such as the EU Dravet Foundation, the Phelan-McDermid Syndrome Foundation and Rett Syndrome database) should be consulted.

In Conclusion, Ashley Winslow endorsed the need for a 'scoping' study which would consult widely, consider examples of successful rare disease databases from other organisations, evaluate what already exists for CDKL5 and take into account future developments. "Crucially we need to keep parental needs at the heart of the process," said Winslow. One possibility, she suggested, would be to establish a patient

portal with information about research and how data is used.

“As preclinical research blossoms there‘s a growing need to overcome obstacles and put together a powerful international patient data base or registry.”

The obstacles for databases include parental frustration with data input (including multiple requests/ types of data), the fact that parents and clinicians are time-poor, data soon gets out of date, and rivalries between different research groups and patients groups competing for data. Solutions identified included making parent groups central to the creation of the database, paying research assistants to input data, providing long term funding to create longitudinal, sustainable databases, and developing a collaborative consented approach where databases would not be owned by any one group. She said there was no clear solution for the bureaucracy associated with databases such as Institutional Review Boards, but this will need to be carefully considered.

WORKSHOP C: DISEASE MODELLING AND RESEARCH TOOLS

Moderator: Sean Clark, Amicus Therapeutics

Discussions of the ‘Disease Modelling and Research Tools’ workshop centred on existing and desired reagents and other resources.

Research Quality: There was general agreement that the field was not yet mature in terms of research quality. Too few studies, for example, have statistical validity, so the literature reports ‘trends’ rather than ‘results’. While journal editors and reviewers have some responsibility here, so do funders, including the Loulou Foundation, which could push for deeper levels of scientific insights. For example, it could insist that the research it supports uses blinding and randomisation, and provides detailed reporting that permit reproduction. There was also a need for more access to data, and for more reporting of negative results.

Human Tissue: With an estimated 1000 to 1500 CDKL5 patients worldwide (around 150 of whom have been described in the literature) it was felt inappropriate that the available cell lines were derived from just six or seven people. There is a clear need for more material to be available, which in turn would lead

to a more diverse and representative knowledge base. Currently, investigators are not seeing the full range of phenotypes, from the slight to the very severe, in available cell lines. The lack of diversity reduces ability to ask the right questions and structure thinking about ways of reversing these effects. There was also a case for creating and curating other types of cell, for example human neuronal cells knocked down to present symptoms of CDKL5. There was substantial interest in the development of a new platform for cell distribution, with the work of the Michael J Fox Foundation into research on Parkinson's identified as an excellent model. Another requirement was for the development of a list of key antibodies and other reagents needed in CDKL5 research, and for financial support to produce and validate them.

Animal Models: With a wide range of animal models for CDKL5 (including knock-in and rescue models) less concerns were expressed about this resource. There was a short debate on whether higher animal models would be of value, perhaps in cats or pigs, but this theme was not taken up. Such models might have value for industry when treatments are approaching human use.

Neurons: A strong case was made for developing better tools allowing individual neurons to be observed in action (perhaps by calcium indicators or optogenetic methods). The need for a centralised electrophysiology resource was identified. Improved tools and material availability would allow better hypotheses to be generated. For example, some neurons involved in epilepsy have been modelled and others have not, with the result that existing hypotheses tend to emphasise those with more data. There was felt to be a need for a central repository of material, available cheaply or free to non-commercial users that would be free of intellectual property complications. It is well understood that commercial interests become active once a drug is in development, and that contractual negotiations can slow development long before commercial considerations become a factor.

In conclusion, Winslow emphasized the valuable role that could be played by the CDKL5 portal in communicating information. "There are potential tools that the community could benefit from, which hopefully they can share. The CDKL5 Forum Portal is one way to make that knowledge public. We want to link people up collaboratively," she said.

WORKSHOP D: NOVEL THERAPEUTICS

Moderator: Prof David Rowitch, University of Cambridge

To stimulate discussion the Novel Therapeutics workshop opened with five short presentations.

David Rowitch highlighted the UK's regional approach (where central neurological hubs offer the opportunity for whole genome sequencing and other diagnostic tests to identify larger populations for clinical studies) as offering a major research advantage. He also raised the issue of brain delivery systems for novel therapeutics, which from his personal experience in the condition of monogenic leukodystrophy, he said was likely to prove challenging in gene and other therapeutic approaches for CDKL5. Finally he drew attention to the DECIPHER database which allows phenotypic and genotypic data to be shared across the clinical community.

Dr Stuart Cobb (University of Glasgow) considered CDKL5 from the perspective of Rett syndrome, a closely related disorder. He highlighted the fact that the level / activity of the protein / pathway may be critical and that activating the system too strongly might prove detrimental. The mosaic nature of the disorder might therefore be an important consideration and challenge for developing pharmacotherapies. There was also a need, he said, to establish which features of CDKL5 were fundamentally 'reversible', as a best case exemplar for comparing novel therapies. He highlighted challenges around CDKL5 being an ultra-rare disorder.

Prof Nickolas Tonks (Cold Spring Harbor Laboratory) explored the potential for inhibiting the protein tyrosine phosphatase PTP1B as an approach for treating Rett syndrome. Disruption of MECP2 in mouse models of Rett, he said, has been associated with elevated levels of PTP1B. His lab has shown that treating Rett mouse models with small molecule inhibitors of PTP1B extends life span of male mice and

re-establishes aspects of the wild-type behavioural phenotype in females. PTP1B exerts its effects on the BDNF receptor, TRKB, and the downstream AKT signalling pathway, which is also disrupted in CDKL5 iPS cells. Although PTP1B inhibitors were without effect in CDKL5 iPS cells, pervanadate, a pan-inhibitor of the PTP family of enzymes, restored normal signalling. He suggested that this points to a role for a different member of the PTP family in CDKL5 deficiency and that such an enzyme may be an attractive therapeutic target.

Prof David Segal (UC Davis Genome Center) presented information about use of zinc finger transcription factors in Angelman syndrome, with the therapeutic goal of reactivating the silenced paternal UB3A gene. One strategy for crossing the blood brain barrier, he suggested, would be to attach a cell penetrating peptide. Short protein half-lives, he said, would lead to the need for repeat administration.

Dr Elisabeth Ciani (University of Bologna) considered novel therapeutic approaches for treating CDKL5 disorders, including influencing ATK signaling with IGF1 and GSK3-B inhibitors. The recent discovery that CDKL5 interacts, phosphorylates and regulates histone deacetylase 4 (HDAC4) activity, an important molecule in brain development, suggests a role for HDAC4 in CDKL5 disorder. The discovery of LMK235, a selective inhibitor of HDAC4 and HDAC5, she added, provides a potential approach to treat pathologies due to altered HDAC4 activity. Ciani also considered the feasibility of protein substitution therapy, compensating for the lack of CDKL5 function by targeting a functional recombinant CDKL5 protein into the brain.

The following themes were discussed.
Early Detection: Introduction of regional or national prospective systems for identification of CDKL5 deficiency in infants, it was suggested, would enable early detection of CDKL5. Such an approach could be used to establish the population incidence of CDKL5 and create 'virtual cohorts' of patient outcomes to support ultra-rare clinical studies. Additionally, the ability to identify children at a young age, would enable 'therapeutic windows' for clinical studies of early interventions to be established.

Delivery Systems: The delivery of drugs was recognized as presenting a particular challenge for drugs in the field of rare neurological diseases. Direct systems for targeted delivery, may be required, with other suggestions including use of additional agents to enhance brain delivery. Protein replacement approaches were may have issues around short half-lives and immunogenicity.
Prioritization of Targets: It was recognized that gain of function approaches might exacerbate certain monogenic rare disorders (such as RETT and PMD). Animal models, it was felt, were needed to determine whether benefits resulted from gain-of-function approaches.

Cell-based Therapy: Given the complexity of regulating gene expression, it was felt that CDKL5 iPSC derived gene corrected cells might confer advantages, although indications for the approach were felt to be uncertain. There may be inflammatory components to CDKL5, raising the possibility for cell-based immunomodulation as a potential treatment. It was also suggested gene engineered microglial might be used to deliver CDKL5 after HSCT.

Trial Design: With regard to trial design, it was felt valuable to engage with regulators. Given the shortage of patients with CDKL5, placebo controlled studies were recognised to be unfeasible. Instead, open label, cross-over observation study designs was preferable.

Additional Points: These included recognition of the value of adding CDKL5 cases to the DECIPHER database, and the importance of harvesting surgical corpus callosumal resection samples from CDKL5 patients so treated for epilepsy. To identify targets for therapy there is a need to undertake a comprehensive analysis of the transcriptome, proteome, and other omes dysregulated in human CDKL5.

In Conclusion, Winslow highlighted the challenge of developing good delivery systems. "Depending on the therapeutic approach sometimes the delivery mechanism can be really hard. So let's not wait until the clinic, but try to start developing things early."

KEY TAKE AWAYS FROM WORKSHOP DISCUSSIONS

From the four workshops Ashley Winslow identified the following new roles and suggestions:

- Funders should push for higher standards of study design (including statistical power, blinding and randomization). There is a need for dependable data.
- Sufficient details of studies should be provided by investigators to enable study replication.
- Open access (potentially through the portal) to all data including negative findings.
- Development of reagent repositories.
- Surgical samples being made available to tissue banks for pathological studies.

In addition:

- There was a clear commitment to tackling patient database problems in CDKL5, and recognition of the importance of keeping clinical and parental communities engaged to contribute data.
- Working groups would be established to provide a Go/No-Go decision process for translational of animal studies. There is a need to discuss current animal models and consider new models on the horizon.
- The community needs to be engaged to use the CDKL5 Forum Portal as a resource, and encouraged to share negative data. There should be opportunities to post pictures of antibodies, and share protocols on the portal.
- Centralization of data from different studies would allow meta-analyses to be undertaken.

Winslow concluded her presentation by asking delegates to email her or the CDKL5 Forum with any further ideas or important gaps that had not been highlighted in her summaries.

DINNER AND ANNUAL PRIZE AWARDS

In the evening of Day One, the Loulou Foundation hosted the CDKL5 Forum dinner for delegates in the historic setting of the Dorchester Library at the Royal College of Physicians.

After the meal a short film was presented featuring interviews with Ashley Winslow and key CDKL5 investigators including, Joe Zhou, Peter Kind, and John Rouse, who have all been awarded research grants from the Loulou Foundation.

The 2016 CDKL5 Forum Awards for Excellence were announced:

- The award for the 'Lab of the Year' went to Orrin Devinsky (New York University). "Orrin is a fantastic clinician and leader in his field who has made such a huge difference in the world of epilepsy in general and CDKL5 deficiency in particular. He has played a big role in making the PTC trial for nonsense mutations in CDKL5 possible as the sponsor investigator." Receiving the award Devinsky said: "The idea that doctors and scientists are driving forward the world of medicine is an old paradigm. It's parents like Majid and Lynn who are the drivers and I'm very proud to be with you."
- The award for 'Company making a difference' went to PTC Therapeutics. "PTC Therapeutics have taken a leap of faith to have the first trial for CDKL5 targeting the underlying kinase deficiency at the genetic level".
- An award for 'Outstanding Contribution' also went to Michael Jasulavic, a CDKL5 parent, who founded Miamed, a company dedicated to developing protein replacement for CDKL5 deficiency, which was acquired by Amicus Therapeutics. Jasulavic was also instrumental in persuading PTC Therapeutics to undertake the new trial in CDKL5 nonsense mutations. "We'd like to recognise an unsung hero."

The collaboration prize for the lab making greatest use of the CDKL5 online portal was not to be awarded, since there had so far been insufficient traffic to the site. Instead the Loulou Foundation will fund five new CDKL5 Forum Junior Fellowships of $10,000 each for post doc or doctoral students, and invited nominations. "It's very important that we start looking at young investigators and encouraging them to make CDKL5 a big part of their career." The criteria for the awards will be work ethic, track record and commitment to CDKL5 research.

The hosts thanked speakers, moderators and panellists, as well as organisers of the Forum. "Your work is life changing for the families. Let's keep going and hopefully come to something very soon,"

The first day of the Forum, said Loulou Foundation Co-Founder Majid Jafar, had far exceeded their expectations. "The mix between clinicians, academic researchers, patient groups and the corporate sector is the magic of this Forum. Last year I set the goal of treatment within five years and cures within 10 years. From what I've seen today I believe this to be achievable. A huge thank you to all of you helping us along this journey."

CLOSING DISCUSSION

In the closing discussion, moderated by Sir Colin Blakemore, delegates raised the following issues:

- The importance of studying the 'natural history' of CDKL5 disorder was emphasized, with recognition of the role such data plays in drug testing. A University of Hamburg initiative, involving collection of clinical genomic data from 25 countries, was cited as a good example of this approach. The initiative also functions as a virtual repository where scientists' list resources that they are prepared to share. There is a particular need, it was felt, to share information about the functional consequences of specific mutations at the protein level. A current initiative, funded by the IFCR, has correlated 150 different CDKL5 mutations with phenotypes (study in press).
- Efforts are needed to identify new biomarkers for CDKL5, in addition to seizures. Visual evoked potentials, it was suggested, might provide a suitable candidate as a CDKL5 biomarker

Addressing the subject of the CDKL5 Forum portal, it was felt the site represented an ideal place to share negative results. There should also be facilities on the portal for uploading publications and linking to papers that are not open access. The CDKL5 community could be asked to provide regular brief updates about research progress. One approach taken by GSK, who sponsor research at over 30 universities, is to require quarterly reports from investigators and to host annual meetings. Once a study with negative results is identified, sharing such information allows other investigators to withdraw from the field of work. It was recognised that investigators placing data in the public domain of the portal prior to publication needed adequate acknowledgment of their work. Suggestions for increasing traffic to the portal included creating email alerts, and introducing 'Facebook' type triggers to encourage people to return to the portal.

- There was felt to be a need to improve the reliability of data and ensure that preclinical studies were powered adequately. It was recognised that the introduction of uniform standards for experimental design would be valuable. In Rett syndrome, for example, NIH published a white paper detailing phenotypes to be tested, the agents and animal models used and how the cellular analysis should be performed. Such an approach, it was felt, would make systematic reviews easier. It was also suggested that the CDKL5 Forum portal could help to set standards for statistical analysis. It was recognised as important to have studies that were sufficiently powered.

The 2016 CDKL5 Forum meeting had been 'very productive', said Sir Colin, who went on to thank the Loulou Foundation for hosting the meeting. "The fact that it went like clockwork conceals the extent and quality of the organisation that has gone into to bringing so many people together from all round the world," he said.

At the close of the meeting Majid Jafar thanked delegates for the 'fantastic discussions'. "The whole point of the forum is that it is a 365 day discussion, so please do remain engaged. The idea of the portal is to continue the discussion," he said.

The date for next year's CDKL5 Forum will be Wednesday 29 and Thursday 30 November 2017, at a location on the East Coast of the USA. Each year, in future both the CDKL5 patient conference and the CDKL5 Forum meetings would be held on alternate years in the US and Europe to ensure one meeting in each every year.

NEXT STEPS

- Funders should encourage higher standards of study design (including statistical power, blinding and randomization), and sufficient details of studies should be provided by investigators to enable study replication.
- There is an urgent need to improve the clinical research enterprise with the goal of improving 'clinical trial readiness' before launching into human trials
- Establishment of scientific working groups to provide a Go/No-Go decision process for translation of animal studies. There is a need to understand the translational relationship of animal models and biomarkers to hard clinical endpoints, and to educate the FDA about feasible endpoints
- Develop a new nomenclature system for naming CDKL5 gene products that encompasses the range of transcript isoforms and predicted protein isoforms
- Investigation of the significance of CDKL5 and its substrate in neuronal development
- Further work to develop and characterise CDKL5 antibodies which are essential to establish where in the cell CDKL5 is located and whether it redistributes during development or in response to stimulation.
- More work to develop a set of reliable phenotypes and biomarkers that have clinical relevance with outcome measures that have high predictive validity
- Make surgical samples from CDKL5 patients available to tissue banks for pathological studies.
- Make use of open access resources which can relate patient clinical information to different mutations to help understand gene functionality and whether there are distinct types of CDKL5. The DECIPHER database has information on 14,000 neurological disorder patients (just 3 have CDKL5) and allows sharing phenotypic and genotypic data across the clinical community
- Address the critical need of an international CDKL5 patient database by undertaking a 'scoping' study that would consult widely, considering examples of successful rare disease databases from other organisations, and crucially keeping parental needs at the heart of the process.
- Undertake more studies on identifying existing drugs that can potentially be repurposed, by matching the genetic profiles of patients with CDKL5 to drugs already on the market eg tianeptine
- Undertake more work to engage the community to use the CDKL5 Forum Portal as a resource, and encouragement to share information and communicate negative as well as positive results.

Ends