CDKL5 *RESEARCH* TIMES

Road to a Cure

IFCR and your research dollars at work!

Breaking News

The First CDKL5 Mouse model created!

Dr. Elena Amendola and Dr. Cornelius Gross at the European Molecular Biology Laboratory in Monterotondo, Italy in a project co-funded by IFCR and IRSF have reported that the first mouse model of CDKL5 has been developed. In an abstract presented at the Federation of European Neuroscience Societies Forum of Neuroscience meeting in Barcelona, Spain on July 15, it is reported that the knockout mouse shows characteristics of limb clasping, motor and respiratory disturbances at approximately six weeks of age. Also dendritic arborization was noted to be reduced in cortical neurons. This is a major advancement for CDKL5 research and promises to add significantly to the progress in finding therapeutic modalities.

IN THIS ISSUE

2012 CDKL5 Scientific Meetings

2012 has proven to be a groundbreaking year for gathering scientists from around the world to meet and collaborate on CDKL5 research.

IFCR's First Annual Research Symposium on June 27, 2012 was attended by over 30 scientists and clinicians from around the world!

In April, the CDKL5 Voluntary Association in Italy hosted a 3 day congress for families, clinicians, researchers and therapists in Bologna, Italy. There were over 20 professionals in attendance as well as many families from Italy, and even a few from the UK, Norway and the US. Many updates on CDKL5 and Rett Syndrome research were presented and discussed.

The **International Foundation for CDKL5 Research** hosted it's First Annual Research Symposium June 27, 2012 in New Orleans which was attended by over 30 scientists and clinicians from the US, Australia, China, Italy, Spain and Israel. It was a successful inaugural meeting that fostered open discussion and sharing of ideas for the advancement of CDKL5 research. The following is a summary of the discussions that took place in New Orleans:

The meeting started with Bruria Ben-Zeev, M.D who is the director of the neurologic institute at Sheba Medical Center in Israel. She described the current clinical understanding of CDKL5 and a summary of the published cases. Currently, it is estimated that only about 30% of cases are published in the medical literature. It was interesting to see how much we have learned from these published cases in a relatively short period of time, but also important to understand how many clinical symptoms, such as cortical vision impairment and various gastrointestinal issues, went unrecognized in the early publications.

Bologna, Italy April 29, 2012

This led to the importance of the discussions by John Christodoulou, M.D, Ph.D from the University of Sydney and Stephanie Fehr, a Ph.D candidate under the supervision of Dr. Helen Leonard at Telethon Institute for Child Health Research in Western Australia. Dr. Christodoulou first discussed the intriguing results of one of the first described cases of CDKL5, which also happens to involve one of the rare families affected with CDKL5. In this family, three of five children (identical twin sisters and their older brother) are affected by an identical mutation of the CDKL5 gene. The identical twins, now 28 yrs old, have extremely different phenotypes with one being symptomatic with severe seizures and neurologic impairment while the other is described as highly functional with a moderate to high functioning type of autism. X-inactivation studies were not different between the girls and this leads strongly to the hypothesis of modifier genes being involved.

To describe more fully the characteristics, history and longitudinal course of CDKL5 it is imperative that we have a comprehensive database. Ms. Stephanie Fehr described her Ph.D project she has been working on (in cooperation with IFCR) to develop family and clinician questionnaires for the international database registry. Overwhelmingly, the researchers agreed on the absolute necessity of having a longitudinal description of characteristics and changes that take place in individuals affected by CDKL5, over their lifetime. One of the key outcomes of this database will be the identification of measurable features (end points) that can be used when testing drugs and treatments in clinical trials. We hope the database will also provide significant clues to help us develop early strategies for improving the quality of life for those living with CDKL5. The CDKL5 International Registry will begin in September, 2012.

Dr. Christodoulou and Ms. Fehr also addressed a project they have been working on, which was just accepted for publication, describing physical features of children effected with CDKL5 as well as how characteristics differ from those with MECP2 mutations (see Eye On It, page 8). Dr. Bruria remarked that she has made a few diagnoses of CDKL5 based on physical characteristics in context with other clinical features . This information may lead to more clinicians thinking of CDKL5 as part of their differential when evaluating new patients.

Dr. Christodoulou also discussed current methods of testing for mutations in the CDKL5 gene. These methods should now include special screening for the 16B exon recently described, as well as exon 18. Many recent reports have shown large deletions in the CDKL5 gene, and therefore if sequencing does not show a mutation then MLPA should be done to evaluate for deletions.

Interestingly, a few parents who attended the family symposium earlier in the week described two cases of CDKL5 involving large deletions of the gene, yet the young children had good cognitive abilities and walking skills. Typically these large deletions are predicted to cause more symptomatic cases. Descriptions of these mildly affected children highlight the importance of *every case of CDKL5* being reported in the upcoming database to provide researchers and clinicians as much information as possible so they can unravel the mysteries of why these differences take place. Capturing all the cases of CDKL5 is crucial to understanding how CDKL5 works, and will ultimately lead to future therapies.

The basic science discussions led off with Nicoletta Landsberger, Ph.D from the University of Insubria, Italy. Her work focuses on the role and function of the CDKL5 protein, and she was the first to describe the potential relationship between CDKL5 and MECP2. She discussed this work as well as current progress on the role of CDKL5 and shuffling of the CDKL5 protein between the nucleus and the cytoplasm. She also described preliminary results regarding the modulation, localization and expression of CDKL5 by NMDA receptors and suggested regulation of CDKL5 by cell death pathways. This leads to several new theories and possible

molecular modifiers of this activity. Dr. Landsberger's work may also play an important role in extrapolating the mechanisms involved with CDKL5 to other neurological disorders.

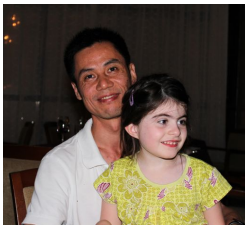
Continuing with this discussion was Zhiqi Xiong, Ph.D from the Institute of Neuroscience, Shanghai Institutes for Biological Sciences, People's Republic of China. Dr. Xiong discussed his work which identified a neuron-specific splicing variant of CDKL5 whose expression was markedly induced during postnatal development of the rat brain. He demonstrated that a decrease in CDKL5 inhibited neurite growth and dendritic arborization, potentially through a cytoplasmic mechanism. Importantly, Dr. Xiong also found CDKL5 formed a protein complex with Rac1, a critical regulator of actin remodeling and neuronal morphogenesis and that CDKL5 was required for BDNF induced activation of Rac1.

This work is important for future studies and lead into the talk by Ilaria Meloni, Ph.D, the post-doctoral fellow from Dr. Alessandra Ranieri's laboratory at the University of Siena in Italy. Dr. Meloni described the early characterization from induced pluripotent stem (iPS) cells from 3 patients with CDKL5. While there is more work to do on this model it has shown promise for further description of the morphogenesis of CDKL5- affected neurons, and has already led to clues of cellular issues shared between CDKL5 and MECP2. Drs. Meloni and Ranieri definitely showed that with a little more work these iPS cells will be appropriate models to help identify compounds for future study. These kinds of studies will allow researchers to then apply results to mouse models of CDKL5.

Zhaolan Zhou, Ph.D from the University of Pennsylvania opened the discussion of the various types of CDKL5 mouse models currently in development. He described the work his laboratory has been doing on creating CDKL5 mouse models including the R59X knock-in, which together with the iPS cell development of two patients with this same mutation should allow for more correlative research outcomes.

The meeting concluded with a discussion led by Dr. David Frame from IFCR as to where CDKL5 research goes next. Plans to further identify the kinase activity and the molecules which CDKL5 phosphorylates were discussed. Also up for discussion was the need for additional animal models to help us move quickly into translational research. The benefit of these modeling approaches are to quickly screen a vast library of drugs and compounds which could then be tested in mice and moved rapidly into clinical development. It was emphasized that while *IFCR's philosophy is to maintain the highest scientific integrity, it is also to promote and create scientific endeavors that will lead to the most rapid approach to bringing treatments and a cure into clinical trials.*

This inaugural meeting in New Orleans was an important milestone for CDKL5 research! IFCR sincerely thanks each and every researcher and clinician who attended and enthusiastically participated in the discussions. IFCR especially acknowledges all of the speakers for sharing their important research, both published and unpublished, in the spirit of cooperation and collaboration.

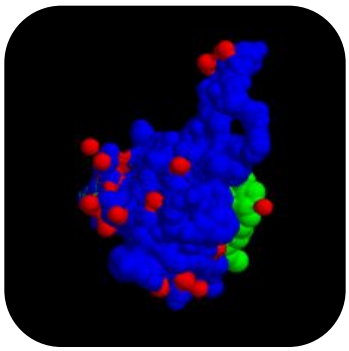


News from 7th Rett Syndrome World Congress

The International Rett Syndrome Foundation (IRSF) has recently announced two exciting therapeutic trials for Rett Syndrome. The first is the Phase 2 trial of IGF-1. After completing the phase I trial for safety and tolerability, the Phase 2 study will be a double-blind placebo controlled cross-over trial in 30 Rett Syndrome patients with the MECP2 mutation. A very important correlative study that may help with future studies in CDKL5 is the development of novel biomarkers of cortical and autonomic function. Currently there are no true biomarkers to help evaluate response and this would be a major advancement in the field. IFCR is also planning to evaluate IGF-1 in vitro for effects on CDKL5 neurons to determine if this may be a compound that is applicable for future studies in CDKL5 patients.

The second trial is in conjunction with Neuren Pharmaceuticals Ltd., a biopharmaceutical company from New Zealand that is developing drugs for brain injury and neurodegeneration. Neuren currently has three drugs in development for different neurologic conditions. One of these agents, NNZ-2566, has been in development for mild traumatic brain injury. It is a synthetic analogue of the endogenous N-terminus tripeptide, Glycine-Proline-Glutamate (GPE, Glypromate®; Neuren Pharmaceuticals), which is proteolytically cleaved from IGF-1 in the brain. GPE has been shown to cross the blood-brain barrier and protect against cell death and has been shown to have potent neuroprotective effects in animal models of neurodegenerative disease. The agent appears to have several activities including potential anti-seizure effects, anti-inflammatory effects as well as anti-apoptotic (cell death) effects. In mid-May Neuren held a pre-Investigational New Drug (IND) meeting with the FDA Division of Neurology Products to discuss clinical development plans for the study of NNZ-2566 in the treatment of Rett Syndrome and it hopes to be able to begin a clinical trial in the fall. IFCR has had preliminary discussions with Neuren in order to evaluate this drug in vitro for CDKL5 as well.

Understanding IGF-1 and CDKL5



Can IGF-1 be used for CDKL5?

The molecule Insulin-like Growth Factor-1 (IGF-1) is currently in trials in patients with Rett Syndrome who have MeCP2 mutations (see previous article).

IGF-1 is a normal hormone protein that is made in humans with a variety of purposes and its structure is similar to that of insulin. IGF-1 is a primary mediator of Growth Hormone and thus IGF-1 plays an especially important role in childhood growth. Interestingly, IGF-1 also promotes growth in most cells including the skin, muscle, bone, kidney, liver, lungs and nerves. In 2005 the United States FDA approved a synthetic analog of IGF-1 (mecasermin, Increlex™) for use as replacement therapy for severe primary IGF-1 deficiency. This is the same drug being tested in the Rett Syndrome trials.

The International Rett Syndrome Foundation (IRSF) recently announced that the Phase 1 portion of the trial was completed in 12 girls between the ages of 2- 12 years. A Phase 1 study is designed to evaluate the safety of the drug in order to be able to carry out larger studies to evaluate efficacy. The Phase 1 study did prove IGF-1 to be safe and thus a Phase 2b trial is starting in order to evaluate the efficacy compared with placebo in 30 girls with MeCP2 mutations. This is designed as a placebo controlled cross-over study in which either drug or placebo will be administered for 20 weeks then the same child will be "crossed over" to receive the opposite therapy (if they started on placebo they get IGF-1, if started on IGF-1 they will get placebo) for the second 20 weeks. Thus the outcomes of this trial will not be known for about 12 to 18 months.

A question often asked is *could IGF-1 possibly have benefit in children with CDKL5 mutations?* IFCR is working with researchers to explore the effects of IGF-1 on neuronal characteristics in CDKL5 cultured cells. This will give us our first glimpse as to whether or not IGF-1 could make a difference. If these studies report promising results it would allow us to try the therapy in CDKL5 mice. If we see encouraging results in a mouse model we could then move to a human clinical trial. However, there is a concern of IGF-1 possibly increasing or causing seizures. The first trial that reported using IGF-1 in Rett Syndrome occurred in Italy with 6 female patients. Three of these girls did not have any seizures going into the trial, but 2 out of 3 of those girls developed seizures during the trial. This has been of some concern, therefore if this new IGF-1 Phase 2b Rett Syndrome trial proves to increase seizure risk it will be more difficult to design a trial in those with CDKL5 mutations.

2012 Research Grants

*In 2012, IFCR is proud to announce we have given close to **$200,000** in research grants to the following recipients:*

Alysson Muotri, Ph.D and **Priscilla Negraes , Ph.D**
University of California San Diego

IFCR has funded a 2yr post-doctoral position for "Modeling CDKL5 Syndrome using Human Neurons". The main goal is to examine specific neuronal gene expression profiles, with the aim to accelerate the discovery of novel therapeutic drugs to treat CDKL5 syndrome.

Sila Konur, Ph.D
University of California San Francisco

"Identification of CDKL5's Phosphorylation Targets" Dr. Konur plans to use a recently developed kinase substrate identification method to reveal the substrates of CDKL5 and the phosphorylation sites to gain valuable information on the synaptic and molecular functions of CDKL5.

Zhaolan Zhou, Ph.D
University of Pennsylvania

"Development of antibodies against CDKL5"

Dr. Helen Leonard and Stephanie Fehr
Telethon Institute for Child Health Research, University of Western Australia

As part of the "The Natural History of the CDKL5 Disorder" The Australian Pediatric Surveillance study will help determine the incidence and prevalence of CDKL5 in Australia thereby highlighting the impact CDKL5 disorder has on families, caregivers and society.

SPOTLIGHT ON RESEARCHERS

Alysson Muotri, Ph.D

Alysson Muotri, Ph.D earned a BSc in Biological Sciences from the State University of Campinas in 1995 and a Ph.D. in Genetics in 2001 from University of Sao Paulo, in Brazil. He moved to the Salk institute as Pew Latin America Fellow in 2002 for a postdoctoral training with Dr. Fred H. Gage on the fields of neuroscience and stem cell biology. He then became an Assistant Professor at University of California in San Diego since 2008. His research focuses on human brain development and evolution, exploring mobile elements as generators of neuronal diversity. Dr. Muotri's lab is also interested on modeling neurological diseases, such as Autism Spectrum Disorders, using human induced pluripotent stem cells. He received several awards, including the NIH Director's New Innovator Award and the Emerald Foundation Young Investigator Award.

IFCR is proud to support Dr. Muotri's efforts to study CDKL5. Since 2011, IFCR has awarded Dr. Muotri with two research grants for the development of iPS cells and characterization of CDKL5 neurons and modeling of the CDKL5 disorder.

EYE ON IT

CDKL5 in the scientific literature

The past 12 months

- **CDKL5** ensures excitatory synapse stability by reinforcing NGL-1-PSD95 interaction in the postsynaptic compartment and is impaired in patient iPSC-derived neurons. *Nat Cell Biol. 2012 Sep*
- CDKL5, a novel MYCN-repressed gene, blocks cell cycle and promotes differentiation of neuronal cells. *Biochim Biophys Acta. 2012 Aug*
- The CDKL5 disorder is an independent clinical entity associated with early-onset encephalopathy *European Journal of Human Genetics 2012 Aug*
- A novel transcript of cyclin-dependent kinase-like 5 (**CDKL5**) has an alternative C-terminus and is the predominant transcript in brain. *Hum Genet*, February 2012
- Historic, Clinical, and Prognostic Features of Epileptic Encephalopathies Caused by **CDKL5** Mutations. *Pediatr Neurol, February 2012*
- Genes of early-onset epileptic encephalopathies: from genotype to phenotype. *Pediatr Neurol*, *January 2012*
- Clinical Phenotype of 5 Females With a **CDKL5** Mutation. *J Child Neurol*, *January 2012*
- iPS cells to model **CDKL5**-related disorders. *Eur J Hum Genet, December 2011*
- Extrasynaptic N-methyl-D-aspartate (NMDA) receptor stimulation induces cytoplasmic translocation of the **CDKL5** kinase and its proteasomal degradation. *J Biol Chem, October 2011*
- **CDKL5** alterations lead to early epileptic encephalopathy in both genders. *Epilepsia*, *October 2011*
- Oligogenic heterozygosity in individuals with high-functioning autism spectrum disorders. *Hum Mol Genet*, *September 2011*
- Mutation screening of the **CDKL5** gene in cryptogenic infantile intractable epilepsy and review of clinical sensitivity. *Eur J Paediatr Neurol*, *September 2011*
- Early-onset seizures due to mosaic exonic deletions of **CDKL5** in a male and two females. *Genet Med*, *May 2011*
- Identification of a novel **CDKL5** exon and pathogenic mutations in patients with severe mental retardation, early-onset seizures and Rett-like features. *Neurogenetics*, *May 2011*
- **CDKL5** gene-related epileptic encephalopathy: electroclinical findings in the first year of life. *Dev Med Child 2011Neurol*, *April 2011*

A Child's Legacy

A family's courageous and selfless act to benefit others living with CDKL5

We write this with the utmost sadness and respect. We grieve the loss of a 5 year old child with the CDKL5 mutation while at the same time honoring the courageous life of this child and the contribution the child's family made to advance our understanding of this disorder. An autopsy has resulted in the first look at the human pathology associated with CDKL5. The contribution this will make to science and to the further understanding of CDKL5 is enormous.

The report was electronically published ahead of print in the journal Neuropathology and Applied Neurobiology, July 19, 2012. The authors acknowledge that the findings are difficult to interpret between changes that are seizure related, treatment related and actually due to the CDKL5 mutation. One particular finding, the formation of islands of heterotopic neurons in the cerebellar vermis have not been associated with epilepsy and the authors conclude this may be related to CDKL5.

We pay great tribute to this family for the courage and dedication to allow their loss to be a catalyst for the better understanding of CDKL5. This type of medical study is essential to help unravel the mysteries of this disorder.

The death of a child is tragic, and no parent wants to consider the unthinkable. When a loved one passes, their legacy can be the gift of hope to those who continue to struggle with CDKL5 and to future generations. IFCR is collaborating with IRSF and the Harvard Brain Bank in order to allow autopsy samples to be safely stored for future investigation. While we hope and pray this never needs to be utilized, the time to consider this option is before you ever have to.

We want to hear from you!

- Please send any research related questions to dframe@cdkl5.com
- Select questions may be used in future newsletters
- We welcome your feedback and would like to hear any suggestions or answer any questions you may have.

Upcoming Events:

- Society for Neuroscience Annual Meeting
  October 13-17, 2012
  New Orleans, LA USA
- NORD / DIA's US Conference on Rare Diseases and Orphan Products
  October 22-24, 2012
  Washington, DC USA
- Rare Disease Day
  February 28, 2013
  Worldwide
- IFCR's 2nd International CDKL5 Scientific Symposium
  October 2013
  The Netherlands

International Foundation For CDKL5 RESEARCH℠ FROM CAUSE TO CURE

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