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International Rett Syndrome Foundation Awards \$2M for Cutting-Edge Rett Syndrome Research

Foundation announces new funding mechanisms to fast-track therapies (Cincinnati, Ohio) The International Rett Syndrome Foundation (IRSF) announced today that it is awarding grants totaling \$2 million to support 18 innovative research projects in 2009. Each project will explore bold new ideas that have the potential to drive the field forward and speed the translation of research into treatments and a cure for Rett syndrome. With this announcement, the foundation has cumulatively funded over \$20M in high quality, peer-reviewed grants that have contributed to the most significant advancements in the field to date.

New awards will be provided for the development of the first neuronal assays for drug screening derived from human-induced pluripotent stem cells (iPSCs). Additional grant awards will be provided to move forward 5 high impact translational research projects which focus on the development of therapeutics to treat or reverse Rett syndrome. Furthermore, a number of the projects chosen in this funding cycle may result in the identification of new drug targets that can be exploited for therapeutic intervention.

IRSF will shortly provide funding through the launch of two new grant mechanisms focused directly on translational research. The HELP ACCCELERATE RETT THERAPEUTICS (HEART) grant awards will provide seed funding to foster early stage translational research programs. The ADVANCED NEUROTHERAPEUTIC GRANT OF EXCELLENCE (ANGEL) award mechanism will provide larger grant awards to more mature drug discovery and development programs that are further along the path to a clinical application. *“The HEART and ANGEL awards will be provided to fast track research that is moving new therapies closer to the clinic”* said Dr. Antony Horton, Chief Scientific Officer of IRSF.

Dr. John McCall, a drug discovery expert and member of the IRSF’s Scientific Advisory Board commented: *“A roadmap is now emerging that will lead to significant advances in therapeutic development, leading to treatments that will improve quality of life and could provide the path to an eventual cure for Rett syndrome.”* Dr. Horton added: *“These new studies will generate fresh insights that will enable us to better understand the mechanisms underlying Rett Syndrome. While there are no quick fixes, we will continue to be aggressive in our search for new treatments for the eventual pharmacological reversal of Rett Syndrome.”*

Regular Research Grants:

Nurit Ballas PhD, Stony Brook University

Analyzing the contribution of MeCP2-deficient oligodendrocyte-lineage cells to Rett syndrome neuropathology

Previous findings suggest that non-neuronal cell populations of the brain may be involved in causing the full spectrum of Rett syndrome symptoms, given that knocking out MeCP2 in neurons results in only a partial model of Rett syndrome. To date, studies have focused on neurons as the primary cause of Rett syndrome due to their high levels of MeCP2 and the difficulty to detect MeCP2 in glial cells in the brain. New detection techniques have been used in preliminary studies that suggest MeCP2 is present in certain glial cell types and that they may contribute to neuronal dysfunction in Rett syndrome by secreting factors that are potentially harmful to neurons. This proposal seeks to assess the role of

MeCP2 loss in glial cells called oligodendrocytes and study how it contributes to the neuropathology of Rett syndrome. Developing the appropriate cellular models will help prove or disprove the real contribution of glial cells in Rett syndrome pathogenesis. In addition the study aims to further investigate possible secondary factors that could be manipulated to positively affect the course of Rett syndrome.

James Eubanks PhD, Toronto Western Research Institute

Assessing Phenotypic Improvement of MeCP2-Deficient Mice By Preservation or Reactivation of Functional MeCP2 in Catecholaminergic Cells

Dr. Eubanks proposes to determine the impact of MeCP2 loss of function by selectively knocking-out MeCP2 function in a specific population of neurons followed by rescue of function in the same discrete populations of neurons. In Rett syndrome, the catecholaminergic system may have a disproportionate impact on the development of disease. These experiments are therefore very relevant to understanding the neurological underpinnings of the disease. In addition, given the recent reversal experiments, it is clear that MeCP2 levels within cells must be carefully regulated to avoid harmful consequences of over-expression or under-expression. The expected beneficial effects of restoring MeCP2 function in specific cell populations is of great importance for this reason and could highlight new treatment strategies for Rett syndrome.

Paul H. Patterson PhD, California Institute of Technology

Regulation of MeCP2-mediated gene expression by I κ B kinase alpha

MeCP2 biology is complicated because MeCP2 has been described as both a repressor and as an activator of gene expression. Maintaining critical levels of MeCP2 appears to be required for normal brain function. However, the molecules regulating MeCP2 expression itself are poorly understood. This grant proposes to test whether a cellular signaling molecule (IKK α), is involved in regulation of MeCP2 function. IKK α is responsive to a wide range of stimuli and is implicated in other neurological diseases. Thus, understanding the link between IKK α and MeCP2 may prove to be crucial for understanding Rett syndrome neuropathology and provide a link to other neurodevelopmental disorders. Future directions include identifying other MeCP2-regulated targets that are controlled by IKK α , which will likely reveal new drug target molecules and signaling pathways related to Rett syndrome. This is a novel proposal that seeks to understand the relevance of a signaling pathway that may be controlling the activity of MeCP2.

Steven Gray PhD, University of North Carolina at Chapel Hill

rAAV-Mediated Replacement of the MeCP2 Gene in a Rett Syndrome Mouse Model

Gene-replacement therapy of MeCP2 is a potential future treatment option for Rett syndrome patients. The proposed study focuses on overcoming two critical issues that are likely to impede clinical translation of MeCP2 gene-replacement therapy using viral vectors. These are; transferring enough of the gene into the brain to make a difference and limiting the likely adverse impact of MeCP2 over-expression. Dr. Gray is a talented young investigator who has clearly demonstrated his ability to perform the proposed work and is associated with an excellent team in the area of virus-mediated gene transfer for CNS diseases.

Heekyung Hong PhD, Northwestern University

Genetic Dissection of Rett Syndrome: a Screen for Modifiers of MeCP2 in the mouse

This proposal seeks to discover potential genes that could modify the course and/or severity of Rett syndrome in a *Mecp2*-null mouse model. The project will examine novel signaling elements that may

alter MeCP2-dependent transcriptional regulation. This is a project that has a potentially high-impact since it may yield novel therapeutic targets for the treatment of Rett Syndrome.

Daniel Kilpatrick PhD, University of Massachusetts Medical School

Transcriptional Dys-Regulation in Rett Syndrome

This proposal will investigate a gene which is involved in regulating the downstream targets of MeCP2. Previous work has shown that the gene, called *Rest*, is upregulated in Rett syndrome and is thought to contribute to the spectrum of down-regulated genes in the CNS of Rett patients and in *Mecp2*-deficient mice. Dr. Kilpatrick will generate new 'knock-out' mouse models targeting the *Rest* gene and will use other techniques to decrease levels of the gene, with the goal of rescuing features of Rett syndrome in MeCP2 mice. This project could therefore present a novel therapeutic target for Rett syndrome treatments.

Jeffrey Neul MD, PhD, Baylor College of Medicine

Characterization of cardiac abnormalities in Rett syndrome

This project will thoroughly investigate cardiac conduction abnormalities previously identified by Dr. Neul's team in Rett syndrome mice, which accurately model defects in Rett syndrome patients. The studies involve long-term, periodic heart monitoring coupled with a correlative analysis of potential MECP2 target genes. These studies may explain the long QT syndrome seen as a consequence of Rett syndrome. Dr. Neul will further this work by developing a conditional knock-out mouse to better determine if MeCP2 loss in neuronal vs. cardiomyocyte cell types is the origin of the observed conduction defects. The innovative aspect of the study is its focus on evaluating the effects of *Mecp2* dysfunction outside the CNS, in particular in the heart, an aspect that has not yet been thoroughly explored. In addition, it could help to understand the basis for the proposed autonomic dysfunction that may be the cause for sudden unexplained death in this population.

N. Carolyn Schanen, MD, PhD

Alfred I. DuPont Hospital for Children/Nemours Children's Clinic at the University of Delaware

Suppression of Rett Nonsense Mutations by Pharmacological Agents

Dr. Schanen will conduct a study that seeks to better understand the pharmacological properties of drugs which suppress nonsense mutations. A nonsense mutation is an alteration in the genetic code that prematurely halts the synthesis of an essential protein. The study will examine a lead series of "read-through" compounds, and will provide valuable information on their mechanism of action within cells. These read-through compounds hold great promise for the treatment of individuals who possess nonsense mutations in the *MECP2* gene.

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Preclinical testing of nonsense suppression in a rodent model of Rett syndrome

Dr. Schanen has previously developed mouse "knock-in" models of the R168X and R255X non-sense Rett syndrome mutations. The project will further generate colonies of the two knock-in mouse models and ultimately the new mouse models will be used to test a lead series of read-through compounds. The successful testing of these drugs could potentially result in a pharmacological reversal of Rett syndrome. This study would have an enormous impact upon the field and has clear implications for treatment of the disease.

Yi Eve Sun PhD, University of California at Los Angeles, David Geffen School of Medicine

The role of microRNA dysregulation in MeCP2-deficient neurons

The field of epigenetics has recently identified a set of small molecules called “micro-RNAs.” These are thought to be critically important regulators that fine tune and modulate the function of different proteins and essential cellular processes. The aim of this project is to test the novel hypothesis that MeCP2 might regulate different sets of “micro-RNAs” in different neuronal subsets within the brain and which may have functional consequences as a whole. The proposal by Dr. Sun presents a clear set of experiments that will further our understanding of the basic mechanisms underlying Rett syndrome and could identify novel targets for therapeutic manipulation.

John Vincent PhD, The Centre for Addiction & Mental Health, University of Ottawa

Comparative Functional Studies Of The Two MeCP2 Isoforms, MeCP2_e1 and MeCP2_e2

This is a research proposal that aims to address the important question of which of the two common forms of MECP2 is most critical for brain development and function in the mouse. This is an interesting proposal that addresses a key aspect of the underlying biology of Rett syndrome and the findings have the potential to change the focus of research to a single MECP2 species. If successful, the results of this study will also help guide future developments with regard to *MECP2* gene-targeted approaches to the treatment of Rett syndrome. Dr. Vincent proposes to compare the contribution of each variant form of MeCP2 to Rett syndrome. He will further explore the effect on gene expression, neuronal differentiation and maturation, development, cognition, and behavior in transgenic mice.

John Williams PhD, Oregon Health Sciences University

Dopamine cells and movement disorders: identifying a cellular basis for Rett syndrome

This proposal aims to look at cellular mechanisms that may underlie the motor deficits in Rett syndrome. Specifically, the investigators will examine the cellular structure and physiology of dopamine neurons in a region of the brain called the striatum. The investigator seeks to define the specific characteristics that distinguish the neurons that are *Mecp2* positive and those that are *Mecp2* negative. In Rett syndrome patients these two cell populations could be independently influencing different characteristics in one another. The investigator will then examine whether re-introducing *Mecp2* to *Mecp2* negative neurons improves their physiological characteristics. This proposal is well thought out, backed by strong preliminary data and feasible; the PI and environment are outstanding. It addresses an aspect of Rett syndrome that has not received a lot of attention, namely, the rapid regression of previously acquired motor skills and the onset of stereotyped and non-functional hand movements.

Xinyu Zhao PhD, University of New Mexico School of Medicine

Role of Mecp2-regulated microRNAs in the pathogenesis of Rett Syndrome

This is a strong collaborative proposal that seeks to understand how the dysregulation of one or more micro-RNAs may be involved in the defects in neuronal maturation in Rett syndrome. This is a novel line of investigation with potentially high-impact results. The investigators have expertise in MeCP2 biology and strong preliminary data on two specific micro-RNAs that suggests these are the key microRNAs that are dysregulated in Rett syndrome with functional consequences and are very relevant to neuronal development. In the final phase of her project, Dr. Zhao will also conduct experiments that are aimed at functional rescue which lends a therapeutic aspect to this study.

Post Doctoral Fellowships

Cassiano Carromeu, PhD University of California San Diego (Mentor Alysson Muotri PhD)

Modeling Rett Syndrome with human pluripotent stem cells

Dr. Carromeu's project will be carried out in the laboratory of Dr. Alysson Muotri who has recently developed induced pluripotent stem cells (iPSCs) from Rett patients'skin cells. These iSPCs have been derived from patients with different MeCP2 mutations, and with different clinical phenotypes. The reprogrammed cells can generate a virtually unlimited supply of human neurons and other neural cell types of interest. In preliminary work, Dr. Carromeu showed that neurons generated this way displayed reduced synaptic contacts which are consistent with neuronal alterations seen in Rett syndrome. This model system will now be used for testing drugs to rescue these cellular alterations and to investigate developmental changes seen in Rett syndrome. Dr. Carromeu's mentor is the recent recipient of an NIH "New Innovator award." Dr. Muotri's research program seeks to use Rett syndrome as a model for other autism-spectrum disorders, this may reveal common molecular and cellular mechanisms present in different types of autism.

Jun Ren PhD, University of Alberta (Mentor John Greer PhD)

Investigation of Respiratory Dysfunction in a Mouse Model of Rett Syndrome

Dr. Ren's project addresses a very important complication of Rett syndrome, seeking to better understand the specific neuronal components, pathways and circuits controlling respiratory dysfunction. The study focuses on the respiratory circuit rhythm generator which is thought to function abnormally in Rett syndrome. While this rhythm generator can function normally during sleep, during wakefulness, it is believed that certain neuronal circuits are dysfunctional and fluctuations within specific regions of the cortex can destabilize the basic respiratory rhythm. The PI will analyze potential sources of central respiratory pattern instabilities to identify the specific circuits and pathways involved.

Yin Shen PhD University of California, San Diego (Mentor Bing Ren, PhD)

Genomic analysis of MECP2 Function

Dr. Shen will conduct an investigation into the action of MECP2 at its various different target-binding sites across the entire human genome. The study will specifically examine MeCP2 binding in stem cells both before and after these cells are encouraged to become neuronal cells. Dr. Shen will study the consequences of MECP2 knockdown at different target sites with a view to identifying critical target genes. The investigator will examine early stage neuronal precursors, and developing neurons to evaluate the epigenetic interactions of MECP2 at successive stages of development.

Anirban Paul PhD, Cold Spring Harbor Laboratory (Mentor Z. Josh Wang, PhD)

Novel Function of MeCP2 in mRNA Regulation and Implication in Rett Syndrome

Dr. Paul's project is aimed at testing a novel hypothesis related to the function of MeCP2. The investigator's preliminary data presents an entirely new facet of MeCP2 function which is that MeCP2 can function by binding directly to messenger RNA (mRNA) and potentially regulates the synthesis of downstream proteins. The project may provide new insights into the role of MeCP2 and mRNA is an ideal target for drug discovery because it is a key regulatory molecule in the cell.

Annamaria Lilienkamp PhD, University of Illinois at Chicago
(Mentor Alan Kozikowski, PhD)

The Role of Histone Lysine Methylation Marks in Rett Syndrome – Identification of Novel Histone Lysine Methyltransferase Modulating Agents

Dr. Lilienkamp will conduct a novel drug discovery project based on interactions between MeCP2 and key enzymes called histone lysine methyltransferases (HKMTs) which are known to regulate transcription. Small molecules that can manipulate this interaction called HKMT inhibitors will be studied to better understand if they alter the developmental course and rescue the pathology of Rett syndrome. The first series of HKMT inhibitors could eventually be tested as new drugs for intervention in

Rett syndrome but they could also help guide the process of identifying new therapeutic agents within the same lead series.

Additional Grants Awarded in 2009

Omar Khwaja, MD, PhD Children's Hospital Boston - ANGEL AWARD RECIPIENT

Pharmacological Treatment of Rett Syndrome by Stimulation of Synaptic Maturation with IGF1

The study will test an experimental treatment based on the administration of insulin like growth factor 1 (IGF1) in a pilot clinical study in 30 patients with Rett syndrome. The trial is based on preliminary findings in mice which suggest that targeting the IGF1 signaling axis may partially reverse Rett symptoms by boosting synaptic maturation of neurons in the brain. The treatment to be used in the study is an FDA approved drug called Increlex, a bioengineered form of the IGF1 protein. The study drug has previously been used to treat growth and has a known safety and tolerability profile in a pediatric population. Although this is a pilot clinical trial in 30 patients, if successful the study will have significant impact on the field and may lead to a new disease-modifying treatment for Rett syndrome.

Alan Kozikowski PhD, University of Illinois, Chicago

Synthesis and preliminary (ADME) testing of selective HDAC inhibitors for treatment of a mouse model of Rett Syndrome

Histone deacetylase (HDAC) enzymes are proteins that regulate gene expression and are involved in brain cell degeneration in neurodegenerative diseases. HDAC inhibitors are drugs which block the action of HDAC enzymes and have shown promise as potential treatments for neurodegenerative diseases such as Parkinson's, Motor Neuron and Huntington's diseases. These drugs have also recently been suggested as potential treatments for neurodevelopmental disorders such as Fragile X, Rubinstein–Taybi syndrome and Rett syndrome. Dr. Kozikowski was awarded a short-term contract to fund preliminary predictive tests to select the best candidate drug from a lead series for testing in a Rett mouse model.

IRSF conducts thorough due diligence on all research grants and for clinical studies involving patients with Rett syndrome, since safety is of paramount importance. We accomplish this through a rigorous peer-review process, drawing on the external advice and expertise of leading experts and specialists in the fields of Rett syndrome, neuroscience, autism, drug discovery and development and other relevant disciplines as appropriate.

IRSF will shortly announce additional detail on the two new funding mechanisms and will solicit research programs through an online grants submission process. Dr. Horton commented "*With the launch of these new award mechanisms, we will have greater power and flexibility to accelerate drug discovery and to develop and test new treatments. These awards will bring us another step closer to finding a cure through the pharmacological reversal of Rett syndrome.*"

According to Stephen Bajardi, Executive Director of IRSF, *"It is through the combined efforts of our parent volunteers, generous contributors and dedicated staff members that we are able to make this exciting announcement and continue to provide the necessary funding to the world's top investigators and research labs. Our primary goal is to identify approaches that will make treatments available for the thousands of girls and women affected by Rett syndrome."*

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About Rett Syndrome

Rett syndrome (RTT), a brain disorder affecting development in childhood, has been identified almost exclusively in females. RTT results in severe movement and communication problems following apparently normal development for the first six months of life. The characteristic features include loss of speech and purposeful hand use, occurrence of repetitive hand movements, abnormal walking, abnormal breathing, and slowing in the rate of head growth. Current treatment for girls with RTT includes physical and occupational therapy, speech therapy, and medication for seizures. No cure for Rett syndrome is known. In 2007, researchers heralded a major breakthrough by reversing RTT symptoms in mouse models. Rett syndrome is recognized as the "Rosetta Stone" of other neurological disorders, with genetic links to other disorders like autism and schizophrenia.

About the International Rett Syndrome Foundation

*IRSF is the world's leading private funder of basic and clinical Rett syndrome research, funding over **\$20M** in research grants and quality research programs to date. Annually, IRSF hosts the world's largest gathering of global Rett researchers and clinicians to establish research direction and priorities while exchanging ideas and the most recent information. IRSF is the most comprehensive non-profit organization dedicated to providing thorough and accurate information about Rett syndrome, offering informational and emotional family support, and stimulating research aimed at accelerating treatments and a cure for Rett syndrome and related disorders. To learn more about IRSF and Rett syndrome, visit www.rettysyndrome.org or call IRSF at 1-800-818-RETT (7388).*