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Ballas, Nurit

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Ballas, Nurit, State University of New York at Stony Brook, 2 years, \$100,000

Title: Neuronal:Glial Interactions Underlie Rett Syndrome

Lay Summary:

Rett Syndrome is a severe neurological disorder in humans that results from mutations in the gene expressing the transcription factor MeCP2. It was shown recently that mice deficient for MeCP2 develop Rett like Syndrome that can be rescued by MeCP2, suggesting that the damage to the nervous system is reversible. We show that at least part of the damage to neurons in the nervous system of RTT mice is likely due to the lack of MeCP2 in astroglia, which represent a large number of cells in the brain. Our data suggests that astroglia that lack MeCP2, likely secrete a toxic factor(s) that negatively affects neurons. In the proposed research, we plan to identify the nature of this secreted factor(s) as well as the defects in astroglia that result from the lack of MeCP2. The proposed studies offer the potential for a new treatment, through pharmacological inhibition of potential glial secreted inhibitory factor(s), of this devastating neurological disorder that strikes one in 10,000-15,000 girls.

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Carlson, Greg, Children's Hospital of Philadelphia, 2 year, \$97,865

Title: Development of Cortical Hyperexcitability in Rett Syndrome

Lay Summary:

Following the initial characteristic symptoms of Rett syndrome, most girls with Rett syndrome and their families are confronted with epilepsy at around age four. This can be particularly devastating part of the disease. Understanding the emergence of epilepsy and the physiological reasons for the seizures in Rett syndrome is important to identifying and testing potential treatments for this part of the disease. The proposed work will determine whether mouse models of Rett syndrome can be used to study seizure susceptibility, and its development in Rett syndrome. To do this, we will measure changes in seizure susceptibility between two different mouse models of Rett syndrome in the whole animal, compared with control animals over the period that Rett symptoms occur and develop in these mice. More broadly, we plan to explore whether abnormal excitability is a common feature of Rett syndrome. Using a novel in vitro imaging approach and single cell assays of excitability, we ask: how does this abnormal excitability affect the way the brain processes specific inputs? In preliminary data, we find large differences in how some areas of the brain control these inputs, producing abnormally prolonged periods of excitation in the Rett mouse models. We believe that this work could have implications beyond the question of epilepsy in Rett syndrome. The work could have broader impact because some of the neuronal changes that underlie the development of epilepsy may also interfere with other normal brain functions, contributing to the development of other Rett symptoms.

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Ellis, James & Dirks, Peter, The Hospital for Sick Children, 2 years, \$100,000

Title: Gene therapy with Cotranscriptional Knockdown for Rett syndrome

Lay Summary:

Rett syndrome (RTT) is caused by mutations in the MECP2 gene. This type of genetic disease may be treated by "gene therapy" that introduces the normal gene into affected cell types. RTT is reversible in mouse models, but gene therapy will be challenging because too much MeCP2 causes neurological disease. Here, we propose to deliver into brain cells an exciting new design of lentivirus vector that preserves normal MeCP2 levels. It functions by expressing a therapeutic human MECP2 gene while an shRNA specifically degrades equivalent amounts of mouse Mecp2 transcripts. These cotranscriptional lentivirus vectors have: 1) a single internal promoter to direct expression to all cell types (EF1OX promoter) or specifically in neurons (Mecp2 promoter), 2) human MECP2E1-myc or MECP2E2-myc coding sequences, 3) a B-globin gene intron in which to place an shRNA, and 4) an shRNA that specifically degrades only mouse Mecp2 transcripts. This cotranscriptional strategy using a B-globin intron embedded shRNA has been shown to cure Sickie Cell

Anemia in mice. Published and novel shRNA sequences will be incorporated into the vector and tested for their ability to knockdown mouse MECP2 in neurons while coexpressing human MECP2 from the same promoter. To correct RTT symptoms, we will infect Neural Stem Cells (NSC) from mutant mice and deliver these modified NSC back into the brains of fetal mice by in utero ultrasound guided injection. The NSC or highly concentrated virus will also be injected into postnatal mouse brains. RTT symptoms in surviving mice will be evaluated prior to determination of MeCP2 expression levels in neurons. The best vector will be introduced into the mutant mouse germline to demonstrate rescue of RTT symptoms when it is expressed in all neurons. Our anticipated outcome is a proof of principle that gene therapy with cotranscriptional lentivirus vectors produces neurons that express correct levels of normal MeCP2. In summary, these studies will test the promise of lentivirus gene therapy to ameliorate RTT phenotypes in vivo.

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Jin, Peng, Emory University, 2 years, \$100,000

Title: Role of Small Rnas Regulated By Mecp2 In Rett Syndrome

Lay Summary:

Rett syndrome (RTT) is a neurodevelopmental disorder mainly caused by mutations in the x-linked gene Methyl-CpG-binding protein (MECP2) and primarily affects females. MeCP2 is thought to selectively bind methyl-CpG dinucleotides in the mammalian genome and block gene expression. Recent studies have shown that small RNAs (~ 20 nucleotides long) play important roles in diverse cellular pathways. Mutations in MeCP2 affect its ability to block gene expression and may lead to aberrant patterns of gene expression in RTT. The predominant manifestation of central nervous system dysfunction in RTT suggests that MeCP2 plays critical roles in the development and stability of Neurons. Our preliminary studies have suggested that MeCP2 could regulate the expression of small RNAs. In this proposal, we are going to examine whether the misregulation of small RNAs in the absence of functional MeCP2 could contribute to the pathogenesis of Rett syndrome. Those micro-RNAs could potentially play macro-roles in Rett syndrome.

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Katz, David, Case Western Reserve University, 2 years, \$100,000

Title: Ampakine Treatment of Respiratory Dysfunction in a Mouse Model of Rett Syndrome

Lay Summary:

Respiratory dysfunction is a major challenge for patients with Rett syndrome (RTT) and may account for more than 25% of deaths in this population (Kerr et al., 1997). Despite this, relatively little is known about underlying mechanisms and there are currently no treatments available. Recent studies, however, suggest a potential role for alterations in Brain Derived Neurotrophic Factor (BDNF) signaling in respiratory control and other neurologic problems in RTT. Indeed, genetically engineered mice lacking *Mecp2* (*Mecp2^{tm1-1Jae}* null mice; Chen et al., 2001), the gene mutated in RTT, exhibit marked deficits in BDNF levels in the brain and genetic restoration of BDNF levels delays the onset of RTT-like symptoms and extends lifespan (Chang et al., 2006). The earliest and most significant deficits in BDNF expression in the *Mecp2* null mouse brain are found in neural structures important for cardiorespiratory control, including the nodose cranial sensory ganglion and brainstem (Wang et al., 2006). Because BDNF is essential for development and function of respiratory neurons and normal breathing behavior (Katz, 2003, 2005), we hypothesize that deficits in BDNF contribute to the RTT-like respiratory phenotype of *Mecp2* null mice. Moreover, these observations raise the possibility that BDNF is a potential therapeutic target for treatment of respiratory dysfunction in RTT. Experiments performed in our laboratory demonstrate that treatment of adult *Mecp2* null mice with CX546, an ampakine drug, can partially restore levels of BDNF in nodose ganglion cells in vivo, raising the possibility that treatment with ampakines could improve respiratory function in these animals. In support of this hypothesis we recently found that treatment with CX546 restores normal mean breathing frequency and minute volume in adult *Mecp2* null mice with RTT-like respiratory dysfunction (Ogier et al., submitted). However, CX546 treatment does not completely restore normal breathing in *Mecp2* null mice; moreover, we have not examined the ability of any ampakine to improve cognitive and motor function or extend lifespan. Because the ampakines constitute a diverse family of molecules, many of which exhibit distinct potencies for increasing expression of BDNF, and target different cell groups (Kessler et al., 1998; Lauterborn et al., 2000, 2003), further studies are required to determine which ampakine(s) are most effective at increasing BDNF expression and treating RTT symptoms in *Mecp2* null mice. Therefore, the aim of the current proposal is to perform a more detailed and comprehensive analysis of the therapeutic potential of the ampakine family of compounds for RTT, using *Mecp2* null mice as a model system.

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Luger, Karolin, Colorado State University, 2 years, \$99,979

Title: The Interaction of *Mecp2* with Histones and with the Nucleosome

Lay Summary:

Rett syndrome (RTT) is a common disorder that leads to severe mental retardation in girls. Mutations in the X-linked gene encoding Methyl CpG binding protein (MeCP2) have been linked to ~ 80% of the known cases of RTT. Because the gene is encoded on the X chromosome, RTT is usually lethal in boys. The function of the healthy protein in neurons is largely unknown, and information on its three-dimensional structure is extremely limited. In order to understand why changes in this enigmatic protein result in a crippling neurological disorder, it is imperative to investigate how it interacts with components of the cell's nucleus. MeCP2 acts through binding with specifically marked regions on the DNA, thus determining the type of information that the cell reads from the DNA. One newly identified function of MeCP2 is to help package cellular DNA to allow it to physically fit within the constricted confines of the cell's nucleus. This interaction regulates access to the information encoded on the DNA. All eukaryotic DNA exists in a complex with an equal mass of proteins to form chromatin. The basic building blocks of chromatin are nucleosomes. These are tiny spools around which two turns of DNA are tightly wrapped. Our preliminary data indicate that MeCP2 regulates chromatin structure by directly binding to nucleosomes. A characterization of the interaction of MeCP2 with the nucleosome and with its protein components, as proposed here, is essential to understand this newly emerging function of MeCP2. We will also investigate the novel hypothesis that several of the RTT-associated mutations impair the ability of the protein to interact with nucleosomes or its protein components. The experiments proposed here will ultimately allow us to determine the three-dimensional structure of MeCP2 in complex with the nucleosome at the atomic level. Such a structure would

permit us to directly visualize the role of amino acids whose character has been changed by mutations, thus causing Rett syndrome. This information will be essential to figure out how mutations in MeCP2 cause Rett syndrome, ultimately paving the way to a cure.

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Macklis, Jeffrey, Harvard/Boston's Childrens Hospital, 2 years, \$99,990

Title: In Vivo Investigation of Mecp2 Target Genes Centrally Involved in the Pathogenesis of Rett Syndrome

Lay Summary:

Recent research has revealed that a defect in a gene called MECP2, which encodes a protein that suppresses activation of other genes ("transcriptional repressor"), causes Rett syndrome. The discovery of MECP2 mutation as the cause of Rett syndrome enabled a new era of cellular and molecular analysis and understanding of mechanisms of Rett syndrome. An important next research goal will be to find the specific genes that MeCP2 regulates in individual affected nerve cells ("neurons"), because MeCP2 normally regulates its "target" genes during development and function of the nervous system, and abnormal activation of target genes directly or indirectly causes Rett syndrome. Over the past few years, several MeCP2 target genes have been identified. Although these genes meet two minimum criteria as MeCP2 target genes (their abnormal expression in Mecp2 mutant neurons and MeCP2 direct interaction with them), it is unclear whether their abnormal regulation is involved in Rett syndrome. Lack of this evidence limits understanding of Rett syndrome pathology. Our previous work demonstrates that MeCP2 is involved in the maintenance and maturation of brain neurons, including their connections, and the stabilization of neurons with long axons, rather than the early development or movement of neurons as the brain is initially formed. These previous results show "pyramidal neurons" that connect the two sides of cerebral cortex in Mecp2 mutant mice are specifically smaller and their dendrites (which receive information) are less complex than those in normal mice (Kishi and Macklis, 2004). In addition, our recent work using genetic and physical "chimeric" mice (mixing one type of neuron with another type of brain), shows that a normal environment does not eliminate the abnormalities of transplanted Mecp2 mutant neurons, indicating that MeCP2 in neurons themselves (rather than MeCP2 in surrounding cells) is the central reason for their abnormalities (Kishi and Macklis, submitted, 2007). Based on our previous work, we are pursuing two complementary approaches for the identification and molecular analysis of target genes of MeCP2 in the specific cortical neurons, using both gene "microarrays" (look at tens of thousands of gene activations at once) and "chromatin immunoprecipitation (ChIP)/ChIP-on-chip" approaches. By comparing gene activation patterns in normal neurons with those in neurons from Mecp2 mutant mice, we can find which genes are abnormally activated in Mecp2 mutant mice. ChIP is an approach to determine the location on chromosomes where molecules like MeCP2 bind, and thus, which genes they regulate. Using these approaches, we have already identified new MeCP2 target genes. One of them shows an effect on only neuronal maturation, suggesting this gene may be involved in Rett syndrome. In this proposal, we propose to extend our ongoing funded analyses to further our MeCP2 target identification using these specifically affected neurons. Then we will analyze the abnormal regulation of MeCP2 target genes in the brain using new ways to raise and lower activation of desired genes in the brain. Our proposed experiments will not only add to our understanding of molecular and pathological mechanisms of Rett syndrome, but will also potentially contribute to future therapeutic strategies.

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Nelson, Sacha, Brandies University, G.E.A.R. Grant, 2 years, \$200,000

Title: Physiological Genomics of the Locus Coeruleus in a Mouse Model of RTT

Lay Summary:

Rett syndrome is caused by malfunctions of the gene MeCP2, but the biological functions and brain structures most involved are still not known. Symptoms of Rett Syndrome include altered learning and memory, seizures, abnormal movement, anxiety, and disorders of the regulation of breathing and heart rate. One neural structure implicated in all of these functions is the locus coeruleus (LC), a group of neurons in the brain stem that release the neurotransmitter norepinephrine. We found that the expression of genes and the physiological properties of LC neurons is abnormal in mutant mice that lack normal MeCP2. Unlike neurons in the cerebral cortex which are hypoactive, LC neurons in these mice are hyperexcitable. However, because these neurons are inhibited by the norepinephrine, they release this hyperexcitability could reflect reduced function of these neurons. We will use a variety of drugs and physiological methods to determine whether norepinephrine release is elevated or reduced. We will also try to determine whether this is a late feature of the disease caused as a reaction to other changes in the brain or whether it is an early change that might participate in causing subsequent changes in the brains of these mice and of Rett syndrome patients. The results of these experiments may suggest drugs which could be of use in treating some of the symptoms of Rett syndrome. Specifically, we will test the possibility that drugs that enhance or block the ability of LC neurons to be inhibited by their own neurotransmitter may normalize the physiology. We will also search for molecular differences between LC neurons and other neurons in the brain that use norepinephrine in order to be able to more precisely target those key neurons and control their activity.

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Rosenmund, Christian, Baylor University, 2 years, \$100,000

Title: The Role of Mecp2 Levels on Synaptic Function

Lay Summary:

Patients with Rett syndrome exhibit a wide variety of behavioral and learning deficits. One prevailing theory of how these wide ranging problems may occur is that the delicate balance of excitation and inhibition in the brain is disturbed. The protein that is mutated in Rett patients may play a crucial role in healthy people controlling the excitation-inhibition balance. We are testing this idea by examining mice that have either too little or too much of this protein, and studying how these changes affect the balance of excitation and inhibition in individual nerve cells. We also want to know if those changes occur at the site of the sender or that of the receiver of nerve cell signals, and whether those nerve cells specializing in excitation are differentially affected than nerve cells that mediate inhibition. We believe that uncovering the origin of the problem will help to define more rational treatments for girls with Rett syndrome.

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Steen, Judith A., Harvard/Boston Children's Hospital, 2 years, \$100,000

Title: A Modification/Structure/Function Analysis of MeCP2 Using Biochemistry and Mass Spectrometry

Lay Summary:

Proteins are machines that carry out a number of essential functions in cells. These proteins, which are encoded by the genetic material in the cell, consist of long chains of amino acids, strung together much like a chain of irregularly shaped beads. Each chain of beads folds to form a structure that can perform one or more functions. Different types of chains will therefore adopt unique structures, each of which is able to perform a different function. Often, these functions need to be modulated in order for the cell, or living system, to respond to its environment on an immediate basis. The activity of these proteins can be modulated by changing the shape of particular beads/amino acids by adding other molecules such as phosphates to the amino acids. These modifications are called post-translational modifications and can control the action of the protein machinery in very short time frame i.e. within (sub-)seconds of a stimulus. We know that mutations in MeCP2 prevent normal responses to stimuli from occurring. Thus, patients who have changes to specific amino acids in MeCP2, carry the disease, resulting in improper function of the protein in the brain. To better understand how MeCP2 functions in the brain we will: 1) map the activity dependent changes in MeCP2 modifications; 2) determine how these modifications affect the structure of the protein; 3) mutate in MeCP2 at amino acid sites that are modified, and then study how these mutations affect the function of MeCP2 by characterizing the phenotypes of neurons and by performing biological assays. These studies will provide an understanding of how MeCP2 functions in the brain, which could lead to new therapies for Rett syndrome.

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Sun, Yi (Eve), University of California, Los Angeles, 2 years, \$100,000

Title: Functional Study of Post-translational Modifications of MeCP2 in Human and Mouse Neurons

Lay Summary:

Rett syndrome (RTT) is a severe neurodevelopmental disorder that is caused by mutations in Mecp2, a transcriptional repressor that binds to methylated DNA. It is unclear how Mecp2 mutations lead to dysfunction of the nervous system in RTT, and no effective treatments for RTT are available. Recent studies from our group and others have demonstrated that the MeCP2 protein is post-translationally modified via phosphorylation and glycosylation, in a rather dynamic and

complex manner to neurons, in response to neuronal activity. However, the functional relevance of these dynamic MeCP2 modifications is unknown. The overall objective of this proposal is to establish a human neuronal culture system where MeCP2 deficiency causes dramatic alterations in the neuronal network property from excitation to inhibition. Using this robust bioassay system, we will rescue the electrophysiological phenotype of cultured MeCP2-deficient human neurons by re-introducing wild-type and various mutants of MeCP2, which are deficient for site specific phosphorylation/glycosylation event, in order to identify which phosphorylation/glycosylation event(s) are critical for MeCP2 function as a regulator of the neuronal network property. Using this information we will further delineate the cellular/molecular mechanism by which MeCP2 regulates the neuronal network. Our study will not only advance our understanding of RTT etiology but also facilitate potential establishment of drug screening for RTT.

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Vincent, John, Center for Addiction and Mental Health (Canada) 2 Years, \$97,802

Title: Characterization of the MeCP2 Isoform, Mecp2_e1

Lay Summary:

The gene responsible for most cases of Rett syndrome, MECP2, is now known to exist as two similar forms, which encode proteins with different N-terminal ends. As the MeCP2 protein is believed to regulate the expression of specific genes that may be important for neuronal and synaptic development, it is crucial to know whether one MeCP2 isoform or the other, or possibly both, regulate these genes.

In addition, we believe that some mutations in Rett girls specifically affect only the new isoform of MeCP2, leaving the previous isoform fully functional. If we can demonstrate this clearly using molecular biological techniques, we can show that the new MeCP2 isoform is the isoform relevant to Rett syndrome. Understanding the differences between the two MeCP2 isoforms will have important implications, particularly for understanding the neurobiology of the disease, and for the development of future treatments for Rett.

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Post Doctoral Fellowship Awards

Gamliel, Amir

Gray, Steven

Guan, Qiaoning

Hong, Heekyung

Matagne, Valerie

Su, Junda

Voituron, Nicolas

Gamliel, Amir, University of California, San Diego, 2 years, \$100,000

Title: MeCP2 and Nuclear Architectural Modulation in the Critical Postnatal Period

Lay Summary:

Understanding how mutations of MeCP2 causes serious dysfunction of neurons and other cell types given its multiple functions that have already been identified, has presented many difficult investigational problems. It has also become quite clear in the past two years that there are complex interaction between chromosomes in the cell nucleus that are key for regulating the machinery of gene expression. MeCP2 exhibits several biochemical and functional features that suggest it is well positioned to play important roles in these processes. Thus, I hypothesize that a key role of MeCP2 and a critical aspect of the disease observed with MeCP2 mutations resides in the precise mediation of critical alterations in the early postnatal period in specific neuronal populations. Therefore, I will explore the roles of MeCP2 in modulating the complex interactions between chromosomes in the cell nucleus by defining the chromosomal interactions of the critical developmental states in mouse, employing a genetically modified mouse which will express a tagged version of MeCP2 which will allow highly efficient identification of interacting DNA sequences. I will also employ and assist in developing a novel method to identify the long range interactions between chromosomes in the cell nucleus that are dependent on MeCP2 and may be key for the proper regulation of specific gene programs. I propose that these studies will lead to a better understanding of the biology of MeCP2 function, and may identify effectors of MeCP2 which could potentially be targeted to ameliorate the effects of MeCP2 mutations.

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Gray, Steven, University of North Carolina, Chapel Hill, 2 years, \$100,000

Title: Development of a rAAV Vector Capable of Highly Efficient, Broad Transduction of Neurons

Lay Summary:

Rett syndrome, a form of autism, is a severe neurological disorder that affects about 1 in 10,000 girls. Rett syndrome is usually caused by a defect in a single gene, called MeCP2, that is required for proper brain function. There is no cure for Rett syndrome. However, a recent article in the journal Science from Adrian Bird's laboratory has demonstrated that

if you can activate a MeCP2 gene in mice that were born without it, the symptoms of Rett syndrome can be reversed. This study demonstrated that a therapy for Rett syndrome is possible if a correct MeCP2 gene can be delivered to cells in the brain. However, this landmark study also indicated that a low amount of MeCP2 activation has only a modest positive effect, and eventually the mice die prematurely. Other research has shown that too much MeCP2 can also kill mice. From these findings, two major obstacles remain before a MeCP2 gene replacement therapy for Rett syndrome can be attempted. 1) One must be very careful about how active the MeCP2 gene is once it is put into brain cells. 2) One needs to be able to deliver the MeCP2 gene to the greater majority of brain cells for any therapy to be effective. Our lab is currently working on ways to control how active a gene is once it is put into an animal, which will help with the first obstacle. As far as the second obstacle, there currently is not a way to deliver the MeCP2 gene to the brain to the extent that is needed. Our lab specializes in modifying a non-disease-causing virus called AAV to deliver therapeutic genes to specific tissues in order to treat genetic disorders. For example, a modified AAV virus that our lab made is currently in Phase I human clinical trials to treat Duchenne's Muscular Dystrophy. We propose to use our extensive expertise to make a modified AAV virus that can efficiently deliver the MeCP2 gene to the brain in mice, with the eventual goal to develop this into a therapy for Rett syndrome patients.

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Guan, Qiaoning, University of California, Berkeley, 2 years, \$100,000

Title: Nutritional Epigenetics: Impact of Folate on Genomic DNA Methylation, Birth Defects, and Neurological Disorder

Lay Summary:

DNA methylation is an epigenetic mechanism in eukaryotes that is a strong candidate for playing decisive roles in Rett syndrome (RTT) given that the disease results from defects in a protein that binds to the major epigenetic mark in the human genome. Changes in DNA methylation can arise through genetic mutation and/or in response to the external environment (such as nutrition in the case of mammals), with the latter case being reversible by a different environmental treatment. For example, the incidence of RTT, a neurological disorder affecting one in ~ 10,000 girls, and neural tube defects (NTDs), one of the most common forms of birth defects, is influenced by both genetic variations and dietary folate intake. A human polymorphism with the potential to be mechanistically linked to RTT and NTDs causes reduced activity of an enzyme in the folate metabolism pathway, and consequently a reduced level of the major methyl donor for all methylation reactions, including DNA methylation (which provides the binding sites for a protein that plays a key role in RTT). Preliminary studies in the Rine lab indicate the polymorphism is folate-remediable by high levels of dietary folate. We hypothesize that humans carrying this polymorphism in both copies of their gene harbor a high frequency of epigenetic marks across the genome due to the reduction in DNA methylation. Folate levels would play a key role in establishing and/or maintaining the epigenetic program in these individuals by suppressing or enhancing the DNA methylation deficiency, depending upon the level of folate. Genetic and genomic studies are proposed to test the hypothesis. The results of this project will lead to an evaluation of whether the changes in gene expression associated with this human polymorphism has the potential for an epigenetic basis, and to the causes of RTT, NTDs, and other related diseases. If so, the project should identify candidate genes with potential for causing the disease. Insights gained through this study will contribute to medical applications in clinic, such as the identification of preventive and therapeutic strategies and the development of epigenetic drugs.

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Hong, Heekyung, Northwestern University, 2 years, \$100,000

Title: Genetic Dissection of Rett Syndrome: A Screen for Modifiers of MeCP2 in the Mouse.

Lay Summary:

Since the identification of mutations in the MECP2 gene as the cause of Rett syndrome, significant progress has been made in unraveling the developmental and biological basis of the disease. Despite this breakthrough however, treatment for the disorder is essentially symptomatic and supportive, and will likely remain so without identification of additional aspects of MECP2 that might suggest therapeutic targets. In most if not all biological processes, proteins function by interacting with one another, which then initiate numerous cascades of cellular events. For MeCP2, only a few interacting proteins, or targets, have been identified thus far, and we are only just beginning to understand how MeCP2 works at the cellular level. Thus, investigating the MeCP2 cellular network and identifying its partners would be critical to the design of therapeutic interventions. In this project, we propose to use a genetic technique known as mutagenesis screening to identify MeCP2 partners (also called “modifiers”) that are not yet known. Specifically, we will use mutagenesis screening techniques to build on the current MeCP2 mouse model, generating novel mouse models that may help us to further understand the disease process while also providing insights into the development of therapeutic approaches for Rett syndrome.

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Matagne, Valerie, Oregon Health and Sciences University, 2 years, \$100,000

Title: Contribution of FXYDI, a MeCP2 Target Gene, to the Neuropathology of Rett Syndrome

Lay Summary:

Rett Syndrome (RTT) is a disorder of brain development that predominantly affects girls, and that becomes clinically evident 8-18 months after birth. Most RTT patients carry a defective gene called MECP2. This gene encodes a protein that normally “silences” other genes. Identification of these genes is important, as it may allow researchers to devise therapeutical strategies to treat the disease. We found that the brains of RTT patients, and that of mice lacking MeCP2, express more of a gene termed FXYDI, which encodes a protein that regulates cell excitability. The FXYDI gene is directly repressed by MeCP2; in the absence of MePC2, the FXYDI gene is activated resulting in loss of a cell membrane-bound enzyme activity essential for brain cells to recover after responding to other neurons. Brain neurons overexpressing FXYDI Show morphological abnormalities similar to those of RTT patients, further suggesting that an excess of FXYD1 contributes to the neuropathology of RTT. We propose to test this hypothesis using mice that are deficient in MeCP2, but are unable to produce FXYDI. We will determine if any of the behavioral, electrophysiological or morphological abnormalities displayed by animals lacking MeCP2 are ameliorated by genetically preventing the FXYD1 response to MeCP2 deficiency.

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Su, Junda, Georgia State University, 2 years, \$100,000

Title: Disruption of CO2 Chemosensitivity of Brainstem Neurons in *mecp2*-knockout Mice and Underlying Mechanisms

Lay Summary:

The Rett syndrome is known to have several breathing disorders that contribute to the developmental abnormalities of other parts of the body and sudden death. The breathing activity arises from the brainstem and is also regulated by other parts of the brain such as cerebral cortex. The central breathing activity is automatically regulated depending on several feedback controlling systems. One of them is the CO2 detection by brainstem neurons, providing the brainstem neurons with continuous information of CO2 levels in the blood stream, a central process known as central CO2 chemoreception. The breathing becomes stronger with high CO2 levels. Disorders in these CO2 chemosensitive neurons and their communication with other brainstem neurons will lead to abnormal respiratory rhythm. Several mechanisms can cause abnormal CO2 chemosensitivity. The sensor molecules in these neurons may be present in very low level, or they are not normally regulated by other molecules. Therefore, we have proposed experiments to address: 1) whether the CO2 chemosensitivity of brainstem neurons is disrupted in the *mecp2*-defected mice, and 2) whether the CO2 chemosensitive molecules that we have identified previously are mis-regulated in the mice, or their density in neuronal membrane becomes abnormal. Several pieces of information that we are going to generate in these proposed experiments will help to understand the disease processes of Rett syndrome and to assist physicians to design better therapeutical methods in the treatment of breathing disorders of the Rett patients.

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Voituron, Nicolas, CNRS, France, 2 years, \$100,000

Title: Pharmacological Treatments Improving Breathing and Life Span in *Mecp2* Deficient Mice: Possible Implications to Breathing Dysfunction in Rett Syndrome

Lay Summary:

Rett Syndrome is a severe neurological disorder accounting for up to 10% of severe mental retardation of genetic origin in women. After of a normal development until 6 -18 months, a number of clinical signs appear such as a regression of acquisitions, behavioral troubles, and life-threatening respiratory symptoms which could lead to frequent and

unexpected sudden deaths. Autopsies of Rett patients revealed alterations of bioaminergic metabolism (catecholamines and serotonin). Mutations in the MECP2 gene have been identified in most Rett patients and a mouse model has been recently created by genetic invalidation of this gene. The mutant Mecp2-deficient mice develop some symptoms during the postnatal period that are resembling those of Rett Syndrome (Guy et al., Nature 2001). In Mecp2-deficient mice, we have shown the postnatal development of bioaminergic alterations, followed by severe respiratory alterations which are lethal (Viemari et al., J. Neuroscience 2005). We hypothesize that the bioaminergic alterations of Mecp2-deficient mice are responsible for their respiratory disorders and that compensating for these bioaminergic deficits could alleviate their respiratory disorders. Therefore, we performed preliminary experiments in Mecp2-deficient mice adding an old drug of clinical use to the drinking water. This drug that is known as efficient to compensate noradrenaline functional deficits, significantly improves breathing and life span of Mecp2-deficient mice. The aim of our project is to check the efficiency of our pharmacological treatment on a large sample of Mecp2deficient mice, to understand the mechanisms of action of the drug with the view to further improve its efficiency and finally to test its safety. In conclusion, our project in mice may be relevant to identify some putative pharmacological treatments to alleviate respiratory deficits of Rett patients.

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Ad hoc Awards

Justice, Monica

Nadeau, Joe

Justice, Monica, Baylor College of Medicine, G.E.A.R. Grant, 2 years \$260,000

Title: Identifying genetic modifiers of Mecp2 in the mouse

Abstract: The primary goal of this proposal is to understand the function of Mecp2, mutations in which are the primary cause of Rett Syndrome in the human. We plan to use the mouse supermutagen, N-ethyl-N-nitrosourea (ENU), in a genetic modifier screen to isolate suppressors of the mutant phenotype. Phenotype assays will be used to better understand the pleiotropy of the Mecp2-/Y phenotype, as well as genetic modification of the Mecp2308/Y phenotype. Such information will help to identify and study the suppressors. New mutations will be mapped to identify the gene responsible for the mutation. Our data on new mutants will be shared immediately with the Rett Syndrome Research Foundation Scientific Community, so that labs that are already working on a protein or gene, or that have expertise in chromatin studies or transcriptional complexes, may carry out further work. We also hope that the information gleaned from our work will lead to non-toxic, effective treatment strategies for Rett Syndrome.

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Nadeau, Joe, Case Western Reserve University, Pilot Study, 1 year \$10,000

Title: Genetic modifiers of behavioral and neurological properties in a mouse model of RETT syndrome

Abstract: The clinical characteristics of genetic disease in humans and mouse models are readily suppressed by the action of variants at other non-disease genes. Although usually dismissed as 'genetic noise', these genetic modifiers often completely suppress disease without adverse side-effects. These genetic variants should provide important insights not only into the mechanisms of disease but also about new ways to treat and perhaps even prevent disease. Preliminary studies suggest that behavioural and neurological properties of mouse models of RETT syndrome are subject to the action of genetic modifiers. We are conducting pilot studies to confirm these observations. With these results, we will then undertake studies to identify several of these modifiers and evaluate their potential as treatment modalities.

The long-term goals are

- to identify modifier genes that might be therapeutic targets
- to characterize models to learn about pathogenesis

This combination of genetic and functional characterizations may lead to effective and safe ways to treat and perhaps prevent Rett Syndrome and other related conditions.

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