

Research Awardees: 2001

Research Awards

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Research Awards

Tony Charman, Ph.D.

Institute of Child Health, London, UK

"A Detailed Genotype-Phenotype Correlation Study in Rett Syndrome"

2-Year Award: \$99,437

Research Sponsor: Family Friends of Jessie Lebson

Final Report (November 2004)

A detailed genotype-phenotype correlation study in Rett syndrome

This study aimed to examine associations between the type and position of mutations in the MECP2 gene and clinical characteristics in a large cohort of patients from the UK with Rett syndrome, specifically attempting to recruit a large number of 'atypical' cases who may be most informative in terms of establishing such associations. We adopted a novel approach to dissecting genotype-phenotype relationships (focusing on just 3 main aspects, or 'dimensions', of the phenotype - the 'typicality' of the disorder, the severity and the age-of-onset) and to subdividing groups of mutations (missense, early truncating and late truncating groupings only). Effects of individual mutations on phenotype were also examined, as was the effect of X chromosome inactivation (XCI) ratio.

We recruited 191 families to the study. This included 140 cases with classic Rett syndrome and 51 cases with atypical

Rett syndrome. 135 cases had identified mutations in MECP2.

The main findings were that cases with early onset of regression and seizures, and those with clinical features that might indicate alternative causes of the disorder, were more likely to have no identified mutations. Individuals with late truncating mutations had a less typical presentation than cases with missense and early truncating mutations, presumably reflecting greater residual function of MECP2 protein. Individuals with early truncating mutations had a more severe outcome than cases with missense and late truncating mutations. It was confirmed that different common individual mutations were associated with more severe and less severe presentations. There was evidence suggesting that the X-inactivation ratio (XCI) moderated the effect of genotype on phenotype.

In summary, the approach we used allowed us to identify novel genotype-phenotype associations that may aid our understanding of the pathogenesis of Rett syndrome and also contribute to clinical knowledge.

We hope that information from this study will help both families and doctors of Rett syndrome patients around the world in their understanding of how the degree of severity of the disorder is related to the underlying genetic cause in individual patients.

Lay Summary

Research into Rett Syndrome, a disorder that profoundly affects brain development in females, moved into a new era with the recent discovery that up to 80% of cases are caused by mutations in a specific gene (MECP2). The extent to which the presence or absence of a mutation in this gene, or the precise nature of the mutation, affect the natural history of the clinical disorder is not yet clear. We aim to address this question by carrying out mutation detection screens in 3 large groups of Rett Syndrome females in whom the clinical presentation has been detailed with greater precision than has been the case in previous studies. We hope that the findings will contribute to our understanding of the pathology that underlies Rett Syndrome (holding out the possibility for gene therapeutic strategies in the future). However they will also inform clinical decision making by improving diagnostic accuracy and advice on prognosis and likely outcome as well as choosing the most efficacious strategies for each individual patient.

Abstract

MECP2. This protein binds to methylated CpG dinucleotides in DNA and probably functions in the control of transcription competence of numerous chromosomal loci. The effect of mutations in MECP2 on the function of the protein are beginning to emerge, but the extent to which the presence or absence of an MECP2 mutation, or the precise nature of the mutations, affect the natural history of the clinical disorder is not yet clear. Previous studies that have attempted to identify genotype-phenotype correlations have been limited by low sample sizes and poor characterization of the physical, developmental and behavioral phenotype. Few consistent associations have thus far emerged. We aim to address this question by carrying out mutation detection screen in MECP2 and measuring X-inactivation ratios, in 3 large cohorts of Rett Syndrome patients. Using a combination of detailed systematic clinical assessment information and parental questionnaire information (including information from a newly-developed questionnaire with proven ability to discriminate between typical Rett Syndrome behaviors and those found in other individuals with severe and profound developmental disability) we will be able to characterize the phenotype more accurately than has been previously achieved. In addition the size of the cohorts available (N=100 with clinical assessment information, N=300 with questionnaire information) and the number of classic (N=220) and atypical/variant (N=80) cases will allow for a stronger test of genotype-phenotype associations than in previous studies. The identification of phenotypic differences between patients with different mutations will aid in the development of models of the neuropathological processes that occur in the disorder (holding out the possibility for gene therapeutic strategies in the future). It will also aid clinicians now to improve diagnostic and prognostic accuracy, and decide on the best therapeutic strategy for each individual patient.

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Jeffrey C. Hansen, Ph.D.

University of Texas Health Science Center at San Antonio

"Solution-state Characterization of Native and Mutant MeCP2 Interactions with Chromatin and Sin3p"

2-Year Award: \$99,000

Research Sponsor: Gardner Family Foundation

Final Report (November 2004)

Mutations in the protein, MeCP2, lead to RTT. The studies funded by this RSRF research grant have discovered a new functional property of MeCP2 that may be related to RTT. By studying how purified MeCP2 interacts in the test tube with chromosomal fragments, we showed that MeCP2 possesses the ability to profoundly alter the local and global structure of chromosomes. Using two different mutated forms of MeCP2 found in RTT, we further demonstrated that both mutants possessed an altered ability to influence chromosome structure relative to normal MeCP2. These studies have opened up an entirely new line of investigation into the molecular basis of MeCP2 function. Further studies will help determine the extent to which genome architecture is one of the molecular factors that contributes to MeCP2 function in normal and RTT disease states.

Lay Summary

The cells in our body contain a protein, called MeCP2, that when mutated leads to the development of Rett Syndrome in affected individuals. Now that this molecular link to Rett Syndrome has been discovered, it is important to determine what molecular functions of MeCP2 are altered in the disease state. With today's technology, it is possible to produce and isolate normal MeCP2 and Rett Syndrome-associated MeCP2 mutants in bacteria. These studies will exploit this technology to determine how normal and mutant purified MeCP2s interact with the chromatin fiber, and with the regulatory protein Sin3p. Chromatin is the genetic material of higher organisms, and consists of an assemblage of DNA and histone proteins. Although MeCP2 is thought to be a chromatin-associated protein, no direct studies of MeCP2-chromatin interactions have been performed. A second important function of MeCP2 is to interact with the regulatory protein Sin3p. We will examine the binding of normal and mutant MeCP2s to Sin3p. This information will allow us to better understand the role of MeCP2-Sin3p, and HDAC. The latter is a chromatin modifying enzyme that is associated with repression of gene expression. In summary, the proposed studies will fill gaps in our knowledge of two key functions of MeCP2 in healthy individuals, and how these functions are affected by Rett Syndrome-associated MeCP2 mutations. Together, the results of the proposed research will significantly improve our understanding of the molecular basis of Rett Syndrome.

Abstract

MeCP2 is a methyl CpG binding protein implicated in Rett Syndrome (RTT). It consists of two key domains, termed the methyl CpG binding domain (MBD) and the transcriptional repression domain (TRD). The first objective of this proposal is to determine the interactions of normal and RTT-associated MeCP2 mutants with the chromatin fiber. The second objective is to quantitatively analyze native and mutant MeCP2-Sin3p interactions. Both objectives test whether Rett Syndrome associated MeCP2 mutants exhibit altered interactions with functionally important macromolecules that bind to native MeCP2. The information obtained from the proposed studies will test the hypotheses that Rett Syndrome mutations in the MeCP2 MBD will disrupt interactions with chromatin, while MeCP2 mutations in the TRD will abolish interactions with Sin3p. These studies will provide insight into the molecular effects of Rett Syndrome-associated MeCP2 mutations, which in turn will allow for a better understanding of the molecular pathogenesis of Rett Syndrome.

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Aharon Razin, Ph.D.

The Hebrew University of Jerusalem-Hadassah Medical School, Israel

"MeCP2 and Rett Syndrome"

1-Year Award: \$50,000

Research Sponsor: Rett Syndrome Association of Illinois / Adam (Buddha) Lavey

Final Report (November 2003)

In 80% of Rett syndrome patients a mutation in the MeCP2 gene had been identified, leaving around 20% of Rett syndrome cases undiagnosed genetically. It is possible that these cases are inflicted with a mutation outside the gene, or that they carry a deletion of the entire gene. However, it cannot be ruled out that in some Rett Syndrome cases the MeCP2 gene is intact, a normal protein is produced, but one or more of its target genes are impaired. Our first attempt to examine these possibilities was to search for possible mutations in the promoter of the gene. To this end, we have analyzed samples from over 100 Rett syndrome patients from many countries around the world. We failed so far to identify any mutations in promoter sequences in these samples. However, we have identified in two Israeli Rett syndrome patients a bona fide mutation within another non coding sequence. This mutation (a deletion of one adenine residue) was found at a position which is believed to be essential for processing the gene product. We will study this mutation and try to verify its relevance to Rett Syndrome. We have already detected no MeCP2 protein in cells from this patient. If we will be convinced that this mutation is relevant to the Rett Syndrome, we will screen for this mutation in all the samples of the Rett syndrome patients that we analyzed for promoter mutations.

In parallel, we have generated a lymphoblast cell line from another Rett patient with no detectable mutation in the MeCP2 coding region. These cells have an intact MeCP2 protein and this may suggest that genes other than MeCP2 are involved in the disease. Such genes may be targets of MeCP2 activity or, alternatively, genes that code proteins that interact with MeCP2. A mutation in one of these genes could theoretically result in the Rett syndrome phenotype.

To identify MeCP2 target genes we have generated two immortalized lymphoblast cell lines originating from a Rett syndrome patient that carries a mutation in the coding sequence of the MeCP2 gene. One cell line expresses the MeCP2 protein (M10) and the other (M12), is MeCP2 deficient. We performed microarray analysis to compare the expression profile of 30,000 genes in these MeCP2 deficient and normal cell lines. This analysis revealed 90 genes with elevated expression levels in M12 compared to M10. Twelve genes with the largest change in expression level were chosen for further analysis.

The next step will be to try to restore the normal expression levels of these genes by introducing the normal MeCP2 gene into the MeCP2 deficient M12 cell line. If expression can be restored, it will prove that the observed changes are reversible and the Rett phenotype can be rescued.

Lay Summary

Rett Syndrome is in most cases caused by a mutation in the MECP2 gene that is located on chromosome X. This gene encodes an important protein that participates in a major regulatory mechanism that is responsible for silencing of a group of genes. We have studied the production of this protein in early mouse development and found that the protein is not present in embryos before implantation to the mother's womb. We propose to study the onset of the production of this protein and discover when in development of the embryo the protein is required. The second part of the proposed

research will be devoted to examine the possibility of a rescue of the disease phenotype by introducing a normal MECP2 gene into MeCP2-deficient mice.

Abstract

MECP2, the affected gene in Rett Syndrome patients, will be studied in mouse early embryo development to determine the onset of its synthesis and assembly of the MeCP2-Sin3A-HDAC multiprotein corepressor complex. Introduction of a MeCP2 expression vector by transfection into MeCP2 deficient cells in culture will provide a tool to study the possibility of complementation of the MeCP2 mutation. The repressor activity will be studied on a suitable target gene that will be selected from genes discovered in comparative microarray assays. The results of the transfection experiments will be used to set the stage for more sophisticated experiments in vivo. In these experiments MeCP2 transgenic lines will be established on a WT and a MeCP2 ^{-/-} mouse background. These mice will be used to examine whether the mutated phenotype can be rescued and will allow us to answer the question whether Rett Syndrome results from MeCP2 loss of activity, accumulation of a MeCP2 aberrant protein or both.

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Bryan M. Turner, Ph.D.

University of Birmingham Medical School

"Histone Deacetylase Complexes Assembled on Wild-Type and Mutant MeCP2: Functional Properties and Genomic Distribution"

1-Year Award: \$49,940

Final Report (November, 2003):

The DNA-binding protein MeCP2 has a role in controlling the expression of many different genes. It is believed that the mutations in MeCP2 that cause Rett Syndrome, exert their effects by altering patterns of gene expression and thereby disrupting normal pathways of brain development. Mutations in MeCP2 are commonly found in either the region of the protein that binds to DNA, or in a region (called the TRD) that is believed to associate with other specific proteins to assemble a multi-protein gene regulatory complex. In our project we used molecular genetic techniques to make MeCP2 proteins that had mutations the same as those found in Rett patients. We were able to demonstrate that MeCP2 with mutations in the protein-binding domain can assemble a gene regulatory complex with the same constituents as that assembled by normal MeCP2, but does so less efficiently. We used microscopical analyses to show that these mutant complexes are targeted to the same DNA regions as normal MeCP2 complexes. In contrast, MeCP2 mutated in the DNA-binding domain, was not properly targeted. Our results suggest that mutations in the protein-binding region of MeCP2 may lead to the symptoms of Rett syndrome by rather subtle, quantitative effects.

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Dean F. Wong, M.D., Ph.D.

Johns Hopkins University School of Medicine

"Dysfunction of High-Affinity $\alpha 4/\beta 2$ Nicotinic Acetylcholine Neuronal Receptors in Rett Syndrome"

1-Year Award: \$50,000

Research Sponsor: Family and Friends of Chelsea Coenraads

Lay Summary

Several lines of evidence suggest that abnormalities of the high-affinity $\alpha 4/\beta 2$ neuronal nicotinic acetylcholine receptors (nAChRs) in the brain play an important role in Rett Syndrome (RTT). The objective of this study is to identify the binding of nAChRs in specific brain regions of ten participants with RTT and ten age and sex-matched normal control subjects. The binding of nAChRs will be determined by the utilization of single photon emission computed tomography (SPECT) scans after the administration of the novel nAChR radioligand (S)-5[123I] iodo-3-(2-azetidylmethoxy)pyridine (51A), which localizes high-affinity $\alpha 4/\beta 2$ nAChRs in the brain. Determination of binding on nAChRs in the brain of people with RTT may provide the basis for the development of safe and effective supportive treatments including nicotine patches and sprays. Additionally this technique may provide the basis to determine the effectiveness of other interventions for RTT.

Abstract

The central methodology of this study will be the examination of the distribution of high-affinity $\alpha 4/\beta 2$ neuronal nicotinic acetylcholine receptors (nAChRs) in the brains, the ascertainment of plasma, salivary, and urinary levels of nicotine and cotinine, and the abnormal involuntary movements, and the psychological functioning of people with RTT and control groups. Two groups of neuroleptic-na.ve subjects, ten patients with RTT who were never exposed to nicotine and ten age and sex-matched normal control subjects without RTT who never smoked cigarettes and never had other nicotine exposure, will be assessed utilizing a protocol consisting of (A) single photon emission computed tomography (SPECT) utilizing the nAChR radioligand (S)-5[123I] iodo-3-(2-azetidylmethoxy)pyridine (51A), which localizes high-affinity $\alpha 4/\beta 2$ nAChRs (Musachio, et al., 1999, 2001), (B) determination of plasma, salivary, and urinary levels of nicotine and cotinine, and (C) structured assessment of psychiatric and movement disorders. Because the parameters measured by the instruments may not be normally distributed, nonparametric tests of significance including the Mann-Whitney test (Conover, 1999) will be used to analyze the results. Data will be analyzed utilizing statistical procedures appropriate for the small sample sizes. If the data are from normally distributed populations, then paired t tests will be used to analyze the results.

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Post-Doctoral Fellowship Awards

Carolyn Ellaway, Ph.D.

The Children's Hospital at Westmead, Sydney, Australia

"Assessment of Central Autonomic Nervous System Function in Rett Syndrome"

Mentor: John Christodoulou, Ph.D.

2-Year Award: \$88,000

Lay Progress Report (August 2002)

Start Date: October 2001

Duration: 2 years

The future of Rett syndrome research will be directed towards understanding the underlying pathogenesis of the disorder and the development of specific treatments, ideally prior to the onset of symptoms or early in the evolution of the disorder. It is therefore vitally important to establish objective methods for assessment of the clinical status of patients with Rett syndrome, prior to commencement of clinical trials and to be able to accurately monitor changes over time. The central autonomic nervous system, which controls the activity of the nerves controlling the function of the heart and lungs has been shown to function abnormally in Rett syndrome subjects. The abnormalities include irregular breathing patterns, extreme agitation with large pupils and flushed face, vacant spells, abnormal brain wave activity, seizures and poor circulation. A relatively new instrument for evaluating the central autonomic nervous system, the Neuroscope™ can measure and monitor the activity of the central autonomic nervous system. Information derived from the assessment of the autonomic nervous system will provide a valuable tool for monitoring Rett syndrome patients during clinical treatment studies.

The main aim of my study is to establish the methods for assessing and monitoring central autonomic nervous system function in Rett syndrome patients.

The first six months of my Rett Syndrome Postdoctoral Research Fellowship involved the preparation and submission of my research application to the Ethics and Scientific Advisory Committees of the Children's Hospital at Westmead. In December 2001 the two committees approved the project. The Neuroscope™ was then ordered and delivered in February 2002. Dr Peter Julu visited Sydney at the time and I commenced the intensive training program required to utilise the equipment. The training program continued for an additional week with Dr Peter Julu at the Central Middlesex Hospital, London. During my visit to London I also attended a two-day meeting held at The Institute of Child Health, Great Ormond Street Hospital, London. The meeting was entitled 'Providing for People with Rett syndrome' and chaired by the Dr Alison Kerr. The trip to London was funded by The Children's Hospital at Westmead.

I have now recruited ten females with Rett syndrome to have central autonomic nervous system monitoring, ranging in age from 4 years to 18 years. The procedure is quite time consuming as the actual monitoring takes at least one hour and the set up procedure can take up to 30 minutes or longer. There have been a few minor technical problems which have delayed progress, but which I hope have now been corrected. The data analysis is complex and can take up to four hours per patient. It appears that the families of my Rett syndrome patients are very keen to be involved in the research project.

I will be taking maternity leave from the end of August 2002, so the research project will be suspended until I return to work next year some time.

Lay Summary

The future of Rett Syndrome research will be directed towards understanding the underlying pathogenesis of the disorder and the development of specific treatments, ideally prior to the onset of symptoms or early in the evolution of the disorder. It is therefore vitally important to establish objective methods for assessment of the clinical status of patients with Rett Syndrome, prior to commencement of clinical trials and to be able to accurately monitor changes over time. The central autonomic nervous system, which controls the activity of the nerves controlling the function of the heart and lungs, has been shown to function abnormally in Rett Syndrome subjects. The abnormalities include irregular

breathing patterns, extreme agitation with large pupils and flushed face, vacant spells, abnormal brainwave activity, seizures and poor circulation. A relatively new instrument for evaluating the central autonomic nervous system, the Neuroscope TM can measure and monitor the activity of the central autonomic nervous system. Information derived from the assessment of the autonomic nervous system will provide a valuable tool for monitoring Rett Syndrome patients during clinical treatment studies. In addition an individual treatment plan can potentially be developed with the ultimate aim of improving patient care and quality of life. It has been suggested that abnormal function of the autonomic nervous system could be the cause of abnormal heart beat rhythms and sudden death. Since at least 25% of deaths in Rett Syndrome are sudden and unexpected, it would be reasonable to establish objective measures of disordered breathing in Rett Syndrome. Since the discovery of mutations in the MECP2 gene as a cause of Rett Syndrome, numerous studies have attempted to correlate the type of mutation (gene error) in the MECP2 gene (genotype) with clinical characteristics (phenotype). The Neuroscope TM, a reliable clinical method of characterizing autonomic nervous system function, may assist these studies in Rett Syndrome.

Abstract

The future of Rett Syndrome (RTT) research will be directed towards understanding the underlying pathogenesis of the disorder and the development of targeted therapeutic interventions, ideally in the pre-symptomatic stage or early in the evolution of the disorder. It is therefore vitally important to establish objective methods for assessment of the clinical status of patients with RTT, prior to commencement of clinical trials and to be able to accurately monitor clinical changes over time. A relatively new neurophysiological approach to the evaluation of brainstem function has been developed. The Neuroscope TM can measure and monitor the activity of the central autonomic nervous system, in particular brainstem function, cardiac vagal tone and respiratory patterns. Information derived from the assessment of the autonomic nervous system will be valuable for monitoring RTT patients during clinical intervention trials. In addition the characterization of autonomic dysfunction will enable a patient outcome oriented treatment plan to be developed, with the ultimate aim of improving patient care and their quality of life. Since the discovery of mutations in the MECP2 gene as a cause of RTT, numerous studies have attempted to identify phenotype-genotype correlations. It has become apparent that the clinical method of characterizing the phenotype in RTT is difficult and unreliable. The study of phenotype-genotype correlations in RTT may be assisted by the Neuroscope TM, a reliable clinical method of characterizing the phenotype.

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Joel M. Harp, Ph.D.

The University of Tennessee - Oak Ridge National Laboratory

"X-ray Crystallographic Studies of MeCP2 on a Chromatin Substrate"

Mentor: Gerard J. Bunick

2-Year Award: \$100,000

Final Report (November 2003)

The MeCP2 protein recognizes a chemical signal on DNA and binds to it. Portions of the MeCP2 protein then recruit other proteins to change the structure of chromatin and silence gene expression. Chromatin is the form in which our DNA is packaged and consists of repeating units called nucleosomes. Questions of how MeCP2 binds to DNA on nucleosomes and then modifies the nucleosomes are very difficult to answer. While graduate student at the University of Tennessee and the Oak Ridge National Laboratory, I worked on the structure of the nucleosome core particle using the techniques of macromolecular crystallography. I received a fellowship from RSRF while still at Tennessee with hopes of solving the structural basis of how MeCP2 recognizes methylated DNA on the nucleosome and how it then affects the structure of chromatin. While this work is still ongoing, the RSRF Fellowship has allowed me to move twice.

Once to the University of Virginia where I worked with Drs. David Allis, Sepideh Khorasanizadeh, and Fraydoon Rastinejad. There I had an opportunity to work on proteins involved in the histone code as well as work on my primary interest in MeCP2. The second move has been to Vanderbilt University where I am now Director of the Macromolecular Crystallography Facility. This position provides opportunities to continue the studies begun during the term of the RSRF Fellowship and, hopefully, to begin new collaborations with Rett Syndrome researchers.

Lay Summary

Final Report (November, 2003):

The MeCP2 protein recognizes a chemical signal on DNA and binds to it. Portions of the MeCP2 protein then recruit other proteins to change the structure of chromatin and silence gene expression. Chromatin is the form in which our DNA is packaged and consists of repeating units called nucleosomes. Questions of how MeCP2 binds to DNA on nucleosomes and then modifies the nucleosomes are very difficult to answer. While graduate student at the University of Tennessee and the Oak Ridge National Laboratory, I worked on the structure of the nucleosome core particle using the techniques of macromolecular crystallography. I received a fellowship from RSRF while still at Tennessee with hopes of solving the structural basis of how MeCP2 recognizes methylated DNA on the nucleosome and how it then affects the structure of chromatin. While this work is still ongoing, the RSRF Fellowship has allowed me to move twice. Once to the University of Virginia where I worked with Drs. David Allis, Sepideh Khorasanizadeh, and Fraydoon Rastinejad. There I had an opportunity to work on proteins involved in the histone code as well as work on my primary interest in MeCP2. The second move has been to Vanderbilt University where I am now Director of the Macromolecular Crystallography Facility. This position provides opportunities to continue the studies begun during the term of the RSRF Fellowship and, hopefully, to begin new collaborations with Rett Syndrome researchers.

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Noriyuki Kishi, M.D., Ph.D.

Children's Hospital, Harvard Medical School

"Molecular Controls Over Differentiation of Cortical Projection Neurons from Neural Precursors: Normal and MECP2 -/-"

Mentor: Jeffrey Macklis, M.D., D.HST

2-Year Award: \$110,000

Research Sponsor: Festival of Food and Wine

Final Report (November 2004)

Rett syndrome is a disease of brain development that causes mental retardation and autistic behavior in girls (one in every 10,000 to 15,000 girls), as well as in a small group of boys. Recent research has revealed that a defect in a gene called the methyl-CpG-binding protein 2 (MECP2), which encodes a protein that suppresses expression of other genes (transcriptional repressor), causes Rett syndrome. MECP2 mutant mice display several symptoms that are similar to those of Rett patients. Although it is evident that the symptoms of these mutant mice are attributable to the lack of the MECP2 gene in the brain, we know little about how the MECP2 gene works in the brain, and why mutation of the MECP2 gene causes predominantly neurological symptoms, despite the fact that the MECP2 gene is expressed throughout the entire body.

My work has added invaluable insights in the role of MECP2 in nerve cell maturation in the brain. I investigated how

mutation in MECP2 gene may cause the symptoms of Rett syndrome using MECP2 genetically modified mice which lack MECP2 gene. First, I investigated which cells express MECP2 in the brain, using mouse brains and cells grown in dishes in the laboratory. I found that MECP2 is expressed in nerve cells (neurons), not in glia. Furthermore, my studies show that MECP2 is expressed in more mature neurons rather than in immature moving neuronal precursors, indicating that MECP2 is involved in the maturation and maintenance of neurons.

Although my data strongly indicate that MECP2 is involved in the maturation and maintenance of neurons, MECP2 has also been detected at low levels during embryonic development, and experiments using frog eggs showed that MECP2 controls production of neurons. However, the idea that MECP2 controls cell fate decisions does not mesh with the symptoms of Rett syndrome, because no report shows that there is decreased number of neurons in the patients. To investigate whether MECP2 is involved in events of initial neural development in mammals, I developed a mouse neural progenitor/stem cell culture system that can investigate these events. My results, using cells from MECP2 mutant mice, indicate that MECP2 mutation does not affect initial neural development events in mice, suggesting that MECP2 plays a different role in mammals (including humans) than it does in frogs.

I next investigated the thickness of the high level brain area called the cerebral cortex (or neocortex) in MECP2 mutant mice. I measured the thickness of the neocortex, which is composed of six layers and is responsible for high level cognitive functions. There is significant reduction of thickness in the neocortex of MECP2 mutant brains. Why do MECP2 mutant brains appear to have a thinner cortex? Two possibilities are 1) loss of neurons; and 2) reduced size and/or complexity of neurons. When I measured the cell density in each layer of each genotype, the cell densities of layers II/III, IV, V, and VI in MECP2 mutant mice are significantly higher than those in wild-type mice, suggesting that the reduced thickness of cortex in MECP2 mutant mice is due to reduced size of neurons, rather than to loss of neurons. In agreement with this hypothesis, I performed direct cellular analysis showing that pyramidal neurons in layer II/III in MECP2 mutant mice are smaller and their dendrites are less complex than those in wild-type mice.

In addition, I am finalizing neuronal transplantation experiments to further investigate potential abnormalities of both cell size and amount/quality of branches they send (dendritic complexity) by neurons of MECP2-null mice. By transplanting MECP2 mutant neuroblasts genetically labeled with a special genetic green color (GFP fluorescence) into wild-type brains, I can address whether the cell size and dendritic complexity of MECP2 mutant neurons is comparable or abnormal compared with wild-type neurons, and, if abnormal, determine whether this is due to MECP2 in neurons themselves, or dependent on the environment. Although some of the results are not yet final, transplanted MECP2 mutant layer II/III pyramidal neurons are smaller and less complex even in the wild-type environment than those of transplanted wild-type neurons, indicating that wild-type environment does not rescue the phenotype of transplanted MECP2 mutant neurons. Thus, MECP2 in neurons themselves is the central reason for their abnormalities.

Taken together, my data indicate 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. I am now beginning experiments to investigate these issues directly at the molecular level.

Lay Summary

Rett syndrome is a disease of brain development that causes mental retardation and autistic behavior in girls. Recent experiments have revealed that a defect in a gene called the methyl-CpG-binding protein 2 (MECP2) causes Rett syndrome. There is evidence from human autopsies that neurons in the cerebral cortex that make long-distance connections between the two hemispheres of the brain express MECP2 at high levels during brain development. In addition, recent work using MECP2 mutant mice has shown that mutation of the MeCP2 protein in neurons is sufficient to cause neuronal dysfunction in various parts of the brain including the cerebral cortex; these animals manifest symptoms that are similar to those of Rett syndrome. However, the specific neuronal abnormalities have not been elucidated. The cerebral cortex is the most complex structure of the brain and is responsible for high functions unique to humans; even a subtle abnormality of connections of this complex structure can cause diseases affecting behavior and cognition, such as Rett syndrome. The development of cortical connection neurons is critical to cortical function; the role of MECP2 mutation in this process may be central to understanding the neurobiological basis of Rett syndrome. The molecular mechanisms underlying development of cortical connection neurons, and the specific role of MECP2 in these processes, remain largely unclear. Therefore, I propose to investigate directly the molecular

mechanisms of cortical connection neuron development and effects of MECP2 mutation on this process. I propose to: 1) investigate the effects of MECP2 on development of connections between the two hemispheres of the cerebral cortex using MECP2 mutant mice and analysis of MeCP2 production in select populations of cortical connection neurons during normal development; and 2) investigate developmental events that follow MeCP2 production and function, using precursor cells and purified cortical connection neurons in cell culture experiments. This knowledge will not only add to our understanding of the neurobiological basis of Rett syndrome and the mechanisms underlying connection neuron development in the cerebral cortex, but also may contribute to the potential for molecular and therapies for neurological disorders involving neuronal compromise and loss, including Rett syndrome.

Abstract

Rett syndrome is a neurodevelopmental disorder and one of the causes of mental retardation and autistic behavior in girls. Recent work has revealed that a defect in the methyl-CpG-binding protein 2 (MECP2) gene causes Rett syndrome. There is evidence from human autopsy material that cortical projection neurons express MECP2 at high levels during development. In addition to the gene discovery, recent mouse genetic work has demonstrated that, in MECP2 mutant mice, a MeCP2 deficiency in neurons is sufficient to cause neuronal dysfunction in neocortex, hippocampus, and cerebellum, with manifesting symptoms that mimic those of Rett syndrome. However, the specific neuronal abnormalities have not been elucidated. The neocortex is the most complex structure of the brain and gives us unique talent, but even a subtle disruption of cortical connectivity can cause behaviorally and cognitively significant diseases, such as Rett syndrome. The development of cortical projection neurons is critical to cortical connectivity; the role of MECP2 mutation in this process may be central to understanding the neurobiological basis of Rett syndrome. The molecular mechanisms underlying development of cortical projection neurons, and the specific role of MECP2 in these processes, remain largely unclear. Therefore, I propose to investigate directly the molecular mechanisms of callosal projection neuron development and effects of MECP2 targeted gene deletion ("knockouts") on this process. I propose to: 1) investigate the effects of MECP2 on development of interhemispheric connections using MECP2 knockouts and analysis of MeCP2 expression in select populations of projection neurons during normal development; and 2) investigate developmental events downstream of MeCP2 expression, using cultured neural precursors and purified cortical projection neurons. This knowledge will not only add to our understanding of the neurobiological basis of Rett syndrome and the mechanisms underlying projection neuron development in neocortex, but also may contribute to the potential for molecular and cellular therapies for neurological disorders involving neuronal compromise and loss, including Rett syndrome.

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Tomoki Yokochi, Ph.D.

National Cancer Institute (NIH)

Recipient of the Alan P. Wolffe Memorial Fellowship

"Chromatin Activities in Neurons: Structural and Functional Analysis of the MeCP2 and HDAC Complexes"

Mentor: Keith D. Robertson, Ph.D.

2-Year Award: \$100,000

Final Report (November 2003)

In this research, I have attempted to identify MeCP2 and its interacting partners in the brain. Previous reports suggested that MeCP2 interacts with a group of proteins called histone deacetylases (HDACs) in the tissue culture (tissue cells that were isolated and kept in medium on a culture dish). In the native mammalian brain tissue, I found that there are two protein complexes containing MeCP2. These complexes also contain several proteins other than MeCP2. One of

the common components is Sin3A, which is regarded as the structural framework for these protein complexes. Neither HDAC1 nor HDAC2 was identified in these complexes, which is inconsistent with previous reports. Thus, it is assumed that MeCP2 plays a unique role in the brain tissue with a distinct group of proteins. To further understand the structural difference of MeCP2s in tissue culture and in brain tissue, I have identified the MeCP2 complex by an alternative approach, immunoprecipitation. Just recently, it was reported that there are two forms of MeCP2 in cultured nerve cells. One receives modification at particular position with a phosphate group, whereas the other was not modified. In contrast, I found that MeCP2 in native brain tissue appeared to have a different modification status. These results suggest that the structural modification of MeCP2 proteins may impart it with a brain-specific function in native brain tissue.

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