

RS Paper Flags 2006 and 2007 Composite

Group 1

1. Moretti P, Levenson JM, Battaglia F, Atkinson R, Teague R, Antalffy B, Armstrong D, Arancio O, Sweatt JD, Zoghbi HY. Learning and Memory and Synaptic Plasticity Are Impaired in a Mouse Model of Rett Syndrome. *J Neuroscience* 2006;26:319-327.

In a mouse model of Rett syndrome, these authors demonstrate abnormalities in the ability to search for targets of interest. Rather than go to a specific target area, the mice wandered about in a fashion reminiscent of the motor apraxia in girls with Rett syndrome indicating a problem in learning to address targets in their environment. Mutant mice were also unable to learn to avoid unpleasant or fear-provoking experiences and did not engage in social behaviors as well as normal mice. One brain region associated with such learning, the hippocampus, had abnormalities in structure and function of the synapses or nerve to nerve connections. These findings extend the known abnormalities in brain from motor and sensory areas to involve those involved in learning and memory.

2. Paterson DS, Thompson EG, Belliveau RA, Antalffy BA, Trachtenburg FL, Armstrong DD, Kinney HC. Serotonin Transporter Abnormality in the Dorsal Motor Nucleus of the Vagus in Rett Syndrome: Potential Implications for Clinical Autonomic Dysfunction. *J Neuropath Exp Neurol* 2005;64:1018-1027.

Considering the clinical problems associated with autonomic function in Rett syndrome, these authors examined the possible role of serotonin. In brainstem from individuals with Rett syndrome, serotonin transporter binding did not demonstrate the expected change with increasing age compared to controls. This failure to modulate vagus nerve response to serotonin may play a significant role in the known abnormalities in function of the vagus nerve including regulation of gastrointestinal and cardiac responses. The authors suggest that more detailed examination of these findings in mouse models could be very informative.

3. Adachi M, Keefer EW, Jones FS. A segment of the *Mecp2* promoter is sufficient to drive expression in neurons. *Human Molecular Genetics* 2005;14:3709-3722.

These authors examined the regulatory region that promotes expression of *Mecp2* in mice in an effort to understand why the gene product is expressed mainly in brain tissues. They identified the specific region involved and demonstrated that this 'regulator' is active in brain, but not in non-neural tissue.

This finding could have important implications for future attempts at gene therapy.

4. Abuhatzira L, Makedonski K, Galil YP, Gak E, Ben Zeev B, Razin A, Shemer R. Splicing mutation associated with Rett syndrome and an experimental approach to genetic diagnosis. *Human Genetics* 2005;118:91-98.

Since the identification of mutations in exon 1 and large deletions of *MECP2*, >95% of individuals fulfilling consensus criteria for Rett syndrome will have an coding abnormality in the gene associated with Rett syndrome. These authors have identified yet another mechanism that would escape diagnostic detection by standard laboratory methods. They identified a mutation in the control region for *MECP2* that blocks the normal coupling of coding information for this gene and results in an absence of the MeCP2 protein. It is suggested, that such defects should be evaluated in those individuals meeting Rett syndrome diagnostic criteria but lacking a mutation by standard diagnostic testing.

Group 2

1. Chang Q, Khare G, Dani V, Nelson S, Jaenisch R. The Disease Progression of *Mecp2* Mutant Mice Is Affected by the Level of BDNF Expression. *Neuron* 2006;49:341-348.

The authors demonstrate that male mice lacking the gene associated with Rett syndrome (*MECP2*) have reduced levels of a growth factor that is essential for survival of nerve cells in the brain. This factor is called BDNF or brain-derived neurotrophic factor. They then show that mice lacking this factor demonstrated some of the same features as mice lacking the gene associated with Rett syndrome. By creating a double mutant mouse, that is male mice lacking both the gene for Rett syndrome and the nerve cell growth factor, onset of disease was earlier and survival was less than in mice lacking only the gene associated with Rett syndrome. Finally, increasing the expression of this important nerve cell growth factor by inserting this gene into male mice lacking the gene associated with Rett syndrome produced significant improvements. Although this added amount of growth factor did not increase brain weight, it did delay the onset of Rett-like behaviors and the spontaneous activity of brain cells in these animals. These studies were conducted in animals lacking the gene associated with Rett syndrome. It will be important to conduct similar studies in animals in which the gene is present but contains mutations known to be associated with Rett syndrome. Further, it will be important to conduct similar studies in female mice that have a normal gene in addition to the mutant gene and thereby more closely resemble the human disorder in terms of genetic make-up.

2. Ormazabal A, Artuch R, Vilaseca MA, Aracil A, Pineda M. Cerebrospinal Fluid Concentrations of Folate, Biogenic Amines and Pterins in Rett Syndrome: Treatment with Folinic Acid. *Neuropediatrics* 2005;36:380-385.

The authors detected reduced levels of the active form of folate in cerebrospinal fluid in 8 of 16 females with Rett syndrome. Of this group, eight had mutations in the gene associated with Rett syndrome and three of these eight had values below the control range, although, in one the difference was minimal. Greater reductions appeared to be associated with seizures, especially those resistant to treatment. Improved behavior and seizure control was noted following dietary supplementation with folinic acid as had been shown previously by others in Europe and the US. Reduced folate levels, however, were not seen in a large cohort of US females, more than 80% of whom had *MECP2* mutations.

3. Young JI, Hong EP, Castle JC, Crespo-Barreto J, Bowman AB, Rose MF, Kang D, Richman R, Johnson JM, Berget S, Zoghbi HY. Regulation of RNA splicing by the methylation-dependent transcriptional repressor methyl-CpG binding protein 2. *PNAS* 2005;102:17551-17558.

The authors have identified an important alternative mechanism by which the *MECP2* gene protein product, methyl-CpG-binding protein 2, exerts its effect and provide an alternate explanation of how abnormalities in this gene lead to Rett syndrome. In essence, this protein interacts with the cell machinery that determines how other genes are expressed. In simple terms, the nuclear coding material, DNA, is transcribed to the corresponding RNA which in turn directs the formation of the proper protein. It has been known that methyl-CpG-binding protein 2 interacts with and regulates DNA expression. These new results indicate that it also interacts with and modulates RNA function. The authors suggest that by having this dual function, methyl-CpG-binding protein 2 has an even more complex and coordinated role in the oversight or control of how genes including those related to Rett syndrome are expressed.

4. Viemari J-C, Roux J-C, Tryba AK, Saywell V, Burnet H, Peña F, Zanella S, Bévençut M, Barthelmy-Requin M, Herzing LBK, Moncla A, Mancini J, Ramirez J-M, Villard L, Hilaire G. Mecp2 Deficiency Disrupts Norepinephrine and Respiratory Systems in Mice. *Neurobiology of Disease* 2005;25:11521-11530.

The authors demonstrate abnormalities in respiratory control pathways in the brainstems (medulla) of male mice lacking the gene associated with Rett syndrome. In addition, levels of the key neurotransmitters, norepinephrine and serotonin, are reduced in these regions. Addition of norepinephrine to preparations from medulla stabilized the respiratory control network. These results increase our understanding of the functional abnormalities associated with irregular breathing in individuals with Rett syndrome. As with other studies in mice lacking the gene associated with Rett syndrome, it will be important to

conduct similar studies in animals in which the gene is present but contains mutations known to be associated with Rett syndrome. Further, it will be important to conduct similar studies in female mice that have a normal gene in addition to the mutant gene and thereby more closely resemble the human disorder in terms of genetic make-up.

Group 3

1. Galvão TC, Thomas JO. Structure-specific binding of MeCP2 to four-way junction DNA through its methyl-CpG-binding domain. *Nucleic Acid Research* 2005;33:6603-6609.

The authors show that MeCP2 binds to unmethylated DNA and may represent an additional role for this protein in the regulation of gene activity. The authors suggest that the specific mutation R133C that lies within the methyl-binding domain does not alter binding to methylated DNA. However, Kudo et al. (*Brain and Development* 2001;23:165-173) had previously shown in a functional assay that R133C has little effect on MeCP2 binding to both methylated and unmethylated DNA. Other mutations (R106W, F155C, and T158M) do alter binding to methylated DNA in that functional assay and could be tried in the system utilized in this paper.

2. Sherren N, Pappas BA. Selective acetylcholine and dopamine lesions in neonatal rats produce distinct patterns of cortical dendritic atrophy in adulthood. *Neuroscience* 2005;136:445-456.

In a study with relevance to Rett syndrome, the authors demonstrate that reducing the levels of neurotransmitter input (either acetylcholine or dopamine or both) to cortical neurons resulted in reductions in cortical neuron size and dendritic branching, findings that are seen in human brain from individuals with Rett syndrome. These findings support the important role of these neurotransmitters on proper development of cortical neurons.

3. Pelka GJ, Watson CM, Radziewicz T, Hayward M, Lahooti H, Christodoulou J, Tam PPL. *Mecp2* deficiency is associated with learning and cognitive deficits and altered gene activity in the hippocampal region of mice. *Brain* (advanced access) 2006;1-12.

The authors generated null mutant mice for *Mecp2* and examined the capability of these mice using measures of motor coordination and response to painful stimulus. The mutant mice had diminished motor skills and diminished fear response, felt to represent abnormalities of hippocampus and amygdala. Similar studies were reported by Moretti et al. in 2005 (*J Neuroscience* 2006;26:319-327) in a knock-in mouse model, in which case a known Rett syndrome mutation

was introduced into *Mecp2*. Pelka and co-authors also demonstrated significant down-regulation of two genes (*Gap43* and *Kif1b*) in hippocampus, both associated with normal neuronal function.

4. Asaka Y, Jugloff DGM, Zhang L, Eubanks JH, Fitzsimonds RM. Hippocampal synaptic plasticity is impaired in the *Mecp2*-null mouse model of Rett syndrome. *Neurobiology of Disease* 2006;21:217-227.

The authors demonstrate neurophysiological abnormalities in hippocampus of mutant mice lacking *Mecp2*. These findings are consistent with abnormal neuronal plasticity and support the published findings on human brain suggesting a disturbance of maturation and maintenance of cortical synapses. Similar findings were reported by Moretti et al. in 2005 (*J Neuroscience* 2006;26:319-327) in a knock-in mouse model, in which case a known Rett syndrome mutation was introduced into *Mecp2*.

Group 4

1. Robertson L, Hall SE, Jacoby P, Ellaway C, de Klerk N, Leonard H. The Association Between Behavior and Genotype in Rett Syndrome Using the Australian Rett Syndrome Database. *Am J Med Genet* 2006;141B:177-183.

The authors utilized a previously published Behavior Questionnaire (Mount et al., *J Child Psychol Psychiatr* 2002;43:1099-1110) to compare specific behaviors associated with the eight common *MECP2* mutations in order to examine possible phenotype-genotype correlations. The authors first demonstrated that results from their cohort were similar to those reported from the UK across the respective behaviors. Although variability within each common mutation was noted, certain observations were of interest. Abnormalities of mood tended to be associated with R294X and stereotypies of hand and face were associated with R225X and R270X. Mutations generally associated with milder overall involvement, R133C and R306C tended to be associated with heightened anxiety and fear. The knock-in mouse model has among its behaviors a reduction in fear or avoidance behavior. In the latter two RS mutations, the milder phenotype may reflect a modification of this characteristic. In this study, for each mutation analyzed, the number of individuals ranged from 10-16. The authors stressed the importance of studying a larger cohort within each mutation to strengthen the validity of their results.

2. Saywell V, Viola A, Confort-Gouny S, LeFur Y, Villard L, Cozzone PJ. Brain magnetic resonance study of *Mecp2* deletion effects on anatomy and metabolism. *Biochem Biophys Res Commun* 2006;340:776-783.

The authors utilized both imaging and spectroscopic capabilities of magnetic resonance techniques to study brain structure and metabolism in the knock-out

mouse model of Rett syndrome. Not surprisingly, brain size was reduced as noted in both human and animal gross anatomical studies. To avoid variation introduced by X-inactivation in females, male mice (age 5-8 weeks) were studied. The significant changes detected in brain chemistry involve reductions in important neuron components (N-acetylaspartic acid or NAA and glutamine/glutamic acid). Given the known neuronal involvement, the reduction of NAA is expected. In humans, glutamate levels are increased in young girls, but appear to be decreased in later years. The rapid pace of disease progression in these male mice may compare more to the findings in older age groups in humans. The authors also noted reduced levels of ATP, the high energy supply for our cells. This finding correlates with previous studies in humans indicating reduced cerebral blood flow. The authors comment on 'cumulative neuronal damage' in Rett syndrome. However, no convincing evidence of this has been noted in Rett syndrome. The use of male mice does result in progressive disease as all neurons are affected rather than the theoretically 'equal' number of normal neurons in females. In addition, this model is not truly representative of Rett syndrome as the gene is completely missing rather than having a specific mutation as in the knock-in mouse model. It would be of interest to conduct comparative studies in the knock-in model. Behaviors in the two models are somewhat different. For example, the knock-out mice clasp their hind-limbs whereas the knock-in mice clasp their fore-limbs, more in keeping with Rett syndrome.

3. Lin C, Franco B, Rosner MR. CDKL5/Stk9 kinase inactivation is associated with neuronal developmental disorders. *Hum Molec Genet* 2005;14:3775-3786.

The X-linked cyclin-dependent kinase-like 5 (CDKL5/STK9) has been associated with children with a severe neurodevelopmental disorder featuring intractable seizures in infancy including infantile spasms along with motor and communication deficits and cognitive impairment. In some instances, these children demonstrate features of the early-onset seizure variant form of Rett syndrome. Thus, these studies are quite relevant to Rett syndrome. This kinase is presumed to transfer a phosphate group to other proteins resulting in alteration of their function. These authors demonstrate that CDKL5 is found in all tissues where it localizes to the nucleus, just as MeCP2 does. The authors did not reveal whether CDKL5 is restricted to neurons or occurs in other brain cells as well. Although MeCP2 is bound to CDKL5, it does not appear to be one of the proteins that receive a phosphate group from CDKL5. It remains possible that MeCP2 and CDKL5 interact in other ways that explain the association of CDKL5 with the early-onset seizure variant.

4. Buoni S, Zannolli R, Colamaria V, Macucci F, di Bartolo RM, Corbini L, Orsi A, Zappella M, Hayek J. Myoclonic encephalopathy in the CDKL5 gene mutation. *Clin Neurophys* 2006;117:223-227.

The authors conducted a detailed clinical and EEG study of children with mutations in the *CDKL5* gene. These children may fulfill criteria for the early-onset seizure variant form of Rett syndrome. Thus, it is important to be able to differentiate the two both clinically and by gene testing. Children with the *CDKL5* mutation typically present within the first two months of life with intractable epilepsy including infantile spasms in some instances. These authors report the new observation that these early seizures evolve into myoclonic epilepsy. This study provides important information that should allow the clinical differentiation from variant Rett syndrome.

5. Perini G, Tupler R. Altered gene silencing and human disease. *Clin Genet* 2006;69:1-7.

This review covers the key aspects of gene regulation through silencing and its relationship to human illness. The review highlights Rett syndrome, among others, as an example of a human disorder in which gene silencing is important. More importantly, this review represents a broadening recognition of Rett syndrome among the scientific community.

Group 5

1. Coon H. Current Perspectives on the Genetic Analysis of Autism. *Am J Med Genet* 2006;142C:24-32.

The author describes in detail the strategies utilized to link autism to specific chromosome regions and specific potential neural mechanisms that may underlie the autistic phenotype.

2. Moy SS, Nadler JJ, Magnuson TR, Crawley JN. Mouse Models of Autism Spectrum Disorders. *Am J Med Genet* 2006;142C:40-51

The authors describe existing mouse models of disorders that have autistic features associated with them. The two main models are those for Fragile X and Rett syndromes. Indeed, in Fragile X syndrome, more than 50% of individuals may meet criteria for autism and the mice may reflect this. In contrast, despite the early appearance of features suggestive of autism in Rett syndrome, these children do not meet criteria for autism and socialization and communication dysfunction in this disorder actually improve, such that the Rett model may not be a particularly good one for studying autism. Interestingly, the pathobiology of both disorders does include altered dendritic spines, suggesting some commonality.

3. Filion GJP, Zhenilo S, Salozhin S, Yamada D, Prokhortchouk E, Defossez P-A. Mol Cell Biol 2006;26:169-181.

The authors describe another group of proteins that have the capacity to alter gene transcription and are highly expressed in brain. However, whether they share common DNA binding patterns with MeCP2 remains to be determined.

4. Berton O, McClung CA, DiLeone RJ, Krishnan V, Renthal W, Russo SJ, Graham D, Tsankova NM, Bolanos CA, Rios M, Monteggia LM, Self D, Nestler EJ. Essential Role of BDNF in the Mesolimbic Dopamine Pathway in Social Defeat Stress. Science 2006;311:864-868.

These authors show that BDNF is integrally related to the development of social isolation following repeated exposure to aggressive behavior. Elimination of BDNF in specific brain neurons involved in a specific dopamine pathway block this antisocialization response. Interestingly, antidepressant medications were also able to block this interference with socialization.

5. Villard L, Roux J-C. Un déficit en noradrénaline à l'origine des troubles respiratoires dans un modèle animal du syndrome de Rett. Medicine/Sciences. 2006;22:81-83.

This paper is in French. The basic message is that deficiency of norepinephrine underlies the breathing irregularities in the mouse model deficient in Mecp2.

6. Miller FA, Begbie M, Giacomini M, Ahern C, Harvey EA. Redefining Disease? the nosologic implications of molecular genetic knowledge. Perspect in Biol Med 2006;49:99-114.

This paper is a provocative and informative discussion of how molecular diagnoses might change the definition or categorization of specific disorders, using Rett syndrome as an example. The authors conclude that while a molecular definition might be appropriate, the fact that mutations in many genes are associated with multiple phenotypes, and that Rett syndrome is a prime example of this, indicates that such molecular categorization is not advisable at this time.

7. Phillippe C, Villard L, De Roux N, Raynaud M, Bonnefond JP, Pasquier L, Lesca G, Mancini J, Jonveaux P, Moncla A, Chelly J, Bienvenu T. Spectrum and distribution of *MECP2* mutations in 424 Rett syndrome patients: a molecular update. Europ J Med Genet 2006;49:9-18.

The authors review the current status of *MECP2* mutation testing throughout France, reporting on 121 different mutations from 424 individuals, more than

(90% found in exon 4). Large alterations accounted for nearly 6% of the total whereas mutations in exon 1 were rare. Comparisons with RettBase (not contained in the paper) revealed overall similarity in the distribution of the 8 most common mutations, although R270X and R255X were most common in this study whereas T158M and R168X were most common in larger sample comprising RettBase.

8. Elefant C, Wigram T. Learning ability in children with Rett syndrome. *Brain Dev* 2005;27:S97-S101.

Utilizing a music therapy approach in 7 girls with Rett syndrome, the authors demonstrated the ability to acquire new information and to develop a communication facility. In this study, comparison with other motivational techniques was not conducted.

9. Bissonnette JM, Knopp SJ. Separate Respiratory Phenotypes in Methyl-CpG-Binding Protein 2 (Mecp2) Deficient Mice. *Pediatr Res* 2006;59:513-518.

The authors examined the respiratory phenotypes in two null mutant mice strains for *Mecp2*, one lacking Mecp2 expression, the other lacking Mecp2 expression only in neurons. In both strains, hyperventilation was induced by placing them in an environment with low oxygen levels. Interestingly, only mice lacking Mecp2 expression developed depressed respiratory activity after hyperventilation. This depression in breathing was eliminated by including carbon dioxide in the low oxygen environment, suggesting that the respiratory depression was related to blowing off carbon dioxide during the hyperventilation. While the authors attribute the difference to Mecp2 deficiency in lung tissues in mice lacking any Mecp2 expression, further work needs to be performed to explain why the animals with Mecp2 deficiency restricted to neurons did not develop the respiratory depression. The authors refer to an elevation in BDNF expression in the relevant brainstem nuclei as a potential explanation. More recent data suggest that BDNF expression is actually reduced in this mouse model.

10. Acampa M, Guideri F. Cardiac disease and Rett syndrome. *Arch Dis Child* 2006;91:440-443.

The authors provide a complete review (and an extensive bibliography) of the cardiac issues associated with Rett syndrome.

Group 6

1. Segawa M, Nomura Y. Rett syndrome. *Curr Opin Neurol* 2005;18:97-104.

This annual review by two experienced physician-scientists highlights the 2004 publications related to Rett syndrome across a range of topics from patient-oriented to laboratory based research.

2. Tsai, S-J. Lithium and antidepressant: Potential agents for the treatment of Rett syndrome. *Med Hypotheses* 2006;67:626-629.

Based on the possible role of BDNF (brain-derived neurotrophic factor) in the pathobiology of Rett syndrome, the author proposes that medications such as lithium and antidepressants that are known to increase brain BDNF protein levels could be effective therapeutic agents. The author further proposes investigating these agents in available animal models to test this hypothesis.

3. Pelka G, Watson CM, Radziewicz T, Hayward M, Lahooti H, Christodoulou J, Tam PPL. *Mecp2* deficiency is associated with learning and cognitive deficits and altered gene activity in the hippocampal region of mice. *Brain* 2006;129:887-898.

Working in the mouse model of Rett lacking *Mecp2* (the so-called null or knock-out mutant), the authors demonstrate abnormal learning in related to amygdala and hippocampal-based functions. Similar findings had been reported in the mouse model containing a mutant *Mecp2* (so-called knock-in mutant). In addition, the authors identified a number of genes that were altered in these mutant mice. As the mice became sicker, the extent of alteration of these other genes increased. One gene in particular had reduced activity in the null mice, *GAP43*. This gene directs the production of the Gap43, growth-associated protein, that is highly important during the developmental of neuronal processes known as axons and in nerve terminals, especially in highly active regions such as the hippocampus. Mice deficient in Gap43 exhibit learning difficulties similar to those deficient in *Mecp2*.

4. Lugtenberg D, de Brouwer APM, Kleefstra T, Oudakker AR, Frints SGM, Schrandt-Stumpel CTRM, Fryns JP, Jensen LR, Chelly J, Moraine C, Turner G, Veltman JA, Hamel BCJ, de Vries BBA, van Bokhoven H, Yntema HG. Chromosomal copy number changes in patients with non-syndromic X linked mental retardation detected by array CGH. *J Med Genet* 2006;43:362-370.

The authors confirm the association of duplication of *MECP2*, that is, an extra copy of this gene, in association with X-linked mental retardation in males. Based on the available reports, this duplication of *MECP2* may be one of the most common causes of mental retardation in males. These findings are consistent with abnormalities noted in male mice when this gene is over-expressed, another example of too much of an essential component being detrimental, not beneficial.

5. Archer HL, Evans JC, Millar DS, Thompson PW, Kerr AM, Leonard H, Christodoulou J, Ravine D, Lazarou L, Grove L, Verity C, Whatley SD, Pilz DT, Sampson JR, Clarke AJ. *NTNG1* Mutation Are a Rare Cause of Rett Syndrome. *Am J Med Genet* 2006;140A:691-694.

The authors examined 52 individuals with the early onset seizure variant of Rett syndrome for mutations in the netrin G1 gene (*NTNG1*), but failed to find any mutations as had been reported previously. This gene produces a protein product involved in neuronal process development.

6. Gemelli T, Berton O, Nelson ED, Perrotti LI, Jaenisch R, Monteggia LM. Postnatal Loss of Methyl-CpG Binding Protein 2 in the Forebrain is Sufficient to Mediate Behavioral Aspects of Rett Syndrome in Mice. *Biol Psychiatry* 2006;59:468-476.

The authors describe development of a version of the null or knockout model for Rett syndrome (no *MECP2*) that is restricted to the forebrain (cerebral hemispheres) after birth. These animals have features typical for RS. These results regarding postnatal expression of the mutation are not surprising as this gene has its principal role after birth. The described results are also somewhat contradictory. The authors describe impaired motor coordination and normal locomotor activity. It is unclear to this reviewer how this is possible. What is clear is that significant differences exist between the knockout mouse model and the knocked-in mutant from the Zoghbi lab that contains a mutation derived from a child with RS.

7. Nelson ED, Kavalai ET, Monteggia LM. MeCP2-Dependent Transcriptional Repression Regulates Excitatory Neurotransmission. *Curr Biol*. 2006;16:710-716.

The authors describe studies in cell cultures of hippocampal neurons from normal and MeCP2 knockout mice. They demonstrate that neurons from the mutant mice have diminished spontaneous excitatory (or stimulatory) nerve cell to nerve cell activity compared to those from normal mice. The same effect could be produced in neurons from normal mice by blocking the enzyme cascade that MeCP2 uses to regulate the activity of other genes. The results are similar to those described others in intact hippocampal slice preparations.

8. Bienvenu T, Chelly C. Molecular genetics of Rett syndrome: when DNA methylation goes unrecognized. *Nature Reviews (Genetics)* 2006;7:415-426.

This excellent review paper describes the important contributions of prior research related to the molecular biology of Rett syndrome and includes 94 references for the inquiring reader.

9. Kriaucionis S, Paterson A, Curtis J, Guy J, MacLeod N, Bird A. Gene Expression Analysis Exposes Mitochondrial Abnormalities in a Mouse Model of Rett Syndrome. *Mol Cell Biol* 2006;26:5033-5042.

The authors examine the effect of the knockout mutation in the mouse model of Rett syndrome on the expression of other genes. They demonstrate among others a significant increase in expression of a mitochondrial protein and using biochemical methods demonstrate a corresponding increase in energy generation through these mitochondria. The results are quite provocative. It is unclear how this increase in mitochondrial activity would relate to onset of the RS phenotype. However, one could imagine how this increase could lead to mitochondrial fatigue over time. The studies were performed in male mice lacking any Mecp2 activity and have a relatively short lifespan. It would be of interest to examine mitochondrial in aging female mice that would have cells with normal Mecp2 and cells lacking Mecp2. The authors refer to microscopic mitochondrial changes in females with RS. However, these findings have not been found uniformly. Also, previous reports of changes in the chemicals, lactate and pyruvate, in spinal fluid appear to relate to the presence or absence of significant breathing abnormalities as they are not found in those with RS lacking significant breathing problems. Nonetheless, the present results are intriguing. It will be interesting to determine if similar findings are demonstrated in the Mecp2 knock-in mice.

Group 7

1. Ventura P, Galluzzi R, Bacca SM, Giorda R, Massagli A. A novel familial *MECP2* mutation in a young boy: Clinical and molecular findings. *Neurology* 2006;67:867-868.

The authors report a pathogenic *MECP2* mutation in a boy with seizures, cognitive impairment, and autistic-like behaviors. His mother also demonstrated psychomotor abnormalities in the form of poor cognitive performance, abnormal gait, and other motor difficulties.

2. Bienvenu T, Philippe C, De Roux N, Raynaud M, Bonnefond JP, Pasquier L, Lesca G, Mancini J, Jonveaux P, Moncla A, Feingold J, Chelly J, Villard L. The Incidence of Rett Syndrome in France. *Pediatr Neurol* 2006;34:372-275.

The authors noted a prevalence of Rett syndrome in France of about 1 in 18,000 or similar to other prevalence reports worldwide and an incidence of between 1 in 15,000-23,000, also in keeping with published reports.

3. Tejada M-I, Peñagarikano O, Rodriguez-Revenge L, Martinez-Bouzas C, Garcia B, Bádenas C, Guitart M, Minguez M, García-Alegría E, Sanz-Parra A,

Beristain E, Milá M. Screening for *MECP2* mutations in Spanish patients with an unexplained mental retardation. *Clin Genet* 2006;70:140-144.

The authors studied 294 individuals with cognitive impairment and noted only 1 individual with a *MECP2* mutation, this being a girl with features of atypical Rett syndrome. The authors suggest a rigorous clinical assessment before proceeding to *MECP2* testing.

4. Nectoux J, Heron D, Tallot M, Chelly, Bienvenu T. Maternal origin of a novel C-terminal truncation mutation in *CDKL5* causing a severe atypical form of Rett syndrome. *Clin Genet* 2006;70:29-33.

Mutations in the gene, *CDKL5*, have been noted in individuals with infantile spasms or intractable epilepsy of infancy in association with features of atypical Rett syndrome. The authors indicate the importance of *CDKL5* testing in females meeting variant Rett syndrome criteria who have an infantile epileptic encephalopathy and lack a mutation in *MECP2*.

5. Donzel-Javouhey A, Thauvin-Robinet C, Cusin V, Madinier N, Manceau E, Dipanda D, Dulieu V, Mugneret F, Huet F, Teyssier J-R, Faivre L. A New Cohort of *MECP2* Mutation Screening in Unexplained Mental Retardation. *Am J Med Genet* 2006;140A:1603-1607.

The authors examined *MECP2* status in 146 individuals with cognitive impairment, 68 of whom had other neurological impairments. None of the 100 males was noted to have a *MECP2* mutation whereas two of 46 females had previously described pathogenic *MECP2* mutations. The authors note the importance of maintaining a high index of suspicion in females with some features consistent with Rett syndrome whereas such studies in males with otherwise uncomplicated cognitive impairment is not typically rewarding.

6. Moog U, Van Roozendaal K, Smeets E, Tserpelis D, Devriendt K, Van Buggenhout G, Frijns J-P, Schrandt-Stumpel C. *MECP2* mutations are an infrequent cause of mental retardation associated with neurological problems in male patients. *Brain & Development* 2006;28:305-310.

The authors studied 72 males with cognitive impairment and other neurological abnormalities and found a pathogenic mutation in a single male who had cognitive impairment, seizure, and autistic-like features. As with previous authors in this set of papers, the authors note the relative infrequency of *MECP2* mutations in otherwise uncomplicated cognitive impairment and suggest caution in such mutation testing.

7. Motil KJ, Schultz RJ, Abrams S, Ellis KJ, Glaze DG. Fractional Calcium Absorption in Girls with Rett Syndrome. *J Pediatr Gastro Nutr* 2006;42:419-426.

The authors studied ten girls with Rett syndrome with an equal number of matched controls found a greater absorption of calcium in the girls with Rett syndrome. Despite this finding, bone mineralization in these girls is markedly reduced. The authors indicate that the effectiveness of providing calcium supplementation remains an open question.

8. Jugloff DGM, Logan R, Eubanks JH. Breeding and maintenance of an *Mecp2*-deficient mouse model of Rett syndrome. *J Neurosc Methods* 2006;154:89-95.

The authors describe their experience with maintaining a colony of null *Mecp2* mice including reduced survival to weaning from heterozygous mothers, smaller litter size, and reduced frequency of null-mice. The authors have developed strategies to improve efficiency of colony productivity.

Group 8

1. Archer HL, Whatley SD, Evans JC, Ravine D, Huppke P, Kerr A, Bunyan D, Kerr B, Sweeney E, Davies SJ, Reardon W, Horn J, MacDermott KD, Smith RA, Magee A, Donaldson A, Crow Y, Hermon G, Miedzybrodzka Z, Cooper DN, Lazarou L, Butler R, Sampson J, Pilz DT, Laccone F, Clarke AJ. Gross rearrangements of the *MECP2* gene are found in both classical and atypical Rett syndrome patients. *J Med Genet* 2006;43:451-456.

In this letter, the authors reinforce the known importance of searching for large deletions in *MECP2* when standard sequencing fails to identify a mutation in females meeting clinical criteria for Rett syndrome, both the typical and variant forms.

2. Jordan C, Francke U. *Ube3a* expression is not altered in *Mecp2* mutant mice. *Hum Mol Genet* 2006;15:2210-2215.

Individuals with Rett and Angelman syndromes may have an overlap of clinical features. In contrast to previous reports, the authors noted no differences in expression of the *Ube3a* gene, mutations of which have been associated with Angelman syndrome, in brains from mice with *Mecp2* null mutations. These results call into question the postulated role of this Angelman syndrome-related gene in the pathogenesis of Rett syndrome.

3. Metcalf BM, Mullaney BC, Johnston MV, Blue ME. Temporal Shift In Methyl-CpG Binding Protein 2 Expression In A Mouse Model of Rett Syndrome. *Neurosci* 2006;139:1449-1460.

The authors examined the expression pattern of *Mecp2* in cortical neurons from mice with null mutations of *Mecp2*. Over time, the percentage of cells in mutant mice expressing wild type protein increased from 50-70%, suggesting a change in X-chromosome inactivation status with increasing age. The authors suggest that this change in *Mecp2* expression could explain, at least in part, the pattern of stabilization and improved interaction in older girls with Rett syndrome.

4. Peddada S, Yasui DH, LaSalle JM. Inhibitors of differentiation (ID1, ID2, ID3, and ID4) genes are neuronal targets of MeCP2 that are elevated in Rett syndrome. *Hum Mol Genet* 2006;15:2003-2014.

In differentiating human neuroblastoma cells, the authors identified the members of this known family of DNA-binding inhibitors as likely MecP2 targets, showing binding of MeCP2 near the promoter regions of these four genes and an increase in expression of each gene in brains from *Mecp2* null mice mutants. In these brains as well as in human brain from individuals with Rett syndrome, levels of each protein were elevated. The authors implicate these four genes in the pathobiology of Rett syndrome.

5. Kriaucionis S, Paterson A, Curtis J, Guy J, MacLeod N, Bird A. Gene Expression Analysis Exposes Mitochondrial Abnormalities in a Mouse Model of Rett Syndrome. *Mol Cell Biol* 2006;26:5033-5042.

The authors demonstrate an upregulation of mitochondrial activity in null mutant mice for *Mecp2* in association with overexpression of a nuclear gene encoding a cytochrome c reductase. The authors propose a link between these results and mitochondrial dysfunction in Rett syndrome. However, evidence suggesting mitochondrial dysfunction in humans with Rett syndrome is inconclusive and in some instances related to patterns of periodic or irregular breathing, making it unclear whether this is cause and effect or a chicken and egg scenario. These studies bear wider examination in other model systems.

6. Ragione FD, Tiunova A, Vacca M, Strazzullo M, González E, Armstrong J, Valero R, Campanile C, Pineda M, Hulten M, Monros E, D'Esposito M, Prokhortchouk E. The X-linked methyl binding protein *Kaiso* highly expressed in brain but is not mutated in Rett syndrome patients. *Gene* 2006;373:83-89.

In order to account for individuals with Rett syndrome lacking a *MECP2* mutation, these authors examined another X-linked gene, *Kaiso*, whose protein product is a methyl-CpG binding protein. In a study of *MECP2* mutation negative individuals

with Rett syndrome, no pathogenic mutations were identified in the *Kaiso* gene making it an unlikely candidate as a cause for otherwise unexplained Rett syndrome.

7. Levenson JM, Sweatt JD. Epigenetic mechanisms: a common theme in vertebrate and invertebrate memory formation. *Cell Mol Life Sci* 2006;63:1009-1016.

The authors provide a stimulating review of the role of epigenetics, that is, the field of heritable changes apart from those associated with the primary coding sequences in DNA, and its role in long-term memory. In other words, epigenetic mechanisms regulate gene expression through modification of DNA and chromatin structure. These in turn have an impact on stabilizing recurring processes such as those involved in preserving patterns of behavior or learned response, much as in the generally stable mechanisms underlying cellular differentiation and maturation.

8. Callinan PA, Feinberg. The emerging science of epigenomics. *Hum Mol Genet* 2006;15:R95-R101.

The authors provide a timely review of the emerging field of epigenomics. This field will provide a link between epigenetic features of inheritance and the primary alterations of DNA resulting from study of the genome, whether human or other organisms. The convergence of informatics and microarray technologies, among others, will likely advance this field rapidly.

9. Chelly J, Khelifaoui M, Francis F, Chérif B, Bienvenu T. Genetics and pathophysiology of mental retardation. *Eur J Hum Genet* 2006;14:701-713. This is an excellent review on cognitive impairment in children emphasizing the successful identification of many of its genetic causes and the increase in our understanding of the underlying pathobiology.

Group 9

1. Quenard A, Yilmaz S, Fontaine H, Bienvenu T, Moncla A, des Portes V, Rivier F, Mathieu M, Raux G, Jonveaux P, Philippe C. Deleterious mutations in exon 1 of *MECP2* in Rett syndrome. *Europ J Med Genet* 2006;49:313-322.

The authors confirm mutations in exon 1 of the gene associated with Rett syndrome including four individuals with large rearrangements covering exon 1. Mutations in exon 1 were found in 1% of their clinical population confirming previous reports that exon 1 mutations represent a rare cause of Rett syndrome.

2. Evans JC, Archer HL, Whatley SD, Clarke A. Germline mosaicism for a *MECP2* mutation in a man with two Rett daughters. *Clin Genet* 2006;70:336-338.

The authors identify two half-sisters with the same *MECP2* mutation. The father did not carry this mutation in blood or cells from the inside of his cheek. However, he did carry this mutation in approximately 5% of his semen DNA. The authors discuss the importance of offering prenatal testing where the father has previously had a daughter with Rett syndrome.

3. Ventura P, Galluzzi R, Bacca SM, Giorda R, Massagli A. A novel familial *MECP2* mutation in a young boy: Clinical and molecular findings. *Neurology* 2006;67:867-868.

The authors describe a male with moderate cognitive impairment, epilepsy, and features of autism together with a novel missense mutation at C964T (P322S). His mother, who has significant neurological findings, carries the same mutation. She was noted to be clumsy with gait difficulties, tremor, and abnormal speech. Her cognitive level was in the low borderline range. X chromosome inactivation studies revealed balanced X inactivation, that is, equal presence of both X chromosomes. However, the position and type of mutation might be predicted to produce a relatively mild phenotype.

4. Baptista PM, Mercadante MT, Macedo EC, Schwartzmann JS. Cognitive performance in Rett syndrome girls: a pilot study using eyetracking technology. *J Intell Disab Res* 2006; 50:662-666.

Using eyegaze technology, the authors show that 6 of 7 girls with Rett syndrome were able to follow verbal commands and respond with a correct answer more often than not. The authors conclude that this technology could be useful in assessing cognitive performance in this population of girls who lack proper hand skills or verbal capabilities to respond to standard cognitive measures.

5. Wilfong AA, Schultz RJ. Vagus nerve stimulation for treatment of epilepsy in Rett syndrome. *Dev Med Child Neurol* 2006;48:683-686.

The authors report their experience with vagal nerve stimulation in the management of medically refractory epilepsy among 7 females with Rett syndrome. Six of these had at least a 50% reduction in seizure frequency after 12 months with increased alertness, but no change in communication skills.

6. Williamson SI, Christodoulou J. Rett syndrome: new clinical and molecular insights. *Europ J Hum Genet* 2006;14:896-903.

The authors provide in this review a comprehensive strategy for the molecular diagnosis of individuals with clinical features of Rett syndrome including the approach to further molecular studies when mutations in *MECP2* are not identified.

7. Stauder JEA, Smeets EEJ, van Mil SGM, Curfs LGM. The development of visual- and auditory processing in Rett syndrome: An ERP study. *Brain Dev* 2006;28:487-494.

The authors use sophisticated EEG recordings to evaluate responses to auditory or visual cues in 17 females with Rett syndrome. Responses among the group with Rett syndrome were delayed and showed less organized processing compared to the control group. Further, individuals with Rett syndrome do not follow the typical developmental pattern of maturation of these responses. Stach et al. had demonstrated similar auditory processing changes in the early 1990s. Taken together, these results support the clinical notion that responses to visual or spoken cues are delayed in Rett syndrome requiring the teacher or observer to exercise patience in noting the anticipated response. This has important implications for all intervention programs in Rett syndrome.

8. Tsai S-J. Lithium and antidepressants: Potential agents for the treatment of Rett syndrome. *Med Hypoth* 2006;67:626-629.

The author builds on findings in *MECP2*-null animals of altered BDNF levels and suggests that a trial in these animals of known modulators of BDNF levels such as lithium or antidepressants could provide useful information. Unfortunately, similar BDNF changes were not noted in the *MECP2* knock-in animal model. Further study is needed prior to launching such a trial.

9. Wang H, Chan S, Ogier M, Hellard D, Wang Q, Smith C, Katz DM. Dysregulation of Brain-Derived Neurotrophic Factor Expression and Neurosecretory Function in *Mecp2* Null Mice. *J Neurosci* 2006;26:10911-10915.

The authors report abnormal cell secretion of BDNF from neurons and catecholamines from adrenal chromaffin cells in the null mouse model of Rett syndrome. These provocative results support the notion of a general abnormality of neurosecretory signaling in Rett syndrome. It will be important to identify similar changes in the knock-in mouse model that may be more representative of Rett syndrome.

10. Zhou Z, Hong EJ, Cohen S, Zhao W, Ho HH, Schmidt L, Chen WG, Lin Y, Savner E, Griffith EC, Hu L, Steen JAJ, Weitz CJ, Greenberg ME. Brain-Specific Phosphorylation of MeCP2 Regulates Activity-Dependent *Bdnf* Transcription, Dendritic Growth, and Spine Maturation. *Neuron* 2006;52:255-269.

The authors provide important data regarding the mechanism by which the addition of phosphate to a specific amino acid (serine at position 421 in MeCP2) in response to nerve stimulation impacts the ability of nerve cells to form functional connections with each other, specifically dendritic development and spine formation. These results offer an important window on the basic mechanisms underlying Rett syndrome and point the way for further studies specifically focusing on this phosphorylation site.

Group 10

1. Laurvick CL, Msall, ME, Silburn S, Bower C, de Klerk N, Leonard H. Physical and Mental Health of Mothers Caring for a Child With Rett syndrome. *Pediatrics* doi:1542/peds.2006-0439.

The authors surveyed 135 mothers of children with Rett syndrome using a standardized instrument of physical and mental well-being (SF-12). Factors associated with good physical health were work outside the home, high school education, health insurance, and strong financial resources. Negative factors were breathing problems in the child and home-based therapies. Mental health was affected positively by a strong marriage, low stress scores, and outside employment. Negative factors were fractures within the past 2 years and facial stereotypies or involuntary movements.

2. Setoguchi H, Namihira M, Kohyama J, Asano H, Sanosaka T, Nakashima K. Methyl-CpG Binding Proteins Are Involved in Restricting Differentiation Plasticity in Neurons. *J Neurosci Res* 2006;84:969-979.

The authors show, in a study of neuronal and astrocytic precursors, that methyl-binding proteins including MeCP2 have an important effect on differentiation of neurons. They further demonstrate that MeCP2 appears to interact with the *GFAP* (glial fibrillary acidic protein) gene to allow neurons to differentiate even late in gestation. GFAP is a marker for astrocytes, suggesting that MeCP2 interacting with the *GFAP* gene may suppress astrocyte formation in favor of neurons.

3. Zwiller J. Fluoxetine and Cocaine Induce Epigenetic Factors MeCP2 and MBD1 in Adult Rat Brain. *Mol Pharmacol* 2006;70:487-492.

The authors show that repeated doses of fluoxetine or cocaine induce MeCP2 in neurons involved in the gamma-aminobutyric acid (GABAergic) system. As both fluoxetine and cocaine elevate serotonin levels, the authors suggest that serotonin produces gene silencing in these GABAergic neurons.

4. Fatemi M, Wade PA. MBD family proteins: reading the epigenetic code. *J Cell Sci* 2006;119:3033-3037.

In this useful commentary on the function of methyl-binding proteins, the authors point out the variability of transcriptional regulation within this family of proteins, specifically pointing out that MeCP2 appears to be highly selective in the genes that it regulates as opposed to other proteins in this family that have broader effectiveness in gene regulation.

5. Archer HL, Evans J, Edwards S, Colley J, Newbury-Ecob R, O'Callaghan F, Huyton M, O'Regan M, Tolmie J, Sampson J, Clarke A, Osborne J. *CDKL5* mutations cause infantile spasms, early onset seizures, and severe mental retardation in female patients. *J Med Genet* 2006;43:729-734.

The *CDKL5* gene has been found in females with infantile spasms and some features of Rett syndrome. In a systematic study of females with infantile spasms and early seizure onset (first 6 months of life), the authors found *CDKL5* mutations in 7/42 females (17%), but no males, with early onset seizures. Only one of these females had Rett-like features, suggesting that a search for mutations in this gene should be conducted in females with developmental delay and early-onset seizures regardless of whether they exhibit features of Rett syndrome.

Group 11

1. Hirtz D, Thurman DJ, Gwinn-Hardy K, Mohamed M, Chaudhuri AR, Zalutsky R. How common are the "common" neurologic disorders. *Neurology* 2007;68:326-337.

The authors provide information on incidence and prevalence of 12 neurologic disorders. Of interest, the prevalence for autism spectrum disorders was 1 per 170 children. RS is considered within this group. Although a misrepresentation overall for RS, the recognition of this group of disorders as highly prevalent will provide a focal point for generating research interest and, one hopes, appropriately targeted federal funding.

2. Ishii T, Makita Y, Ogawa A, Amamiya S, Yamamoto M, Miyamoto A, Oki J. The role of different X-inactivation pattern on the variable clinical phenotype with Rett syndrome. *Brain & Dev* 2001;23:S161-S164.

The authors provide support for the modifying role of skewed or disproportionate X-inactivation to explain the discordant presentation of RS in one set of monozygotic (identical) twins and in two sisters.

3. Kim I-J, Kim Y-J, Son B-H, Nam S-O, Kang H-C, Kim H-D, Yoo M-A, Choi O-H, Kim C-M. Diagnostic mutational analysis of *MECP2* in Korean patients with Rett syndrome. *Exp Mol Med* 2006;38:119-125.

The authors report mutations in 26 of 43 (61%) children (41 females and 2 males) with RS. The authors used sequencing, but not MLPA analysis, explaining in part the low frequency of mutation detection. However, the analyses were not restricted to typical RS, also leading to the lower detection rate. Mutations were not identified in the males, whose phenotypes were not detailed.

4. Huppke P, Maier EM, Warnke A, Brendel C, Laccone F, Gärtner J. Very mild cases of Rett syndrome with skewed X inactivation. *J Med Genet* 2006;43:814-816.

Skewed X chromosome inactivation accounted for milder, non-Rett phenotypes in three individuals with common mutations generally associated with typical RS (pR306C, pP225R, and a c-terminal deletion), in all cases the normal gene expression occurred at a level of 75% or greater. Ordinarily, one would expect a ratio closer to 50:50.

5. Smeets EEJ, Julu POO, van Waardenburg D, Witt-Engerström I, Hansen S, Apartopoulos F, Curfs LMG, Schrander-Stumpel CTRM. Management of a severe forceful breather with Rett Syndrome using carbogen. *Brain & Dev* 2006;28:625-632.

The authors report the improvement of hyperventilation with the use of a breathing mixture of 5% carbon dioxide and 95% oxygen.

Group 12

1. Guy J, Gan J, Selfridge J, Cobb S, Bird A. Reversal of Neurological Defects in a Mouse Model of Rett Syndrome. *Science* 2007;315:1143-1147.

Using a cleverly engineered gene construct in their mouse model of Rett syndrome, the authors provide substantial evidence that the abnormal features in these mice are reversible even after the neurological abnormalities are well-advanced. While not providing a specific therapeutic intervention for humans, this work does support the notion that treatment would not have to be initiated prior to or very soon after onset of neurodevelopmental abnormalities in girls with Rett syndrome.

2. Ager S, Fyfe S, Christodoulou J, Jacoby P, Schmitt L, Leonard L. Predictors of Scoliosis in Rett Syndrome. *J Child Neurol* 2006;21:809-813.

The authors evaluated 242 individuals with RS for the presence of scoliosis and noted that 75% developed scoliosis at a mean age of 9.8 years. Predictors of early onset of scoliosis were abnormal development prior to age 6 months, reduced mobility prior to 10 months, and failure to walk at all. Among the common mutations in *MECP2*, the R294X mutation appeared to be associated with the lowest chance for developing scoliosis.

3. Li M-r, Pan H, Bao X-H, Zhang Y-Z, Wu X-R. *MECP2* and *CDKL5* gene mutation analysis in Chinese patients with Rett syndrome. *J Hum Genet* 2007;52:38-47.

The authors surveyed 121 individuals with RS and found *MECP2* mutations in 102 (94/107 or 88% typical RS and 8/14 or 57% atypical RS). Only one mutation was noted in *CDKL5*, indicating that this gene is rarely etiologic in children with Rett-like phenotypes.

4. Friez MJ, Jones JR, Clarkson K, Lubs H, Abuelo D, Bier J-AB, Pai S, Simensen R, Williams C, Giampietro PF, Schwartz CE, Stevenson RE. Recurrent Infections, Hypotonia, and Mental Retardation Caused by Duplication of *MECP2* and Adjacent Region in Xq28. *J Pediatr* 2006;118:1687-1695.

The authors identified duplication of *MECP2* in six families with several males affected with severe mental retardation in association with hypotonia progressing to spasticity, seizures, absence of speech, and recurrent respiratory infections. Highly skewed X chromosome inactivation was found in their mothers. In 5 families, the duplication also involved the L1 cell adhesion molecule gene. This paper suggests the need to perform duplication analysis in males who fit this clinical profile.

5. McGill BE, Bundle SF, Yaylaoglu MB, Carson JP, Thaller C, Zoghbi HY. Enhanced anxiety and stress-induced corticosterone release are associated with increased *Crh* expression in a mouse model of Rett syndrome. *PNAS* 2006;103:18267-18272.

The authors provide convincing evidence of enhanced corticosterone release and corticotropin-releasing hormone (*Crh*) expression in their knock-in mouse model of RS. Enhanced expression was noted not only in the primary releasing site, the hypothalamus, but also in the amygdala and stria terminalis, structures associated with anxiety and fear. *MeCP2* binds to the *Crh* promoter as potential regulator of its expression whereas mutant *MeCP2* does not, suggesting an important role of the hypothalamic-pituitary-adrenal axis and these other neural structures in the behavioral manifestations of RS. The results also suggest therapeutic interventions to address anxiety directly.

Group 13

1. Moser SJ, Weber P, Lütschg J. Rett Syndrome: Clinical and Electrophysiological Aspects. *Pediatr Neurol* 2007;36:95-100.

The authors highlight the challenges in identification and management of 'seizures' in individuals with Rett syndrome. In this study, 3 of 11 did not have EEG events that correlated with the clinical events. When questions exist about the nature of the clinical events, video-EEG correlation is critical. This could avoid the unnecessary use of medications.

2. Roux J-C, Dura E, Moncla A, Mancini J, Villard L. Treatment with desipramine improves breathing and survival in a mouse for Rett syndrome. *Eur J Neurosci* 2007;25:1915-1922.

The authors build on studies in the null model of RS showing reduced norepinephrine (NE) content and tyrosine hydroxylase expressing neurons in medulla (brainstem). Desipramine, an inhibitor of NE reuptake, not only improved breathing abnormalities and prolonged survival, but also increased the number of neurons with tyrosine hydroxylase in this region. The treatment group had a 5 week period of ~ 75% reduction in breathing irregularities compared to the placebo group. At that point, the breathing irregularities accelerated in the treatment group until their death in another 5-6 weeks. The study is quite provocative, but is limited for reasons described by the authors to the use of male animals. Nonetheless, a clinical trial in females with RS should be considered.

3. Archer H, Evans J, Leonard H, Colvin L, Ravine D, Christodoulou J, Williamson S, Charman T, Bailey MES, Sampson J, de Klerk N, Clarke A. Correlation between clinical severity in patients with Rett syndrome with a p.R168X or p.T158M MECP2 mutation, and the direction and degree of skewing of X-chromosome inactivation. *J Med Genet* 2007;44:148-152.

The authors demonstrate a relationship between the percent of mutant MECP2 vs. normal MECP2 and clinical severity for these two common mutations in girls with RS. The percent of mutant MECP2 ranges from less than 10% to ~65%. The actual number of girls analyzed in each group, due to issues with XCI determination, is rather small (20 for R168X and 23 for T158M) and the scatter of severity scores is large, especially for the T158M group. Other factors including genetic background and other epigenetic factors may play a role as well.

4. Giacommetti E, Luikenhuis S, Beard C, Jaenisch R. Partial rescue of MeCP2 deficiency by postnatal activation of MeCP2. *PNAS* 2007;104:1931-1936.

Unlike the recent paper reporting reversal of the RS phenotype in the null mouse model, this paper, employing a different postnatal activation strategy, reported partial improvement in male mice. Female animals were not studied. Nonetheless, the results provide proof of concept regarding possible reversibility in humans with RS.

5. Miyake K, Nagai K. Phosphorylation of methyl-CpG binding protein 2 (MeCP2) regulates the intracellular localization during neuronal cell differentiation. *Neurochem Int* 2007;50:264-270.

The authors demonstrate that MeCP2 is localized initially in the cytoplasm of neuron precursors and enters the nucleus following differentiation. The cytoplasmic component is phosphorylated [added phosphate group(s)], but the nuclear component is not. These results support the role of MeCP2 modification in its cellular functioning within the nucleus.

Group 14

1. Weese-Mayer DE, Lieske SP, Boothby CM, Kenny AS, Bennett HL, Silvestri JM, Ramirez J-M. Autonomic Nervous System Dysregulation: Breathing and Heart Rate Perturbation During Wakefulness in Young Girls with Rett Syndrome. *Pediatr Res* 2006;60:443-449.

The authors examined cardiorespiratory function in 47 awake girls with Rett syndrome and mutations in *MECP2* and 47 matched controls. Significant differences were noted in the girls with Rett syndrome with excessive increase heart rate in association with breathholding. Even during apparently normal breathing, heart rate was abnormal compared to controls. The specific mutations are not described. It does not appear that the authors analyzed the altered cardiorespiratory regulation with respect to specific mutations; that is, were these changes more prevalent in girls with particular mutations.

2. Villard L. *MECP2* mutations in males. *J Med Genet* 2007; JMG Online First, 10.1136/jmg.2007.049452.

The author reviews the occurrence of *MECP2* mutations in males and notes three distinct mutation groups: 1) severe encephalopathy or more typical RS in boys with Klinefelter syndrome (XXY) or somatic mosaicism in association with mutations commonly seen in girls with RS; 2) mental retardation with or without motor abnormalities in association with mutations not seen in girls with RS; and 3) mental retardation, absent speech, abnormal gait, and recurrent respiratory infections in association with duplications of *MECP2* and in some instances adjacent genes. Among males with mental retardation, the frequency of mutations in *MECP2* is 1.3-1.7%.

3. Zahorakova D, Rosipal R, Hadac J, Zumrova A, Bzduch V, Misovicova N, Baxova A, Zeman J, Martasek P. Mutation analysis of the *MECP2* gene in patients of Slavic origin with Rett syndrome: novel mutations and polymorphisms. *J Hum Genet* 2007;52:342-348.

The authors report *MECP2* mutations in 68 of 87 (78%) girls with typical Rett syndrome. The report represents the first large scale survey in girls of Slavic origin.

4. Sampieri K, Meloni I, Scala E, Ariani F, Caselli R, Pescucci C, Longo I, Artuso R, Bruttini M, Mencarelli MA, Speciale C, Causarano V, Hayek G, Zappella M, Renieri A, Mari F. Italian Rett Database and Biobank. *Hum Mutat* 2007;28:329-335.

The authors present a large series of individuals from Italy with Rett syndrome and the construction of an impressive and accessible database (www.biobank.unisi.it) for analysis of mutation type and frequency. *MECP2* mutations were identified in 113 of 126 (90%) with typical RS, in 27 of 61 (44%) with atypical RS, in 17 of 18 (94%) without a determined diagnosis, and in 5 individuals with Rett-like features. In addition, the authors have developed a bank of DNA and cell lines from a large number of individuals. Both resources will be extremely valuable for purposes of further study and analysis.

5. Stettner GM, Huppke P, Brendel C, Richter DW, Gärtner J, Dutschmann M. Breathing dysfunctions associated with impaired control of postinspiratory activity in *Mecp2*^{-/-} knockout mice. *J Physiol* 2007;579:863-876.

The authors provide important information on motor control during breathing in males mice lacking the *Mecp2* gene and note dysregulation of voluntary breathing after inspiration. The authors attribute these abnormalities to failure of sensory feedback circuits to regulate the inspiratory/expiratory cycle.

6. Amaral M, Chapleau CA, Pozzo-Miller L. Transient receptor potential channels as novel effectors of brain-derived neurotrophic factor signaling: Potential implications for Rett syndrome. *Pharmacol Therapeut* 2007;113:394-409.

The authors review neurotrophin signaling, among else exploring transient calcium channels as BDNF targets. In disorders, such as Rett syndrome, where synaptic development and maintenance is impaired, these BDNF-responsive channels may provide a window on potential therapies.

7. Dragich JM, Kim Y-H, Arnold AP, Schanen NC. Differential Distribution of the *Mecp2* Splice Variants in the Postnatal Mouse Brain. *J Comp Neurol* 2007;501:526-542.

The authors examine the temporal and spatial distribution of the two *Mecp2* isoforms. Their results suggest that the e1 isoform predominates outside the thalamus and cortical layer V where the e2 isoform is abundant.

Group 15

1. Chadwick LH, Wade PA. MeCP2 in Rett syndrome:transcriptional repressor or chromatin architectural protein. *Curr Opinion Genet Develop* 2007;17:121-125.

The review describes two roles for the Rett syndrome-related MeCP2 protein. The traditional role has been as a transcription regulator of such protein as BDNF, CRH (corticotrophin releasing hormone), and AVP (arginine vasopressin). Now, information is reviewed indicating that MeCP2 is an integral component of the complex nuclear structure known as chromatin and important for imprinting of specific genes.

2. Freilinger M, Kalisch D, Muehl A, Haas O, Moritz A, Bodamer O. Methylation Status in Females With Rett Syndrome. *J Child Neurol* 2007;22:635-638.

The authors measured methionine, homocysteine, and other metabolites in 29 females with RS and found no abnormalities. Nonetheless, the authors suggest that supplementation of methyl-group donors could be important in RS.

3. Nag, N, Berger-Sweeney JE. Postnatal dietary choline supplementation alters behavior in a mouse model of Rett syndrome. *Neurobiol Dis* 2007;26:473-480.

Choline supplementation in the null mouse model for RS demonstrated improved motor performance in both male and female mice whereas no effect on fear conditioning was noted.

4. Tsai S-J. Semax, an analogue of adrenocorticotropin (4-10), is a potential agent for treatment of attention-deficit hyperactivity disorder and Rett syndrome. *Med Hypoth* 2007;68:1144-1146.

The author notes that Semax could improve attention by enhancing stimulant-type medications. Semax also is said to enhance BDNF production. As such, he proposes further testing in animal models of RS to assess possible therapeutic benefits.

5. Harvey CG, Menon SD, Stachowiak B, Noor A, Proctor A, Mensah AK, Mnatzakanian GN, Alfred SE, Guo R, Scherer SW, Kennedy JL, Roberts W, Srivistava AK, Minassian BA, Vincent JB. Sequence Variants Within Exon 1 of *MECP2* Occur in Females With Mental Retardation. *Am J Med Genet* 2007;144B:355-360.

The authors screened a population of individuals with autism and cognitive impairment for sequence changes in Exon 1 of *MECP2*. Among the 401 individuals with autism, no abnormalities were noted. Among 1410 individuals with cognitive impairment 13 females had sequence variations. However, the same variation in 6 of these individuals was found in 3 individuals in the control group, suggesting that this is a benign change. Variations in the remaining 7 individuals (~0.5%) bear further study and could explain a small per cent of females with cognitive impairment.

6. Campos Jr M, Abdalla CB, Santos-Rebouças CB, Vaz dos Santos A, Pestana CP, Domingues ML, Mendonça dos Santos J, Pimentel MMG. Low significance of *MECP2* mutations as a cause of mental retardation in Brazilian males. *Brain Dev* 2007;29:293-297.

In this *MECP2* sequencing study from Brazil on 239 males with cognitive impairment, only a single pathogenic mutation was identified. Five non-pathogenic changes were found along with two previously unreported sequence alterations of uncertain significance. The authors conclude that *MECP2* mutations are a rare cause of cognitive impairment in males in Brazil.

7. Lesca G, Bernard V, Bozon M, Touraine R, Gérard D, Edery P, Calender A. Mutation screening of the *MECP2* gene in a large cohort of 613 fragile-X negative patients with mental retardation. *Europ J Med Genet* 2007;50:200-208.

The authors studied 442 males and 171 females with cognitive impairment in France and identified eleven sequence alterations, nine of which were non-pathogenic polymorphisms. The remaining two include one female lacking RS features but possessing a two nucleotide change in the C-terminal region leading to mild or borderline cognitive impairment. The other sequence in a female with mild cognitive impairment and behavioral problems was a variation of uncertain significance. Nonetheless, the authors conclude that *MECP2* mutations are a rare cause of cognitive impairment in this population.

8. Coutinho AM, Oliveira G, Katz C, Feng J, Yan J, Yang C, Marques C, Ataíde A, Miguel TS, Borges L, Almeida J, Correia C, Currais A, Bento C, Mota-Vieira L, Temudo T, Santos M, Maciel P, Sommer SS, Vicente AM. *MECP2* Coding Sequence and 3'UTR Variation in 172 Unrelated Autistic Patients. *Am J Med Genet* 2007;144B:475-483.

The authors evaluated 172 individuals in Portugal with the diagnosis of autism by sequencing methodology. Fifteen sequence variations were noted. One is in the coding region that is described as pathogenic. However, this variation was transmitted in the maternal line suggesting that it may not be pathogenic. Twelve occur in the 3'UTR. In four of these, the messenger RNA (mRNA) levels

were reduced suggesting instability of these transcripts. No mutations were identified in Exon 1.

9. Deng V, Matagne V, Banine F, Frerking M, Ohliger P, Budden S, Pevsner J, Dissen GA, Sherman LS, Ojeda SR. *FYXD1* is an MeCP2 target gene overexpressed in the brain of Rett syndrome patients and *Mecp2*-null mice. *Hum Molec Genet* 2007;16:640-650.

The authors report that the *FYXD1* protein production is increased in RS and in the null-mutation mouse model. This protein modulates the activity of an important enzyme involved in brain dendrite maturation, Na⁺,K⁺-ATPase. The authors therefore have uncovered yet another target of MeCP2 regulation and suggest abnormal function of this enzyme as one of the pathogenic bases for RS.

10. Stearns NA, Schaevitz LR, Bowling H, Nag N, Berger UV, Berger-Sweeney J. Behavioral and Anatomical Abnormalities in *MECP2* Mutant Mice: A Model For Rett Syndrome. *Neuroscience* 2007;146:907-921.

The authors describe abnormal motor function and increased anxiety as well poor cognitive function in male and female null-mutant mice for *Mecp2*. The females, due to having some *Mecp2*, are more mildly involved. In addition, in males, brain volumes were reduced in areas of the temporal lobe and basal ganglia associated with these functions. The identification of similar problems in females is important and more representative of RS in humans.

11. Itoh M, Ide S, Takashima S, Kudo S, Nomura Y, Segawa M, Kubota T, Mori H, Tanaka S, Horie H, Tanabe Y, Goto Y-i. Methyl-CpG-Binding Protein 2 (a Mutation of Which Causes Rett Syndrome) Directly Regulates Insulin-Like Growth Factor Binding Protein 3 in Mouse and Human Brains. *J Neuropathol Exp Neurol* 2007;66:117-123.

The authors demonstrate that MeCP2 regulates expression of the insulin-like growth factor binding protein 3 gene in human and mouse brain leading to elevated expression of IGF-binding protein 3 in brain. These elevated levels may adversely affect brain maturation. Importantly, these studies were conducted both in male and female mice. The female null-mutants mice had IGF-binding protein 3 levels in brain intermediate between normals and null-mutant males. Interestingly, these changes were quantitatively similar to those noted for BDNF in the same mice.