Rett Syndrome: Recent Research Progress

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Recent progress in our understanding of Rett syndrome has been dramatic. Against the background that the clinical features of Rett syndrome may be reversible, in part or in whole, substantial optimism has emerged regarding possible therapies. As such, it is timely to update recent research progress. This update summarizes research advances during the past 18 to 24 months in terms of clinical and translational research, as well as basic research.

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Recent progress in our understanding of Rett syndrome has been dramatic. Notable optimism has emerged regarding possible therapies relative to the background that the clinical features of Rett syndrome may be reversible, in part or in whole. Under the auspices of the International Rett Syndrome Association, the recent compilation of the North American Database of patients with Rett syndrome will be beneficial to the process of launching clinical trials. In brief, the North American Database, derived principally from members of the International Rett Syndrome Association, represents the first comprehensive compilation of information in the United States and Canada on individuals with Rett syndrome or with another diagnosis in association with an MECP2 mutation. The North American Database contains specific information by diagnosis, mutation status, and mutation type and frequency on 1928 participants. Among 1928 cases, 85.5% were typical, 13.4% were atypical, and 1.1% had MECP2 mutations but did not have Rett syndrome. MECP2 testing has been completed in 1165 subjects, for whom results are known in 1059 (90.9%). Overall, MECP2 mutations were identified in 914 of 1059 subjects (86.3%), but when stratified by diagnosis, 799 of 870 subjects (91.8%) with typical Rett syndrome had an MECP2 mutation, 94 of 162 subjects (58.0%) with atypical Rett syndrome had an MECP2 mutation, and all 21 subjects whose diagnosis was not Rett syndrome had an MECP2 mutation. Missense-type mutations (39.0%) were slightly more common than nonsense-type mutations (35.1%). Individual frequencies for the 8 common mutations varied from 11.9% for T158M to 4.4% for R106W; large deletions accounted for 6.4%, and C-terminal truncations occurred in 8.8%. The remaining mutations (14.3%) occurred singly or in small numbers. This database provides a unique resource for expansion of our understanding of Rett syndrome, for comparison with other national databases, and for future study, including clinical trials based on the expected emergence of fundamental therapies. This update summarizes research advances during the past 18 to 24 months in terms of (1) clinical and translational research and (2) basic research.

Clinical and Translational Research

Diagnostic Strategies

How do molecular diagnoses change the definition or categorization of specific disorders such as Rett syndrome? Although a molecular definition might be appropriate, many single-gene disorders are associated with multiple phenotypes, as occurs in Rett syndrome, suggesting that molecular categorization is not advisable at this time.2 Since the identification of mutations in exon 1 and of large deletions of MECP2, more than 95% of individuals fulfilling consensus criteria for Rett syndrome will have an identifiable pathogenic mutation. Large deletions represent 6% to 10% of the total cases. Exon 1 mutations account for only about 1%, as recently confirmed.3 Identification of an MECP2 control region mutation that blocks the normal coupling of coding information for this gene and results in an absence of MeCP2 protein provides yet another mechanism that would escape diagnostic detection by standard laboratory methods. Application of this method to individuals meeting Rett syndrome diagnostic criteria but lacking a mutation by standard diagnostic testing may provide additional sensitivity to molecular confirmation.4

The following 5 country-specific reports indicate global expansion of MECP2 testing. (1) In Korea, mutations were identified in 26 of 43 (60.5%) children (41 girls and 2 boys)
with Rett syndrome.\(^7\) The use of sequencing, but not multiplex ligation-dependent probe amplification analysis for detecting large deletions, explains in part the low rate of mutation detection. The inclusion of typical and atypical Rett syndrome will also lower the detection rate. Mutations were not identified in the boys, whose phenotypes were not detailed. (2) MECP2 mutation testing throughout France among 424 individuals indicated 121 different mutations, more than 90% of which were found in exon 4. Large alterations accounted for almost 6%, whereas mutations in exon 1 were rare. Comparisons with RettBASE (http://mecp2.chw.edu.au) revealed overall similarity in the distributions of the 8 most common mutations (data not shown in the article), although T158M and R168X were most common in the larger sample comprising RettBASE. (3) Among 121 individuals in China, 102 subjects (94 of 107 with typical Rett syndrome [87.9%] and 8 of 14 with atypical Rett syndrome [57.1%]) were found to have MECP2 mutations.\(^7\) (4) The first large-scale survey among girls of Slavic origin with typical Rett syndrome revealed MECP2 mutations in 68 of 87 girls (78.2%).\(^8\) (5) In Italy, MECP2 mutations were identified in 113 of 126 patients (89.7%) with typical Rett syndrome, in 27 of 61 patients (44.3%) with atypical Rett syndrome, in 17 of 18 patients (94.4%) without a determined diagnosis, and in 5 individuals with Rett syndrome–like features.\(^9\) The data are detailed in an impressive and accessible database (http://www.biobank.unisi.it) for analysis of mutation type and frequency, along with a bank of DNA and cell lines from a large number of individuals.

The occurrence of MECP2 mutations in boys segregates into the following 3 distinct mutation groups: (1) typical Rett syndrome in boys with Klinefelter syndrome (XXY) or somatic mosaicism or severe encephalopathy in association with mutations commonly seen in girls with Rett syndrome, (2) mental retardation with or without motor abnormalities in association with mutations not seen in girls with Rett syndrome, 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 male patients with mental retardation, the frequency of MECP2 mutations ranges from 1.3% to 1.7%.\(^10\) Duplication of MECP2 was examined in 6 families in which several men were affected with severe cognitive impairment, seizures, absence of speech, recurrent respiratory infections, and hypotonia progressing to spasticity.\(^11\) Skewed X-chromosome inactivation was noted in their mothers. In 5 families, the duplication also involved the L1 cell adhesion molecule gene. This association was reported in another group of male patients with X-linked mental retardation.\(^12\) MECP2 duplication may be one of the most common causes of cognitive impairment in male subjects. Overexpression of MECP2 in male mice was shown previously to be detrimental.\(^13\)

The identification of MECP2 mutations in individuals with cognitive impairment has prompted large-scale assessments in this population. Overall, the yield has been modest. Among 416 individuals with cognitive impairment, 68 of whom had other neurological impairments, none of 100 male subjects were noted to have an MECP2 mutation, whereas 2 of 46 female subjects had previously described pathogenic MECP2 mutations.\(^14\) Nonetheless, expansion of molecular testing to include MECP2 among individuals with cognitive impairment is reasonable. For example, a boy with moderate cognitive impairment, epilepsy, and features of autism was found to have a novel missense mutation at C964T (P322S).\(^15\) His mother, who has notable neurological findings (including clumsiness, gait difficulties, tremor, abnormal speech, and a cognitive level in the low borderline range), carries the same mutation. Her X-chromosome inactivation was balanced, but the position and type of mutation predict a mild phenotype.

**Phenotype-Genotype Studies**

Numerous reports provide insights into phenotype-genotype correlation. A behavior questionnaire\(^16\) was used to compare specific behaviors associated with the 8 common MECP2 mutations. Despite variability within each mutation, certain observations were of interest. Abnormalities of mood tended to be associated with R294X, and stereotypies of hand and face were associated with R255X and R270X. R133C and R306C, mutations generally associated with milder overall involvement, tend to be associated with heightened anxiety and fear.\(^17\)

The modifying role of skewed or disproportionate X-chromosome inactivation explained the discordant presentation of Rett syndrome in monozygotic (identical) twins and in 2 sisters.\(^18\) Skewed X-chromosome inactivation also accounted for milder non–Rett syndrome phenotypes in 3 individuals with common mutations generally associated with typical Rett syndrome (R306C, P225R, and a C- terminal deletion); in all instances, the normal gene expression occurred at a level of 75% or greater.\(^19\) In another X-chromosome inactivation study,\(^20\) the percentages of mutant MeCP2 ranged from less than 10% to approximately 65%. Factors likely to affect phenotypic expression include genetic background and regional differences in mutant MeCP2 expression in brain.

**Neurophysiologic and Neurotransmitter Studies**

To elucidate the possible role of neurotransmitters regarding the clinical problems associated with autonomic dysfunction in Rett syndrome, serotonin transporter binding in brainstem from individuals with Rett syndrome was compared with that in control subjects.\(^21\) Rett syndrome tissues failed to demonstrate the expected developmental progression with
increasing vagus nerve response to serotonin in Rett syndrome may underlie dysregulation of gastrointestinal and cardiac responses.

Cardiorespiratory function was examined in 47 awake girls with Rett syndrome and MECP2 mutations and in 47 matched control subjects. Substantial differences were noted in the girls with Rett syndrome, including an excessive increase in heart rate in association with breath-holding. Even during apparently normal breathing, heart rate was abnormal compared with that of controls. Unfortunately, the cardiorespiratory alterations were not analyzed with respect to specific mutations (ie, were these findings more prevalent in girls with particular mutations?).

Seizure identification and management in Rett syndrome can be challenging. In a recent study, 3 of 11 participants did not have electroencephalographic events that correlated with clinical events. When questions exist about the nature of clinical events, video-electroencephalography correlation is critical. This could avoid the unnecessary use of medications.

Cognitive and Behavioral Studies

Assessment of cognition and behavior in Rett syndrome is also challenging. Music therapy in 7 girls with Rett syndrome facilitated the ability to acquire new information and to develop communication skills. A comparison with other motivational techniques was not conducted.

Eye-gaze technology was examined in 7 girls. Six were able to follow verbal commands and to respond with a correct answer more often than not. This technology could be useful in assessing cognitive performance in Rett syndrome, for which proper hand skills or verbal capabilities required to complete standard cognitive measures are lacking.

Sophisticated electroencephalographic recordings were used to evaluate responses to auditory or visual cues in 17 female subjects. Responses were delayed and showed less organized processing compared with that of a control group. Furthermore, individuals with Rett syndrome did not follow the typical developmental pattern of maturation for these responses. Stach et al 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 waiting for the anticipated response. This delay in responsiveness has important implications for all intervention programs.

Medical Studies

Medical management in Rett syndrome continues to evolve. Compared with control subjects, 10 girls with Rett syndrome showed greater calcium absorption, despite having markedly reduced bone mineralization. The effectiveness of providing calcium supplementation remains an open question.

One hundred thirty-five mothers of children with Rett syndrome completed a standardized instrument of physical and mental well-being (12-Item Short Form Health Survey). Factors associated with good physical health were work outside of 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 positively affected by a strong marriage, low stress scores, and outside employment. Negative factors were fractures within the past 2 years and facial stereotypes or involuntary movements.

Another study addressed unremitting hyperventilation. Improvement followed the use of a breathing mixture of 5% carbon dioxide and 95% oxygen.

Scoliosis is also a potential problem. Among 242 individuals with Rett syndrome, 75% developed scoliosis at a mean age of 9.8 years. Predictors of early-onset scoliosis were abnormal development before age 6 months, reduced mobility before age 10 months, and failure to walk at all. Among common MECP2 mutations, the R294X mutation seemed to be associated with the lowest likelihood for developing scoliosis.

Prenatal diagnosis has been performed, although the risk for recurrence is exceedingly low. In an informative instance, the father of 2 half-sisters with the same MECP2 mutation did not carry this mutation in blood or buccal mucosa but had the mutation in approximately 5% of his semen DNA, indicating the importance of offering prenatal testing in this setting.

Basic Research

Mouse Models

As with other genetic disorders, the availability of animal models provides important opportunities to examine the pathobiology of Rett syndrome and to evaluate potential therapies. A knockin mouse model of Rett syndrome demonstrated abnormalities in the ability to search for targets of interest. Rather than going to a specific target area, the mice wandered about in a fashion reminiscent of motor apraxia in girls with Rett syndrome, indicating a problem in the ability to address targets in their environment. These 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. Abnormalities were noted in the structure and function of hippocampal synapses. These findings extend the known brain abnormalities from motor and sensory areas to those involved in learning and memory.

Similarly, knockout mice had diminished motor skills and fear response, believed to represent abnormalities of the hippocampus and amygdala. Notable down-regulation of 2 genes (Gap43 and Kif1b) in the hippocampus associated with normal neuronal function was also demonstrated. As the mice became sicker, the extent of alteration of these
other genes increased, raising the suspicion of a secondary effect.

Male mice lacking Mecp2 have reduced levels of brain-derived neurotrophic factor (BDNF). Mice lacking the Bdnf gene demonstrated some of the same features as mice lacking Mecp2. By creating a double-knockout mutant, the onset of disease was earlier, and survival was less than that in mice lacking only the Rett syndrome gene. Increasing BDNF expression produced notable improvements. Although the brain weight did not increase, the onset of Rett syndrome–like behaviors was delayed. Similar studies in mice with Mecp2 knockin mutations would be interesting, but most important, studies in female knockin mice that more closely resemble the human disorder in terms of genetic makeup should be considered.

Abnormal secretion of BDNF from neurons and of catecholamines from adrenal chromaffin cells was also demonstrated in the knockout mouse, supporting the notion of a general abnormality of neurosecretory signaling in Rett syndrome. Again, demonstrating similar changes in a knockin mouse model that may be more representative of Rett syndrome would be of interest.

Several studies examined respiratory control in the knockout model. Abnormalities in respiratory control pathways in brainstem (medulla) of male knockout mice were associated with reduced levels of the key neurotransmitters norepinephrine and serotonin. The addition of norepinephrine to preparations from medulla stabilized the respiratory control network. These results expand our understanding of the functional abnormalities associated with irregular breathing in Rett syndrome. As with other studies in knockout mice, it would be important to conduct similar studies in knockin mutant animals, particularly in female knockin mice.

Respiratory phenotypes were examined in 2 null mutant mice strains for Mecp2, one lacking any Mecp2 expression and the other lacking Mecp2 expression only in neurons. In both strains, hyperventilation was induced in an environment with low oxygen levels. Only mice lacking Mecp2 expression in both nonneural tissues and neurons developed depressed respiratory activity after hyperventilation. This depression in breathing was eliminated by including carbon dioxide in the low-oxygen environment, suggesting that respiratory depression was related to reduced carbon dioxide during hyperventilation. Although the authors attribute the difference in mice lacking any Mecp2 expression to Mecp2 deficiency in lung tissues, further work needs to be performed to explain why the animals with Mecp2 deficiency restricted to neurons did not develop respiratory depression. Elevated BDNF expression in the relevant brainstem nuclei is suggested as a potential explanation. As already noted, recent data suggest that BDNF expression is actually reduced in this mouse model.

Motor control during breathing in male mice lacking Mecp2 is associated with dysregulation of voluntary breathing after inspiration. The findings are attributed to failure of sensory feedback circuits regulating the inspiratory-expiratory cycle.

Building on findings in the knockout model showing reduction of norepinephrine content and tyrosine hydroxylase–expressing neurons in medulla (brainstem), a study demonstrated that desipramine hydrochloride, an inhibitor of norepinephrine 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 an approximate 75% reduction in breathing irregularities compared with the placebo group. At that point, the breathing irregularities accelerated in the treatment group until their death in another 5 to 6 weeks. The study is provocative but is limited for reasons associated with the use of male animals that are described by the authors. Nonetheless, a pilot clinical trial in female animals with Rett syndrome might be considered.

Neurophysiologic abnormalities in the hippocampus of knockout mice are consistent with abnormal neuronal plasticity and support findings in the human brain. This suggests a disturbance in maturation and maintenance of cortical synapses, as well as similar findings in a knockin mouse model already noted.

In hippocampal neuronal cell cultures from normal and knockout mice, neurons from the mutant mice displayed diminished spontaneous excitatory (or stimulatory) cell-to-cell activity compared with normal mice. The same effect was produced in normal neurons by blocking the enzyme cascade that Mecp2 uses to regulate the activity of other genes. Overall, the results are similar to other findings in intact hippocampal section preparations.

A version of the knockout model restricted to the forebrain (cerebral hemispheres) after birth has features typical of Rett syndrome. These results are not surprising because Mecp2 has its principal role after birth. The results are also somewhat contradictory. The authors describe impaired motor coordination and normal locomotor activity. It is unclear how this is possible. What is clear is that notable differences exist between the knockout mouse model and the knockin mutant described by Moretti et al.

The expression pattern over time of Mecp2 in cortical neurons from female mice with knockout mutations shows an increase of cells expressing wild-type protein from 50% to 70%, suggesting altered X-chromosome inactivation status with increasing age. This change in Mecp2 expression could explain, at least in part, the pattern of stabilization and improved interaction in older girls with Rett syndrome.

Examination of the expression of other genes in the knockout mouse model of Rett syndrome demonstrated a notable increase in the expression of a nuclear gene encoding cytochrome c reductase. Using biochemical methods, a corresponding increase in energy generation through these mitochondria was shown. The results are provocative. It is unclear how this increase in mitochondrial activity would
relate to onset of the Rett syndrome phenotype. However, one can imagine how this increase could lead to mitochondrial fatigue over time. The investigations were performed in male mice lacking any Mecp2 activity and having a short lifespan. It would be of interest to examine mitochondrial function in aging female mutant mice. The authors refer to microscopic mitochondrial changes in female mice with Rett syndrome. These findings have not been uniformly found. Also, previous reports of changes in the spinal fluid analytes lactate and pyruvate seem to relate to the presence or absence of notable breathing abnormalities. These cerebrospinal fluid analytes are not elevated in girls with Rett syndrome lacking substantial breathing problems. Nonetheless, the results are intriguing. It will be interesting to conduct similar studies in MeCP2 knockin mice.

Enhanced corticosterone release and corticotropin-releasing hormone (CRH) expression was demonstrated in a knockin mouse model of Rett syndrome. 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. Wild-type Mecp2 binds to the Crh promoter as a 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 Rett syndrome. The results also suggest the possibility of therapeutic interventions to address anxiety directly.

In a cleverly engineered gene construct in a knockout mouse model, the Rett syndrome--like features could be reversed in male and female mice even after advanced neurological involvement. Although not offering a specific therapeutic intervention for humans, this work provides proof of principle that treatment would not have to be initiated before onset or soon after onset of neurodevelopmental abnormalities. In contrast, the use of a different postnatal activation strategy provided partial improvement in male mice. Female animals were not studied. Nonetheless, these results also provide proof of concept regarding possible reversibility in humans with Rett syndrome.

**MECP2 Function**

Increased understanding of MECP2 function remains an important target for unraveling the pathobiology of Rett syndrome. The temporal and spatial distribution of the 2 MeCP2 isoforms in brain is varied. The e1 isoform predominates outside of the thalamus and cortical layer V, where the e2 isoform is abundant. It was recently shown that MeCP2 is localized initially in neuronal precursor cytoplasm and enters the nucleus following differentiation. The cytoplasmic component is phosphorylated, but the nuclear component is not. These results suggest the role of MeCP2 modification before its cellular localization within the nucleus. In addition, phosphorylation of serine at position 421 in MeCP2 in response to nerve stimulation affects 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 fundamental mechanisms underlying Rett syndrome and suggest a need for further studies on this phosphorylation site.

Examination of neuronal and astrocytic precursors demonstrated that methyl-binding proteins, including MeCP2, have an important effect on differentiation of neurons. MeCP2 seems to interact with the glial fibrillary acidic protein gene (GFAP) to allow neurons to differentiate even late in gestation. GFAP is a marker for astrocytes, suggesting that the MeCP2-GFAP interaction may suppress astrocyte formation in favor of neurons. In another study, the regulatory region that promotes expression of MeCP2 was found to be active in brain but not in nonneural tissue in mice, a finding that could have important implications for future attempts at gene therapy.

The MeCP2 protein product, methyl-CpG-binding protein 2, exerts its effect by interacting with the cell machinery that determines how other genes are expressed. Additional explanation of how abnormalities in this gene could lead to Rett syndrome involves MeCP2 action at the RNA level. In simple terms, the nuclear coding material, DNA, is transcribed to the corresponding RNA, which in turn directs the formation of the proper protein, suggesting that potential regulation is dual sided. It is known that MeCP2 interacts with and regulates DNA expression by binding to methylated DNA. In disorders such as Rett syndrome, are expressed.

MeCP2 was shown to bind to unmethylated DNA, representing yet another potential regulatory mechanism. The authors suggest that the specific Rett syndrome mutation, R133C, within the methyl-binding domain does not alter binding to methylated DNA. However, Kudo et al previously showed in a functional assay that R133C has little effect on MeCP2 binding to methylated and unmethylated DNA. In the same functional assay, other mutations (R106W, F155C, and T158M) altered binding to methylated DNA.

**Rett Syndrome--Related Studies**

Neurotrophin signaling involves transient calcium channels, including those targeted by BDNF. In disorders such as Rett syndrome in which synaptic development and maintenance are impaired, these BDNF-responsive channels may provide a window on potential therapies. Following repeated exposure to aggressive behavior, BDNF is integrally related to the development of social isolation. The elimination of BDNF in a specific dopamine pathway blocks this antisocialization response. Antidepressant
medications were also able to block interference with socialization. A reduction in the levels of neurotransmitter (acetylcholine chloride, dopamine hydrochloride, or both) input to cortical neurons resulted in decreases in cortical neuron size and in dendritic branching, findings noted in Rett syndrome.58

Repeated doses of fluoxetine hydrochloride or cocaine hydrochloride induce MeCP2 in neurons involved in the γ-aminobutyric acid (GABA) system.59 Because fluoxetine and cocaine elevate serotonin levels, the authors suggest that serotonin produces gene silencing in these GABAergic neurons.

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References


