RETT SYNDROME: OF GIRLS AND MICE—LESSONS FOR REGRESSION IN AUTISM

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INTRODUCTION

Rett syndrome (RTT) is a neurodevelopmental disorder occurring almost exclusively in females. Regression is a defining feature of RTT. During the regression stage, RTT girls display many autistic features, such as loss of communication and social skills, poor eye contact, and lack of interest, and initially may be given the diagnosis of autism. The discovery of the genetic cause of RTT, mutations in the MECP2 gene, a transcriptional repressor, has promoted the early diagnosis of RTT and development of mouse models. The phenotype of one mouse model includes features such as regression and abnormal behavioral and social interactions. The timing of the period of regression in RTT—during ages 1 to 2 years—parallels the period of intense synaptic development. The effects of the MECP2 mutation also increases concomitantly with peak synaptogenesis. Neuropathological findings in Rett include the selective reduction of dendritic spines in the pyramidal cells of RTT brains; this feature has also been reported in autism. Studies have observed that MECP influences the expression of brain-derived neurotrophic factor and thus may influence synaptic plasticity. Abnormalities in synapse maintenance and modulation may contribute to regression in RTT and autism. Studies of the clinical aspects of the regression period and of the mouse model may be useful in understanding the pathophysiology of RTT and other neurodevelopmental disorders such as autism. Although the genetic background and certain clinical features differ in RTT and autism, a similar mechanism involving MeCP2 regulation and expression may contribute to regression.

Rett Syndrome Clinical Characteristics

Rett syndrome, an X-linked dominant neurodevelopmental disorder, is a leading cause of neurological dysfunction in females. RTT was first described in 1966 by Andreas Rett, but went unrecognized until 1983 when Hagberg et al. described 35 patients [Hagberg et al., 1983]. RTT affects all ethnic groups and there is a reported incidence of 1/10,000 to 1/20,000 live female births [Hagberg, 1985; Kozinetz et al., 1993]. Survival to 30–40 years is the rule rather than the exception. RTT is characterized by an initial period of apparently normal psychomotor development, followed by loss of communication skills and purposeful hand movements. Next, hand stereotypes and gait abnormalities become apparent. Deceleration of head growth is evident in most RTT females and may occur as early as 2–4 months. Other problems include: growth failure, epilepsy, wakeful breathing abnormalities (including hyperventilation and breath holding); bruxism; motor dysfunction, including dystonia and scoliosis; and chronic constipation [Glaze and Schultz, 1997]. Although the genetic basis for RTT is known, MECP2 mutations are associated with a broad spectrum of phenotypes in girls and boys [Amir et al., 1999; Shahbazian and Zoghbi, 2001]. The diagnosis of RTT remains a clinical one. Recently, revised criteria have been suggested [Hagberg et al., 2002], indicating features of regression such as loss of purposeful hand skills, social withdrawal, and communication dysfunction with loss of spoken language skills.

Rett and Regression

Regression is a defining feature of the RTT disease profile. However, regression is limited and the characteristic RTT profile is illustrated in the four-stage model as presented by Hagberg and Witt-Engerstrom [1986]:

- **Stage I:** Early-onset stagnation. Stagnation occurs between 6 and 18 months of age during which there are nonspecific developmental delays. The developmental pattern is typically perceived as being in the broad normal range. This stage typically lasts weeks to months.
- **Stage II:** Rapid developmental regression. The onset of regression is between 1 and 3 years and is characterized by the loss of acquired purposeful hand and communication skills. Duration is weeks to as long as 1 year. RTT females may appear “in another world.” Some females may experience periods of inconsolable crying and an appearance of extreme fear and distress.

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Stage III: Pseudostationary period. This period appears after the female passes through Stage II. There is no further cognitive decline. Slow neuromotor regression may occur. The RTT female appears more interactive and sociable and may regain communication skills, albeit not useful spoken language skills. This stage may last years to decades.

Stage IV: Late motor deterioration. This stage is characterized by the loss of ambulation in previous walkers and further motor impairment, including Parkinsonian features, worsening scoliosis, and distal wasting and deformities.

Although hand skills and verbal and nonverbal communication skills are the most common features lost during the regression stage, play and motor skills are lost in half of cases. Some RTT females may show regression prior to 6 months or after 36 months. Over two-thirds of RTT females have a history of preregression developmental delays or abnormalities. The age of regression has not been demonstrated to correlate with severity of symptoms. The most frequent signs of early developmental problems are low-tone and delayed motor milestones [Charman et al., 2002]. Early signs may include delay in social and play skills, not recognizing familiar adults, and appearing “cut off” [Charman et al., 2002]. A retrospective study observed that subtle signs such as transitory tremulous neck movements, hypotonia, and low intensity in contact appeared during the first 6 months in 80% of RTT girls [Witt-Engerstrom, 1993]. This study also reported that at 10 months, some RTT patients experienced delays in balancing the body in the upright position and locomotion. Flapping or fisting of the hands above the shoulders was observed in some RTT girls at this age. Optimal development was achieved at 11–12 months and included single words and opposed thumb or pincer grasp. By 2 years all RTT girls in this study had stereotyped hand movements and had no purposeful hand use or spoken communication skills. At this age, too, contact and emotional responses were impaired. With the onset of Stage III, there was increased activity and efforts to use residual functions, including word-pieces, hand movements, and gross motor functions. Contact improved and the girls used eye pointing as a form of communication [Witt-Engerstrom, 1993].

Seizures are reported to occur in 50–80% of RTT females, and nearly 100% of RTT patients at some time have abnormal EEGs that are frequently characterized by epileptiform abnormalities [Glaze et al., 1987, 1998; Glaze and Schultz, 1997; Glaze, 2002]. Interestingly, seizures are more prominent in Stage III, following the regression phase. Correlation of the EEG abnormalities and clinical stages indicate that during Stage I (preceeding the period of regression), the EEG is normal in many RTT girls, although in some RTT patients mild slowing is evident. During the period of regression (Stage II), the EEG features are characterized by slowing of the background activity and the recording of occasional epileptiform discharges primarily in the central or parietal regions. However, following the regression, a more dramatically abnormal EEG pattern is frequently evident. Further slowing of the background and loss of expected developmental and sleep characteristics are evident. During this time, the EEGs of most RTT girls are characterized by the frequent recording of epileptiform abnormalities, including multifocal spikes and sharp waves, generalized slow spike and slow wave abnormalities, and even a pattern of hypsarrhythmia [Glaze et al., 1987]. That these features are typically evident after the period of regression suggests that RTT is not an “epileptic regression” disorder.

Regression is a defining feature of Rett syndrome (RTT).

RTT and Autism: Clinical Overlap

Phenotypic overlap between RTT and autism is well recognized [Mount et al., 2003]. Many individuals with RTT are first regarded as autistic [Percy et al., 1988, 1990]. Although certain behaviors such as hand stereotypies and wakeful breathing abnormalities differentiate RTT from autism, other features appear common to both. These include the timing of the onset of symptoms and regression between ages 6 and 36 months. Regression is characteristic of RTT and occurs in many children with autism. Regression in RTT and autism is typically characterized by impairment of communication and social skills. Frequently reported behaviors observed in RTT girls, including indifference to persons, sleep abnormalities, and features of anxiety and low mood or mood fluctuations, may appear similar to those observed in autistic children [Mount et al., 2003]. Features of autism are reported to occur more commonly in RTT than in groups of males with fragile-X syndrome [Mazzocco et al., 1998]. Mount and associates used the Autistic Behavior Checklist to measure autistic symptoms in a sample of girls with RTT and a comparison group of girls with severe or profound mental retardation [Mount et al., 2003]. Some, but not all, features of autism were noted more frequently in the RTT group. Girls with RTT scored more highly on the Sensory and Relating subscales. There were no group differences on the Body and Object Use, Language, and Social and Self-help subscales. Clearly, there are distinct differences between RTT and autism. Defining features of RTT, such as loss of hand skills, hand stereotypies, gait ataxia, deceleration of head growth, wakeful breathing abnormalities, and increasing motor dysfunction including scoliosis, are rarely if ever seen in individuals with autism [Percy et al., 1988, 1990]. Features of autism that include social rejection, excessive attachment to objects, complex stereotypies, and stereotypic play are not typically observed in RTT [Percy et al., 1988, 1990; Mount et al., 2003]. RTT is recognized as a distinct neurogenetic disorder, whereas autism is usually recognized as a behavioral phenotypic endpoint of a wide variety of etiological pathways [Mount et al., 2003]. The genetic etiologies of RTT and autism are different. Although a mutation in a single gene causes the vast majority of RTT, the genetic predisposition for autism is polygenetic. MECP2 mutations are rarely the cause of autism [Lam et al., 2000; Beyer et al., 2002; Carney et al., 2003; Vourc’h et al., 2001; Lobo-Menendez et al., 2003]. Most RTT cases are sporadic and are not familial. However, the phenotypic similarities of RTT and autism suggests that understanding the characteristics and degree of symptom overlap might contribute to models of the neuropathological development of both diseases [Mount et al., 2003]. Regression that characterizes all cases of RTT, but only some cases of autism, may be consequent to similar processes [Mount et al., 2003].
RETTSYNDROMEANDMECP2

In more than 80% of females with RTT, mutations in the Methyl-CpG-binding protein-2 (MECP2) gene are found [Amir et al., 1999; Huppke et al., 2003]. The MECP2 gene encodes for the Methyl-CpG-binding protein-2 (MeCP2); which is believed to be involved in the silencing of genes by functioning as a methylation-dependent repressor. MeCP2 is a putative transcriptional repressor of downstream genes [Lewis et al., 1992; Meehan et al., 1992]. Mutations in this gene could lead to “gain of function” by overexpression of certain genes at critical developmental time points and disrupt the normal cascade of gene silencing and activation critical to development. However, the genes that are regulated by MeCP2 are unknown and the mechanisms by which mutations in MECP2 cause RTT have not been discovered. MeCP2 is expressed in all tissues, but is more abundant in brain tissue than most peripheral tissue. MeCP2 is expressed in neurons, not glia, and is localized to cell nuclei. MeCP2 levels increase in cortical neurons throughout development [Shahbazian et al., 2002a; Mullaney et al., 2004; Akbarian et al., 2001]. In the rat brain at early postnatal ages, regions having neurons that were generated early and are more mature have the strongest MeCP2 expression. Late developing structures, including the cortex, hippocampus, and cerebellum, exhibit the most significant increases in MeCP2 expression. The timing of MeCP2 expression in the granule cells of the cerebellum is coincident with the onset of granule cell synapse formation. The degree of MeCP2 expression in the cortex and hippocampus correlate with synaptogenesis in both regions [Mullaney et al., 2004]. This expression pattern provides one possible explanation for the postnatal onset of symptoms and delay of the expression of the RTT phenotype and suggests that MeCP2 may maintain or modulate neuronal and synaptic maturity and plasticity [Zoghbi, 2003].

Although mutations in MECP2 gene are the cause of the majority of RTT, there is a broad range of phenotypes. Variant forms of RTT have been described in RTT that lack the “essential” characteristics. These variants include forme fruste, late regression RTT, congenital RTT, early seizure onset RTT, and preserved speech variant RTT [Hagberg et al., 2002]. RTT is a clinically and not genetically defined condition. Factors influencing the phenotype include mutation type and X-chromosome inactivation (XCI). Females with favorably skewed XCI can be asymptomatic or have mild learning problems; others may manifest more severe neurodevelopmental disorders such as autism and later-onset RTT. Mutations that cause the RTT phenotype in females cause severe neonatal encephalopathy in males, with death by the second year. X-linked mental retardation with seizures, tremors, spasticity, macrocephaly, and/or bipolar disorders are seen in males with other types of MECP2 mutations that cause little or no problems in female carriers [Zoghbi, 2003].

The discovery of the MECP2 mutation as the cause of RTT provides new opportunities to characterize the clinical overlap of RTT and autism and to elucidate the neuropathological processes underlying regression in RTT and other neurodevelopmental disorders. Previous clinical studies comparing RTT and autism generally have been conducted in older postregression RTT girls. Prior to the genetic discovery, a diagnosis with certainty could not be made until the girls passed through the regression phase. MECP2 mutational analysis, along with the knowledge that most RTT girls have early developmental abnormalities, should allow early diagnosis even prior to the period of regression. Such early diagnosis should make possible careful clinical, neurophysiological, and neuroimaging characterization of the regression phase in RTT.

THE MOUSE MODEL OF RTT

The genetic discovery also provides the opportunity to develop animal models recapitulating the clinical RTT phenotype. A mouse model has been developed that has a truncated MECP2 mutation similar to one found in classic RTT (MeCP2

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Neurons are small and densely packed, consistent with a reduction in the development of axons and synaptic connections. Reduction in MAP2 staining suggests abnormal development of dendrites [Kaufman et al., 2000]. Reduced dendritic branching and a paucity of dendritic spines in pyramidal neurons further suggest abnormal synaptic development [Armstrong et al., 1995]. Similar neuropathological findings, including small neuronal size, increased packing density, and decreased dendritic branching, have been reported to occur in autism [Kemper and Bauman, 1998; Raymond et al., 1996].

Microarray analysis of gene expression in postmortem frontal cortex of RTT patients observed increases and decreases of many synaptically related genes [Johnston et al., 2001]. Both excitatory and inhibitory receptors tend to be expressed in higher than normal numbers in young RTT patients. In postmortem brain tissue, glutamate receptors, NMDA type glutamate receptors, and GABA receptors were reported elevated in RTT girls younger than 8 years; however, in those girls over 8 years, those markers were lower than in controls [Johnston et al., 2001]. Altered homeostasis of excitatory and inhibitory factors consequent to over expression of specific genes and secondary compensatory effects may contribute to disordered modulation and maintenance of synapses.

Expression of MeCP2 during development, and the relationship of MeCP2 expression to synaptogenesis, have been studied in the olfactory system which displays postnatal neurogenesis [Ronnett et al., 2003; Cohen et al., 2003]. In the intact system, MeCP2 expression increases postnatally and localizes to mature olfactory receptor neurons. Removal of the olfactory receptor neurons is associated with neurogenesis. After this lesion, MeCP2 expression in olfactory receptor neurons remains postnatally pacified at levels as cells mature. Unilateral olfactory bulbectomy removes the olfactory receptor neuron target. After this procedure, neurogenesis takes place, but functional synaptogenesis cannot occur. Following bulbectomy, MeCP2 expression is not completely restored. Thus, MeCP2 expression appears to correlate with the maturational status of the olfactory receptor neurons and to precede synaptogenesis. Based on these results, the authors hypothesize that MeCP2 is critical to maintaining and modulating synapses [Cohen et al., 2003].

Two recent reports suggest MeCP2 is important in the regulation of brain-derived neurotrophic factor (BDNF) [Chen et al., 2003; Martinovich et al., 2003], a substance that influences neuronal survival, development, and plasticity. The results of these studies suggest that increased BDNF transcription involves dissociation of MeCP2 from its promoter. Chen et al. [2003] observed that MeCP2 binds selectively to BDNF promoter III and functions to repress the expression of the BDNF gene. Calcium influx induces release of MeCP2 from BDNF promoter III and thereby leads to relief of repression of the promoter. Based on these results, Chen et al. [2003] suggest that since activity-dependent transcription plays an important role in synaptic development and plasticity, MECP2 may regulate aspects of synaptic development and maturation.

**R TT, A UTISM, A ND M ECP2**

MeCP2 expression may be abnormal in neurodevelopmental disorders without MECP2 mutations, as well as in RTT. Samaco et al. [2004] quantified MeCP2 expression on a tissue microarray containing frontal cortex samples from RTT patients, patients with other neuromuscular disorders including autism, and age-matched controls. Deficiency in MeCP2 protein expression was demonstrated in MECP2 and non-MECP2 mutation RTT individuals. Significant decreases in MeCP2 expression were also observed in three of four autism samples, a patient with Angelman’s syndrome, and four patients with Prader-Willi syndrome. In addition, an increase in MeCP2 expression was observed in one PDD sample and one autism sample. These findings suggest that both decreased and increased expression of MeCP2 may be associated with abnormal developmental phenotypes. Disregulation of MeCP2 expression may be a common downstream consequence of many neurodevelopmental disorders [Samaco et al., 2004]. Although mutations in the MECP2 gene are rarely identified in autism, other mutations or defective pathways may act to modify MeCP2 expression in these disorders.

Perhaps relevant to the studies of MECP2 mutations is the recent report of mutations in two X-linked genes encoding neuroligins, NLGN3 and NLGN4, in individuals with autism [Laumonnier et al., 2004]. Such mutations result in suppression of the transmembrane domain and sequences important for the dimerization of neuroligins, which is required for proper cell interactions through binding to beta-neurexins. The NLGN-4 gene belongs to the neuroligin family made of neuronal cell-surface proteins located in the synaptic structures. They may specifically be targeted to excitatory synapses. Intracellular neuroligin/neurexin adhesion complexes are able to trigger formation of functional presynaptic elements leading to axon specialization. Laumonnier et al. [2004] propose that NLGN4 deficiency in the brain may contribute to abnormal development of synaptic structures with resultant deficits in cognitive development and communication processing.

**CONCLUDING REMARKS**

Regression is a defining feature of RTT. In this disorder, regression is limited and is followed by some recovery of communication, social, and hand-use skills. Frequently, as RTT girls age, increasing motor dysfunction is apparent. This disease profile and other characteristic features of RTT (including hand stereotypies, gait ataxia and apraxia, and wakeful breathing abnormalities) distinguish RTT from other neurodevelopmental disorders, including autism. However, certain features of the regression stage of RTT, including impairments in communication and social skills, are observed in autistic children.

Characterization of regression in RTT may provide insight into other neurodevelopmental disorders including autism, and age-matched controls. Deficiency in MeCP2 protein expression was demonstrated in MECP2 and non-MECP2 mutation RTT individuals. Significant decreases in MeCP2 expression were also observed in three of four autism samples, a patient with Angelman’s syndrome, and four patients with Prader-Willi syndrome. In addition, an increase in MeCP2 expression was observed in one PDD sample and one autism sample. These findings suggest that both decreased and increased expression of MeCP2 may be associated with abnormal developmental phenotypes. Disregulation of MeCP2 expression may be a common downstream consequence of many neurodevelopmental disorders [Samaco et al., 2004]. Although mutations in the MECP2 gene are rarely identified in autism, other mutations or defective pathways may act to modify MeCP2 expression in these disorders.

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