Chapter 9

Rett Syndrome: A Model of Genetic Neurodevelopmental Disorders

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Additional information is available at the end of the chapter

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1. Introduction

Neurodevelopmental disorders, sometimes referred to as disorders of intellectual disability (ID), are a large family of conditions of genetic, acquired, or environmental origin that are characterized by deficiencies in cognitive and behavioral functions. While many of these disorders share similar behavioral phenotypes, they are often accompanied with other features specific to each disorder. One disorder with a unique progression is Rett Syndrome (RTT; Online Mendelian Inheritance in Man #312750; http://www.ncbi.nlm.nih.gov/omim/). RTT is the leading cause of severe ID in females, with approximately 1:10,000 females worldwide affected by this disorder. Mutations in the gene encoding the transcriptional regulator, methyl-CpG-binding protein 2 (MeCP2), located on the X chromosome (Xq28), have been confirmed in more than 95% of individuals meeting diagnostic criteria for RTT. RTT is characterized by an uneventful early infancy followed by stagnation and regression of growth, motor, language, and social skills later in development.

RTT was first described by Dr. Andreas Rett, an Austrian developmental pediatrician in the 1960's, and was also recognized about the same time by Dr. Bengt Hagberg, a Swedish child neurologist. Dr. Rett was the first to publish initial cases on a number of girls that he was following in his practice that had the same repetitive hand-washing motion with a similar neurodevelopmental phenotype (Rett 1966). Dr. Rett initially described this disorder as an association with increased ammonia levels, but it was latter discovered that this characterization was incorrect due to an improperly calibrated measurement system. These observations and subsequent characterization by Dr. Rett would go unnoticed by the broader medical community in large part related to the publication of the manuscript in a German-language medical journal. A chance meeting of Hagberg and Rett in 1981 in Toronto led to the first widely read

English language publication by Hagberg and colleagues from France and Portugal that attributed the disorder to Rett's early work. Thus, it was not until 1983, in an English-language medical journal, that Bengt Hagberg and colleagues reported 35 females that had a similar disease progression; onset around 7 to 18 months of age, with developmental stagnation followed by rapid worsening of numerous neurological functions (Hagberg, Aicardi et al. 1983).

RTT is typically diagnosed early in the life of a female and based on strict diagnostic criteria that have been extensively established (Neul, Kaufmann et al. 2010); (Percy, Neul et al. 2010). These criteria are based on the unique disease progression, which begins with stalled developmental progress after an apparently normal pregnancy and an uneventful first 6–18 months of life. After development becomes abruptly stagnated, frank regression occurs in growth, motor, language, and social skills that leads to either partial or complete loss of these skills. The next period of RTT consists of stabilization and recovery of socialization skills by age four to five that persist into adulthood. While the research into pathology and genetics of RTT has made great strides from Dr. Rett's initially characterization, a number of things have stayed constant. First, RTT is still an observational disorder; certain hallmark features that differentiate this disorder from other neurodevelopmental disorders. Second, RTT has no cure at present and treatment is based on health issues that are specific for each person.

2. Clinical features

The general overview of this disorder from a clinical perspective is a disease associated with phenotypes that vary depending on the age of the child. The developmental profile is a distinct feature of RTT that includes three unique stages. The first stage is an apparently normal gestational period and a generally ordinary period of development typically occuring until approximately 6-18 months of age. The next developmental period may last up to 4 years of age, characterized first by the stagnation of development and then the frank regression in growth, motor, language, and social skills (Percy 2002). This is the most detrimental period in the progression of the disorder with the prominent loss, partial or complete, of cognitive, social, and motor skills. The last period of RTT consists of stabilization and recovery of socialization skills by age four to five with persistence into adulthood. Due in part to better clinical understanding and immense effort that has been put forth to study this disorder, recent projections predict that individuals with RTT will live into adult life with average survival just over age 50 (Kirby, Lane et al. 2010).

In most individuals with RTT, the single, most observable and characteristic feature consists of stereotypic hand movements. These stereotypies, of yet unknown neurological origin, may consist of hand washing, wringing, squeezing, clapping, tapping, or rubbing and are evident in approximately 94% of individuals (figure 1) (Percy and Lane 2005), (Carter, Downs et al. 2010). Furthermore, they are continuous during waking hours; only subsiding when the individual falls asleep. Another motor difficulty that these individuals display is difficulty maintaining balance and ambulation. Most girls walk at some point in their life (~80%), however gait and balance issues arise, presumably from poor motor control and significant anxiety, such that about 25% lose this ability.

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Figure 1. Image of two females with Rett Syndrome, displaying stereotypical hand behavior

Behavioral issues, in addition to cognitive and social regression, are another hallmark of RTT. Heightened anxiety and mood disturbances are well-known features in many girls with RTT, and demonstrated by behaviors including difficulties maintaining posture and breathing irregularities (Mount, Charman et al. 2002; Robertson, Hall et al. 2006). RTT is considered an Autism Spectrum Disorder (ASD) due in part to the girls being withdrawn from social contact, thus demonstrating communication dysfunction (Percy 2011). It is during this period of regression that features consistent with autism emerge. They often lack the ability to respond to commands and lose the ability for verbal communication. For this reason, RTT is commonly compared to autism. However, the autistic features are generally transient in RTT, typically subsiding after the age of three-four years. At this time point, while language acquisition might not ever occur, eye gaze returns and becomes the primary form of communication with the outside world. It is believed that the description of RTT as an autistic disorder and the ability to develop non-verbal communication could be used as a diagnostic sign.

3. Diagnostic criteria

RTT is diagnosed based on observable criteria. While MeCP2 mutation occurs in an overwhelming majority of individuals diagnosed with RTT, positive mutations of the gene are not part of the diagnostic checklist, but as a supporting piece of information. In 2009 investigators from the RettSearch Consortium, an international group of clinicians and researchers revised the diagnostic criteria in order to clarify and simplify the diagnosis of classic (typical) and variant (atypical) forms (Neul, Kaufmann et al. 2010). Listed in table 1 is the set of definitive diagnostic criteria for classic RTT.

Inclusion and exclusion criteria for diagnosis of classic RTT
Period of developmental regression
Period of recovery or stabilization
Evidence of all main criteria
1. Partial or complete loss of acquired purposeful hand skills
2. Partial or complete loss of spoken language
3. Gait Abnormalities
4. Stereotypic hand movements
5. Exclusion of brain injury secondary to trauma or grossly abnormal psychomotor development in first 6 months of life

Table 1. Consensus criteria for the diagnosis of classic RTT

Additionally analysis of the RTT population through the many years of following these individuals in clinics throughout the world have noted a wide variability in severity where individuals might present with some, but not all clinical features that would signify a diagnosis of classical RTT. For this reason, a number of recognized atypical forms have been characterized and documented based on unique characteristics (Neul, Kaufmann et al. 2010). The atypical forms that have been established consist of preserved speech, early seizure, and congenital variants (Hagberg and Skjeldal 1994). The RettSearch Consortium provided consensus criteria and core features essential for diagnosis of atypical forms of RTT (table 2).

Diagnosis of atypical forms of RTT
Period of developmental regression followed by period of stabilization
Meet at least 2 of 4 phenotypes from the main criteria
Meet at least 5 of 11 supportive criteria
Supportive Criteria
1. Abnormal muscle tone
2. Breathing disturbances when awake
3. Bruxism
4. Diminished response to pain
5. Growth retardation
6. Impaired sleep pattern
7. Inappropriate laughing or screaming spells
8. Intense eye communication
9.Peripheral vasomotor disturbances
10. Scoliosis/kyphosis
11. Small/cold hand and feet

Table 2. Consensus criteria and core features essential for diagnosis of atypical forms of RTT; preserved speech, early seizure, and congenital variants

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4. Physical features and pathophysiology

From an initial observation, individuals with RTT tend to be very small in terms of height for their age, nearly 2-3 standard deviations from normal (Hagberg, Stenbom et al. 2001). Furthermore, a deceleration of the rate of height and weight is also a characteristic of this disease (Schultz, Glaze et al. 1993). Given that individuals with RTT tend to be very small, it is no surprise that their feet and hand growth are also often stunted.

Many studies have been conducted that have examined head size and brain growth. Head circumference in RTT individuals is also very small for their age and head growth deceleration occurs as early as 1.5 months of age (Tarquinio, Motil et al. 2012 *in press*). Similar to body height, head circumference was also found to be 2-3 standard deviations below values estimated in healthly individuals (Hagberg, Stenbom et al. 2001). Furthermore, RTT brains typically weigh less than those from unaffected individuals (Armstrong 2005). Microscopic studies of brains from autopsy samples have demonstrated that neurons from numerous cortical areas, including the hippocampus and thalamus, are smaller in size and densely packed, without evidence of neurodegeneration or gliosis (Bauman, Kemper et al. 1995; (Armstrong 2005).

It has long been suggested that disorders related to ID have been associated with structural irregularities in neuronal connectivity (Fiala, Spacek et al. 2002) (Kaufmann and Moser 2000). This well supported notion was developed from pioneering studies in the 1970's that described striking abnormalities in the dendritic morphology of cortical neurons obtained from postmortem brain samples from individuals with a number of different disorders associated with ID (Huttenlocher 1970; Marin-Padilla 1972; Huttenlocher 1974; Purpura 1974). From this standpoint, a number of studies have been conducted that provide evidence to suggest that reduced or altered neuronal connectivity at synapses is a characteristic of RTT. To support this notion, magnetic resonance imaging demonstrated a reduction in gray matter volume in the parietal lobe in addition to reductions in cortical white matter (Bauman, Kemper et al. 1995; Carter, Lanham et al. 2008). Consistent with these findings, dendritic growth and arborization are reduced in RTT (Belichenko, Oldfors et al. 1994; Armstrong, Dunn et al. 1995; Chapleau, Calfa et al. 2009).

Another fascinating discovery that was uncovered during these series of observations from the 1970's was the description of alterations in dendritic spines on the postsynaptic side of excitatory synapses. Postmortem observations described reductions in dendritic spine densities in addition to changes in their shape, commonly referred to as "spine dysgenesis" (Purpura 1974). Confocal imaging studies using postmortem brains from individuals diagnosed with RTT or control individuals, have demonstrated a decrease in the density of dendritic spines in several areas of the brains including the somatosensory cortex and hippocampus (Belichenko, Oldfors et al. 1994; Armstrong, Dunn et al. 1995; Chapleau, Calfa et al. 2009). Molecular analysis of postmortem brains further supports this notion, as studies have found that the expression of microtubule-associated protein (MAP-2), a protein involved in microtubule stabilization and a key cytoskeletal component of dendrites, is reduced in the cortex of RTT individuals (Kaufmann, Naidu et al. 1995; Kaufmann, Taylor et al. 1997). Further-

more, expression levels of cyclooxygenase, a protein enriched in dendritic spines is also reduced in RTT cortex (Kaufmann and Moser 2000). With regard to density of synapses, reduced levels of the synaptic vesicle protein, synaptophysin, were detected in the motor, frontal and temporal cortices by immunofluorescence (Belichenko, Hagberg et al. 1997). Intriguingly, glutamate receptor density has a differential distribution during RTT development, where younger individuals with RTT have a higher density compared to controls, while older individuals have a lower density compared to controls (Blue, Naidu et al. 1999). These results suggest that an increase in glutamate receptor density may be a compensation for the reduction in dendritic spines.

In addition to these various anatomical brain findings, a number of alterations have been discovered in its neurochemistry. Specific areas of the brain including the midbrain and caudate, which produce a number of neurotransmitters, have been shown to have reduced volume compared to healthy individuals (Reiss, Faruque et al. 1993). In support of this finding, tyrosine hydroxylase staining was reduced in the substantia nigra, an important region for dopamine production (Jellinger, Armstrong et al. 1988). Furthermore, cerebrospinal fluid (CSF) measurements of RTT individuals, demonstrated a reduction in the dopamine metabolite, homovanillic acid, and the serotonin metabolite, 5-hydroxyindoleacetic acid (Samaco, Mandel-Brehm et al. 2009).

A number of other structural and physical abnormities have been demonstrated. One striking physical finding can be made upon observation and by touching an individual's feet. In many individuals with RTT, feet are not only cold to touch, but may appear blue or purplish in color. It is assumed that defective autonomic function is the pathological culprit; however the exact mechanism for this has yet to be determined. Another finding that might relate to dysfunction in autonomic signaling is abnormal cardiac conduction. A feature that has been observed in a number of individuals diagnosed with RTT is a prolonged QT interval, where the timing between ventricle depolarization and repolarization is delayed, which is estimated to occur in approximately 20% of individuals (McCauley, Wang et al. 2011) (Sekul, Moak et al. 1994). For this reason, at the time of the diagnosis of RTT (and every one to two years thereafter), one of the first tests to be ordered is an electrocardiogram (EKG), in order to guard against the sudden death risk with prolonged QT intervals.

Another condition that arises in the RTT population, presumably because of altered metabolic requirements, lack of physical mobility, and their small stature, is osteopenia, a condition characterized by reduced bone mineralization and increased likelihood of fractures (Haas, Dixon et al. 1997). Reduced bone size and lower bone mass have been detected in the RTT population (Roende, Ravn et al. 2011). Lastly, irregularities in the motility of the gastric region and esophageal tract lead to the development of gastroesophageal reflux, which is a major cause of pain and discomfort (Motil, Schultz et al. 1999). Abnormalities in gallbladder function, in addition to the accumulation of gallstones, have been identified as a serious concern in the RTT population and may require surgical assessment (Percy and Lane 2005).

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5. Genetics and MeCP2

A number of clinical observations linked this disorder to a genetic mutation of *de novo* origin. First, RTT predominantly affected female individuals; second, RTT rarely recurred in the same family, and third, RTT was a worldwide disorder that affected all racial and ethnic groups, not commonly associated with a pattern of inheritance. In the 1990's after much early speculation, numerous studies determined that RTT was indeed caused by a genetic mutation. First, mapping studies using RTT families mapped the mutation to the X chromosome with subsequent analyses localizing the likely gene at Xq28, confirming an X-linked dominant pattern of inheritance (Archidiacono, Lerone et al. 1991; Ellison, Fill et al. 1992; Sirianni, Naidu et al. 1998). After this lengthy study of the X chromosome, a hallmark paper was published in 1999 identifying the gene encoding methyl-CpG-binding protein 2 (*MECP2*) (Amir, Van den Veyver et al. 1999).

More than 95% of individuals with classic RTT carry a *de novo* mutation in the gene encoding *MECP2*. To date, more than 250 different mutations of *MECP2* have been identified in the RTT population, with about 60% of the mutations coming from 8 specific point mutations involving the following amino acid changes (R106W, R133C, T158M, R168X, R255X, R270X, R294X, R306C) (Williamson and Christodoulou 2006). Interestingly, mutations on *MECP2* have only been identified in approximately ~75% of atypical or variant RTT (Percy, Lane et al. 2007). Characterizations of *MECP2* mutations through genetic testing provide molecular confirmation of the diagnosis of RTT but should not be used as the sole diagnostic factor, as roughly 5% of the classical RTT population do not have defined mutations of MeCP2, but meet the clinical criteria (Neul, Fang et al. 2008). While the reason that the *MECP2* gene is susceptible to mutations is not understood, the overwhelming majority of RTT cases are a result of spontaneous *de novo MECP2* mutations in the paternal X chromosome germlines (Trappe, Laccone et al. 2001). However, some families do exist where *MECP2* mutations are present throughout multiple generations (Augenstein, Lane et al. 2009).

MeCP2, a member of the methyl-CpG-binding domain (MBD) family of transcriptional regulator proteins, is encoded by a ~76kb gene located on chromosome Xq28 and constructed from four exons associated with two protein isoforms, MECP2_e1 and MECP2_e2. The MeCP2 protein has two major functional domains; the methyl-binding domain (MBD), consisting of 85 amino acids that binds specifically to DNA at methylated CpG's, and the transitional repressing domain (TRD), consisting of 104 amino acids, that is responsible for recruiting other proteins to form complexes that mediate transcription of various genes (Guy, Cheval et al. 2011). MeCP2 binds specifically to A/T rich sites in close proximity to CpG-methylated DNA sites, working with other proteins in recruiting co-repressors and histone deacetylase complexes, thereby altering the structure of genomic DNA and modifying the transcription of specific target genes (Klose and Bird 2006). Recent studies have demonstrated that MeCP2 has both repressor and activator transcription activities (Chahrour, Jung et al. 2008). MeCP2 has been shown to be tightly bound to DNA at all times and its transcriptional control activity is regulated by post-translational

modifications such as phosphorylation and acetylation, that alter the conformational shape of DNA, enabling or repressing transcription of a potential target gene (Skene, Illingworth et al. 2010; Cohen, Gabel et al. 2011). Furthermore, a role of MeCP2 in RNA splicing has also been hypothesized due to its ability to interact with the RNA-binding protein, Y box-binding protein (Young, Hong et al. 2005).

6. MeCP2 function

The precise mechanism of dysfunction of mutated MeCP2 that is responsible for RTT symptomatology remains unknown. MeCP2 is highly expressed in the brain and is critical for the development and maturation of neurons (Akbarian, Chen et al. 2001);,(Jung, Jugloff et al. 2003);,(Mullaney, Johnston et al. 2004). Recent reports suggest that the MeCP2 is also expressed in glial cells and altered function of glial cells might be another reason for disease progression (Ballas, Lioy et al. 2009);,(Maezawa, Swanberg et al. 2009);,(Maezawa and Jin 2010). Expression of MeCP2 in humans and mice increases with neuronal development and maturation (Shahbazian et al., 2002). In addition, the expression levels of MeCP2 control the development of excitatory synapses early in postnatal development (Chao, Zoghbi et al. 2007). Since MeCP2 expression in cortical areas increases during neuronal development, it is assumed that MeCP2 is crucial for axonal and dendritic differentiation during the first 6–18 months of age leading to proper synapse formation and maturation. Furthermore, new data suggests that MeCP2 expression is also important in adulthood, as MeCP2 removal during later stages of postnatal/adult development, altered the density of excitatory synapses and the expression of synaptic proteins involved with maintaining synapses (Nguyen, Du et al. 2012).

The functions of MeCP2 have been shown to extend beyond its importance in the development and maintainence of synapses in the brain. It has been shown that MeCP2 regulates the balance between excitatory and inhibitory transmission. It is unknown exactly how MeCP2 governs the balance between glutamate and GABA, but recent studies suggest that altered MeCP2 might also be expressed on non-neuronal brain cells (i.e., oligodendroglia and astrocytes) that regulate the uptake of glutamate (Maezawa and Jin 2010). Astrocytes from mutant *Mecp2* mice co-cultured with wild-type neurons caused significant dendritic damage to the neurons. In support of these findings, recent studies using hippocampal slices from *mecp2* mutant mice demonstrate these tissues to be extremely hyperexcitable (Calfa, Hablitz et al. 2011). Furthermore, studies in null mice demonstrated that GABAergic synaptic transmission is weakened in the ventrolateral medulla region of the brain stem (Chao, Chen et al. 2010).

Another important finding related to MeCP2 function relates to the concept of homeostatic plasticity, a type of plasticity whereby an optimal level of transmission is regulated by modulating the strength of excitatory and inhibitory synapses. Recent evidence has demonstrated that MeCP2 functions in synaptic scaling, a form of homeostatic plasticity that regulates the strength of excitatory synapse currents in response to neuronal activity (Qiu, Sylwestrak

et al. 2012). These data suggest that MeCP2 is involved with balancing network excitability. Since glutamate levels tend to be increased in individuals with RTT, in addition to the common occurrence of seizures (Glaze, Percy et al. 2010), mechanisms responsible for modulating glutamate transmission or increasing GABAergic transmission need to be explored as a treatment option.

7. Disease severity

As we discussed previously, wide variability in phenotypic severity is observed in RTT. Since the gene for MeCP2 resides on the X-chromosome, the balance of X-chromosome inactivation (XCI) plays an important factor in disease severity. Studies have shown that individuals with classical RTT have XCI that has a random distribution, whereas nonrandom XCI is associated with milder phenotypes (Amir, Van den Veyver et al. 2000). However, a dramatic example of the importance of XCI in RTT comes from a family in which the same *MECP2* mutation was present in several family members (Augenstein, Lane et al. 2009). The mother, had a 44bp mutation, passed the mutation to a daughter with classical RTT and a son with a progressive neurological disorder. The mother, who had some cognitive deficits, does not have any features of RTT and has an XCI ratio that favors the "good" X-chromosome (89:11).

In addition to XCI, another factor to consider is the genotype-phenotype relationship. Various mutations or types of mutation tend to be associated with different phenotypes. For the most part, nonsense mutations, mutations that cause a premature stop transcription, tend to produce more severe effects than missense mutations. For instance, the R133C mutation is associated with a less severe phenotype compared to the R168X mutation, one of the most severe mutations as individuals tend not to walk, use hands, or speak (Neul, Fang et al. 2008). Furthermore, missense mutations in the TRD tend to have a milder phenotype (Schanen, Houwink et al. 2004).

8. Treatment options

Therapeutic management for these individuals can be quite complicated when taking into account their specific neurodevelopmental phenotype. Research into the clinical management of RTT relies on focusing on present day treatment using FDA approved agents and future research using newly found molecules. Moreover, the present day treatment research focuses on reviewing the entire spectrum of symptoms that relate to RTT, while future treatment options focus on discovering molecules that could cure the entire disorder. Conducting clinical trials in this population is challenging as stratification of participants requires careful planning. Additionally as a result of the difficulty in performing clinical trials in these individuals, few results have come from previous clinical trials that have provided unquestionable therapeutic recommendations. An issue in performing studies in the RTT pop-

ulation is the huge variability in disease severity, which makes comparison between groups an extremely challenging task. Trials using the drug naltrexone to blocked the observed increase in beta-endorphin expression, appeared to diminish motor behavior overall (Budden, Myer et al. 1990) (Percy, Glaze et al. 1994). Other clinical trials have been conducted in the RTT population to examine the potential effectiveness of the vitamin, folate, on disease progression. Results from the study demonstrated no major improvement in objective measurements (Glaze, Percy et al. 2009).

Numerous strategies to cure RTT have been proposed that are based on preclinical evaluation of potential pharmacological agents or based on the proposed mechanism of MeCP2 dysfunction. One issue to address in treatment options is to determine if a specific point in development exists where treatment has to be initiated to rescue function. Studies have shown symptom resolution can be accomplished in MeCP2 null transgenic mice by simply turning on the expression of MeCP2 in adult mice. Using an insertion of the *lox-stop* cassette into the Mecp2 gene of mice, where tamoxifen administration removed the stop cassette causing *Mecp2* expression to be activated fully, symptomatic mice were noted to have markedly diminished severity of RTT-like phenotypes. (Guy, Gan et al. 2007).

These studies suggest that for a potential curative agent, therapy could be initiated at any time point in development and lead to potential benefit. Potential therapies that could be developed stem from these basic properties of MeCP2. The first option revolves around the lifespan of mutant proteins. Transgenic mice were created with a mutation in the methyl binding domain of the MeCP2 protein (T158A), causing a reduction in MeCP2 binding affinity to methylated DNA and reducing the protein's half-life (Goffin, Allen et al. 2011). Thus a potential therapeutic option is to increase either the protein's expression or half-life, granted that mutant MeCP2 proteins do not lead to a gain of function deficit. Another potential therapy relates to the location of the *MECP2* gene and the modulation of XCI. When XCI is randomly distributed in RTT, individuals tend to be more severely affected compared to situations of nonrandom XCI where a greater percentage of cells express the normal allele (Amir, Van den Veyver et al. 2000). By taking advantage of this process, if the normal allele is turned on and the mutate allele is turned off, it is possible that symptom improvement would occur. In an overwhelming majority of instances, *de novo MECP2* mutations have been identified in the paternal X chromosome (Trappe, Laccone et al. 2001). If this is the case, by identifying the locus of the mutation, a practical approach could be developed to turn off the mutant allele and activate the normal allele. This is a technically challenging, but potentially exciting strategy.

Another potential therapy that revolves around modulating the existing genetic environment is by using a molecule that allows read-through of premature STOP codons in mutant genes. Nonsense mutation in *MECP2* resulting in premature transcription termination occurs in approximately 35% of North American RTT patients (Percy, Lane et al. 2007). Aminoglycosides are antibiotics that are used today against resistant gram-negative bacteria, but studies have shown that they also have potential as pharmacological agents to overcome transcriptional termination caused by nonsense mutations (Rowe and Clancy 2009),(Zingman, Park et al. 2007). In various models, the full-length MeCP2 protein was discovered in different cell cultures expressing nonsense mutations after incubation with an aminoglyco-

side antibiotic. (Brendel, Klahold et al. 2009),(Brendel, Belakhov et al. 2011),(Popescu, Sidorova et al. 2010). From a functional standpoint, iPSC-derived neurons from RTT patients expressing nonsense mutations, when treatment with the aminoglycoside gentamicin was employed, demonstrated increased dendritic spine density (Marchetto, Carromeu et al. 2010). While a potentially useful molecule has yet to be tested in transgenic mouse expressing a nonsense MeCP2 protein, this particular approach might be of great promise for this subset of the RTT population.

Another option revolves around the use of the growth factor, BDNF, a member of the neurotrophin family of growth factors that have essential roles in neuronal survival and differentiation in early development and a strong modulator of synaptic transmission and plasticity in the mature brain (Amaral, Chapleau et al. 2007). BDNF protein levels measured by ELISA were found to be lower in brain samples of *Mecp2* mutant mice (Chang, Khare et al. 2006). Intriguingly, crossbreeding *Bdnf* heterozygous mice with *Mecp2* mutant mice exacerbated the onset of the RTT-associated phenotypes.(Chang, Khare et al. 2006) More importantly, BDNF mRNA levels are lower in brain samples from RTT patients, similar to the finding described in MeCP2 mutant mice (Deng, Matagne et al. 2007).

Promising work has shown that the genetic overexpression of BDNF can rescue some of the deleterious consequences of MeCP2 dysfunction. For example, crossbreeding *Bdnf* overexpressing mice with *Mecp2* mutants alleviated numerous phenotypes, including motor hypoactivity, reduced activity of cortical neurons, in addition to extending their lifespan (Chang, Khare et al. 2006) Consistently, *BDNF* overexpression in neurons transfected with RTT-associated *MECP2* mutations or with *Mecp2* shRNA to knockdown its expression reversed dendritic atrophy in primary hippocampal neuron cultures (Larimore, Chapleau et al. 2009). While the genetic overexpression of BDNF is promising in rodent models, the administration of BDNF is not a useful clinical approach due to its short half-life and inability to cross the blood–brain barrier.(Kingwell 2010) However, small molecules that can mimic BDNF's effects or that can increase the levels of endogenous BDNF are attractive potential therapeutic options. Small molecules that act like BDNF, so called "BDNF mimetics," are blood-brain barrier permeable factors and have shown promise to reverse RTT-like features in experimental mouse models. Heterozygous female *mecp2* mutant mice, treated with LM22A-4, rescued breathing abnormalities and increased TrkB phosphorylation in areas of the brain central for respiration, medulla and pons.(Schmid, Yang et al. 2012) 7,8-DHF delayed body mass deficits and improved wheel running and breathing impairments in mutant male mice.(Johnson, Lam et al. 2011) While these agents present limited supporting research, they do offer hope of targeting BDNF without administering the actual protein.

Another potential option that has recently gained much attention as therapeutic treatment of RTT individuals is the pleiotropic growth factor insulin-like growth factor-1 (IGF-1). Unlike BDNF, IGF-1 crosses the blood-brain barrier and gains access to the CNS. IGF-1 stimulates proliferation of neural progenitors, neuronal survival, neurite outgrowth, and synapse formation (D'Ercole, Ye et al. 1996),(D'Ercole, Ye et al. 2002). Consistent with these BDNF mimetic actions, daily injections of the active tri-peptide fragment of IGF-1 improved motor function, breathing rhythm and cardiac irregularities, in addition to increased brain weight

in *Mecp2* mutant mice (Tropea, Giacometti et al. 2009). The active tri-peptide also improved a number of synaptic features, including dendritic spine density in pyramidal neurons of layer V of the motor cortex. In the same region of the cerebral cortex, IGF-1 restored the motility of dendritic spines, a process crucial for synaptic development and plasticity (Landi, Putignano et al. 2011). Since the full-length IGF-1 is approved by the Food and Drug Administration for the treatment of growth failure in children that were unresponsive to treatment with growth hormone,(Fintini, Brufani et al. 2009) a clinical trial is currently underway to determine if administration of Mecasermin (Increlex®), a synthetic analog of full-length IGF-1 improves the symptoms and health of RTT patients (ClinicalTrials.gov identifier: NCT01253317).

9. Conclusions

It should also be noted that other genes have been linked to variant RTT, including cyclin-dependent kinase-like 5 (CDKL5), FOXG1, and the Netrin G1 genes. Future research examining MeCP2 dysfunction needs to consider the influence of MeCP2 mutations on other disorders.

The greatest challenge at present is to develop translational research that implements unique options developed at the basic science level and moves them efficiently and smoothly to the clinical setting. Furthermore, since many common neurobiological mechanisms exist in the spectrum of neurodevelopmental disorders, understanding the key components might hasten the progress of novel treatment for all these unique and devastating disorders. As the clinical and basic science understanding of RTT has unfolded, key points of interaction have been targeted to approach its understanding and potential management. From the clinical perspective, the array of medical issues includes epilepsy, periodic breathing, altered growth patterns, gastrointestinal dysfunction, and significant orthopedic issues including scoliosis. From the basic science viewpoint, the epigenetic role of *MECP2*, with emphasis on known elements such as brain derived neurotrophic factor and corticotrophin releasing hormone, and the broad impact on neural function have led to exciting opportunities for therapeutic intervention currently receiving intense scrutiny at the translational level at the same time that clinical investigation and intervention, particularly related to augmentative communication strategies are increasing. Future research regarding the treatment of RTT must rely on determining which dysregulated genes contribute to a specific symptom or symptom cluster, and what drug therapy might overcome this dysfunction.

List of abbreviations

ASD autism spectrum disorder

CDKL5 cyclin-dependent kinase like 5

CSF cerebrospinal fluid

ID Intellectual disability

MAP-2 Microtubule-associated protein-2

MBD methyl binding domain

MeCP2 methyl-CpG-binding protein 2

Human gene: MECP2

Human protein: MeCP2

Mouse gene: Mecp2

Mouse protein: Mecp2

RTT Rett syndrome

TRD Transitional Repressor Domain

XCI X chromosome inactivation

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