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Evaluation of Current Pharmacological Treatment Options in the Management of Rett Syndrome: From the Present to Future Therapeutic Alternatives

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Abstract

Neurodevelopmental disorders are a large family of conditions of genetic or environmental origin that are characterized by deficiencies in cognitive and behavioral functions. The therapeutic management of individuals with these disorders is typically complex and is limited to the treatment of specific symptoms that characterize each disorder. The neurodevelopmental disorder Rett syndrome (RTT) is the leading cause of severe intellectual disability in females. Mutations in the gene encoding the transcriptional regulator methyl-CpG-binding protein 2 (*MECP2*), located on the X chromosome, have been confirmed in more than 95% of individuals meeting diagnostic criteria for classical RTT. RTT is characterized by an uneventful early infancy followed by stagnation and regression of growth, motor, language, and social skills later in development. This review will discuss the genetics, pathology, and symptoms that distinguish RTT from other neurodevelopmental disorders associated with intellectual disability. Because great progress has been made in the basic and clinical science of RTT, the goal of this review is to provide a thorough assessment of current pharmacotherapeutic options to treat the symptoms associated with this disorder. Furthermore, we will highlight recent discoveries made with novel pharmacological interventions in experimental preclinical phases, and which have reversed pathological phenotypes in mouse and cell culture models of RTT and may result in clinical trials.

Keywords

MeCP2; IGF-1; BDNF; read-through aminoglycosides

1. Introduction

Neurodevelopmental disorders are associated with a prevailing deficiency in cognitive function and behavioral adaptations. While many neurodevelopmental disorders share similar behavior phenotypes, each condition is very much a disease unto itself. These syndromes are often accompanied with other features specific to each disorder. Thus therapeutic management for these individuals can be quite complicated when taking into account their specific neurodevelopmental disorder.

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One disease 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 intellectual disability in females, with approximately 1:10,000 females worldwide affected by this disease and without predisposition to a particular race or cultural ancestry. The disease affects only females due to a mutant gene on the X chromosome that is essential for *in utero* development. This review will discuss the genetics, pathology, and symptoms that characterize RTT. The goal of this review is to provide a complete evaluation of current therapeutic options for RTT individuals. Specifically, we will discuss selected aspects of this disease and treatment plans that need to be addressed when caring for these patients. Lastly, we will review the recent history of discoveries that have been made in RTT research focusing on therapies that have reversed pathological phenotypes in mouse and cell culture models of RTT.

2. Diagnosis and Phenotype

RTT is typically diagnosed early in the life of a female. The RettSearch Consortium has provided an extensive review of the diagnostic criteria that define typical cases of RTT, in addition to atypical or variant forms.[1,2] Generally, birth and the milestones of a female's early development are uneventful and occur apparently normally. However, two distinct developmental periods occur that lead to the diagnosis of RTT. At approximately 6–18 months of age, a delay in development occurs: this time point is characterized by the halted progression of growth, motor, language, and social skills. However, a second phase occurs after the initial stagnation of development and may extend up to 4 years of age, when a rapid period of developmental regression occurs.[3] At this point in time, acquired cognitive, social, and motor skills are rapidly lost. A period of symptom stabilization occurs thereafter, but the established deficits persist as the child develops into adulthood. From this symptomatology, RTT is believed to be a disease of arrested development.

Other features of the disease include stereotypic hand movements, periodic breathing, gait dysfunction, dystonia, and increasing rigidity.[4] In addition, cognitive regression is a hallmark of RTT: similar to patients diagnosed with autism-spectrum disorders, RTT individuals are typically withdrawn from social contact and experience communication dysfunction during the regression period.[5] However, this social dysfunction seems to be temporary in nature, because social contact and eye gaze are markedly improved after three years of age and allow rich non-verbal communication with their caregivers. RTT individuals typically survive into middle age, and current projections estimate they may survive even longer.[6]

3. The Genetics of RTT and MeCP2

It is estimated that more than 95% of individuals with RTT carry a mutation in the gene encoding methyl-CpG-binding protein 2 (*MECP2*).[7] MeCP2 is a nuclear protein encoded by a gene located in chromosome Xq28.[8] To date, more than 200 mutations have been identified in *MECP2*, but only 8 mutation types account for approximately 60% of RTT cases.[4] The overwhelming majority of RTT cases are *de novo MECP2* mutations in the paternal X chromosome expressed in sperm.[9] However, some families do exist where *MECP2* mutations are present throughout multiple generations.[10]

MeCP2 was originally discovered as a methylated DNA binding protein with transcriptional repressing activity, working with other proteins to alter the structure of genomic DNA.[11] Recent studies have demonstrated that MeCP2 has both repressor and activator transcription activities.[12] MeCP2 has other functions besides those of a classical transcriptional regulator in the nucleus, such as RNA binding and regulation.[13] For example, MeCP2 might also be involved in regulating RNA binding and splicing of reporter minigenes, as it has been shown

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to interact with the RNA-binding protein, Y box-binding protein (YBX1). [14] Recently, it has been suggested that MeCP2 is tightly bound to DNA all the time and its transcriptional control activity is regulated by post-translational modifications such as phosphorylation and acetylation, very much like a histone.[15] MeCP2 is highly expressed in the brain and is critical for the development and maturation of neurons.[16,17,18] 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.[19,20,21] While mutations in MeCP2 are highly correlated with the diagnosis of RTT, the specific functions of MeCP2 that are impaired by mutations and are responsible for RTT symptomatology remain a mystery.

4. RTT Neuropathology

Head circumference in RTT individuals is normal at birth, but a period of abnormal head growth deceleration occurs as early as 1.5 months of age (Tarquinio, Lane & Percy, personal communication). In autopsy material, RTT brains typically weigh less than those from unaffected individuals, after parameters of age and height are normalized. Neither neurodegeneration nor gliosis have been described in RTT.[22] At the macroscopic level, imaging studies have demonstrated a reduction in gray matter in the cerebral cortex.[23] Consistently, RTT neurons are smaller in size and more densely packed in cortex, including the hippocampus, and the hypothalamus.[24,25] Furthermore, dendritic growth and arborization are reduced, suggesting abnormal neuronal connections.[26] In addition, the density of dendritic spines, the postsynaptic side of excitatory synapses, is reduced in several brain regions including cortex and hippocampus.[27,28] 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. Furthermore, expression levels of cyclooxygenase, a protein enriched in dendritic spines is also reduced in RTT cortex.[29] Together, these results strongly suggest that RTT is associated with deficient dendritic arborization and neuronal connectivity at synapses.

5. Clinical Management of RTT symptomatology

In the next sections, we will highlight areas of RTT symptomatology and what therapeutic interventions can be used to manage the clinical presentation. Recent projections predict that RTT patients will live into adult life, indicating the need for careful planning for long-term care of these individuals.[6] In the future, as early diagnosis and clinical management of RTT improves, it is possible that longevity will be extended even further. Thus, the need for careful planning for the future care of these women needs to be addressed. Our goal in this review is to provide a complete evaluation of current pharmacotherapeutic options to treat the symptoms that are associated with RTT. We will discuss specific aspects of this disease and treatment plans that need to be addressed when caring for these patients. Lastly, we will review the recent history of discoveries that have been made in RTT research, focusing on future therapies that are currently in pre-clinical investigation.

5.1 Sleep Disorders

Sleep is often disrupted in RTT individuals. They may have difficulty falling to sleep or they may suffer from frequent awakenings during the middle of the night.[30] When presented with sleep deficits, a number of disease states need to be ruled out before determining if medication for sleep would be appropriate. Otitis media needs to be ruled out as this infection can prevent sleep. Otitis media infections should be treated with an appropriate antibiotic.

Respiratory rhythm is quite irregular in the waking state but typically normal during sleep; however, a sleep consultation is required to rule out possible airway obstructions.[31] Other

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disease states that need to be considered are gastrointestinal dysfunction (see below). Gastrointestinal pain as a result of constipation or gastroesophageal reflux disease (GERD) is something that needs to be considered when determining reasons why sleep is disrupted.

Proper sleep hygiene is essential for better general health in RTT individuals. A number of medications are effective to treat sleep disturbances in the RTT population. Trazodone, a serotonin receptor modulator, is safe to initiate sleep. Also, the sedating agent chloral hydrate can be used safely to aid sleep induction. The liquid formulation of chloral hydrate is the most common preparation, which has a strong bitter aftertaste making it unpalatable to some patients. Liquid chloral hydrate can be made less bitter with the addition of flavoring agents formulated by compounding pharmacies (C. Chapleau, L. Wolsoncroft, J. Lane & A. Percy, personal communication).

Melatonin has been shown to be a potential treatment option for the management of sleep in RTT. Melatonin is an endogenously produced hormone that is secreted by the pineal gland and has long been demonstrated to be important for maintaining circadian cycles. The use of melatonin appears to be suitable for the initiation of sleep, not for sleep maintenance.[32] After the first three weeks of melatonin treatment, RTT patients with poor sleep hygiene, appeared to have improved sleep duration.[33] An independent study confirmed these findings and further demonstrated that the discontinuation of melatonin was associated with recurrence of sleep disturbances.[34] An open label trial of L-carnitine, an amino acid derivate of methionine and lysine that is required for energy metabolism, was shown to be beneficial in sleep maintenance in a subset of RTT patients.[35]

5.2 Cardiac Dysfunction

A critical consideration regarding RTT is the incidence of sudden death that occurs in this population. In fact, it is estimated that the rate of sudden death is greater in the RTT population as compared to the non-RTT population.[36] Various possibilities can be attributed to this pathophysiological consequence. One reason is a failure of the respiratory system to function properly. Respiratory dysfunction is common in RTT and is characterized by numerous breathing alterations, including hyperventilation and breath holding. It has been suggested that respiratory malfunction is a direct result of altered synaptic connectivity in the brain stem of RTT.[31] For instance, GABAergic synaptic transmission is weakened in the brain stem (ventrolateral medulla) of Mecp2 null mice.[37] Additional, studies in mutant *Mecp2* mice have demonstrated that various neurochemical signaling factors essential for synaptic connectivity and respiratory function are altered in the brainstem.[38] Furthermore, since respiratory issues are prominent in RTT patients, avoiding medication that lower respiratory rate would prevent possible deadly adverse drug responses.

Another issue that relates to the sudden death risk is irregularities in cardiac contractility in the RTT population. At the time of the diagnosis of RTT, one of the first tests to be ordered is an electrocardiogram (EKG). A prominent feature is the observation of a prolonged QT interval, where the timing between ventricle depolarization and repolarization is delayed in RTT patients as compared to age-matched healthy females.[39] An abnormal rhythm will allow for abrupt therapeutic intervention to management of the prolonged QT interval, thus reducing the risk for sudden death. The prominent treatments for the management of QT prolongation in the general population are beta-blockers.[40] The prolongation of the QT interval has been recapitulated in female and male *Mecp2* null mice. Interestingly, the use of beta-blockers was not effective in preventing altered cardiac rhythm in *Mecp2* null mice and suggests that standard therapy might be insufficient in the treatment of cardiac issues in RTT.

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Avoiding certain drugs in RTT individuals that have developed prolonged QT interval will also help to avoid cardiac dysfunction.[41] For example, macrolide antibacterial agents might not be a first line antibiotic for the treatment of a bacterial infection because of the risk to elicit changes in the QT interval. When treating RTT individuals, reviewing and preventing drug-drug interactions that elicits an increase QT interval will prevent adverse drug reactions from occurring.[42,43] The following website can be utilized to determine a drug's potential to slow the QT interval http://www.qtdrugs.org. Several other accounts of cardiac dysfunction have been observed in the RTT population, which indicates a potential role of MeCP2 in cardiac functioning.[36]

5.3 Bone Mass and Fractures

Osteopenia, a condition characterized by reduced bone mineralization and increased likelihood of fractures, is a concern in the RTT population.[44] Bone loss occurs typically in all people but especially in post-menopausal women, and continues as females continue to age. RTT patients, because of their small stature, have been shown to have reduced bone size and lower bone mass.[45] By some estimates, RTT patients are at 4 times greater risk of sustaining a fracture than the general population.[46,47] Furthermore, RTT patients are prone to sustain low-energy fractures.[48]

The reason RTT patients are prone to reduced bone mass and fracture remains unknown. The lack of ambulation and inadequate diet are two reasons that have been identified. Reduced vitamin D levels are another reason that contributes to an increased risk of osteopenia.[45,49] As a result of its importance for bone formation, vitamin D levels should be constantly evaluated in RTT patients. Anatomical findings from RTT cases have shown a reduction in bone formation, a result consistent with findings of inadequate levels of vitamin D.[50] If vitamin D deficiency is observed, supplementation with vitamin D is required. If risk factors for fracture exist (i.e. inability to walk or inadequate food intake), prophylactic treatment of vitamin D needs to be considered. Another therapy that might be of consideration is the family of drugs called bisphosphonates, which inhibit bone resorption thus increasing the density of mineralized bone. Clinical trials need to be conducted to determine if bisphosphonates prevent degradation of bone and potential fractures in the RTT population. However, these bisphosphonates have adverse side effects, such as osteonecrosis and gastrointestinal pain, as well as increased risk of non-vertebral fractures after long-term use,[51,52] which warrant further risk-to-benefit analyses before prescription to RTT individuals.

Research on MeCP2-based mouse models are beginning to identify mechanisms responsible for decreased bone mass in RTT individuals, such as decreased activity of bone cells responsible for bone formation.[53] Examination of drug-disease interactions is also warranted. For example, anti-epileptic drugs (AED) alter the metabolism of bone, which increases the risk of fracture in individuals on AEDs.[54] Similarly, long-term use of carbamazepine, phenytoin, and valproic acid (but not lamotrigine) reduces blood Ca^{2+} levels.[55] Additional studies report increased the risk of fractures in RTT individuals on valproic acid.[56] Thus, the use of AEDs in a population at risk of reduced bone mass density needs to be closely monitored for alterations in bone structure.

5.4 Behavioral Alterations

Anxious and fearful behaviors, as well as general mood disturbances are prominent features in many girls with this disorder, particularly so with certain *MECP2* mutations (R133C, R294X and R306C).[57,58] Clinical behaviors associated with heightened anxiety in RTT include difficulties maintaining sitting and standing posture and irregularities in their breathing patterns. Mouse models of RTT have provided molecular clues regarding the

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prominence of such behaviors in this disorder.[59] Corticosterone, the cortisol equivalent in rodents, is elevated during restraint experiments by nearly twice as much in *Mecp2* mutant mice than in wild-type controls. Expression levels of corticotrophin-releasing hormone (CRH), the primary hypothalamic signal in the cortisol pathway, is elevated in *Mecp2* mutant mice, suggesting that MeCP2 normally silences *Crh* transcription.[60] In turn, higher CRH levels lead to heightened release of adrenocorticotrophic hormone (ACTH) from the pituitary gland, followed by increased corticosterone release from the adrenal gland, thus creating an imbalance of the hypothalamus-pituitary-adrenal (HPA) axis. Furthermore, genes that are regulated by glucocorticoids are differentially expressed in *Mecp2* null mice.[61]

The most successful management of anxiety and mood behaviors is the use of selective serotonin reuptake inhibitors (SSRIs). SSRIs are thought to modulate the HPA axis and its downstream regulatory targets.[62] In addition, serotonin levels are lower in the hippocampus of *Mecp2* null mice compared to those in wild-type mice. Thus, SSRIs by adjusting serotonin levels might improve mood in RTT individuals. Intriguingly, the classical SSRI fluoxetine increases *Mecp2* mRNA levels in rats.[63] Thus, SSRIs might be beneficial for the management of RTT symptomatology by acting through different mechanisms.

5.5 Gastrointestinal Dysfunctions

Gastrointestinal problems in the RTT population have been well characterized. Basic steps in the digestive process, such as chewing and irregular bowel movements leading to constipation, occur commonly in RTT patients.[4] Furthermore, 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.[64] To complicate things further, difficultly eating and altered metabolism make determining daily intake a difficult task. Lastly, abnormalities in gallbladder function, in addition to the accumulation of gall stones in the gallbladder, have been identified as a serious concern in the RTT population and may require assessment to determine the functional state of the gallbladder.[4] Presently, a survey of RTT patients followed by the International Rett Syndrome Foundation (IRSF) is being conducted to determine the frequency of this issue in this population (Lane & Motil, in preparation).

Since constipation and gastroesophageal reflux disease (GERD) are the main gastrointestinal issues in RTT individuals, several over-the-counter and prescription products can be used to prevent or reduce these issues.[65] Constipation in RTT is believed to be caused by a combination of many factors including physical inactivity, diet, dehydration, and medications to name a few.[66] Medication to treat the symptoms of constipation alleviates the pain associated with these issues, while the use of fiber with adequate fluid intake will promote regular bowel movements. Simethicone can be used to prevent and alleviate the feeling of gas in the stomach and can also help in the symptoms of reflux.[65] Laxatives, such as polyethylene glycol 3350 or magnesium hydroxide (milk of magnesia), can be used to treat and prevent constipation in the RTT patient population. These over-the-counter medications can be used in patients suffering from chronic constipation; however an adequate intake of fluids is required to prevent side effects. Polyethylene glycol and milk of magnesia seem to be equally effective in long-term treatment of children with constipation; however, polyethylene glycol is tolerated better and more palatable than milk of magnesia. [67]

Gastroesophageal reflux disease (GERD), also known as acid reflux, is a painful and irritating condition caused by the upward movement of gastric acid from the stomach to the esophagus. If untreated, it can lead to mucosal damage and serious esophageal pathology, which includes narrowing of the tube, bleeding ulcer and the development of cancerous

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cells. In RTT, GERD has a high incidence.[64] While some non-pharmacologic methods can be used to prevent its symptoms, a number of pharmacologic treatment options exist.[65] Typical first line treatment is the use of an antacid, for example calcium carbonate. If this is not sufficient, a secondary option is the use of histamine H2 receptor blockers. One particular H2 receptor blocker that should not be used in RTT is cimetidine. The pharmacokinetic profile of cimetidine elicits many drug-drug interactions that can be potential harmful. For instance, cimetidine may inhibit the cytochrome P450 mediated breakdown of AEDs, thus increasing their serum concentrations and potentially leading to the development of adverse effects. [68]

If an H2 blocker does not alleviate symptoms of GERD, the next treatment choice would be the use of proton-pump inhibitors (PPI). PPIs irreversibly block the H+/K+ ATPase enzyme system and prevent the overproduction of acid by parietal cells in the stomach. PPIs are effective and popular drug options for the treatment of GERD, however they do not come without warning. Research has associated PPIs use with increased incidence of fracture in postmenopausal women. [69] If PPIs are to be started on RTT patients, since bone composition in these patients may increase their risk for fractures, constantly monitoring bone density and vitamin D levels might prevent future fractures.

5.6 Epilepsy and Seizure Disorders

Epilepsy is considered a major problem in RTT, not only in terms of diagnosis but also in treatment strategies. It is believed that a majority of RTT individuals have some type of seizure disorder. It was recently reported that approximately 60% of RTT individuals reported seizure activity.[70] Furthermore, RTT individuals with epilepsy are typically more severe in their case presentation. A genotype-phenotype analysis demonstrated that the missense *MECP2* mutations T158M and R106W were most frequently associated with epilepsy.[70] In addition, the occurrence of epilepsy appears to be an age related event, as patients 7 to 12 years of age tend to be at highest risk for seizure development.[71]

Seizures have also been observed in *Mecp2* deficient mouse models. [72] *Mecp2* mutant mice have an imbalance of excitation-to-inhibition in brain regions known to contribute to epilepsy and seizure disorders. For example, the hippocampus, the most epileptogenic brain region, is in a hyper-excitable state in symptomatic *Mecp2* mutant mice.[73] Since seizures occur at a high rate in the RTT population, its occurrence in *Mecp2*-based mouse models offers a useful endpoint for pre-clinical therapeutic evaluations.

In terms of treatment, many options exist that alleviate seizures in RTT. Valproate, lamotrigine, and carbamazepine, in addition to oxcarbamazepine, are AEDs that all have been shown be effective in seizure management.[74] The use of levetiracetam was shown to be effective in reducing seizure frequency in RTT patients that suffered from drug-resistant seizures.[75] Clinical judgment determines what therapy to initiate and if changes in mediations are to be made. However, it is critical to determine for certain that the diagnosis of seizures is correct and if medications should be initiated. Since AEDs are associated with many different adverse drug events, for instance the use of lamotrigine may cause a serious rash, limiting their use to RTT patients with an actual seizure disorder would avoid unwarranted side effects. Lastly, since RTT patients may be on many medications, determining drug-drug interactions would also prevent unnecessary harm to the patients. For instance, carbamazepine is a known CYP450 inducer and may cause a drug-drug interaction by increasing the clearance and decreasing the blood concentration of other drugs that the patient might be taking.

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5.7 Breathing Irregularities

As mentioned previously, irregular breathing is one of the most typical features in RTT. Common issues are hyperventilation, apnea, breath holding, and air swallowing that occurs generally while awake. It is believed that breathing issues result from abnormalities in brain stem connections.[76] Hypoxia may cause a reduction in heart rate, when such breathing patterns occur, thus exacerbating the length of the QT interval.[77] The neurobiological basis for altered respiratory function in RTT relate to several mechanisms, although all arise from impaired development of the neuronal circuitry responsible to set the respiratory rhythm. For example, deletion of *Mecp2* selectively in GABAergic interneurons leads to severe respiratory dysfunction.[78]

Successful management of breathing irregularities in RTT remains elusive. A drug-disease interaction that needs to be monitored is medications that delay breathing. The use of opioids or benzodiazepines could further reduce breathing patterns in the RTT population with compromised respiratory function and could lead to cardiac arrest. Mouse models of RTT displaying respiratory dysfunction have been used to study the pathophysiology of the brainstem and determine possible therapeutic interventions.[79] Using this model, it has been demonstrated that administration of the tricyclic antidepressant desipramine, improved respiratory rhythm and longevity in *Mecp2* mutant mice.[80,81] In addition, the neurotrophin brain-derived neurotrophic factor (BDNF) reverses synaptic impairments in the brainstem of *Mecp2* mutant mice, while increasing its expression with a nootropic AMPAkine regularizes the breathing rhythm, suggesting useful treatment strategies. [82,83,84]

6. The Future

Breakthroughs in the treatment of any type of neurodevelopmental disorder seemed impossible decades ago. However, the sequencing of the human genome, with the development of molecular biology and genetics techniques, has revolutionized the study of neurodevelopmental disorders. Furthermore, genetically engineering mice with mutations associated with neurodevelopmental disorders have not only furthered the basic science of the disease pathophysiology, but have also allowed the development and testing of therapeutic interventions. These technologies used with powerful imaging, electrophysiology, and behavioral assays have greatly impacted current research on the science of developmental disabilities. These basic science developments have ushered in a new paradigm for the study of neurodevelopmental disease. What we thought were incurable diseases decades ago, have now become diseases that can be managed successfully with rational therapies based on experimental preclinical science.

6.1 Lessons from Fragile X

An example of this bench-to-clinic approach in the treatment of neurodevelopmental disorders comes from research in the field of Fragile X syndrome (FXS). FXS is the most common form of inherited intellectual disability, with a prevalence of 1 in 4,000 males and 1 in 8,000 females.[85] Common clinical features of the disorder include moderate to severe intellectual disability, delayed speech, long narrow faces, macrocephaly, macro-orchidism in males, and gait ataxia.[86] FXS is caused by a trinucleotide repeat in the *FMR* gene located on the X chromosome, where a repeat of CGG (>200 repeats) occurs in the untranslated region of the gene leading to DNA hypermethylation and reduced transcription of the fragile X mental retardation protein (FMRP).[87]

The main hypotheses regarding the cause of FXS etiology have focused on the up-regulation of group 1 metabotropic glutamate receptors (mGluR1/5).[88,89] *Fmrp1* knockout mice have enhanced mGluR-dependent long-term depression (LTD), a form of plasticity associated with an activity-dependent reduction in synapse strength, in the CA1 region of the

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hippocampus.[90] Since FMRP is a repressor of mRNA translation at synapses, this research demonstrated that FMRP1 regulates mGluR expression and signaling.[91] Based on this work, it was hypothesized that mGluR1/5 antagonists could be used to alleviate FXS phenotypes.[92] Indeed, reduction of mGluR5 expression in *Fmrp1* knockout mice by crossbreeding with mGluR5 knockout mice rescued many defects observed in this FXS mouse model. [93] These genetic manipulations provide support for the development of mGluR antagonists for the symptom management in FXS currently in clinical.[94] For example, the mGluR5 antagonists, fenobam or AFQ056, improve behavioral defects in FXS with limited adverse effects.[95,96] While these clinical trials are just in there infancy, together with the basic research made in the mouse model, this group of drugs has great potential for the successful management of FXS symptomatology.

6.2 Preclinical Investigation in Mouse Models of RTT

The FXS story details how basic science investigators use experimental mouse models to develop and test novel therapies based on rational design. While the molecular interplay contributing to the pathogenesis of FXS is well known, the altered pathways contributing to the pathogenesis of RTT remain unclear. Considering that the best characterized function of MeCP2 is as a transcriptional regulator, numerous gene expression profiles have been conducted on RTT patients and in the available mouse models.[61,97–99] These studies identified several genes with altered expression in RTT, but their role in disease progression remains unknown. Here, we will focus on novel pharmacological treatment for the management of RTT; other research avenues for the alleviation of RTT symptoms in *Mecp2* deficient mice have been recently reviewed.[100]

In vitro manipulations of MeCP2 in neuronal and glial cell cultures have been successfully utilized for testing the efficacy of novel pharmacological agents. More recently, induced pluripotent stem cells (iPSCs) reprogrammed from skin fibroblasts of RTT individuals have been successfully differentiated into neurons that can be used for *in vitro* screening.[101, 102, 103, 104] However, the majority of the studies attempting to identify mechanisms to treat RTT have come from mouse models with mutations or deletions of the *Mecp2* gene.[105] A number of issues exist when using these animals to relate back to the human condition. First, due to the variable and delayed phenotype of female *Mecp2* mutant mice, most research has been done in male mice. Second, disease progression is different from that in humans. Boys with *MECP2* mutations typically present with severe abnormalities soon after birth; male *Mecp2* mutant mice on the other hand, present symptoms approximately at 4 to 6 weeks after birth, while females show symptoms after 6 months or more after birth.[106] Consequently, female mice become symptomatic as they enter adult ages, while the human disease begins 6 to 18 months after birth. Needless to say, the mouse is very much different from the human; however, it remains the very best tool that we have to understand the function of MeCP2 and determine what potential therapeutic options exist for the human condition.

One major issue to address in treatment options of RTT is to determine if a specific point in development exists where treatment has to be initiated to rescue function. Questions regarding the reversibility of brain damage in adult mice have recently been discussed. The laboratory of Adrian Bird engineered a mouse line in which the endogenous *Mecp2* gene was silenced via the insertion of a *lox-stop* cassette under the control of the estrogen receptor antagonist tamoxifen. Upon tamoxifen treatment, the stop cassette is removed and *Mecp2* expression is activated. The activation of *Mecp2* expression before the symptom occurrence prevented the onset of the RTT-like phenotype. Furthermore, activation of *Mecp2* expression in fully symptomatic mice diminished the severity of RTT-like phenotypes.[107] These results demonstrate that several key features of RTT can be reversed even after symptom onset, suggesting that a critical window of treatment does not exist.

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However, maintaining treatment protocols is essential to prevent reappearance of symptoms. Indeed, *Mecp2* deletion in adult mice after a lifetime of normal *Mecp2* expression causes a neurological phenotype similar to that of mice with germ line *Mecp2* deletions, strongly suggesting that proper MeCP2 levels need to be maintained throughout the lifetime.[108]

In the following sections, we address potential therapies now undergoing intensive pre-clinical investigation. These are summarized below and in the accompanying Table.

6.2.1 Brain Derived Neurotrophic Factor and "BDNF-Mimetics"—A targeted molecular approach demonstrated that the *Bdnf* gene is under MeCP2 transcriptional control.[109,110] BDNF is 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.[111] BDNF binds to a specific membrane-bound receptor called tropomyosin-related kinase B (TrkB), organizing precise signaling cascades that control varied neuronal actions such as survival, differentiation, neurite outgrowth, and synaptic function throughout development.[112]

Over the last two decades, BDNF has been linked to the pathogenesis of several neurological and psychiatric diseases.[113,114,115] BDNF has also been implicated in the pathophysiology of RTT. BDNF protein levels measured by ELISA were found to be lower in brain samples of *Mecp2* mutant mice.[116] Intriguingly, crossbreeding *Bdnf* heterozygous mice with *Mecp2* mutant mice exacerbated the onset of the RTT-associated phenotypes.[116] More importantly, BDNF mRNA levels are lower in brain samples from RTT patients, similar to the finding described in MeCP2 mutant mice.[117]

It remains unclear how *MECP2* mutations affect *BDNF* gene transcription, mRNA translation and protein trafficking, even though is clear that deregulation in any of those steps contributes to the RTT symptomatology. Indeed, promising work has shown that 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.[116] 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.[118]

Unfortunately, the administration of BDNF is not a useful clinical approach due to it's short half-life and inability to cross the blood–brain barrier.[119] However, small molecules that can mimic BDNF's effects or that can increase the levels of endogenous BDNF are attractive potential therapeutic options. For example, a class of nootropic drugs called AMPAkines has been used to increase BDNF levels. These compounds reduce the desensitization of AMPA-type of glutamate receptors, thus slightly elevating neuronal activity and thus Ca^{2+}-dependent BDNF expression.[120] Indeed, daily administration of an AMPAkine improved breathing patterns in *Mecp2* mutant mice by increasing BDNF levels in the brainstem respiratory centers.[83, 84] SSRIs also have been shown to enhance BDNF expression.[121] While considerable work has demonstrated that SSRIs increase BDNF levels in experimental models of mood disorders, there is no clinical evidence yet whether such BDNF elevations caused by chronic SSRI treatment improve RTT symptoms.

Another alternative is that of small molecules with TrkB ligand activity, so called "BDNF mimetics." These molecules are permeable to the blood-brain barrier and have been shown to activate TrkB receptors and mimic the biological activities of BDNF by preventing neuronal degeneration and death, having antidepressant effects and improving learning and memory.[122–124] Two recent reports with novel small molecule TrkB agonists, LM22A-4 and

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7,8-dihydroxyflavone (7,8-DHF), 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.[125] 7,8-DHF delayed body mass deficits and improved wheel running and breathing impairments in mutant male mice.[126] While these agents present limited supporting research, they do offer hope of targeting BDNF without administering the actual protein.

6.2.2 Insulin-like Growth Factor-1—The pleiotropic growth factor insulin-like growth factor-1 (IGF-1) has recently gained much attention as therapeutic treatment of RTT individuals. IGF-1 exerts its biological actions by binding to the IGF-1 receptor (IGF-1R), thus activating a receptor tyrosine kinase with downstream signaling cascades similar to the BDNF receptor TrkB actions.[127] Unlike BDNF, IGF-1 crosses the blood-brain and gains access to the CNS. IGF-1 stimulates proliferation of neural progenitors, neuronal survival, neurite outgrowth, and synapse formation.[128,129] 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 increase brain weight in *Mecp2* mutant mice.[130] 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;[131] however, it is currently unknown if full-length IGF-1 has similar actions. In addition, IGF-1 increased synapse number in neurons differentiated from iPSCs cells reprogrammed from skin fibroblasts of RTT individuals RTT neurons far more than in control neurons.[104] Taken altogether, these results suggest that IGF-1 is a good therapeutic option for RTT individuals.

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,[132] 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). Furthermore, IGF-1 has also been suggested for the treatment of cancer, diabetes, and amyotrophic lateral sclerosis.[133,134]

6.2.3 Aminoglycosides antibiotics for Read-through of Nonsense Mutations—Aminoglycosides are antimicrobial agents that inhibit protein synthesis of sensitive bacteria, but also allow read-through premature STOP codons in mutant genes. Since a nonsense mutation in *MECP2* resulting in premature transcription termination occurs in approximately 35% of North American RTT patients, [135] and RTT patients with nonsense mutations have a more severe phenotype compared to individuals with missense mutations that result in a single amino acid substitution,[136] these so-called read-through compounds represent a potentially useful pharmacological approach to overcome transcriptional termination caused by nonsense mutations.[137,138] Indeed, full-length MeCP2 protein was detected in HeLa cells expressing *MECP2* with nonsense mutations after treatment with an aminoglycoside antibiotic.[139] In addition, full-length MeCP2 protein was localized in the nucleus of ear fibroblasts from mice carrying a R168X mutation after treatment with aminoglycosides.[140] Furthermore, aminoglycosides increase translation of full-length MeCP2 protein in a lymphocyte cell line derived from a RTT patient carrying a R255X nonsense mutation.[141] The read-through aminoglycoside gentamicin not only allowed expression of full-length MeCP2 in cells carrying premature STOP codons, but also increased dendritic spine density in IPSC-derived neurons from RTT patients with a Q244X mutation,[104] and increased the expression of BDNF in primary fibroblasts cultured from RTT patients with nonsense mutations,[142] demonstrating the clinical potential of read-

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through compounds. Since aminoglycosides have renal and auditory toxicity, the development of safer read-through compounds will be of great relevance for establishing this model not only for the treatment of RTT but also for other disorders caused by nonsense mutations (e.g. cystic fibrosis).[143]

7. Conclusions

The therapeutic management of individuals with neurodevelopmental disorders is complex and requires reviewing the entire spectrum of symptoms that relate to each specific disorder. RTT is a perfect example of how crucial management of all features of the disease could extend and improve quality of life. In this critical review of past and current literature surrounding RTT and MeCP2, we have evaluated the current treatment choices that arise from observations in the clinic leading to evidence-based clinical research outcomes. Continued clinical research is required to limit the severity of RTT, but also to continue to increase the quality of life for RTT patients and their families.[144] In addition, we have brought attention, summarized in the Table, to the pre-clinical development of novel pharmacological agents that have been effective in ameliorating symptoms in mouse and cell culture models of RTT. We focused on three potential treatment strategies, but several other alternatives exist. Since MeCP2 activates and represses a large number of target genes,[12] dysfunction in the expression in any number of those genes alone or in combination could contribute to RTT symptomatology. More research is certainly needed to determine which deregulated genes contribute to a specific symptom or symptom cluster. The greatest challenge in translational research for the discovery of new rational therapies requires a highly interactive interdisciplinary approach engaging basic science labs and clinicians. Since many common neurobiological mechanisms exist in the spectrum of neurodevelopmental disorders, understanding of the key components might hasten the progress of novel treatment for all these devastating disorders.

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Table

Overview of Putative Pre-clinical Pharmacologic Approaches

Potential Compound	Advantages	Disadvantages
BDNF	• Potent Synaptic Growth Factor • Expression regulated by MeCP2 • Reversed or attenuated phenotypes in mutant *mecp2* mice	• Short half life • Administration • Blood-brain barrier permeability • Specificity of target effect
BDNF-Mimetics	• Similar to BDNF • Crosses blood-brain barrier • Reversed or attenuated phenotypes in mutant *mecp2* mice	• Effects in humans unknown • Potential side effects • Specificity of target effect • New class of compounds with limited research
Insulin-Like Growth Factor-1	• Crosses blood-brain barrier • FDA approval for poor growth • Reversed or attenuated phenotypes in mutant *mecp2* mice	• Mechanism of phenotype reversal unknown • Potential side effects • Specificity of target effect
Read-through Compounds • Aminoglycosides • Small Molecules	• Corrects full length protein deficiency • Reversed phenotypes in cell cultures models of RTT	• Useful for limited RTT population with nonsense mutations • Blood-brain barrier permeability unknown • Renal and ototoxicity is a major problem with aminoglycosides • Specificity for MeCP2 unknown • Mis-expression of other genes that could be harmful