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Review Article

Rett Syndrome: Coming to Terms with Treatment

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Rett syndrome (RTT) has experienced remarkable progress over the past three decades since emerging as a disorder of worldwide proportions, particularly with discovery of the linkage of RTT to *MECP2* mutations. The advances in clinical research and the increasing pace of basic science investigations have accelerated the pattern of discovery and understanding. Clinical trials are ongoing and others are planned. A review of these events and the prospects for continued success are highlighted below. The girls and women encountered today with RTT are, overall, in better general, neurologic, and behavioral health than those encountered earlier. This represents important progress worldwide from the concerted efforts of a broadly based and diverse clinical and basic research consortium as well as the efforts of parents, family, and friends.

1. Introduction: Early History of Rett Syndrome

Rett syndrome (RTT; Online Mendelian Inheritance in Man #312750; http://www.ncbi.nlm.nih.gov/omim/) was first recognized by Andreas Rett, a neurodevelopmental pediatrician in Vienna, more than fifty years ago when he observed two girls in his clinic simultaneously engaged in hand stereotypies [1, 2]. His attempts to raise awareness of this observation among physicians in Europe met with little success in creating interest in expanding understanding of this unique neurodevelopmental disorder. As it happened, most of his written efforts were in German and were not widely circulated beyond Austria. Bengt Hagberg, a Swedish child neurologist, had also identified young girls with virtually identical features, but unlike Rett, he did not report these observations or extend his information beyond Sweden. Rett's single major English publication appeared in the *Handbook of Clinical Neurology* in 1977 [3]. However, a series of extensive metabolic tests on blood and urine in his participants with this disorder had identified hyperammonemia, the subject of this *Handbook* volume. Fortunately, this finding turned out to be spurious. At a gathering of child neurologists in Europe near the end of the 1970s, Hagberg became aware of this change in the association of these clinical features and hyperammonemia, realized that they were observing the same disorder, and planned together with Jean Aicardi, Karin Dias, and Ovidio Ramos to publish their own combined experiences. Shortly thereafter in 1981, Hagberg had a chance meeting with Rett in Toronto and following this discussion elected to name the disorder Rett syndrome. At the time, RTT was scarcely known outside of Europe, but with the 1983 publication in the *Annals of Neurology* this disorder gained immediate prominence as the leading cause of significant cognitive disability among females [4]. Rett led a series of international meetings in Vienna with representatives from throughout the world and energized this group to develop appropriate diagnostic criteria and to identify a causal relationship [5]. Vanja Holm, Hugo Moser, and Alan Percy [6] attended the 1984 meeting which saw the first effort in development of consensus criteria for diagnosis and created the first broad scale efforts worldwide in clinical and research activity [7].

2. Initial Studies

Following this initial clinical exposure to RTT, investigations began to intensify, spurred by efforts of Hugo Moser to convene an international meeting at Johns Hopkins Medical School in 1985 and the subsequent creation of the International Rett Syndrome Association (IRSA) through the leadership of three parents, Kathy Hunter, Gail Smith, and Jane Brubaker. This energized clinical studies at the Baylor College of Medicine led by Alan Percy, Daniel Glaze, and Huda Zoghbi, and Johns Hopkins led by Hugo Moser and Sakkubai Naidu. Important results emerged almost immediately with the identification of reductions in spinal fluid metabolites of the biogenic amines [8, 9], studies on prevalence from a survey of Texas residents [10], and the initiation of broad scale growth assessments by the Baylor group and the corresponding development of PET scanning analyses at Johns Hopkins [11]. This culminated among others in the first large scale growth measurement study from Baylor assessing height, weight, and head circumference [12] and, subsequently, hands and feet [13]. These results clearly demonstrated the profound failure of growth and initiated a long-term effort at defining areas of responsibility including nutrition and gastrointestinal function. In the years leading up to identification of the causal gene, considerable effort was expended in developing a clear understanding of the potential mechanisms. Similarly, significant attention was given to identifying the electrophysiological underpinnings of the common notations of epilepsy [14–18] and periodic breathing [19–21]. These early studies in the US were supported by the efforts of IRSA and the subsequent lobbying of Congress to promote funding streams through the National Institutes of Health. Program projects emerged both at Johns Hopkins and at the Baylor College of Medicine and continuing training grants from IRSA spurred these clinical advances as well as to the successful gene identification. At the same time, prevalence studies emerged ranging from 1 : 10,000 to 1 : 22,000 [10]. More recently, a population-based study from Australia indicated an incidence of about 1 : 10,000 female births [22]. As international interest grew, it became quite evident that RTT occurs worldwide, affecting all racial and ethnic groups with similar frequency.

At the same time, clinical studies identified the existence of atypical forms of RTT including the delayed onset and preserved speech variants among those with better overall function and the early onset seizure and congenital variants among those with poorer overall function [23–27].

Throughout the next decade considerable attention concentrated on identifying a causal mechanism. Among the possibilities, the speculation regarding environmental or medical causes failed to match with the striking occurrence of RTT virtually exclusively in females. This alone suggested a genetic etiology based on an X-linked dominant mechanism. Thus, a series of studies gradually focused attention to Xq28, a very gene-rich region associated with several important human disorders [28–35], culminating in the identification of mutations in the *MECP2* (*methyl-CpG-binding protein 2*) gene [36]. This discovery then led to vigorous and productive basic science investigations.

3. Mutation Analyses

Due to restriction of the initial features of RTT to young girls, the presumption had been made that the molecular abnormality represented an X-linked dominant disorder. Although not uniformly accepted [37], extensive efforts were directed at the X chromosome among girls and their families. The area of interest was narrowed to Xq28 based on DNA samples obtained through the efforts of Dan Glaze, Huda Zoghbi, and Alan Percy at Baylor [34] and Carolyn Schanen [30] at Stanford and from a large family in Argentina who were studied at Johns Hopkins [38]. This region is one very rich in genes associated with human conditions including the genes for adrenoleukodystrophy, X-linked muscular dystrophy, and color blindness. While *MECP2*, which is located at Xq28, was well known having been described previously in the cancer literature as an epigenetic modulator [39], it was not initially regarded as the primary candidate. Nevertheless, as the result of intense efforts by Ruthie Amir in the laboratory of Huda Zoghbi at the Baylor College of Medicine, the association between mutations in *MECP2* and RTT was firmly established [36]. At present, more than 250 different mutations associated with RTT have been identified in this gene [40]. However, as will be described below, these mutations not only have provided the molecular basis for almost all girls with RTT, but also have uncovered a rich and complex array of previously unanticipated disorders [41–43].

4. Clinical Diagnosis

RTT, characterized by partial or complete loss of fine motor and communication skills, remarkable stereotypic movements, principally of the hands, significant cognitive impairment, and pervasive growth failure, has its onset during the first 6–30 months of life following a period of what is considered to be normal development. Intense efforts to establish a biologic marker were uniformly unsuccessful. Although hy- perammonemia was initially described by Andy Rett and colleagues, these findings were not confirmed on subsequent testing. Indeed, it was this early metabolic finding that seemed to differentiate the separate observations of Rett and Hagberg.

With the establishment of obligate requirements for diagnosis in 1984 [7], the diagnosis of RTT was based on meeting specific clinical criteria. A series of revisions in the diagnostic criteria have emerged subsequently, as understanding of RTT has advanced, most notably through the identification of *MECP2* mutations [44–47]. An important reason for these continued revisions is the need to provide precise definitions and avoid any misunderstandings related to nuances of different languages so that application is practiced similarly throughout the world. As such, the most recent revision [46] based on an international consensus panel occurred in 2010 (Table 1). The early periods of pre- and perinatal history are typically normal. Initial development is regarded by families as normal for the first several months of life, but it is clear that the acquisition of early development milestones is often delayed beyond the period accepted as the upper limit of normal. After age of six months, continued developmental

Table 1: Rett Syndrome (consensus criteria—2010) (typical or classic).

Regression followed by recovery or stabilization
Must fulfill all main criteria and all exclusion criteria
Supportive criteria while present not required
Main Criteria
Partial or complete loss of purposeful hand skills
Partial or complete loss of spoken language
Gait abnormalities: dyspraxic or absent
Stereotypic hand movements
Exclusion Criteria
Traumatic brain injury
Neurometabolic disease
Severe infection
Very abnormal development in first 6 months of life

Table 2: Rett syndrome timing of regression period.

Age at regression	Classic RTT	Variant RTT	
		Higher functioning	Lower functioning
<6 months	13 (1.7%)	3 (4.4%)	27 (38%)
6–<12 months	54 (7.0%)	2 (2.9%)	9 (12%)
12–<18 months	281 (36%)	2 (2.9%)	15 (21%)
18–30 months	351 (46%)	26 (38%)	10 (14%)
>30 months	74 (9.6%)	35 (52%)	11 (15%)
Total	773	68	72

progress stagnates and thereafter a frank regression occurs. During this regression period, the partial or complete loss of fine motor skills and the virtually simultaneous occurrence of stereotypic movements are noted along with delays in cognitive development and abnormalities in communication including eye contact and responsiveness to attempts at socialization. The girls ignore spoken language or even loud noises, and profound irritability including inconsolable crying for prolonged periods without apparent explanation is common. It is during this phase that some are regarded as autistic. The typical period of regression is between 12 and 30 months but may be seen as early as age of 6 months age or beyond 30 months (Table 2).

Deceleration in the rate of head growth is often the first clinical sign of RTT. This was noted by Rett and described further in the context of microcephaly [1, 48]. While it is now clear that deceleration in the rate of head growth may be profound and microcephaly is evident in 70% or more, not all have head circumference deceleration to that level and not all girls actually have a remarkable change in rate of head growth such that this deceleration has been removed as an obligate criterion. Based on data from the US Natural History study, abnormal deceleration may be noted as early as one to two months of age [49]. However, the later and nearly simultaneous occurrence of reduction

Figure 1: A 3-year-old girl with Rett syndrome demonstrating midline hand stereotypy and preserved ambulatory skills.

Figure 2: A 13-year-old ambulatory girl with Rett syndrome showing intense eye gaze and prominent midline hand stereotypy.

or loss of fine motor hand function and the appearance of stereotypic movements is often the pivotal point of diagnostic significance. The stereotypies are typically first noted between ages of 1 and 3 years and predominantly involve the hands but may also be seen in the orofacial region and the feet, particularly if the hand movements are suppressed. Hand movements consist principally of hand-washing, hand-wringing, or hand-clapping/hand-patting and may dominate hand function to the exclusion of any effective motor skills (Figures 1–3). In some, prominent hand mouthing or picking at the hair or clothes is the predominant stereotypic movement. These stereotypies typically are noted in the midline but may be asymmetric with one hand in the mouth and the other patting, finger rubbing, or hair twirling. In rare instances, the hand-wringing may occur behind the back. Ambulation is acquired by most (~80%) girls (Figure 1). However, between age of 1 and 4 years the gait is punctuated by significant truncal ataxia and apraxia demonstrating a broad-based,

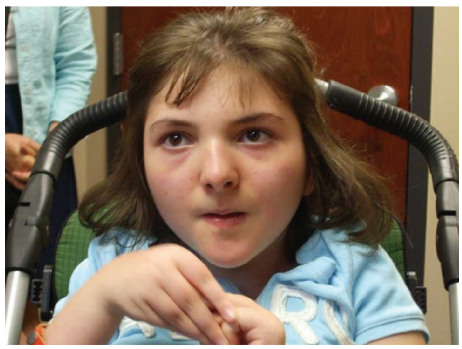

Figure 3: A 12-year-old girl with Rett syndrome who is nonambulatory but has excellent eye contact and a prominent midline hand stereotypy.

wandering, and purposeless character and is often initiated by retropulsion (first stepping backwards). Prominent to-and-fro truncal rocking is common, either side-to-side or back and forth, whether sitting or standing. About 30–40% of those acquiring the ability to walk will lose this independent capability, such that, overall, about 50% have independent gait. The majority of those no longer walking independently are able to walk with assistance. In some this requires only minimal guidance whereas others may require substantial support. Ambulation, whether independent or supported, should be encouraged indefinitely. Anxiety regarding fear of falling, changes in floor pattern or surface, or changes in terrain is evident and simply providing a steadying hand may be sufficient.

The clinical evaluation for RTT rests on a thorough history and current assessment including a neurological evaluation. In particular, careful attention must be accorded to the course of developmental progress from birth and a comprehensive evaluation of growth parameters (height, weight, and head circumference). Particular importance should be given to the temporal sequence of the child's developmental history noting delay in achieving specific developmental milestones, loss of motor or communicative skills, and appearance of hand stereotypies. Comprehensive laboratory tests should assess the complete chemistry profile including triglyceride and cholesterol measures and vitamin D levels. An EEG is recommended both to assess the background activity and to evaluate the presence of epileptiform features. Typically, the EEG may remain normal for the first two years of life but thereafter is marked by profound slowing of background activity and the presence of epileptiform characteristics. Frequently, the EEG may be particularly epileptiform in character during periods of sleep, but definitive seizures may not occur. Behavioral events, occurring daily or several times per week, may resemble clinical seizures. It is essential to corroborate these events by video-EEG assessment as in many cases they are not based on epileptiform activity. Careful attention to this evaluation is essential. Routine neuroimaging (cranial CT or MRI) has not been informative in general in RTT.

Confirmation of the clinical diagnosis of RTT should be based on determination of *MECP2* mutations [50–61]. Initial emphasis should be placed on sequencing the four exons of *MECP2*. In approximately 10% of individuals with the clinical diagnosis of RTT, sequence analysis is normal. Secondary analysis utilizing techniques such as multiplex ligation-dependent probe amplification (MLPA) is required to reveal large deletions involving one or more of the exons. Standard sequence determination will miss these mutations. MLPA testing will also be informative for duplications of *MECP2*, as will be discussed below. In 4-5% of individuals meeting clinical criteria for RTT, no *MECP2* mutation may be found. These individuals may represent phenocopies of RTT or may be the result of currently undefined abnormalities in the *MECP2* gene.

5. Variant Phenotypic Expression

More than twenty years ago, Hagberg recognized the occurrence of variant phenotypic expressions of RTT [23, 24]. Since then, a number of reported variances have been noted. In these, the girls meet some but not all criteria for RTT and are considered to have *atypical* RTT with definite and discernible patterns of involvement. Others have *MECP2* mutations but lack the clinical features of RTT. In these instances, both females and males may be involved. Individuals who function at level higher than that seen in typical RTT may have preservation of some speech and are most commonly associated with a specific *MECP2* mutation, namely, R133C [25]. Others may not have identifiable features consistent with RTT until age of 8–10 years and are termed delayed onset variant or *formes fruste* [23]. Quite distinct from both this group with better function and those with typical RTT, individuals may function at a lower level and generally fall into two groups, a congenital form demonstrating little or no developmental progress and an early onset seizure form featuring a relatively severe epileptic encephalopathy that produces markedly abnormal early development. Criteria [46] for these variant RTT phenotypes are based on fulfilling at least 2 of 4 main criteria and at least 5 of 11 associated features for RTT (Table 3). Hagberg noted that 107/130 (82%) girls with RTT in Sweden fulfilled the classic criteria, 16/130 (12%) displayed formes fruste, and the remaining 7 were either preserved speech or congenital forms [24]. In the RTT Natural History study funded by the National Institutes of Health, of more than 1000 participants meeting criteria for RTT, 85% were classic or typical RTT and 15% were atypical. Of those with atypical RTT, the higher and lower functioning variants were virtually equivalent in number.

Other females with *MECP2* mutations do not meet any criteria for RTT. Some of these have significant cognitive and behavioral problems with autistic features [62, 63]. These individuals generally have mutations in the $3'$-region of *MECP2* and may have favorable skewing of X chromosome inactivation (XCI). Others are quite normal or have mild cognitive impairment or learning difficulties and include families in which a mother expressing the abnormal *MECP2* has skewed XCI. Her offspring may include females with classic RTT and males with variable phenotypes as described

Table 3: Rett syndrome (consensus criteria—2010) (atypical or variant).

Regression followed by recovery or stabilization	
Meet at least 3 of 6 main criteria	
Meet at least 5 of 11 supportive criteria	
Main criteria	
Partial or complete loss of purposeful hand skills	
Partial or complete loss of spoken language	
Gait abnormalities: dyspraxic or absent	
Stereotypic hand movements	
Supportive criteria	
Awake breathing problems	Growth retardation
Bruxism	Small hands and feet
Impaired sleep	Scoliosis and/or kyphosis
Abnormal muscle tone	Increased pain tolerance
Peripheral vasomotor abnormalities	Intense eye contact
Inappropriate laughter or screaming	

below, namely, a rapidly progressive encephalopathy or simply cognitive impairment with or without motor difficulties [64].

6. RTT in Males

Few males have been reported meeting obligate criteria for RTT. These have occurred under two distinctly different scenarios and appear not to exceed 10 males. Males with Klinefelter syndrome (47, XXY) [65–67] and *MECP2* mutations and males with somatic mosaicism [68], that is, two cell populations, and *MECP2* mutations in one cell population may have clinical features typical for RTT. Among males with mutations in *MECP2* who do not meet obligate criteria for RTT, a somewhat larger number has been identified [69]. One group numbering, less than twenty, displays a rapidly progressive encephalopathy and significant apneic episodes leading to markedly shortened (1-2 years) survival [70]. A quite separate group of similar size involves individuals presenting with mutations in the 3′-region of *MECP2*. These males demonstrate a developmental disorder that may include only cognitive delays and inappropriate behaviors or may display developmental delay and a quite significant and steadily progressive dystonia leading to remarkable limitation of motor function [42, 64, 71]. Most of these males failing to meet RTT criteria have only been identified due to the presence of RTT in female siblings. Otherwise, they would have escaped detection in all likelihood because they display a set of developmental abnormalities distinct from RTT.

Although not demonstrating a sequence abnormality in *MECP2*, a separate group of males totaling more than 150 have a completely different defect [41, 72–74]. This group has duplication of the *MECP2* gene, not a mutation. Each duplication is rather unique in terms of its size and the number of other genes involved. Prior animal studies had demonstrated that *MECP2* is tightly regulated with either a loss or gain resulting in unique neurodevelopmental consequences so that the identification of these individuals is not surprising. Males with a *MECP2* duplication demonstrate cognitive impairment, absence of spoken language, epilepsy, and an abnormal shuffling gait and rarely display the growth retardation or hand stereotypies typical for RTT [75–77]. Frequent upper respiratory infections or chronic sinusitis events in some males are felt to relate to a separate gene among those duplicated. Many mothers of these males have the same *MECP2* duplication but appear remarkably normal due to XCI. More detailed analysis reveals that some of these mothers acknowledge depression or obsessive-compulsive behaviors [76].

7. Clinical Profile

Already in 1986, Hagberg had developed a staging system to characterize the clinical progression of RTT [78]. This staging system however was created at a time when current practices regarding nutrition, physical and occupational therapies, and surgical management of orthopedic issues were not broadly emphasized. As greater experience has been gained, it is now recognized that clinical progression requires a different view [79]. It is recognized that motor development and function evolve steadily from initial hypotonia progressing to normal tone and then to an increasing pattern of hypertonia and rigidity reminiscent of parkinsonian features. On the other hand, interpersonal interaction and communication perspectives seem to remain quite stable over time. Therefore, a developmental pattern emerges that fits a clear temporal profile: (1) a period of apparently normal early development, (2) then an arrest of developmental progress typically between age of 6 and 18 months, (3) followed by a period of regression involving a partial or complete loss of social contact and fine motor skills generally between age of 12 and 30 months, and (4) finally a prolonged period of stabilization with markedly improved social interaction, eye contact, and socialization (Figures 2 and 3) contrasting with the gradual evolution of motor function marked by increasing muscle tone, rigidity, and significant dystonic posturing, particularly of the feet, hands, and occasionally the trunk. With the recognition of *MECP2* mutations, a basis was provided for understanding the broad range of clinical involvements associated with the specific *MECP2* mutations and with other genetic factors. Further, the application of broadly based treatment programs for physical and occupational therapy, for dystonic positioning, and for orthopedic concerns has altered the long-term outlook sufficiently that utilization of the original staging system has been clouded and its implementation as structured is not currently recommended. Certainly, the clinical staging format offered a means of assessing clinical progress, but subsequent advances have rendered it less useful.

8. Clinical Issues

Specific systemic problems may alter the landscape of RTT considerably (Table 4). In order to maintain the optimal functional level, proper attention to these is essential recognizing that each individual may be quite variable with respect to the

Table 4: Rett syndrome (clinical issues).

Cognitive impairment	
Epilepsy	
Breathing Irregularities	
Hyperventilation, breathholding, or both	
Gastrointestinal dysfunction	
Poor chewing and swallowing	Gastroesophageal reflux
Delayed gastric emptying	Constipation
Gallbladder dysfunction	
Growth	
Sleep	
Ambulation	
Self-abuse	
Quality of Life	
Longevity	
Other associated features	
Hypertonia	Progressive dystonia
Prolonged QTc interval	Bruxism
Vasomotor disturbances	

specific medical issue. Therefore, the following guidelines are recommended for consideration and include cognitive impairment, epilepsy, breathing irregularities, gastrointestinal function, growth failure, scoliosis, sleep difficulties, ambulation, self-abuse, quality of life, and life expectancy.

Growth failure is pervasive having been recognized early on as a fundamental concern. We now understand that survival may be quite prolonged albeit less than normal. Nevertheless, this raises fundamental issues. Efforts to identify a consistent metabolic abnormality have been fruitless in spite of concerted efforts regarding standard blood chemistries, the amino and organic acids, mitochondrial function, and urea cycle metabolism. In addressing each of the potential clinical concerns, it is important to be cognizant of maintaining an open dialogue with the parents or principal caregivers. For additional information, the International Rett Syndrome Foundation (IRSF), successor to IRSA, is an excellent resource for individuals and families. IRSF provides effective guidance to interested individuals and supports both basic and clinical research investigations (http://www.rettsyndrome.org/). In recent years, patient advocacy groups have developed worldwide offering similar levels of support. These organizations are critical to maintaining a platform for exchanging relevant clinical information and providing updates on scientific advances in support of more effective treatment.

8.1. Cognitive Impairment. Cognitive function in RTT cannot be assessed effectively due to the inability of these girls to demonstrate purposeful hand skills and effective communication. Despite these limitations, available measures provided estimates of mental developmental age at the 8–10-month level and gross motor function ranging from 12 to 18 months. Application of modalities based on visual response has not proved more effective. More recently, advances in computer-based technology utilizing eye gaze tracking have provided a means of communication that suggests a level of interaction and comprehension not previously recognized [80–82]. It is possible that such methodology will provide an effective, reliable assessment of cognitive function in RTT. An important aspect of such modalities is appropriate consideration of the slow response time. More than a few seconds and often as long as half a minute are required to elicit a response to specific requests. Failure to allocate proper response time is likely to doom attempts at determining comprehension. Assessing cognitive function in girls with RTT remains extremely problematic and will require objective assessment of these techniques.

The acquisition of feeding, dressing, and toileting skills is poor at best, requiring assistance from others throughout their lives. Nevertheless, occupational, cognitive, and speech therapies form necessary elements of effective therapeutic management. Utilization of the advanced computer technologies noted above is an essential component of this plan where feasible. The objective assessment of this methodology will be required to substantiate its efficacy.

8.2. Epilepsy. Seizure frequency in RTT has been reported across a broad range from 30 to 80% [83–85]. The electroencephalogram (EEG) is invariably abnormal after age of 2 years marked by slowing of background activity with a reduction or loss of posterior dominant rhythm and recurrent spike and slow spike and wave activity. Particularly during sleep in the young child, the epileptiform pattern may be nearly continuous suggesting hypsarrhythmia or nearly continuous slow spike and wave abnormalities. However, despite these findings, clinical seizure activity may be minimal or absent in the majority of girls. This represents a major challenge particularly when the child demonstrates unusual behaviors resembling clinical epilepsy. The challenge is to differentiate these behavioral patterns or possibly brainstem events from clinical seizures. This requires video-EEG monitoring to provide a satisfactory resolution and is best accomplished when these events occur relatively frequently. In the US Natural History study, 85% of girls were noted to have clinical epilepsy by age of 16, but only 30–35% required medication for their management at any given time. In addition, it was noted that new-onset epilepsy is rarely noted after age of 20 and in many instances the epileptiform EEG pattern is no longer present.

Control of clinical epilepsy in individuals with RTT is usually attained rather easily with single agents such as carbamazepine, oxcarbazepine, sodium valproate, or lamotrigine. While levetiracetam, topiramate, and clobazam have been used with increasing frequency, no evidence of superior control has been noted whereas undesirable side-effects of decreased appetite, irritability, and dull affect have been. When control of epilepsy becomes more challenging, the use of multiple medications together or the addition of alternative strategies such as the vagal nerve stimulator and the ketogenic diet has been employed with efficacy in many instances.

8.3. Breathing Irregularities. Rett noted early on the occurrence of irregular breathing during wakefulness consisting of hyperventilation or breathholding or both as a common feature. In general, this periodic breathing has its onset in early childhood (3–5 years) but is most problematic between age of 5 and 15 years and is commonly worsened by activities that increase anxiety or agitation. Periods of breathholding may be prolonged exceeding one minute and may be accompanied by air swallowing (aerophagia) leading to significant abdominal distension or bloating. The distension tends to subside, particularly during sleep. The periodic breathing may dominate periods of wakefulness and create problems with feeding and other activities. In some girls, the periodic breathing may be very subtle and actually unrecognized by parents and other caregivers. One sign can be the oral expulsion of air or saliva as in a sigh or harsh expiration. After the mid-teenage years, the breathing irregularities may tend to diminish in frequency and intensity. Efforts to arrest or modify the periodic breathing have met with limited success [86]. Buspirone, magnesium citrate, and the opiate antagonist, naltrexone, have been of limited efficacy and nothing has been uniformly beneficial [87, 88]. The selective serotonin reuptake antagonist antianxiety medications, particularly escitalopram, have been effective although not in all girls.

When patterns of irregular breathing or snoring occur during sleep, a sleep study to assess the presence of obstructive apnea or other sleep-related abnormalities is warranted as these are not features typically noted by parents [89].

8.4. Gastrointestinal Function. As stated above, growth is a major issue in RTT. Nutrition is often a specific contributor to this problem. Therefore, we recommend utilization of a nutritionist to assist with this process. Previous studies have suggested that girls with RTT have increased calorie and protein requirements, but beyond that specific issues with chewing and swallowing, recurrent aspiration, prominent gastroesophageal reflux, abnormally slow gastric emptying, and constipation require additional strategies including high calorie liquid supplements and occasionally alternative feeding via gastrostomy tubes (G-tubes) [90–94]. That is, G-tubes may be required to provide sufficient daily caloric intake, to supplement appropriate amounts of fluids, or in the case of recurrent aspiration, to by-pass oral feedings altogether to protect the health of the child. Periodic breathing and aerophagia may also be so prominent as to affect the maintenance of adequate oral intake adversely. In the US Natural History study, about 30% of girls with RTT have required gastrostomy tube placement for preservation of nutrition, adequate fluid intake, or protection of the airway [94].

Gastroesophageal reflux (GERD) is extremely common in RTT. In some instances this may lead to esophagitis, but more commonly, recurrent GERD is so significant to produce unexplained irritability or apparent distress. In these instances, it is essential to refer to a gastroenterologist for evaluation and treatment as indicated. Proton-pump inhibitors have been the most effective medication for GERD, although H2-blockers may provide temporary benefit. One should also be alert to the possibility of *H. pylori* infection. Delayed gastric emptying has also been noted in which case bethanechol or erythromycin has proven efficacious. Marked caution should be observed with implementation of metoclopramide as the occurrence of profound movement disorders represents a potentially serious adverse consequence.

Constipation occurs virtually uniformly in RTT [93]. This relates to the general issue of poor GI function in this disorder and may also result from the tendency for these girls to have poor fluid intake. While multiple strategies including the use of high-fibre foods, enemas, mineral oil, and milk of magnesia have been employed, success has been quite variable. In addition, these strategies may be associated with adverse consequences such as the reliance on enemas leading to dependency on this treatment plan or the interference of mineral oil with proper absorption of the fat-soluble vitamins. In addition, many girls resist even flavored milk of magnesia. Miralax (polyethylene glycol) can be quite effective provided that it is given with adequate fluid volumes as it is tasteless and odorless and may be dissolved in juice. The presence of a G-tube will greatly facilitate its use as well as that of milk of magnesia.

Gallbladder dysfunction has been recognized relatively recently, although the frequency of ~3% is relatively low. It may cause marked agitation and discomfort and has been identified as early as age of 2 years. Assessment by abdominal ultrasound may reveal gallstones, but more commonly the HIDA scan is required to substantiate significant dysfunction with an ejection fraction less than 35–40%. At this point, cholecystectomy should be considered.

8.5. Growth. Growth failure in RTT is pervasive, first noted as early as 1-2 months of age with progressive, abnormal deceleration in the rate of head growth, the median head circumference approaching the second percentile for the normal population by age of 1.5 to 2 years [49]. This is followed by a decline in weight near the end of the first year of life, the median value falling below the 2nd percentile for the normal population between age of 12 and 13 years. Decline in height or length is then noted around 15 months of age, the median values declining to the 2nd percentile for the normal population around age of 12 years. Already in early childhood and continuing into adolescence, acquisition of weight gain is problematic for girls with RTT. Following menstruation weight tends to increase abnormally representing a major shift in concern for mobility and ease of transfer. Therefore, attention is required to maintain acceptable weight gain throughout childhood, during adolescence, and beyond. Similar declines in hand and foot growth are also evident, with the feet ultimately more affected than the hands. The reduction in rate of foot growth appears to parallel that of height. Growth of the hands tends to be more preserved.

8.6. Scoliosis. Scoliosis in RTT demonstrates a profound increase with age [95–101]. It is typically evident by age of 4 when it is noted in ~8% of preschoolers. By age of 16, more than 80% of girls will have some degree of scoliosis. Onset may occur as late as age of 8. Progression of the scoliosis

thereafter may be of sufficient clinical significance to warrant medical or surgical attention. Progression is generally evident through the primary school years and is much more likely to be noted in girls who are nonambulatory and spend most of the day in a seating device. It is essential that positioning is optimal to minimize progression. Bracing should be considered when the curvature (Cobb angle) exceeds 25°, if not before. When the curvature exceeds 40°, surgery is strongly recommended. In the US Natural History study, surgical instrumentation was provided in 13% of girls with definite improvement in their quality of life [102, 103].

8.7. Sleep. Abnormal sleep hygiene, that is, difficulty both with falling asleep and maintaining sleep, is very common in RTT. First, it is critical to rule out medical issues that could impact sleep including GERD, constipation, urinary tract infection, or even a missed bone fracture before considering medical management of sleep. Adequate sleep hygiene is critical for the entire family such that proper attention should be placed on both associated medical issues and sleep itself. An overnight sleep study may be helpful in identifying the specific features. Melatonin will aid sleep onset in some, although its effectiveness in maintaining sleep may be questioned. Antihistamines may be transiently effective but may lose their efficacy over time, so-called tachyphylaxis. Trazodone and clonidine are generally quite effective. Chloral hydrate has long been known to be an effective sedative, but its strong taste may make it undesirable unless it can be satisfactorily compounded. However, if a G-tube is present, it can be regarded as a suitable alternative.

8.8. Ambulation. As noted previously, 80% of girls with RTT are able to walk independently. However, 30% will lose this ability during or after the period of regression such that about 50% of girls overall remain ambulatory. Anxiety is a major problem in many aspects of RTT and will certainly lessen security in maintaining independent gait or even in navigating changes in floor coverings or uneven terrain. With assistance, in some only minor degrees of support, in others significant assistance, another 20% continue to be able to walk. Whether independent or assisted, ambulation should be encouraged as much and as long as possible. For those who do not walk, weight-bearing should be an essential part of therapy, either standing frames or gait-trainers and at least twice daily. In addition to providing effective therapy, this should assist the management of bone undermineralization. In this regard, the utilization of recommended amounts of calcium and vitamin D and the periodic assessment of vitamin D levels are essential elements for optimizing bone health.

8.9. Self-Abuse. Self-abusive behavior in the form of hair pulling, biting the fingers, hands, or other parts of the upper extremities, and head banging may be noted occasionally. Further, aggressive behavior towards others may be seen such as hitting, biting, or hair pulling. While the tendency may be to provide medication for this, medical issues such as underlying infections, gastrointestinal dysfunction (GERD, constipation, or gallbladder dysfunction) as noted above, bone fractures, or the side effect of a medication already in use should first be considered. If such problems are excluded, these behaviors may be reduced by low dose risperidone (0.5 mg BID) or by an SSRI such as escitalopram. Behavioral management may also be considered as these girls do respond to specific modification techniques.

8.10. Quality of Life. As assessed by the Child Health Questionnaire Parent form (CHQ-PF50) in the US Natural History study, quality of life (QOL) in RTT syndrome revealed that individuals with lower motor abilities had less behavioral problems but that individuals with more preserved motor functions had greater behavioral issues [104]. These included aggressive behaviors toward others and a greater risk of potentially dangerous activities such as touching a hot pan or stove, climbing on furniture, or even wandering outside the home. As we are engaged in clinical trials, the idea of improving motor abilities while leading to functional concerns is a matter requiring vigilance.

8.11. Longevity. Previous reports had suggested markedly reduced survival in RTT [105]. However, more recent information indicates that survival into adulthood is more likely. A systematic study in the US revealed that survival was normal up to age of 10 and that median survival exceeded age of 50 years [106]. More recently, this was substantiated by review of data from the US Natural History study. These data not only represent critical information for parents and other caregivers about long-term care but also represent an important issue for the public health authorities. In the USA, the parents continue to provide care for most individuals with RTT. However, contingency plans involving a complex set of issues are required for the future when this is no longer possible.

8.12. Other Associated Features. Hypertonia and progressive dystonia, cardiac conduction QTc interval prolongation [107, 108], bruxism (teeth grinding), and vasomotor disturbances of feet and hands, the former more so than the latter, can be major concerns.

Muscle tone is generally reduced in infancy and early childhood, but this gradually increases with time leading to hypertonia and increased rigidity by teenage years or beyond. Dystonic posturing, particularly in the feet, but also occurring occasionally in the hands and the axial skeleton, is also prominent. These factors represent major therapeutic challenges for physical medicine and orthopedics. In addition, contractures in the lower extremities for those who are nonambulatory and spend most of the day seated or even at the elbows related to the constant midline hand stereotypies are often problematic. Orthotic devices are generally effective in maintaining neutral positions of the distal extremities, but the use of botulin toxin injections may be required.

Already in the early 1990s, prolongation of the QTc interval and an increase in nonspecific T-wave abnormalities were found in girls with RTT compared to healthy age-matched girls. These findings appeared to worsen with increasing age. Examination of QTc intervals in the US Natural History Study

revealed that nearly 20% of girls had prolongation beyond 450 msec [107]. Although most remained asymptomatic and were not treated specifically, a few girls had marked prolongation necessitating medical management, generally with β-blockers and at least one young woman received a pacemaker. Animal model studies have suggested that treatment should involve a sodium channel blocking agent rather than the utilization of a β-blocker [107].

Bruxism tends to be the most prominent during early childhood and less problematic over time. Attempts to modify this often harsh sound have not been overly helpful. Vasomotor disturbances producing cold feet and hands appear to be associated with exaggerated sympathetic tone. Again, effective treatment is elusive.

9. Neuropathology

In keeping with the abnormal deceleration in head growth, the main neuropathologic features on gross inspection are reduced brain weight and reduced volume of frontal and temporal cortex [109–121]. Volumetric MRI confirmed reduction in cortical volume as well as noting reduction in deep gray nuclei [122]. On microscopic assessment, neurons are small and closer together (increased packing density), dendritic arborizations were reduced, and melanin deposition is markedly diminished or absent in the substantia nigra [117]. Remarkably, no evidence of any recognizable disease process is evident. The absence of any progressive neuropathologic features, namely, any evidence of neuronal loss or extensive gliosis, suggests that the fundamental neurobiologic problem in RTT is neurodevelopmental and not neurodegenerative. The brain does appear normal but is generally about 60–70% of expected weight for age. Golgi studies were highly informative revealing shortened and primitive dendritic arborizations supporting the notion of a failure in the proper development and maintenance of synaptic connections. In subsequent animal model studies, similar features were noted, namely, small neurons and deficient dendrites and dendritic spines. Although the fundamental cause is quite different, similar neuropathologic features have been observed in other neurodevelopmental disorders. Dendritic spines are deficient already by 4 months of age in Down syndrome and in Angelman syndrome. Increased packing density and decreased cell size have been noted in autistic spectrum disorder [123].

10. Genetic Basis of Rett Syndrome

As a genetic disorder predominantly affecting females with a different and generally much more aggressive phenotype in some males or even fetal demise, RTT is established definitively as an X-linked dominant disorder. It typically occurs sporadically as a *de novo* mutation in germinal cells. As a majority of individuals with RTT arise from the paternal cell line mutations, these mutations appear to occur predominantly in the more rapidly developing germinal cells, namely, the sperm. Recurrences within families are much less than 1%. Despite this observation, parents desiring additional children should obtain specific information from a genetic counselor and may wish to assess the carrier status of the mother by testing for the same mutation in her peripheral blood. Although a germline mutation is considered unlikely, such testing in either the mother or fathers blood would not exclude its presence. In fact, one instance of recurrent germline mutation in a father has been reported as the result of sperm analysis [124].

Among participants in the US Natural History Study, 8 specific point mutations account for about 60% of the total and specific deletions and insertions account for another 15–18% of mutations. However, over 200 different mutations have been defined in *MECP2* in girls or women with RTT identified to-date worldwide such that numerous mutations occur in only one or a small number of individuals. In the US study, mutations in *MECP2* have been identified in >95% of females with classic RTT. Greater than 75% of females with atypical forms of RTT also have mutations. When considered as specific groups in classic RTT, R133C, R294X, R306C, and 3′-truncations are associated with a milder phenotype and the remaining five common point mutations as well as large deletions are associated with more severe clinical involvement. These data suggest that specific phenotype-genotype correlations exist [125–129]. However, when considering the impact of a specific mutation in a given situation, two girls with exactly the same mutation may have a quite different clinical profile. Several factors are involved in this difference, the most important determinant being variability in X-chromosome inactivation (XCI). Skewing of XCI can lead to milder or more significant clinical involvement regardless of the specific mutation. However, XCI determined in blood may not represent the same distribution in other cell populations such that other factors should be considered. These include the distribution of the mutation in brain cells as it is unlikely to be uniform and may vary significantly both within a given individual and between different individuals. MeCP2 is known to regulate the transcription of other genes. These effects may vary between different individuals. Finally, the involvement of other unknown factors including environmental influences such as activity level, different therapeutic programs, and dietary patterns could also be important contributors to outcome.

The same factors are likely responsible for the broad spectrum of clinical phenotypes associated with *MECP2* mutations. This spectrum may range from completely normal females to autistic spectrum disorder and nonsyndromic mental retardation and may affect both females and males. Thus, *MECP2* mutations have a very broad clinical impact apart from RTT. Following the identification of *MECP2* mutations, subsequent evaluations revealed a quite different process that explained previous observations of chromosomal rearrangement associated with developmental delays and other features as described above Section 6. Inasmuch as the clinical picture differs substantially from RTT, identification of males with duplications represents a much smaller number to-date. However, the increased availability of genome-wide array studies will likely result in increased identification of this disorder.

In recent years, girls with mutations in three other genes have had phenotypes resembling atypical RTT. *CDKL5*, expressing cyclin-dependent kinase-like 5, is located on X-chromosome at Xp22. It has an as yet unknown function but does appear to be a target of MeCP2 transcriptional repression. It produces significant developmental delay and epilepsy in both females and males and some females have the early-onset seizure variant of RTT [130, 131]. *FOXG1*, expressing forkhead box G1, is located at 14q12 and is a transcriptional repressor involved in early embryonic to adult telencephalon function. It has been associated with the congenital or limited development variant of RTT [132, 133]. A more recent and very rare cause of the early onset seizure variant of RTT involves the *NTNG1* gene located on chromosome 1 [134]. *NTNG1* is important for axonal guidance and NMDA receptor function. An attempt to identify additional affected individuals was not fruitful.

11. Functions of MECP2

MECP2, located at Xq28, encodes methyl-CpG-binding protein 2 (MeCP2). *MECP2*, derived from four exons, is ubiquitous in mammalian cells and is highly expressed in brain. It functions principally in the nucleus where it is important in the regulation of gene transcription. The MeCp2 protein occurs as two isoforms, MeCP2_e1 and MeCP2_e2, with the former being more highly expressed in brain. MeCP2 contains two functional domains, the methyl-binding domain (amino acids 78–162) that binds to methylated CpGs in DNA and the transcriptional regulating domain (amino acids 207–311) that recruits other proteins to mediate transcription of other genes. In addition, a nuclear localization signal (amino acids 255–271) directs MeCP2 to the cell nucleus. In the human brain, MeCP2 has a characteristic ontogeny first appearing in the brainstem during the first trimester with a subsequent caudal-to-rostral progression of expression such that, by 35 weeks of gestation, the protein is evident in forebrain and by age of 10 the protein is distributed widely throughout both the forebrain and brainstem. This ontogenetic pattern fits well with the onset of the disorder. Prior to the third trimester, MeCP2 is scarcely present in the forebrain, explaining the absence of abnormality in neuronal proliferation and migration at the cortical level. As MeCP2 expression in forebrain increases more significantly at or after the end of the third trimester, the delay in onset of clinical expression of RTT is not surprising.

MeCP2 was initially felt to be a transcriptional repressor, but more recent findings indicate that it has both activating and repressing functions, actually with more genes being activated than repressed [135]. The precise number and identification of genes affected are not known at present. However, several genes are known to be impacted including *BDNF* [136], expressing brain derived neurotrophic factor, *CRH*, expressing corticotrophic releasing hormone [137], and *FYXD1*, expressing phospholemman [138]. Whether MeCP2 is involved in the transcriptional regulation of additional genes is critical to a complete understanding of its function. Initial emphasis was placed on the role of MeCP2 in normal neuronal function as the principal pathologic changes were noted in neurons, namely, small neurons with abnormalities in dendritic size and complexity, synaptic organization, and axonal arborization. However, the importance of MeCP2 in glial development has received recent attention. Among the important roles of glial cells, the astrocytic role for controlling extracellular glutamate levels appears to be critical.

Table 5: Rett syndrome animal models of Rett syndrome.

Mouse model type	Genetic composition	Reference
	Null mutation	
Tm1.1Bird	Exon 3-4 deletion	[139]
Tml1.1Jae	Exon 3 deletion	[140]
Tm1.Pplt	Methyl-binding domain deletion	[141]
2loxB *TH-Cre*	Dopaminergic/noradrenergic neurons	[142]
2loxB *PET1-Cre*	Serotonergic neurons	[143]
2loxJ *hGFAP-CreT2*	Astrocytes	[144]
	Truncated mutation	
Tm1Hzo	Nucleotide 308 truncation	[145]
	Human point mutation	
Tm1.1Coyle	R168X	[146]
Tm1.1Vnar	A140V	[147]
Tm1.1Hup	R168X	[148]
Tm1.1Joez	T158A	[149]
Tm1.1IRSF	R255X	Unpublished

12. Animal Models

With the identification of *MECP2* mutations being responsible for RTT, the development of animal models advanced dramatically [139]. Prior attempts, before the linkage with RTT, at creating a knockout model, that is, with an absent *Mecp2* gene, were regarded as difficult due to prenatal demise of the null animals. Later efforts proved more successful such that both knockout and knock-in models are now available [139, 150, 151]. Animal models are available that restrict *Mecp2* deletion to the forebrain [140], the hypothalamus [152], and to dopaminergic/noradrenergic neurons [142], serotonergic neurons [143], or astrocytes [144] (Table 5). A key National Institutes of Health consensus conference was held in 2011 resulting in the elaboration of a comprehensive summary of animal models and their clinical phenotypes and clear standards for the design of preclinical studies to include adequate sample size, proper outcome measures, statistical methodologies, reporting of both positive and negative results, and replication of results in multiple animal models with varying genetic backgrounds and in independent laboratories [150]. The goal is to improve on the rather poor overall performance in moving from translational studies in animal models of a number of human disorders to specific clinical trials. Several aspects of this require emphasis related

to RTT. Much of the animal work has occurred in male mice as male siblings tend to be quite similar in onset and character of their disease expression. Secondly, these male mutants are considerably more mature at the onset of symptoms than the corresponding time of onset in humans. Further, female mice are generally several months older with considerable variation among siblings at onset of their symptomatology. Finally, while these male mice do have a characteristic phenotype, by definition, they do not represent the genetic mosaicism seen in RTT in humans. Thus, it is critical that evidence of effective therapies in males be validated carefully in the more appropriate female heterozygotes and with animals that represent different genetic backgrounds and express a variety of *Mecp2* mutations to be certain that effectiveness is evident in the more relevant female phenotype. It has been demonstrated clearly that specific features of RTT may vary with different genetic backgrounds both in the presence or absence of particular features and in the timing of their appearance. Such background effects were noted both with respect to the timing of the appearance of impaired social behavior, prepulse inhibition, and increases in weight and the overall occurrence of stress-related reduction in corticosterone levels [153]. In addition, female mice from at least two different backgrounds did not display the increase in anxiety typically noted in individuals with RTT [153].

Critical research models have explored the role of Mecp2 in specific cell populations. Thus, removal of *Mecp2* from tyrosine hydroxylase-expressing dopaminergic and noradrenergic neurons or tryptophan hydroxylase-expressing serotonergic neurons reduced the levels of their respective metabolites, homovanillic acid (HVA), or 5-hydroxyindole acetic acid (5-HIAA) [142]. This study recapitulated the very early findings in girls with RTT and provided additional evidence for such decreases from an expanded group of individuals with RTT, particularly associated with the R168X mutation. In the HVA deficient animals, abnormalities in motor activity were evident whereas abnormalities in motor learning, anxiety, social interaction, learning, and memory or in breathing function were not noted. This differed from observations in 5-HIAA deficient animals in which motor activity was not reduced whereas increased aggression was noted. In higher functioning individuals with RTT, aggressive behaviors toward parents and siblings, teachers, and classmates are common and often problematic. Interestingly, both of these animal models had a normal life span indicating that Mecp2 function in these cells did not appear essential for determining longevity.

In a related study of serotonin function in male *Mecp2* mutant mice, use of the serotonin reuptake inhibitor, citalopram, resulted in improved carbon dioxide chemosensitivity, suggesting that elevation of serotonin levels has a beneficial effect [154]. The number of animals tested was small and, unfortunately, did not include females. Nevertheless, replication of these results in appropriate numbers and with appropriate inclusion of both sexes is warranted.

In a similar cell-type specific study, *Mecp2* was removed from GABA (γ-aminobutyric acid) neurons resulting in the occurrence of specific features of RTT including stereotypic movements, motor difficulties, compulsive behaviors, and severe breathing abnormalities [143]. When the *Mecp2* removal was limited to forebrain nuclei, the RTT behaviors were preserved, but the abnormalities in breathing were absent, supporting the role of brainstem nuclei as critical for this particular feature of RTT. In addition, this model provided support for the notion of excitatory-inhibitory imbalance in RTT, particularly related to the concept of glutamate hyperexcitability.

More recently, a novel approach to *Mecp2* mutant mice involved the creation of a mutagenesis suppressor screen [155]. One of the five suppressors that resulted produced a stop-codon in the rate limiting enzyme for cholesterol biosynthesis, squalene epoxidase, and this suppressor resulted in a reduction of symptoms in the *Mecp2* mutant animals. Examining cholesterol metabolites in nonsuppressed mutant mice revealed significant abnormalities that were reversed in part along with motor performance abnormalities by the statin drugs, fluvastatin, and lovastatin in male animals and fluvastatin in female animals. This finding was prominent in a mouse strain that is obese and needs to be repeated in other strains as well to allay this concern.

13. Translational Research

The impetus for translational studies in RTT rests in part on the pivotal findings of reversing the abnormality in the null mutant model. Utilizing clever genetic engineering, null mice were produced that contained the *Mecp2* gene lying dormant under the control of the estrogen receptor [156]. At different time points, both male and female mutant animals were treated with the estrogen analogue, tamoxifen. Regardless of the level of disease expression in these animals, the tamoxifen-produced activation of the *Mecp2* resulted in remarkable improvement. Consideration has long been given to the necessity for early diagnosis to allow for earlier implementation of treatment. These findings underscored the notion that RTT represents a neurodevelopmental and not a neurodegenerative disorder and provided proof of principle that if effective therapies could be developed, they could be beneficial. Moreover, this study suggested that treatment could be effective even if implemented later in the course of the disease process. Thus, the long-term goal in RTT research continues to be identification and testing appropriate agents for therapeutic trials in animals or cell-based systems as a prelude to the necessary clinical trials in individuals with RTT. These therapeutic options are outlined in Table 6 and include restoration of the activity of mutant MeCP2, a variety of different symptomatic therapies, and gene replacement strategies. These approaches are already being assessed as described in the following paragraphs. However, additional points should be made. Attempts should be made to target the missense mutations in order to reactivate the full-length protein and with the effective promotion of full-length proteins in nonsense mutations to secondarily reactivate these as well. In total, that would deal effectively with about 70% of participants with known mutations in *MECP2*. Strategies for correcting deletion and insertion truncating mutations are more complicated to resolve in this manner.

Table 6: Rett Syndrome (therapeutic options).

MeCP2 restoration
Missense mutations: reactivate full-length protein
Nonsense (stop) mutations: promote full-length protein; may require "reactivation"
Deletions/insertions: more complicated
Symptomatic therapy
Serotonin reuptake inhibitors: ameliorate anxiety
NMDA receptor blocker: memantine reverse glutamate hyperexcitability
IGF-1: full length and tri-peptide downstream effect in BDNF cascade
BDNF-mimetics: TrkB agonists restore BDNF levels
Read-through compounds: stop mutations produce full length MeCP2
Gene therapy
Gene correction correct only abnormal allele
Stem cell transplant
X chromosome activation of normal allele activate normal allele in all cells

Symptomatic therapies are being addressed as described below. Gene therapy through provision of a normal gene or stem cell transplantation has been attempted. Theoretically, X chromosome activation of the normal allele is feasible but requires that all cells be activated to the normal allele.

While the principal research themes to-date have targeted neurons virtually exclusively, recent efforts have been extended to glia, providing yet another series of important and provocative observations. While RTT had long been considered to represent a disorder of neuronal function, very early studies had actually provided strong support for the role of MeCP2 in glia. Through the use of immunofluorescence and laser scanning cytometry, it was noted that both oligodendroglia and astrocytes express MeCP2 [157]. Certainly, the predominance of behaviors related to neuronal dysfunction was the subject of intense investigation and likely led to an active search regarding the role of neuronal cells in the disease process. However, with the notion that neuronal-glial interactions are critical, more recent attention has been directed to the glial cell population, recognizing that important pathological consequence could arise from glial dysfunction as well. Using an *in vitro* coculture system, *Mecp2* mutant astrocytes produced deleterious effects on dendritic morphology in both mutant and wild-type hippocampal neurons providing evidence for the lack of cell autonomous effects [158]. The authors suggested that these effects represented abnormalities in secretion of soluble factors. Subsequently, work from the same laboratory demonstrated that reexpression of *Mecp2* in astrocytes alone was sufficient to improve motor activity, anxiety, breathing pattern, and survival in null mutant mice and to reverse dendritic abnormalities in neurons from these animals [144]. Thus, unlike the cell autonomous effects noted with neuronal models, the impact of altered Mecp2 function in astrocytes is clearly noncell autonomous. Not considered in this model were the potential adverse effects of excess glutamate on neuronal function. Astrocytes are known to represent an important sink for extracellular glutamate and inasmuch as both brain glutamate levels and NMDA-receptors are increased in young girls with RTT, it is likely that astrocytes, normally responsible for the clearance of extracellular glutamate, are unable to maintain normal expression of relevant proteins in the absence of normal Mecp2 [159–161]. As a result, abnormalities in control of extracellular glutamate levels adversely affect neurons in terms of synapse formation and dendritic morphology. These studies correlate well with evidence that hippocampal slices from *Mecp2* mutant mice are extremely hyperexcitable [162, 163]. While additional work is required to identify the factor or factors responsible for this abnormality in astrocytic function, it is tempting to suggest that a reduction in network hyperexcitability could be achieved by modulating glutamate levels or interfering with glutamate-related synaptic activity. In this regard, the NMDA receptor antagonist, memantine, is capable of restoring two components of short-term plasticity, posttetanic potentiation, and paired-pulse facilitation [164]. As such, the prospect of modulating glutamate levels deserves scrutiny in future preclinical trials.

BDNF (brain-derived neurotrophic factor) has also received significant attention in relation to RTT [136, 165]. BDNF expression is reduced both in autopsy-derived brain samples from individuals with RTT and in *Mecp2* mutant mice. Coupled with the finding that *Bdnf* levels were reduced in Mecp2 mutant mice, excitement has been raised regarding increasing BDNF levels in RTT. However, application of BDNF directly is limited by two factors, one being its short half-life, the other being that BDNF does not readily cross the blood-brain barrier. Thus, preclinical studies have focused on the activation of endogenous BDNF or the application of compounds that are capable of mimicking BDNF. It is known that, when *Bdnf* is overexpressed in *Mecp2* mutant mice or in neurons transfected with relevant RTT *MECP2* mutations, the behavioral phenotypes in animals and dendritic abnormalities in cultured primary neurons can be reversed [166, 167]. Similarly, application of the so-called nootropic AMPAkines, molecules known to increase BDNF expression, has reversed the synaptic impairments in the brainstem of *Mecp2* mutant models with abnormal breathing patterns [167–169]. Finally, the BDNF mimetic drugs have superior blood-brain barrier profiles and have been demonstrated to modulate function in *Mecp2* mutant models. Molecules that act like BDNF, termed "BDNF mimetics," have the capability to pass through the blood-brain barrier and bind to the TrkB receptor. The TrkB agonists, LM22A-4 and 7,8-DHF, are two agents that appear capable of reversing features of RTT in these animal models [170, 171].

Operating within the same pathway as BDNF, a tripeptide of IGF-1 was noted to produce better motor performance, to increase dendritic spine density and motility, and to correct breathing dysfunction in *Mecp2* mutant mice [172]. In a separate study using induced pleuripotential stems cells reprogrammed from skin fibroblasts of individuals with RTT, full-length IGF-1 application resulted in an increase in

synaptic development in neurons derived from this technique [173]. These results led directly to on-going clinical trials in RTT as described below.

A completely different approach has been taken directed at nonsense or so-called STOP mutations. It has been known for some time that the aminoglycosides, a class of potent antibiotics, are capable of reading through the premature STOP codon to achieve a protein of full-length. Although this protein will not have the correct amino acid composition, it is posited that the full-length protein will have greater functional capabilities than its mutant precursor. Knockout mutations occur in approximately 35% of girls with RTT in the US Natural History study such that this approach could target a significant number of affected participants. Animal studies utilizing *MECP2* nonsense mutations have shown promise when exposed to gentamycin as well as to small molecule compounds with similar read through properties [148, 174, 175]. The development of gentamycin analogues lacking the toxic side effects of gentamycin or the class of small molecules is crucial as gentamycin itself is associated with significant ototoxicity and renal toxicity when used chronically. Studies are ongoing to evaluate the effects of these compounds in mutant *Mecp2* animal models with knockout mutations. Similarly, studies using the induced pleuripotential stem cell-derived neurons from an individual with RTT carrying a nonsense mutation indicated that the aminoglycoside, gentamycin, increased levels of MeCP2 although this finding was not reproduced at a higher gentamycin dosage [173].

Efforts at hematopoietic stem cell transplantation (HSCT) have been revealing but not overall successful. Presymptomatic male and female mutant mice did appear to have improved survival and motor performance, but symptomatic males were not benefited and female symptomatic mice were not tested [176]. However, the studies suggested that microglia resulting from the HSCT did have improved function over endogenous microglia from the mutant mice suggesting that pharmacologic manipulation of microglial cells could be a therapeutic target [177]. Unfortunately, efforts to reproduce these findings have not been forthcoming. Furthermore the methodology utilized in HSCT is inherently problematic in and of itself.

Systemic delivery of intact Mecp2 to female *Mecp2* mutant mice using the adenoassociated virus as a vehicle resulted in improved survival and overall motor performance but did not appear to produce resolution of breathing dysfunction [178]. The gene replacement either did not reach critical brainstem nuclei or did not have a brainstem impact. The number of animals treated was small. Further, animals treated by direct cranial injection had only modest improvement and more concerning the appearance of parkinsonian features that were attributed to the scAAV9/cre virus utilized, independent of the Mecp2.

14. Treatment

14.1. Clinical Trials. Clinical trials have been relatively few in RTT. Naltrexone was employed in the premutation era in an effort to ameliorate breathing dysfunction. While the treatment group in this double-blind, placebo-controlled trial did appear to have a reduction in breathing abnormalities, this could not be distinguished from the sedating properties of the opiate antagonist. Further, stratification of participants did not yield balanced groups such that individuals in the treatment group actually progressed more rapidly than those in the control group.

Shortly after identification of *MECP2* mutations as the cause of RTT and with knowledge that one role of MeCP2 is to bind to methylated CpG nucleotides, a double-blind, placebo-controlled trial of folate and betaine was employed. No objective evidence of improvement was noted although some parents of participants in the treated group observed subjective improvement in overall behavior.

Active clinical trials currently involve the use of either IGF-1 (NCT01777542) or a tripeptide fragment of IGF-1 (NCT01703533). The mechanisms of action of the full length IGF-1 and the tripeptide are felt to differ. The full length compound is a 70-amino acid peptide that inserts itself downstream of BDNF at the IGF-1 receptor and activates the same Akt pathway. The tripeptide is only bound weakly to the IGF-1 receptor and has other actions that are independent of the full-length molecule. Based on studies in *Mecp2* mutant mice, the IGF-1 tripeptide was shown to penetrate the blood-brain barrier and to promote increased longevity and improved motor performance as well as improve neurite outgrowth and synaptogenesis. The full-length IGF-1 is approved for use in children with short stature and an IND was obtained to utilize this compound in RTT. Due to its effect on bone growth, IGF-1 is approved for use in RTT participants less than age of 11 years. As a subcutaneous injection, the phase 2 safety trial showed that the agent had no concerns in the RTT participants. An advanced phase 2 double-blind, placebo controlled, crossover design trial is now in progress (two 20-week assessments with an intervening 10-week washout period) with outcome information expected in about one year.

A separate phase 2 trial with the modified initial tripeptide of the IGF-1 molecule is also being conducted. Inasmuch as this tripeptide is being trialed in individuals age of 15–45 years with traumatic brain injury, significant safety data already exist allowing a similar 40-day double-blind, placebo-controlled safety trial to proceed in a two-phase dose escalation protocol. This agent is provided as an oral preparation. It is anticipated that this trial will be concluded before the end of 2104 and at that time additional approval will be received to test this agent in younger participants.

A double-blind, placebo-controlled trial with secondary crossover for both groups to active agent is on-going with the serotonin reuptake inhibitor, escitalopram, to evaluate its anxiolytic properties. This agent is approved for use in children at age of 12 years and older.

An additional trial involves the use of dextromethorphan (NCT01520363). It is a three-month double blind, placebo-controlled trial involving girls, age 2–10 years with classic or atypical RTT. Dextromethorphan is an NMDA receptor antagonist and the trial proposes to evaluate its efficacy in

alleviating glutamate hyperexcitability. This phase 2 safety assessment will examine improvements in cognitive function.

14.2. Clinical Therapies. Current recommendations regarding on-going treatment for individuals with RTT include aggressive physical, occupational, and speech and appropriate interventions as required for nutrition and optimal growth, orthopedic concerns, proper assessment and management of epilepsy and GI dysfunction, and other issues as they become relevant. Despite the limitations in motor performance, physical and occupational strategies should be employed daily. The establishment of optimal communication is also essential. This should take advantage of the improved social interaction and eye contact which develop by school age and involve the application of the recently available computer technologies as best as possible. Optimal growth and nutrition are especially relevant by school age and beyond. An expert in nutrition is often necessary to maintain proper guidance. The principal orthopedic concerns relate to scoliosis and limitation of joint mobility. Scoliosis should be assessed regularly and bracing or surgical intervention with stabilizing rods should be implemented based on these assessments. The presence of epilepsy should be assessed accurately, including the use of video-EEG where necessary. While antiepileptic agents may be warranted, care should be used in selecting the proper agents, being mindful to observe the potential side-effects and to remove drugs that are not effective before adding a new agent. Great care should be taken to avoid multiple drugs that may be ineffective as a group. Inasmuch as GI issues play a major role in the general well-being of those with RTT, careful attention must be paid to all aspects.

As suggested above, long-term care and planning are crucial. Proper attention to other medical and dental issues is essential in view of the potential longevity of individuals with RTT. An ECG for prolongation of the QTc interval and gynecologic assessment should be performed annually. Inasmuch as adult physicians have little experience in dealing with RTT or other neurodevelopmental disorders for that matter, their education must be accelerated to allow for the proper provision of continuity of care.

The principal caregivers must also develop a long-term care plan. When individuals with RTT reach the age of majority, certificates of guardianship must be obtained by their responsible caregivers. As formal school options end, usually by age of 22, the treatment and social programs available during the prior 21 years will cease. It is essential that planning for satisfactory replacements is made well in advance in order to maintain a stable transition to an adult program. This is a major issue from both the public health and societal perspectives. Finally, in planning for the future, a blind trust is necessary in order for individuals with RTT to preserve their resources.

In summary, women with RTT may well survive into middle age. Parents and other caregivers may require guidance and assistance both to promote the well-being of individuals with RTT including socialization and interaction with family and friends and to address the specific issues involving health care and continuing therapies specific to RTT.

15. Future Perspectives

Understanding the complexities of *MECP2* mutations, the dysregulated genes that contribute to specific symptoms of RTT, and rational approaches to effective therapy represent the major challenges for the future. The effective management of RTT will depend on finding and implementing FDA-approved and repurposed agents and investigating novel compounds through continued research. While current emphasis is focused on addressing specific symptoms related to RTT, continued emphasis must be placed on treating the underlying cause in a manner to provide an effective and lasting cure. This will rely on promoting the interaction of both basic and clinical research through major efforts in translational research and the necessary and critical collaboration with pharmaceutical enterprises and regulatory agencies.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

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