Rett syndrome: Neurologic and metabolic aspects
Hagebeuk, Eveline

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Rett syndrome: clinical, laboratory, and therapeutic evaluation. Review of recent developments

Eveline E.O. Hagebeuk¹
Marinus Duran²
Johannes H.T.M. Koelman³
Nico Abeling²
Bwee Tien Poll-The¹

Department of Pediatric Neurology¹, Laboratory Genetic Metabolic Diseases², Department of Neurology and Clinical Neurophysiology³, Academic Medical Center, Amsterdam, The Netherlands

Orphanet Journal of Rare Diseases, Submitted.
Abstract

Rett syndrome (RTT) is a neurodevelopmental disorder, characterized by a regression that affects purposeful hand-, motor-, cognitive- and communication functions. Stereotypic hand movements, ataxia, apraxia, seizures, breathing and sleep disturbances develop over time. The clinical diagnosis is confirmed by a dominant mutation in the gene, encoding methyl-CpG binding protein 2 (MeCP2), on the X chromosome. Because of new diagnostic RTT criteria and the possibility of molecular genetic confirmation, more RTT patients are diagnosed. This review gives an overview of developments which led to a better understanding of the various aspects of epilepsy, cardiorespiratory and sleep disturbances. Recent neurometabolic and intervention studies are described in more detail. Besides this, pathophysiology and genetics in RTT are discussed.

**Key words:** Rett syndrome, Epilepsy, MECP2, Neuropathology, Neurometabolic evaluations, Folates, Cardiorespiratory and sleep disturbances
Definition

Rett syndrome (RTT; OMIM 312750; ORPHA778) is a progressive neurodevelopmental disorder, characterized by apparently normal psychomotor development during the first 6 to 18 months of life, followed by regression that affects speech, motor skills and purposeful hand function, replaced by stereotypic hand movements. Additional features include postnatal deceleration of head growth and motor abnormalities, such as hypertonia and dystonia, apraxia, and broad based gait. Seizures and breathing disturbances belong to the most detrimental clinical phenotype in RTT.\textsuperscript{1-4} Most cases result from mutations in the \textit{MECP2} gene, coding for a methyl-CpG binding protein 2, on the X chromosome.\textsuperscript{5}

Epidemiology

RTT occurs in all ethnic groups at similar rates.\textsuperscript{6} The population-based Australian Rett Syndrome Database (including atypical RTT) showed an increase of birth prevalence of RTT to 0.88 (2004) and an overall prevalence of 0.93 per 10,000 females (1976-2008), ages 0-19 years.\textsuperscript{6-7} The median age at diagnosis has decreased to 3.5 years.\textsuperscript{7}

Diagnostic criteria

The diagnosis of classic RTT was originally based upon eight obligate-, and eight supportive clinical criteria.\textsuperscript{1} The revised diagnostic criteria are now limited to the obligatory presence of a period of regression, and four main criteria, i.e. partial or complete loss of acquired purposeful hand skills and speech, gait abnormalities and stereotypic hand movements (Table 1).\textsuperscript{4} Atypical variants of RTT may be either milder or more severe than classic RTT and not all main RTT criteria are obligatory for diagnosis (Table 1). The late regression variant, in which school performance declines at pre or early school age, is rare. The preserved speech variant is a milder phenotype, including better manual and speech–language abilities, milder intellectual disability, and less frequent seizure or breathing disorders.\textsuperscript{3} Exclusion criteria for classic RTT are any other cause of developmental delay and grossly abnormal psychomotor development in the first 6 months of life.
Table 1. Revised diagnostic criteria for Rett syndrome (RTT) 2010.4

Consider the diagnosis when postnatal deceleration of head growth is observed

**Required for typical or classic RTT**
1. A period of regression followed by recovery or stabilization
2. All main and all exclusion criteria
3. Supportive criteria are not required, although often present in typical RTT

**Required for atypical or variant RTT**
1. A period of regression followed by recovery or stabilization
2. At least 2 of the 4 main criteria
3. At least 5 of the 11 supportive criteria

**Main criteria**
1. Partial or complete loss of acquired purposeful hand skills
2. Partial or complete loss of acquired spoken language
3. Gait abnormalities: impaired (dyspraxia) or absence of ability (apraxia)
4. Stereotypic hand movements such as hand wringing/squeezing, clapping/tapping, mouthing, and washing/rubbing automatisms

**Exclusion criteria for typical RTT are:**
1. Brain injury secondary to peri- or postnatal trauma, neurometabolic/genetic disease, or severe infection that causes neurological problems
2. Grossly abnormal psychomotor development in the first 6 months of life

**Supportive criteria for atypical RTT are:**
1. Breathing disturbances when awake
2. Bruxism when awake
3. Impaired sleep pattern
4. Abnormal muscle tone
5. Peripheral vasomotor disturbances
6. Scoliosis/kypnosis
7. Growth retardation
8. Small cold hands and feet
9. Inappropriate laughing/screaming spells
10. Diminished sensitivity to pain
11. Intense eye communication and eye-pointing behavior

**Staging scales of clinical and electroencephalographic RTT features**

Clinical symptoms have been outlined in a four stages-clinical staging system.1,8 The electroencephalographic (EEG) findings are categorized in four EEG stages, which correlates with the clinical stage of the RTT patient (Table 2) 9. However, it is often difficult to discern the transition between stages.

Supplementary numerical severity classification scales are the symptom severity score (SSS) and the Kerr score.10,11 The validated Rett Syndrome Motor Behavioral Assessment Scale classifies the major clinical symptoms of RTT, such as behavioral and social skills, orofacial and respiratory signs and motor signs, in objective numerical ratings.12
### Table 2: Clinical and EEG stages in Rett syndrome

<table>
<thead>
<tr>
<th>Stage</th>
<th>Age (yrs)</th>
<th>Duration</th>
<th>Clinical stage&lt;sup&gt;1&lt;/sup&gt;</th>
<th>EEG stage&lt;sup&gt;9&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stage I</strong></td>
<td>0.5-1.5</td>
<td>weeks-months</td>
<td>Change in interactive communicability</td>
<td>Normal or minimal slowing of the awake occipital dominant rhythm</td>
</tr>
<tr>
<td>Early-Onset</td>
<td></td>
<td></td>
<td>Postural development progress, but delayed “bottom-shufflers”</td>
<td></td>
</tr>
<tr>
<td>Stagnation</td>
<td></td>
<td></td>
<td>Language development minimal</td>
<td></td>
</tr>
<tr>
<td><strong>Stage II</strong></td>
<td>1-4</td>
<td>weeks-months</td>
<td>Rapid regression of acquired abilities</td>
<td>Slowing of occipital dominant and background rhythm</td>
</tr>
<tr>
<td>Rapid</td>
<td></td>
<td></td>
<td>Previously acquired babbling disappears</td>
<td>Focal spike or sharp wave discharges</td>
</tr>
<tr>
<td>developmental</td>
<td></td>
<td></td>
<td>Loss of fine motor skills and play interest</td>
<td></td>
</tr>
<tr>
<td>regression</td>
<td></td>
<td>(up to 1 year)</td>
<td>Mental retardation</td>
<td></td>
</tr>
<tr>
<td>period</td>
<td></td>
<td></td>
<td>Eye contact preserved</td>
<td></td>
</tr>
<tr>
<td><strong>Stage III</strong></td>
<td>preschool-early school</td>
<td>years-decades</td>
<td>After regression, some stabilization develops</td>
<td>Dominant theta and delta activity</td>
</tr>
<tr>
<td>Pseudo</td>
<td></td>
<td></td>
<td>Stereotypy of hands replaces hand function</td>
<td>No occipital dominant rhythm</td>
</tr>
<tr>
<td>stationary</td>
<td></td>
<td></td>
<td>Gross motor dysfunction and ataxia</td>
<td>Prominent central rhythmic theta activity</td>
</tr>
<tr>
<td>period</td>
<td></td>
<td></td>
<td>Contact and eye-gaze communication</td>
<td>More multifocal epileptiform discharges</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Epilepsy and breathing disturbances</td>
<td>and generalized slow spike-wave pattern</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Loss of NREM sleep characteristics</td>
</tr>
<tr>
<td><strong>Stage IV</strong></td>
<td>decades</td>
<td></td>
<td>Non ambulant (wheelchair dependency)</td>
<td>Loss of occipital dominant rhythm</td>
</tr>
<tr>
<td>Late motor</td>
<td></td>
<td></td>
<td>Scoliosis</td>
<td>Marked slowing background activity (delta)</td>
</tr>
<tr>
<td>deterioration</td>
<td></td>
<td></td>
<td>Growth retardation, cachexia</td>
<td>Multifocal epileptiform discharge, in sleep</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Epilepsy and emotional contact improve</td>
<td>continuous generalized spike-wave activity</td>
</tr>
</tbody>
</table>
**Classic RTT syndrome**

A historical overview of the main developments in classic RTT is presented as a time line figure (Figure 1). Recently, mutations in the genes cyclin-dependent kinase-like 5 (CDKL5, located on the X-chromosome) and forkhead box protein G1 (FOXG1, an autosomal gene) have been reported to resemble clinical features of RTT and are called atypical or congenital RTT, although significant differences from RTT have been documented, such as developmental delay from birth on\(^{13}\), and they should be regarded as a separate entity.\(^{14}\)

The different clinical aspects of RTT have been discussed in several excellent reviews.\(^{2,3,15-18}\) Therefore, we will focus exclusively on the clinical symptoms, neurometabolic evaluations, therapeutic attempts, and future prospects.

**Figure 1: RTT time line**

![RTT time line](image)

**Clinical description**

RTT patients are not dysmorphic and often look like their parents.\(^{19}\) Postnatal deceleration in head growth is not always present and is not required for the clinical diagnosis.\(^{4}\) Although social interaction diminishes, alertness and eye gaze-based communication remain present.\(^{2}\) Hearing is often impaired.\(^{20}\) The typical cold and blue extremities are signs of autonomic nervous system dysfunc-
tion. RTT patients lack speech development in their second year of life, and lose acquired babbling and speech. Purposeful hand function is replaced by midline hand stereotypies such as wringing, clapping and mouthing. In the remaining hand function, hand apraxia develops. In the younger RTT patients hypotonia predominates, subsequently replaced by hypertonia, rigidity, and dystonia. RTT patients may acquire the ability to walk, but after some time gait apraxia and ataxia develop. Together with joint deformities and tight tendon-Achilles, resulting in an equinovalgus/varus position, independent walking is impaired. Locomotor and orthopedic problems in RTT worsen throughout life. Bone mineral deficit and immobility increases the risk of bone fractures (30%). Scoliosis is common in non-ambulant Rett patients and increases with age.

**Seizures**

Epilepsy is very common in RTT. Its prevalence increases with age and correlates with the clinical severity and the type of MECP2 mutation. Notably, seizures are more frequent in patients with large deletions. The median age of seizure onset is 4 years, according to an international internet Rett database. Seizures were rarely reported before the age of 2, but thereafter the proportion affected by seizures was as follows: aged 3-5 years 33%, aged 5-10 years 62%, and in 86% of adults. The seizure frequency varied widely with seizures on a yearly, monthly-, weekly-, and daily basis, in 36, 27, 20 and 11 percent respectively. The most common types reported include complex partial, generalized tonic-clonic and myoclonic seizures. Treatment was unnecessary in 20% of patients, 37% received one anti-epileptic drug, 35% two, and 8% received three or more anti-epileptics. Frequently used anti-epileptic drugs are broad spectrum drugs, such as valproate, lamotrigine, carbamazepine and levetiracetam. A vagal nerve stimulator (13%) and a ketogenic diet (12%) may improve intractable seizures. Early-onset (median age 3 years) and daily seizures may be drug-resistant, and at that time electrical status epilepticus during slow sleep (ESES) is frequently present. Approximately 30% of RTT patients have drug-resistant epilepsy, which is more often present in specific MECP2 mutations such as R106W, R168X and R255X. In those cases, valproate, levetiracetam, zonisamide and lamotrigine are used. The severity and frequency of the seizures decline in adulthood, and discontinuation of treatment is then advisable. Paroxysmal non-epileptic manifestations, such as breath-holding, hyperventilation, staring, myoclonus, head turning, dystonia, and oral fascial dyskinesias are often misdiagnosed as seizures. Prolonged video-EEG monitoring is recom-
mended to differentiate non-epileptic events from actual seizures and to identify unrecognized seizures.\textsuperscript{9,31} The EEG is almost invariably abnormal after the age of 2 (Table 2).\textsuperscript{9} This further emphasizes the importance of correlation of clinical and EEG findings. Despite the presence of electrographic discharges, clinical seizures may be absent and at that time, anti-epileptic drug treatment should not be started.

\textbf{Cardiorespiratory disturbances}
Breathing and sleep disturbances are not obligatory criteria, but are prominent features of RTT. Hypoventilation, central apnea, episodic hyperventilation followed by prolonged apneas, air swallowing, and Valsalva manoeuvres frequently occur. Bradycardia predominates during sleep, whereas during the daytime tachycardia and bradycardia alternate. The severity of these cardiorespiratory disturbances varies,\textsuperscript{32} is more prominent when awake, and can be life threatening.\textsuperscript{33,34} Recurrent breath holds may cause prolonged QT syndrome.\textsuperscript{35} Julu et al. classified the daytime cardiorespiratory disturbances in three groups: 1) forceful breathers have a period style of breathing and fixed low partial pressure of carbon dioxide (pCO\textsubscript{2}); 2) feeble breathers have a weak respiration with a chronic high pCO\textsubscript{2}; and 3) apneustic breathers have long-lasting apneas and accumulate CO\textsubscript{2}.\textsuperscript{36} Brainstem dysfunction is thought to be the cause.\textsuperscript{35,36} This idea is further supported in Rett mouse models.\textsuperscript{39-41} During sleep respiratory disturbances, such as central apnea, hypoventilation and obstructive sleep apnea syndrome have been reported.\textsuperscript{32,35,37,38}

\textbf{Sleep}
Sleep disturbances, affecting at least 80\% of RTT patients, consist of increased total night-time sleep and excessive daytime sleep, without fixed duration of sleep, despite aging.\textsuperscript{42,43} Besides this, irregular sleep/wake patterns and nighttime screaming and laughing are present.\textsuperscript{43} Polysomnography revealed reduced Rapid Eye Movement sleep compared to age-matched healthy controls, frequent arousals and low sleep efficiency.\textsuperscript{44}

\textbf{Gastrointestinal and growth disturbances}
Gastrointestinal dysfunction, such as prolonged feeding time, reflux (39\%), constipation (80\%), dysphagia (92\%), as well as chewing and swallowing difficulties (81\%) frequently complicates RTT, but diminishes with age. In teenage and adult RTT women, growth deficits (45\%) and malnutrition, despite normal dietary intake, are increasingly present.\textsuperscript{21,46} Often gastrostomy feeding is necessary. RTT
patients have no pubertal growth spurt, although the mean BMI is similar to the control population. This growth failure is more prominent in specific \textit{MECP2} genotypes and is associated with disease severity.\textsuperscript{46}

## Biochemical and molecular aspects

\textit{Molecular genetics}

In the majority of patients, RTT is caused by a dominant mutation in the \textit{MECP2} gene. The genomic locus of \textit{MECP2} gene spans approximately 80 kb and consists of 4 exons, from which two different isoforms of MeCP\textsubscript{2} may be transcribed. MeCP\textsubscript{2}e1 (encoded by exon 1, 3 and 4) is the predominant isoform expressed in the brain, and MeCP\textsubscript{2}e2 (encoded by exon 2,3 and 4) is expressed in peripheral tissues as well as in the brain.\textsuperscript{47} At the protein level MeCP\textsubscript{2} contains four functional domains, a methyl-CpG binding domain (MBD) (split between exon 3 and 4)\textsuperscript{48}, a transcriptional repression domain (TRD, exon 4)\textsuperscript{16}, two nuclear localization signals and the C-terminal segment (exon 4).\textsuperscript{17} Most mutations are located in exons 3 and 4 (MBD, TRD), and rarely in exon 1.\textsuperscript{49} No more than 10\% of the mutations are small C-terminal deletions, 6\% complex rearrangements. Although more than 396 different mutations have been described so far (RettBASE, http://mecp2.chw.edu.au), the eight most commonly occurring mutations account for 60\% of all mutations.\textsuperscript{50} Missense mutations (single nucleotide transitions, causing an amino acid change), such as R133C, R106W and T158M, are the most frequent and tend to cluster in the MBD.\textsuperscript{50} However, in the TRD, nonsense mutations (single nucleotide change resulting in a stop codon, and thus a truncated protein) include the frequent mutations R255X, R270X, R294X and R168X and the missense mutation R306C. (http://mecp2.chw.edu.au). Larger deletions or earlier truncations result in a more severe clinical picture. An international RTT Networked database has been set up to pool data for genotype-phenotype correlations (http://rettdatabasesnetwork.org).\textsuperscript{51} However, genotype-phenotype comparisons are complex, especially in an X-linked disease.

It has become clear that females with \textit{MECP2} mutations present with a much broader phenotype than originally envisaged, including unexplained neonatal encephalopathy, mild learning disabilities without RTT features, autism, Angelman syndrome phenotype and variant RTT.\textsuperscript{52} Therefore, the diagnosis of classic RTT rests on clinical criteria.\textsuperscript{4}
**Genetic counselling**

RTT is inherited as an X-linked dominant trait, resulting from a *de novo* mutation in *MECP2* in more than 99%. The missense mutations are almost exclusively on the paternally derived X-chromosome. However, single nucleotide insertions and deletions or early truncating frameshift mutations are more likely maternal. With a negative family history, and if a MECP2 mutation is not detected in the mother, the recurrence risk of RTT to sibs is less than 0.1%. It seems prudent to offer prenatal testing with regard to a next pregnancy to both parents. Another situation occurs in familial RTT. In rare family cases the mother is either a carrier of the mutation or mosaic for the mutation, Recurrence within sibships of *de novo* mutations originating in the maternal meiosis has been described, as well as a mother who has a *MECP2* mutation, by somatic mosaicism, or skewed X-inactivation. These mothers may be symptomatic with mental retardation. When the mother is a known carrier, the risk to her offspring of inheriting the *MECP2* mutation is 50% in each pregnancy. Originally it was thought that the large preponderance of females versus males with RTT was due to early male lethality of the syndrome. To date, It is known that the preponderance of females is sufficiently explained by the large proportion of *de novo* mutations located on the paternal allele. The male phenotype ranges from mild or severe intellectual disability reaching adulthood, to a severe congenital encephalopathy and early death. Mutations in *CDKL5* and *FOXG1* can cause clinical syndromes that mimic RTT and should be considered in the differential diagnosis of certain patients with a normal *MECP2* genotype.

**Neurometabolic evaluations and therapeutic attempts.**

A wide variety of tests to demonstrate a single consistent biochemical abnormality in RTT is presented in Table 3 in a detailed overview.

**Carnitine and mitochondrial dysfunction**

Carnitine is synthesized in the liver and kidney or acquired from the diet. Carnitine deficiency has been seen coincidentally in RTT; no clinical effects of L-carnitine supplementation have ever been observed. It was postulated that the underlying defect in RTT might affect mitochondrial function. However, in a cohort of 44 RTT patients, plasma lactate was consistently normal. On the other hand, electron microscopic evaluation of muscle biopsies, have shown mitochondrial changes.
**Oxidative stress**

Oxidative stress (OS) is an imbalance between the systemic manifestation of reactive oxygen species (ROS) and the inability to detoxify them. The ROS are toxic and may destroy valuable substances, such as the omega-3 polyunsaturated fatty acids (ω-3 PUFAs). Supplementation with ω-3 PUFAs resulted in a significant reduction of OS (trapping of ROS) and significant improvement of the clinical severity score, in particular for motor-, non-verbal communication and respiration. OS biomarkers seems to be related to neurological symptom severity, mutation type and clinical presentation in RTT.

**DNA methylation**

Based on the hypothesis that altering DNA methylation could improve residual MeCP2 function, several trails haven been conducted. For example, the vitamin folate, is essential for DNA synthesis, growth and development of the central nervous system (CNS). The active form 5-methyltetrahydrofolate (5MTHF) is transported to cerebral spinal fluid (CSF) and through intermediate steps, converted into the methyl donor S-adenosylmethionine (SAM). Low levels CSF 5MTHF have been demonstrated in 32-50% of European RTT patients, however this could not be reproduced in an American cohort of 76 patients. Folinic acid restored CSF 5MTHF levels and RTT symptoms. In our randomized placebo-folinic acid trial, there was no change in the CSF SAM/SAH ratio, no clinical improvement and only limited effects in seizure control, and EEG features. Other methyl donors have been attempted, including betaine, but failed to give an effect. Creatine is a methyl group accepter. Plasma and urine creatine evaluations showed conflicting results. Oral creatine increased DNA methylation, however without clinical improvements.

**Biogenic amines and neurotransmitter disturbances**

Rigidity, dystonia, autonomic dysfunction, behaviour and sleep disturbances are all associated with neurotransmitter or biogenic amine disturbances, such as dopamine, serotonin and norepinephrine. Decreased CSF levels of their metabolites, i.e. homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5HIAA), are inconsistent in RTT. Post mortem brain studies showed disturbed levels of dopamine and age-dependent development of the receptors for glutamate, γ-aminobutyrate acid (GABA) and N-methyl-D-aspartate (NMDA), which confirm the importance of MECP2 gene for neurotransmitter regulation. However, alterations of 5-HIAA are common (19%) in patients with neurometabolic or epileptic encephalopathies, and therefore not specific for RTT.
Reduced CSF β-endorphin (an agonist of the opioid receptors), is supplemented by an opiate antagonist (naltrexone), with diminishing of breathing disturbances during the day.\textsuperscript{86}

**Growth factors**

Neurotrophic factors are important regulators of neuronal growth, differentiation and synaptic formation. Nerve growth factor acts on the frontal cholinergic neurons; in RTT Nerve growth factor disturbances are present.\textsuperscript{87,88} Insulin-like growth factor-I treatment in \textit{MECP2} mutant mice diminishes RTT symptoms\textsuperscript{89}, however, Insulin-like growth factor-I levels in RTT patients are normal.\textsuperscript{90} Brain derived neurotrophic factor, which expression is controlled by \textit{MECP2} gene, is the most prevalent growth factors\textsuperscript{18,85} and appears to be a genetic modifier of RTT severity.\textsuperscript{91}

**Neuroimaging and Neuropathology**

Volumetric and multiple-approach MRI studies showed volumetric reduction throughout the brain in RTT, which is less prominent in the cerebellum, whereas there is preferential reduction of the (pre)frontal and anterior temporal regions.\textsuperscript{62,92,93} Diffusion tensor imaging (DTI) MRI studies revealed the presence of white matter tract disturbances in regions including the frontal white matter, corpus callosum, internal and external capsulae.\textsuperscript{93} Proton magnetic resonance spectroscopy (MRS) revealed presence of mild white matter pathology.\textsuperscript{94} The average brain weight of RTT patients (aged 3-42 years) is reduced, 900 gr, comparable with a 1 year old infant. The brain weight is equally reduced in both gray and white matter, does not decrease further with increasing age, and is not caused by atrophy.\textsuperscript{62,33,95} Macroscopic brain evaluation shows absence of altered gyral patterns, vasculature, or cranial nerve abnormalities.\textsuperscript{62,95} At the cellular level, the cortex, thalamus, basal ganglia, hypothalamus and hippocampus, show a reduced neuronal size. Besides this, the size and branching of the dendritic spines of the cortical pyramidal cells are decreased.\textsuperscript{95} Even glia cells play a role in the pathology of RTT.\textsuperscript{55}

**Animal models**

To clarify the pathogenesis of RTT, mouse models of RTT have been made, which show phenotypic abnormalities resembling the human RTT, summarized in an
excellent review by Katz.\textsuperscript{97} Potential treatment can be evaluated in mouse models of RTT, prior to clinical trials.\textsuperscript{97}

**Differential diagnosis**

The differential diagnosis of RTT depends upon the age, gender and clinical stage of presentation and includes many causes of intellectual impairment, autism, inborn errors of metabolism and genetic neurodevelopmental disorders. For example Pitt-Hopkins syndrome patients have dysmorphic features and breathing irregularities, without the loss of speech and hand use. RTT and Angelman syndrome may be difficult to distinguish. However, patients with Angelman syndrome have dysmorphic features. Seizures tend to be much more difficult to manage than in classic RTT, except for the congenital (FOXG1) and early seizure variant (CDKL5). Both RTT like phenotypes, are characterized by developmental delay and hypotonia from birth on, without a regression period, which is obligatory in classic RTT. In all conditions, neurometabolic, neuroimaging and genetic screening is mandatory.

**Prognosis**

Epilepsy is a prominent symptom in RTT and substantially contributes to the morbidity of the disease. The clinical severity can in part be predicted based on the type of mutation (missense versus truncation), its location, and the presence of skewed X-chromosome inactivation.

The mortality rate in RTT is 1.2% per annum, 13% of these patients had prior severe seizures, 26% of these were young adult RTT patients, who died sudden and unexpected, caused by respiratory dysrhythmias and complications such as pneumonia.\textsuperscript{33} Life expectancy is better in individuals with, less severe, variant RTT versus classic RTT, but both have the potential for survival into middle age.\textsuperscript{98} At 25 years of age, 77.8% of Australian RTT patients were still alive.\textsuperscript{6} Individuals with RTT are not capable of independent living. Therefore, long-term care planning within a multidisciplinary team, to improve the care and quality of life of RTT patient, is necessary.
Unresolved questions and future research

Over the past years the genetic basis of RTT has been firmly established. Mutations of the MECP2 gene have been found responsible for the disease in the majority of patients and the nature of the mutation generally predicts the severity of the symptoms. The exact function of the MECP2 gene, apart from its action as a transcription repressor, remains to be established. Moreover, not all the genes or proteins in the central nervous system, which are influenced by mutations in MECP2, are known. Both a proteomic approach and the use of array techniques will eventually lead to the unravelling of the interplay of MECP2 and its target genes and proteins.

The time course of RTT in the majority of patients includes a more or less symptom-free interval prior to the development of the clinical signs with the deceleration of the head growth as one of the most important signs. It can be envisaged that some accumulation of metabolites/storage products has to take place, which then triggers the onset of disease. A metabolomics study of the CNS compartment such as the cerebrospinal fluid or the intracellular content of brain tissue may turn out to give additional clues to the understanding of the disease process. It would be especially useful to perform these studies in the four stages of the disease, thereby throwing light on the development of these stages.

Thus far a number of neurometabolic pathways in RTT have been probed by the analysis of (end)metabolites in the CSF and by the experimental supplementation of vitamins and cofactors. The clinical effects of these attempts at treatment have remained rather disappointing, while conflicting results of the neurometabolic investigations have been achieved, with the exception of the of the evidence of the role of disturbed methylation processes in RTT. Further studies of DNA- and protein methylation might be expected to be fruitful in the future. Several mouse models have been generated and their use has shown some promising results, e.g. in the reversal of symptoms. The translation of the observations in murine tissue to the human counterpart is a challenge, but may yield important answers and may even show the way to gene therapy.

Random X-chromosome inactivation in female RTT patients is a complicating factor in evaluating the progress of the disease as well as the results of therapeutic interventions. This can be overcome by studying male RTT patients who have only one X-chromosome in each cell, which is always active.

Since most RTT patients survive into adult age, in order to cope with the growing burden of multiple clinical problems, a multidisciplinary treatment approach is needed, not only including the paediatrician and pediatric neurologist, but also
an orthopaedic surgeon, rehabilitation physician, physiotherapist, speech therapist as well as an adult neurologist and internist will be needed in the future to ensure an improved prognosis and quality of life in RTT patients.

Conclusions

Following the publication of revised diagnostic RTT criteria and the possibility of genetic confirmation, knowledge of RTT has increased and the diagnosis is made at younger age.

No laboratory findings have proven consistent enough to be diagnostically useful and therapeutic attempts have been disappointing. Issues about the fluctuating clinical course, phenotypic variability and possibility of influencing MECP2 gene expression are still unresolved.

List of abbreviations

RTT (Rett syndrome), MeCP2 (methyl-CpG binding protein), CDKL5 (cyclin-dependent kinase-like 5), FOXG1 (forkhead box protein G1), ESES (electrical status epilepticus during slow sleep), EEG (electroencephalography), SSS (symptom severity score), pCO2 (partial pressure of carbon dioxide), MBD (methyl-CpG binding domain), TRD (transcriptional repression domain), OS (Oxidative stress), ROS (reactive oxygen species), ω-3 PUFAs (Omega-3 polyunsaturated fatty acids), NPBI (non-protein-bound iron), F2-IsopPs (F2-isoprostanes), 5MTHF (5-methyltetrahydrofolate), CSF (cerebrospinal fluid), SAM (S-adenosylmethionine), SAH (S-adenosylhomocysteine), HVA (homovanillic acid), 5HIAA (5-hydroxyindoleacetic acid), GABA (γ-aminobutyric acid), NMDA (N-methyl-D-aspartate), RMBA (Rett syndrome Motor Behavioral Assessment), PWBI (Patient Wellbeing Index), CSS (Clinical severity score), MRS (Proton magnetic resonance spectroscopy), CNS (Central Nervous system).
Table 3. Neurometabolic evaluations and treatment results in RTT patients

<table>
<thead>
<tr>
<th>Neurometabolic evaluations</th>
<th>Author</th>
<th>Study design</th>
<th>Number RTT patients</th>
<th>Biochemical evaluations</th>
<th>Clinical evaluation Scale</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Carnitine deficiency</strong></td>
<td>Philippart 1989</td>
<td>Observational study</td>
<td>NA</td>
<td>Plasma carnitine</td>
<td>-----</td>
<td>Low plasma carnitine</td>
</tr>
<tr>
<td></td>
<td>Plioplys 1993,</td>
<td>Case report</td>
<td>1</td>
<td>Plasma carnitine</td>
<td>-----</td>
<td>Low plasma carnitine</td>
</tr>
<tr>
<td></td>
<td>Plochl 1996</td>
<td>Case report</td>
<td>1</td>
<td>Plasma carnitine</td>
<td>-----</td>
<td>Low plasma carnitine</td>
</tr>
<tr>
<td></td>
<td>Ellaway 1999</td>
<td>Randomized placebo controlled double-blind cross over trail (8 weeks)</td>
<td>35</td>
<td>Plasma carnitine</td>
<td>RMB, PWBI Hand apraxia scale</td>
<td>Increase plasma carnitine; Subjective improvements PWBI scale (&gt; 5yrs), behavioral and respiratory subscale RMB A</td>
</tr>
<tr>
<td><strong>Mitochondrial defect</strong></td>
<td>Naidu 1990</td>
<td>Observational study</td>
<td>44</td>
<td>Plasma lactate, Pyruvate</td>
<td>-----</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>Eeg-Olofsson 1988, 1990</td>
<td>Observational study</td>
<td>2</td>
<td>Muscle biopsy</td>
<td>-----</td>
<td>Electron microscopy (EM) study revealed abnormally swollen and dumb-bell shaped mitochondria;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Observational study</td>
<td>6</td>
<td>Muscle biopsy, Plasma lactate</td>
<td>-----</td>
<td>Muscle biopsy and EM showed abnormal mitochondria</td>
</tr>
<tr>
<td></td>
<td>Dotti 1993</td>
<td>Observational study</td>
<td>2</td>
<td>Muscle biopsy, Plasma lactate</td>
<td>-----</td>
<td>Increased number of abnormal swollen mitochondria</td>
</tr>
</tbody>
</table>
### Omega-3 polyunsaturated fatty acid (ω-3 PUFAs)

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Patients</th>
<th>Test</th>
<th>Scale</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leoncine 2011</td>
<td>Observational and intervention study ω-3 PUFAs therapy</td>
<td>113</td>
<td>Non-protein-bound iron (NPBI), F2-isoprostanes (F2-IsoPs)</td>
<td>Clinical Severity Score (CSS)</td>
<td>Significant improvement of CSS; Reduction in NPBI, F2-IsoPs (oxidative stress markers) levels</td>
</tr>
<tr>
<td>De Felice 2012</td>
<td>Observational and intervention study ω-3 PUFAs therapy</td>
<td>20 (stage 1 RTT)</td>
<td>Non-protein-bound iron (NPBI), F2-isoprostanes (F2-IsoPs)</td>
<td>Clinical Severity Score (CSS)</td>
<td>Significant improved CSS, motor-, nonverbal communication and breathing. Low NPBI, F2-IsoPs</td>
</tr>
</tbody>
</table>

### Folates and DNA Methylation

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Patients</th>
<th>Test</th>
<th>Scale</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ramaekers 2004</td>
<td>Observational and intervention study folinic acid therapy</td>
<td>4</td>
<td>CSF 5MTHF, HVA, 5HIAA neopterin, biopterin</td>
<td>Neurological and General assessment</td>
<td>Clinical and social improvement Low 5MTHF, 5HIAA in 100%, restored by folinic acid;</td>
</tr>
<tr>
<td>Temedo 2009</td>
<td>Observational and intervention study folinic acid therapy</td>
<td>25</td>
<td>CSF 5MTHF, HVA, 5HIAA neopterin, biopterin</td>
<td>Neurological and General assessment</td>
<td>No clinical improvement low 5MTHF in 32% 3 low 5HIAA (fluoxetine therapy)</td>
</tr>
<tr>
<td>Ormazabal 2005</td>
<td>Observational and intervention study folinic acid therapy</td>
<td>16</td>
<td>CSF 5MTHF, HVA, 5HIAA neopterin, biopterin, amino acid</td>
<td>Neurological and General assessment, EEG</td>
<td>Clinical, epilepsy and EEG improvement in 3 out of 6; low 5MTHF in 50%, 2 low 5HIAA</td>
</tr>
<tr>
<td>Nuel 2005</td>
<td>Observational study</td>
<td>76</td>
<td>CSF 5MTHF</td>
<td>------</td>
<td>All, but two, normal 5-MTHF</td>
</tr>
<tr>
<td>Glaze 2009</td>
<td>Randomized placebo controlled double-blind controlled trail</td>
<td>73</td>
<td>Plasma betaine, creatine methionine, homocysteine guanidinoacetate dimethylglycine,</td>
<td>RMBA, PSG, Neurological score, General assessment, Parent questionnaire</td>
<td>No objective clinical change; Subjective improvement &lt; 5 years; Betaine, dimethylglycine increased</td>
</tr>
<tr>
<td>Neurometabolic evaluations</td>
<td>Author</td>
<td>Study design</td>
<td>Number RTT patients</td>
<td>Biochemical evaluations</td>
<td>Clinical evaluation Scale</td>
</tr>
<tr>
<td>----------------------------</td>
<td>--------------------</td>
<td>------------------------------------------------------------</td>
<td>---------------------</td>
<td>------------------------------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>Follates and DNA Methylation</td>
<td>Hagebeuk 2012</td>
<td>Randomized placebo controlled double-blind cross over trail folic acid therapy</td>
<td>11</td>
<td>CSF 5MTHF</td>
<td>Neurological scales RMBA, Hand apraxia Scale, PWBI</td>
</tr>
<tr>
<td></td>
<td>Hagebeuk 2011</td>
<td>Randomized placebo controlled double-blind cross over trail folic acid therapy</td>
<td>12</td>
<td>-----</td>
<td>EEG, seizures</td>
</tr>
<tr>
<td></td>
<td>Hagebeuk 2012</td>
<td>Randomized placebo controlled double-blind cross over trail folic acid therapy</td>
<td></td>
<td>Plasma and CSF SAM, SAH, methionine, homocysteine</td>
<td>-----</td>
</tr>
<tr>
<td>Creatine</td>
<td>Freilinger 2011</td>
<td>Randomized placebo controlled double-blind controlled trail</td>
<td>18</td>
<td>Plasma methionine, homocysteine, SAM, SAH, DNA methylation</td>
<td>RMBA</td>
</tr>
<tr>
<td>Biogenic amines, neurotransmitters</td>
<td>Zoghbi 1998</td>
<td>Observational study</td>
<td>32</td>
<td>CSF biogenic amine metabolites MHPG, HVA,5HIAA</td>
<td>-----</td>
</tr>
<tr>
<td></td>
<td>Perry 1988</td>
<td>Observational study</td>
<td>5</td>
<td>CSF HVA,5HIAA, GABA</td>
<td>-----</td>
</tr>
<tr>
<td></td>
<td>Lekman 1990</td>
<td>Observational study</td>
<td>38</td>
<td>CSF and urine HVA, 5HIAA, MHPG</td>
<td>-----</td>
</tr>
</tbody>
</table>
**Rett syndrome: clinical, laboratory, and therapeutic evaluation. Review of recent developments**

### Neurometabolic evaluations and treatment results in RTT patients

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Number</th>
<th>Evaluations</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hagebeuk 2011</td>
<td>Randomized placebo controlled double-blind cross over trail</td>
<td>12</td>
<td>CSF biogenic amine metabolites HVA, 5HIAA</td>
<td>Reduction of HVA levels (19%), Reductive 5HIAA level (23%)</td>
</tr>
<tr>
<td>Hagebeuk 2012</td>
<td>Randomized placebo controlled double-blind cross over trial</td>
<td>11</td>
<td>Plasma and CSF SAM, SAH, methionine, homocysteine</td>
<td>No change in SAM/SAH ratio in CSF and plasma</td>
</tr>
<tr>
<td>Freilinger 2011</td>
<td>Randomized placebo controlled double-blind controlled trial</td>
<td>18</td>
<td>Plasma methionine, homocysteine, SAM, SAH, DNA methylation</td>
<td>No significant clinical change; methionine, homocysteine = DNA methylation increased</td>
</tr>
<tr>
<td>Zoghbi 1998</td>
<td>Observational study</td>
<td>32</td>
<td>CSF biogenic amine metabolites MHPG, HVA, 5HIAA</td>
<td>Reduced metabolites of dopamine (HVA), serotonin (5HIAA), norepinephrine (MHPG)</td>
</tr>
<tr>
<td>Perry 1988</td>
<td>Observational study</td>
<td>5</td>
<td>CSF HVA, 5HIAA, GABA</td>
<td>All normal</td>
</tr>
<tr>
<td>Lekman 1990</td>
<td>Observational study</td>
<td>38</td>
<td>CSF HVA, 5HIAA, MHPG</td>
<td>All normal</td>
</tr>
<tr>
<td>Blue 1999</td>
<td>Neuropathology study</td>
<td>9</td>
<td>9 post mortem RTT patients and 10 controls GABA, Glutamate, NMDA receptors</td>
<td>GABA and glutamate receptors higher in younger RTT patients, and lower in older RTT patients</td>
</tr>
<tr>
<td>Samanco 2009</td>
<td>Observational study</td>
<td>64</td>
<td>CSF biogenic amine metabolites HVA, 5HIAA</td>
<td>-----</td>
</tr>
<tr>
<td>Naidu 1990</td>
<td>Observational study</td>
<td>31</td>
<td>CSF β Endorphin</td>
<td>-----</td>
</tr>
<tr>
<td>Percy 1994</td>
<td>Randomized placebo controlled double-blind cross over trail of Naltrexone</td>
<td>25</td>
<td>CSF β Endorphin</td>
<td>Clinical stage, RMBA, PSG, EEG</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>No clinical effect on clinical stage, RMBA, EEG, Improvement of disorganized breathing</td>
</tr>
</tbody>
</table>

**Growth factors**

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Number</th>
<th>Evaluations</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Riikonen 1999</td>
<td>Observational study control group autistic patients</td>
<td>11</td>
<td>CSF nerve-growth factor (NGF) levels</td>
<td>Significant reduced levels of CSF NGF levels in RTT patients compared to autistic patients</td>
</tr>
<tr>
<td>Vanhala 2000</td>
<td>Observational study</td>
<td>13</td>
<td>Serum and CSF IGF1, Growth hormone levels</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Legend: NA: Not available. RMBA: 40 item scale to evaluate behavioral/social, respiratory and motor signs in Rett patients. Hand apraxia scale: validated 10 item scale for hand function. PWBI = Patient Well-being Index: 5 item tool for evaluation parents perception of child functioning. Clinical severity score (CSS): a validated clinical RTT, based on 13 individual common RTT features. CSF = Cerebral Spinal Fluid, 5-MTHF = 5- methyltetrahydrofolate, SAM = S-adenosylmethionine, SAH = S-adenosylhomocysteine, metabolites of dopamine (HVA), serotonin (5HIAA), norepinephrine (MHPG), nerve-growth factor (NGF), Insulin-like growth factor-I (IGF1), norepinephrine (MHPG).
References

Rett syndrome: clinical, laboratory, and therapeutic evaluation. Review of recent developments


75. Hagebeuk EEO, Duran M, Abeling NG, Vyth A, Poll-The BT. S-adenosylmethionine and


