Rett syndrome (RTT), a neurodevelopmental condition characterized by delayed-onset loss of spoken language and the development of distinctive hand stereotypies, affects approximately 1 in 10,000 live female births. Clinical diagnosis has been based on symptoms such as loss of acquired purposeful hand skills, autistic behaviors, motor dysfunctions, seizure disorders, and gait abnormalities. RTT is a genetic disease and is caused almost exclusively by mutations in the X-linked gene, MECP2, to produce a phenotype that is thought to be primarily of neurological origin. Clinical reports show RTT patients to have a smaller brain volume, especially in the cerebral hemispheres, and alterations in various neurotransmitter systems, including acetylcholine, dopamine, serotonin, glutamate, substance P, and various trophic factors. Because of its monogenetic characteristic, disruption of Mecp2 is readily recapitulated in mice to produce a prominent RTT-like phenotype and provide an excellent platform for understanding the pathogenesis of RTT. As shown in human studies, Mecp2 mutants also display subtle alterations in neuronal morphology, including smaller cortical neurons with a higher-packing density and reduced dendritic complexity. Neurophysiological studies in Mecp2-mutant mice consistently report alterations in synaptic function, notably, defects in synaptic plasticity. These data suggest that RTT might be regarded as a synaptopathy (disease of the synapse) and thus potentially amenable to rational therapeutic intervention.

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1. Introduction

Rett syndrome (RTT; MIM 312750) was first identified by Dr Andreas Rett in 1966, after he observed 22 patients with similar unique symptoms. The constellation of features that represent RTT became more widely recognized as...
a specific neurodevelopmental disorder after subsequent larger studies by Dr Hagberg et al.\textsuperscript{2} In 1999, Amir et al\textsuperscript{3} discovered the genetic basis of RTT (\textgtrapprox 95\% of cases of classical RTT to date) to be mutations in the gene \textit{MECP2}, thus successfully linking the fields of genetics and neurology. The discovery of \textit{MECP2} as the primary cause of RTT opened into another field, that of molecular biology. Some years earlier, Dr Bird and colleagues\textsuperscript{4} identified \textit{MeCP2} (methyl-CpG binding protein 2) as a novel protein that binds to methylated CpG dinucleotides within the mammalian genome. Methylation of CpGs is associated with gene silencing and alterations in chromatin structure. Although the exact function of \textit{MeCP2} is still not known, it is an abundant nuclear protein and is considered likely to regulate gene expression whether through the silencing or activation of specific genes or through more global regulation (e.g. dampening) of transcriptional processes.\textsuperscript{5–7}

An important feature in the etiology of RTT is the fact that \textit{MECP2} is located on the X chromosome, Xq28.\textsuperscript{8} Most mutations are sporadic and rarely inherited. Moreover, mutations in males typically result in severe infantile encephalopathy because of complete absence of functional \textit{MeCP2}. In contrast, females are heterozygous for the mutation with, because of X chromosome inactivation, approximately one-half of the cells expressing the mutant \textit{MECP2} allele but with the other half expressing a functional allele. Thus, RTT is a disease that is almost exclusively seen in females.

Because of the monogenic characteristic of RTT (i.e. single gene disorder), several MeCP2 knockout models have been generated in mice and other species for investigating RTT-like pathologies and for identifying and testing therapeutic strategies.\textsuperscript{9–13} Such animal studies are important changing the way we view neurodevelopmental conditions, including the tractability of such disorder. The traditional view that abnormalities in brain development will produce aberrations in the nervous system and result in irreversible neurological and psychiatric features has been challenged. Specifically, a number of studies in animal disease models ranging from Down’s syndrome,\textsuperscript{14,15} tuberous sclerosis,\textsuperscript{16–18} fragile X syndrome,\textsuperscript{19,20} Angelman syndrome,\textsuperscript{21} as well as RTT\textsuperscript{10} have demonstrated an unexpected propensity for phenotypic reversal, even in adult mice.\textsuperscript{22} As yet, few of these results have yet been translated to the clinic. The current short review summarizes the main clinical features and genetics of RTT before considering the recent advances in animal model studies in aiming to understand the pathogenesis of RTT and identifying and testing rational therapeutic strategies, thus filling the gap between clinical practice and basic research in RTT.

2. Clinical Features

RTT is a predominantly neurological disorder and a primary cause of severe mental retardation in girls with an incidence of approximately 1 in 10,000 female births.\textsuperscript{23} Patients with RTT appear to develop normally up to 6–18 months of age. They typically achieve normal neurodevelopmental milestones, from gross and fine motor functions to social communication skills. The head circumference of Rett girl is normal at birth; however, it begins to decelerate in its growth at 2–3 months of age.\textsuperscript{24} Distinctive aspects contributing to the diagnosis include developmental regression, with accompanying loss of hand skills, mobility skills, and speech and stereotypic hand movements. As the syndrome progresses, social withdrawal and loss of language become apparent with features reminiscent of autism.\textsuperscript{25} The onset of mental deterioration is accompanied by loss of motor coordination and the development of ataxia and gait apraxia. Associated features such as microcephaly, respiratory/autonomic abnormalities,\textsuperscript{26} seizures, scoliosis, growth deficits and early hypotonia are very prevalent.

Among these symptoms, the most significant one to pediatrician is seizure disorder, which ranges from simple convulsion to intractable epilepsy.\textsuperscript{27} Neurophysiologic evaluations show cortical hyperexcitability on the electroencephalogram (EEG), which represents a loss of expected rhythmic slow activity, primarily in the frontal-central regions. However, most events presumed to be seizures are without EEG correlation during video-EEG recording.\textsuperscript{28} Other associated abnormalities during the postregression phase include teeth grinding, night laughing or crying, screaming fits, low mood, and anxiety episodes elicited by distressing external events.\textsuperscript{29} Most girls with RTT lose mobility and are often wheelchair-bound during the teenage years. Impairment of the autonomic nervous system in RTT is suggested by an increased incidence of long Q–T intervals during electrocardiographic recordings and it can contribute to the higher incidence rate of sudden unexpected death in RTT patients. Other autonomic abnormalities include hypotrophic cold blue feet; severe constipation; oropharyngeal dysfunction; and cardiac abnormalities, including tachycardia and sinus bradycardia. Even with high risk of sudden death because of respiratory and cardiac dysfunctions, several patients survive till the 6\textsuperscript{th} or 7\textsuperscript{th} decade of life with limited mobility.\textsuperscript{30}

3. Genetic Background

RTT cases are usually the result of dominantly acting, sporadic mutations in the X-linked gene \textit{MECP2}, which encodes methyl-CpG-binding protein 2 (MeCP2).\textsuperscript{3} MeCP2 is expressed quite widely throughout the body, with notably high expression in postnatal neurons.\textsuperscript{12,31,32} However, the question remains why disruption of a ubiquitously expressed protein results in a predominantly neurological phenotype.\textsuperscript{32} Most pathogenic mutations in \textit{MECP2} cause RTT in heterozygous females, whereas mutations leading to other phenotypic outcomes are also known.\textsuperscript{33} Because most RTT cases are sporadic, it was difficult to map the disease locus by traditional linkage analysis; instead, using information from rare families, Xq28 region was identified and subsequent screening of candidate genes in RTT patients revealed mutations in \textit{MECP2}.\textsuperscript{3} Boys inheriting a mutant \textit{MECP2} allele are much more severely affected, presenting with infantile encephalopathy and usually not surviving infancy. Because most \textit{MECP2} mutations leading to RTT involve loss of function of the mutant allele, RTT can be modeled using gene knockout mice that recapitulate many of the key clinical signs that characterize RTT in humans.\textsuperscript{10,11}
MeCP2 is a member of a family of proteins that bind regions of DNA enriched with methylated CpG regions. Containing a methyl-CpG binding domain and a transcriptional repression domain, MeCP2 was classically considered a methylation-dependent transcriptional repressor. However, other studies suggest additional or alternative roles, including an enhancer of transcription, a global regulator of chromatin structure, or a global damper of transcriptional noise.

MeCP2 is expressed in a range of tissues but is especially abundant in postmitotic neurons. Mice lacking MeCP2 in neurons show overt RTT-like symptoms, whereas mice in which the expression of MeCP2 is driven in neurons alone are reported to show a normal phenotype. Although MeCP2 is present at low levels in astrocytes and MeCP2 deficiency in these cells may confer subtle noncell autonomous actions on neuronal phenotype, a body of evidence points to the overt RTT-like symptoms being due mainly to MeCP2 deficiency in the nervous system and neurons in particular.

4. Neuropathology in RTT Patients

The average 1-year-old infant brain weight in RTT patients is 900 g. The weight of the RTT brain is significantly less than that of the brain of age-matched controls in autopsy studies. Observation of MeCP2 expression showed it primarily distributed in post-mitotic neurons. The weight of the Rett brain does not decrease significantly with age, so atrophy does not account for the small brain. Instead, RTT is characterized by lack of brain growth, which is not generalized because some structures such as the cerebral hemispheres are affected more than other structures such as the cerebellum. Alterations in brain volume mainly occur in prefrontal, posterior frontal, and anterior temporal regions, with preservation in the posterior temporal and posterior occipital regions.

Neurochemical studies based on RTT patient data are relatively limited. Nevertheless, cerebrospinal fluid and brain tissues have been analyzed with respect to levels of various transmitters, receptors, and additional trophic factors. Abnormalities have been reported in most systems, including in acetylcholine, dopamine, serotonin, glutamate, substance P, and nerve growth factor. The age of the patient with RTT and the severity of the symptoms influence measurements. The reduced levels of acetylcholine and cholinergic markers is one of the most consistent findings. In terms of therapeutenic potential, much interest has surrounded the monoamine systems (see in the following sections).

5. Animal Models of RTT

Several genetic mouse models of RTT have been developed through interruption of murine MeCP2, and these models accurately recapitulate the cardinal signs that characterize RTT in humans. RTT models are proving invaluable in helping to understand the underlying pathology and neuronal dysfunction in RTT as well as providing insights into the pathophysiology of neurodevelopmental disorders more generally. MeCP2-null male mice develop motor impairment, tremor, breathing abnormalities, and limb stereotypes. In most of the models, the null males demonstrate apparently normal early development before the onset of overt signs at about 6 weeks of age, and progression is usually fairly aggressive, with death at about 16–20 weeks. Females heterozygous for MeCP2 show a more delayed onset of symptoms. The symptom severity increases over a period of weeks to months, as in human females, these stabilize and the mice show an apparently normal lifespan.

Three RTT models have been widely used, the first one is the Jaenisch model, which is generated from the cre-lox recombination system in which exon 3 is deleted. In males normal development is noted before 5 weeks following which mice develop nervousness, body trembling, piloerection, and occasional breathing abnormalities. Mice typically experience weight loss at 8 weeks and most die by 10 weeks. Reduced brain weight and neuronal size in hippocampus, cerebral cortex, and cerebellum are observed and have been reported. The second model is the Bird model, which was generated by deletion of exons 3 and 4 of MeCP2 in embryonic stem cells to produce a complete null in terms of the production of MeCP2 protein. Hemizygous males are normal till 3–8 weeks, later develop stiff uncoordinated gait, hindlimb claspin, irregular breathing, and uneven teeth wear. Symptoms progress rapidly after 10 weeks with weight loss and death at around 12–16 weeks. In contrast, the heterozygous females develop inertia and hindlimb claspin at around 3 months and breathing abnormalities (irregular breathing patterns) and decreased mobility after 6 months. They survive longer than males and are fertile. The MeCP2308/y model is a milder model and was generated by insertion of a truncating MeCP2 mutation. A stop codon (at position 308) produces a protein that is truncated yet retains the methyl-CpG binding domain, transcriptional repression domains, and nuclear localization signal motif. Males appear normal till 6 weeks and later develop tremors, motor impairment, hypoactivity, anxiety behavior, seizures, kyphosis, and stereotypic forelimb motions. Survival is much longer than MeCP2 null models with males living to 10 months and being fertile. Females also survive and have milder symptoms than seen in the null (complete absence of protein) models.

Most RTT model studies have focused on global deletion or truncation of MeCP2. Behavioral studies in these mice have revealed altered gait and motor defects, including hypoactivity, paw stereotypes, and balance and swimming impairments. Such mice also display an anxiety phenotype, including reduced exploration, increased thigmotaxis (movement toward or away from mechanical stimulus), and altered plus and zero maze behavior. Motor dysfunction limits the application of certain cognitive tests, but impairments in fear conditioning and novel object recognition have been reported. In the MeCP2308/y model, which generates truncated MeCP2 and displays significantly milder symptoms, mice show impairments in hippocampal-dependent spatial memory as well as social memory.

Like in RTT patients, MeCP2-mutant mice have smaller cortical neurons packed at a higher density than their wild-type littermates. Moreover, pyramidal neurons in the cortex, as well as in hippocampal CA3 region, and granule cells of the dentate gyrus show reduced dendritic
complexity. Furthermore, Jaenisch Mecp2-mutant mice show a disorganized olfactory neuroepithelium indicative of delayed terminal differentiation. However, dendritic branching in Layers III and V pyramidal neurons of the frontal cortex of male Mecp2<sup>306/v</sup> mice was comparable with that in the controls. In the Bird model, pyramidal neurons from the somatosensory cortex of 6-week-old mice show lower spine densities compared with wild-type controls. Newly generated granule cells in the dentate gyrus of 8-week-old Jaenisch model mutant mice also show impaired dendritic spine density and distribution. In female heterozygous mice, the onset of this dendritic spine phenotype is delayed and more severe in Mecp2-lacking neurons than in Mecp2-expressing neurons, suggesting both cell autonomous and non-cell autonomous effects. The intensity of PSD-95 (an abundant postsynaptic protein) is also lower in Layer V pyramidal neurons of the motor cortex of Mecp2-null mice. As to the presynaptic terminals, the motor cortex of Bird model mice show defects in axonal fasciculation. Moreover, the intensity of VGLUT1 (a presynaptic protein) is lower in the dendritic region of the hippocampal CA1 region from Mecp2-null mice; however, the postsynaptic dendritic marker MAP-2 was not different, the authors interpreted that Mecp2 deletion caused a reduction in the number of mature synapses in area CA1, consistent with their results from dissociated neuronal cultures.

### 6. Neurophysiological Studies and Synaptic Plasticity

At the cellular level, studies in mice show subtle changes in neuronal electrical properties within cortical areas and more pronounced changes in other regions such as the brain stem and locus ceruleus. Overt changes in synaptic function include reduced synaptic plasticity and changes in basal inhibitory and excitatory synaptic transmission. Anatomical studies have shown changes in synaptic connectivity and neuronal structure, whereas at the network level, there are changes in network excitability. Interestingly, synaptic plasticity (activity-dependent changes in the strength of synaptic communication) appears normal in young Mecp2-mutant mice but shows impairment when tested in older mice on onset of overt RTT-like signs. Moreover, the degree of impairment appears to correlate with the severity of the RTT-like neurological phenotype (see Figure 1). The precise mechanisms underlying the involvement of MeCP2 in regulating morphological and functional aspects of synaptic signaling remain to be identified. However, synaptic plasticity deficits are one of the most consistent findings and may provide important insights into RTT-like pathogenesis as well as serving as a target system for therapeutic interventions.

### 7. Therapeutic Approaches

It appears that lack of functional MeCP2 results in a nervous system primed to malfunction at a critical point during postnatal brain development. However, function can be restored (including normal plasticity) to a large degree by the reintroduction of MeCP2. In the study by Guy et al., endogenous Mecp2 was silenced by insertion of a lox-stop cassette allowing the mice to develop symptoms (and plasticity deficits) before MeCP2 could be reintroduced into the brain by pharmacological reactivation of the gene. This reactivation resulted in a pronounced improvement in neurological signs and reduced mortality in the mice. Similar strategies to reintroduce or rebalance MeCP2 levels have been adopted by other groups using different genetic approaches and these studies have demonstrated improvements in motor function and a reversal of brain weight and neuronal morphology deficits. Another genetic strategy has been to overexpress the brain-derived neurotrophic factor (BDNF, a potent modulator of synaptic plasticity/function that is dysregulated in MeCP2-mutant mice).
Rett syndrome, from clinics to animal studies

...mice), which again reverses signs such as locomotor defects. Although none of these studies represent a therapeutic strategy that can be applied to human patients, they nevertheless demonstrate the concept of phenotypic reversibility in mouse models of RTT and suggest that the MeCP2-mutant mice represent a viable platform for testing future pharmacological and genetic strategies that can be translated for clinical use.

The most obvious strategy in RTT is one of gene therapy. This approach has been successful in disorders such as thalassaemia, sickle cell disease, cystic fibrosis, and some cancers but the application of gene therapy to CNS disorders is a particular challenge. Although conceptually straightforward, RTT being a monogenic disorder, the application of gene therapy in RTT is likely to be problematic on numerous counts. For instance, it is likely to be necessary to express MeCP2 in the correct cell types and at the correct levels because overexpression of MeCP2 is also known to be detrimental. A related complication is that females with RTT have a mosaic expression of MeCP2 with cells expressing the mutant allele and other cells expressing the normal allele and producing functional protein. It is possible that a targeted strategy would be required whereby the transgene was activated only in the “mutant” cells and avoiding overexpression in the cells with the “healthy” allele, which are presumably functioning normally. This is an active current area of research but there is relatively little published literature as yet. Using lentiviral delivery of Mecp2 driven by the endogenous Mecp2 promoter, Rastegar et al98 achieved natural expression patterns of MeCP2 and concomitant reversal of dendritic maturation phenotypes in vivo but this approach has not yet been tested in vivo.

In contrast to the limited gene therapy literature, a significant number of studies have investigated pharmacological interventions in models of RTT. Several modulators of synaptic function/plasticity have been tested, including the AMPA receptor modulator CX546, the insulin-like growth factor-1 tripeptide, the monoamine reuptake inhibitor desipramine, and the Alzheimer drug memantine. In addition to the pharmacological strategies targeting neuronal mechanisms downstream of the MeCP2 deficiency, another approach is to target the MeCP2 mutation itself. This may be applied to specific but common mutations in MeCP2 leading to premature stop codons. Gentamycin, a widely used antibiotic agent, has this capacity and can provide modest (10–22%) read-through of common nonsense mutations in Mecp2-mutant transfected HeLa cells. Whether this approach can be successfully applied in vivo or indeed in the clinic remains to be established.

The studies focusing on AMPA receptor modulators (the ampakine CX546) are based on the fact that levels of BDNF are considered to be regulated by MeCP2 binding and that aberrant levels of BDNF have concomitant effects on neurite outgrowth and synaptic maturation and maintenance. Mice treated with daily dosing of CX546 showed enhanced levels of BDNF and an improvement in the breathing phenotype (irregular breathing patterns), which is a prominent feature of RTT patients and seen in MeCP2-mutant mice.

Another drug targeting glutamate receptors (and possibly cholinergic systems also) is the Alzheimer’s drug memantine. This drug has recently been shown to be effective in other neurodevelopmental disorder models and is well known to be effective in alleviating synaptic plasticity deficits. Weng et al103 recently reported a synaptic plasticity saturation effect in the hippocampus of Mecp2-mutant mice and that impairment in both short-and long-term forms of synaptic plasticity can be partially reversed by memantine application in vitro when applied at clinically relevant concentrations. However, administration of memantine to MeCP2-mutant male mice was ineffective at preventing the onset of RTT-like signs and survival. Nevertheless, it remains to be tested whether memantine can afford cognitive benefits in females with a milder and stable RTT phenotype and mimic the human genotype.

In addition to BDNF (via ampakines), another growth factor that has received attention is IGF-1, which is a well-known regulator of synaptic maturation and plasticity. The activity of IGF-1 is regulated by a range of IGF-binding proteins. One of these, IGFBP3, has a binding site for the MeCP2104 and MeCP2-null mice and RTT patients express aberrantly high levels of this protein, which would be expected in turn to inhibit IGF-1 signaling. An active tripeptide fragment of IGF-1 has been shown to enhance lifespan, improve locomotor function, and breathing pattern and heart rate abnormalities in MeCP2-null mice. At the cellular level, reversed structural and cortical plasticity deficits were also observed following IGF-1 tripeptide treatment and clinical trials using recombinant IGF-1 are now underway.

For some time, there has been an interest in monoamine systems with respect to RTT. There are consistent reports that levels of monoamine markers are reduced in the RTT brain and in MeCP2-null mice. To counter these deficits, drugs such as desipramine (an inhibitor of monoamine uptake) have been tested in MeCP2-null mice. Repeated administration of desipramine improves breathing and prolongs lifespan in RTT mice and at a cellular level, reverses the depletion in brain stem tyrosine hydroxylase.

8. Conclusion

It has been more than 40 years since girls with Rett phenotype were first reported and the last decade has seen a rapid escalation in basic research. It is clear that deficiency of MeCP2 has a multiple downstream consequences and that a fuller understanding of the molecular actions of MeCP2 will reveal new strategies for targeting the disorder. However, genetically engineered mouse studies have amply demonstrated the concept of phenotypic reversibility and progress is being made on a number of fronts to find ways of reversing synaptic, functional, and behavioral aspects of RTT. It is not unreasonable to expect that some of these approaches will see rational interventions move from laboratory bench to bed in the coming years.

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