

Rett Syndrome and *MECP2* Duplication Syndrome: Disorders of MeCP2 Dosage

Bridget E Collins¹ , Jeffrey L Neul² 

¹Medical Scientist Training Program, Vanderbilt University, Nashville, TN, USA; ²Vanderbilt Kennedy Center, Departments of Pediatrics, Pharmacology, and Special Education, Vanderbilt University Medical Center and Vanderbilt University, Nashville, TN, USA

Correspondence: Jeffrey L Neul, PMB 40, 230 Appleton Place, Nashville, TN, 37203, Tel +1 615-322-8242, Fax +1 615-322-8236, Email jeffrey.l.neul@vumc.org

Abstract: Rett syndrome (RTT) is a neurodevelopmental disorder caused predominantly by loss-of-function mutations in the gene *Methyl-CpG-binding protein 2 (MECP2)*, which encodes the MeCP2 protein. RTT is a *MECP2*-related disorder, along with *MECP2* duplication syndrome (MDS), caused by gain-of-function duplications of *MECP2*. Nearly two decades of research have advanced our knowledge of MeCP2 function in health and disease. The following review will discuss MeCP2 protein function and its dysregulation in the *MECP2*-related disorders RTT and MDS. This will include a discussion of the genetic underpinnings of these disorders, specifically how sporadic X-chromosome mutations arise and manifest in specific populations. We will then review current diagnostic guidelines and clinical manifestations of RTT and MDS. Next, we will delve into MeCP2 biology, describing the dual landscapes of methylated DNA and its reader MeCP2 across the neuronal genome as well as the function of MeCP2 as a transcriptional modulator. Following this, we will outline common *MECP2* mutations and genotype–phenotype correlations in both diseases, with particular focus on mutations associated with relatively mild disease in RTT. We will also summarize decades of disease modeling and resulting molecular, synaptic, and behavioral phenotypes associated with RTT and MDS. Finally, we list several therapeutics in the development pipeline for RTT and MDS and available evidence of their safety and efficacy.

Keywords: neurodevelopmental disorders, epigenetics, DNA methylation, disease modeling, therapeutics

Review

One afternoon in Vienna in 1954, pediatrician Andreas Rett walked into the waiting room of his clinic for children with disabilities and noticed two girls sitting in their mothers' laps, both with their arms gently restrained.¹ The mothers, by happenstance, released their grip simultaneously and both girls began to make unusual, stereotyped movements. With further research, Rett found that these girls shared similar developmental and clinical histories, and, upon analysis of the clinic's records, identified an additional six girls that did too. Rett believed he was observing a previously unrecognized syndrome and reported his findings in a little-known Austrian newsletter one year later, in 1966.² This seminal article, titled "On a remarkable syndrome of cerebral atrophy associated with hyperammonemia in childhood", would remain virtually unrecognized for 15 years, until a group of European researchers would give a name to what pediatric neurologists now recognize as Rett syndrome.³

Rett Syndrome (RTT)

Andreas Rett's 1966 report described 22 young girls, each with an uncomplicated birth history and typical development until approximately 1 year of age.² However, at this age, Rett noted that the girls exhibited a consistent pattern of disruption in typical development, including shared characteristics of absent speech, motor dysfunction, apraxic gait, hand stereotypies, and epilepsy. Rett termed this syndrome "cerebral atrophy associated with hyperammonemia" and published his findings to raise awareness in the pediatric neurology community. Unfortunately, Rett's work was not widely read in the Austrian newsletter *Wiener Medizinische Wochenschrift*, and his finding of hyperammonemia was not replicated by others (and subsequently discovered to be a lab error), preventing broad acknowledgement of this childhood

syndrome. Broad acknowledgement came only in 1983, when Swedish neurologist Bengt Hagberg and colleagues published a synthesis of 35 cases of “developmental stagnation...followed by rapid deterioration of higher brain functions” in young girls from France, Portugal and Sweden.³ Noting similarities to the cases described in Rett’s 1966 report, Hagberg termed this clinical pattern Rett’s syndrome.

In the years following Hagberg’s report, growing attention to this disorder led to the need for improved clinical characterization. With his broad expertise, Hagberg proposed a four-stage system describing the temporal progression of the disease: (1) early onset, (2) regression, (3) plateau, and (4) late motor deterioration (Figure 1).⁴ Prior to onset of symptoms, children with Rett syndrome (RTT) undergo apparently typical development. In the first stage, early onset, girls with RTT may show delays in psychomotor skill progression (developmental delay) and growth, specifically, a decrease in the rate of head circumference growth, with some individuals having acquired microcephaly. The second stage of the disease, regression (termed “rapid deterioration” by Hagberg, but subsequent work determined that regression is not always rapid), involves partial or complete loss of previously acquired skills pertaining to spoken language and hand use. This stage also includes the onset of hand stereotypies such as hand wringing/squeezing and gait abnormalities or the inability to ambulate. Children with RTT may also develop respiratory abnormalities (eg, hypoventilation, apneas) at this time, although these features often do not manifest until the plateau phase. The regression stage has a finite duration, after which affected individuals enter the plateau stage around 3–5 years of age. During the plateau stage, further loss of skills does not occur and behavioral and cognitive function may stabilize, while other medical conditions, such as seizures, gastrointestinal and nutritional problems (notably constipation), other movement disorders, autonomic abnormalities, and other medical issues manifest.^{5,6} Finally, in the late motor deterioration stage, individuals with RTT may experience decreasing mobility, onset of parkinsonian features, and scoliosis, leading to significant motor disability and, in some cases, the loss of the ability to ambulate.⁷ It is important to note that this staging system is a general characterization of clinical progression and that not all girls with RTT will experience each stage or each clinical feature.

The clinical diagnosis of RTT is made according to consensus guidelines published in 2010 by the RettSearch Consortium.⁸ Typical, or classic, RTT is defined by the presence of the characteristic disease progression of RTT (a period of regression followed by recovery or stabilization), with the presence of 4 main criteria: 1) loss of acquired purposeful hand skills; 2) loss of acquired spoken language; 3) development of gait abnormalities or the inability to walk; and 4) presence of characteristic hand stereotypies. With the broader recognition of RTT, clinicians have also identified individuals that display some, but not all, of the features of typical RTT. These individuals are described to have atypical,

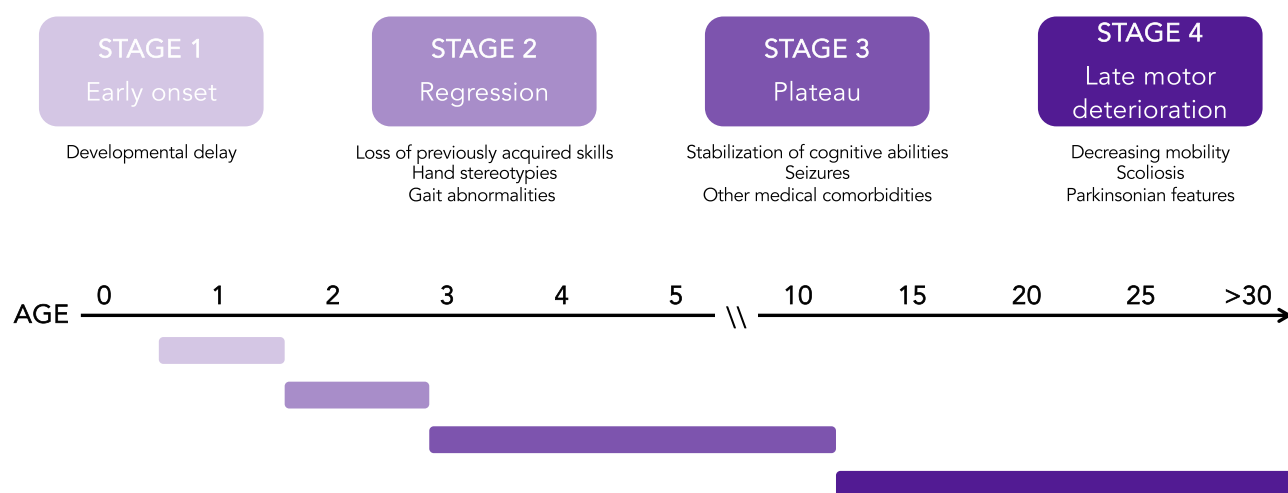


Figure 1 Disease stages in Rett syndrome. The progression of Rett syndrome can be described in four stages of disease. In Stage 1 (early onset), children develop mostly typically with possible developmental delay. In Stage 2 (regression), children undergo developmental regression over the course of weeks, months, or years with loss of previously acquired skills including purposeful hand movements and spoken language. Children also begin to develop breathing and gait abnormalities during this period. In Stage 3 (plateau), children usually experience stabilization of cognitive abilities and have onset of other medical conditions such as seizures. In Stage 4 (late motor deterioration) individuals with RTT experience decreasing mobility and may develop parkinsonian features.

or variant, RTT. Atypical RTT is defined by the presence of a period of regression followed by recovery or stabilization, as well as at least 2 of the main 4 criteria for typical RTT and at least 5 out of the 11 supporting criteria (eg, breathing disturbances when awake, peripheral vasomotor disturbances, intense eye communication – “eye pointing”). Though diagnostic criteria have varied over the almost four decades since RTT was recognized globally, researchers have been able to glean epidemiologic data as to the incidence of RTT. RTT is a rare neurodevelopmental disorder, occurring with a cumulative incidence of 1.09 per 10,000 females by age 12 years.⁹

The nearly exclusive occurrence of RTT in girls led the research community to predict that the disease is caused by an X-linked dominant mutation with lethality in males, the first part of which turned out to be true and the latter not.^{3,10–14} Extensive gene mapping of rare familial cases of RTT narrowed down the genetic region of interest to the Xq28 region, and in 1999 Ruthie Amir and colleagues identified a causative gene.^{13–18} Using a systematic gene screening approach in sporadic cases of RTT, Amir et al identified disease-causing mutations in the *MECP2* gene encoding methyl-CpG-binding protein 2 (MeCP2). Since then, it has been found that *MECP2* mutations account for over 95% of typical RTT cases and approximately 75% of atypical RTT cases.^{19,20} *MECP2* mutations occur largely in a sporadic manner; however, a small percentage of RTT cases occur in a familial manner through parental *MECP2* mutations.^{21,22}

The discovery of a genetic basis to RTT provided insight into the disease’s marked manifestation in girls and women. While the notion of male lethality continues to mistakenly permeate the literature, genotyping of affected individuals and their parents led to the discovery that de novo *MECP2* mutations nearly exclusively derive from the paternal X-chromosome.^{21,23,24} Consistently, a large portion of *MECP2* mutations involve C→T transitions at CpG sites, which, when methylated, are prone to spontaneous deamination and conversion to thymine.²⁵ Male germ cells are highly methylated on the X-chromosome, providing more chance for CpG mutation.²⁶ As the paternal X-chromosome is only inherited by female children, RTT occurs nearly exclusively in girls and women. Occasionally, the *MECP2* mutation is inherited on the maternal X-chromosome.^{12,16,27} In this case, mothers typically carry one allele with a *MECP2* mutation and have skewed X-chromosome inactivation in preference of the non-mutant allele. These mothers often do not fulfill criteria for RTT and often are completely unaffected, but some may display cognitive impairment or a learning disability.

While most individuals with RTT are female, *MECP2* mutations do cause disease in males with an array of clinical phenotypes.¹² These mutations manifest disease in males in one of three ways: (1) inheritance of two X-chromosomes (47XXY, Klinefelter syndrome),^{28,29} (2) development of a postzygotic *MECP2* mutation with resulting somatic mosaicism,³⁰ and (3) inheritance of a *MECP2* mutation leading to RTT.³¹ The array of *MECP2* mutations that causes disease in males also differs from those causing disease in females. Reported mutations in males include those causing RTT in females but also a subset of likely pathogenic mutations not observed in females with RTT. This subset includes A140V, G118E, N126I, F157L, R167W, P176H, R306P, R309W, and P322S.³² The A140V mutation, for example, is not considered to be associated with RTT in females, but can cause cognitive, motor, and psychiatric manifestations in males.³³ *MECP2*-associated disease in males ranges from cognitive impairment to typical and atypical RTT, progressive encephalopathy, and neonatal encephalopathy. Overall, disease tends to be more severe in males than in females, with males more commonly exhibiting abnormal initial development, ventilator dependency, and early death.³² Males tend to have more severe disease because they express *MECP2* from a single X-chromosome, whereas females undergo X-chromosome inactivation and variably express the non-mutant or mutant *MECP2* allele in each cell (therefore retaining some expression of full-length MeCP2). To better classify the different presentation and progression of *MECP2*-associated disease in males, the term “male RTT encephalopathy” has been proposed to describe the subset of males that meet criteria for RTT.³²

MECP2 Duplication Syndrome (MDS)

In the mid-2000s came the clinical discovery that overexpression of MeCP2 in people causes a different *MECP2*-related disorder, called *MECP2* duplication syndrome (MDS).^{34,35} Specifically, Meins et al reported on a boy with severe intellectual disability and features of RTT who was determined to have a submicroscopic ~430 kb duplication within the Xq28 region containing the *MECP2* gene.³⁴ Following this, Van Esch et al reported on four families with individuals displaying intellectual disability that had duplications 400–800 kb in size containing several genes, including at least *LICAM* (L1 cell adhesion molecule) and *MECP2*.³⁵ Several lines of evidence pointed specifically to *MECP2* as the

genetic cause of MDS. First, the duplication of the individual described by Meins et al did not involve *LICAM*, suggesting that *MECP2* duplication is sufficient to cause MDS. Second, mouse modeling work suggested that MeCP2 overexpression leads to a progressive neurological disease involving seizures, spasticity, and premature death, and that higher MeCP2 level is associated with more severe disease.³⁶

The duplications that cause MDS are thought to occur via several molecular mechanisms.^{37,38} The described *MECP2* duplications are non-recurrent, meaning that duplications have variable breakpoints and do not span the same genomic interval across unrelated individuals. In general, the Xq28 region has higher GC content and higher *Alu* repeat density compared to random sequences of the genome, possibly increasing regional genomic instability.³⁷ Furthermore, most duplications have a distal breakpoint within a 215 kb genomic interval (47 kb telomeric to *MECP2*) that contains complex low copy repeat (LCR) sequences, suggesting that these LCRs may have a role in the origin of genomic rearrangement. Analysis of individual duplication breakpoints suggests that multiple mechanisms may be involved. For example, the *MECP2* duplication of one individual was proposed to occur via non-homologous end joining (NHEJ) due to the presence of proximal and distal microhomology.³⁷ However, the duplications of other individuals have been proposed to occur via complex rearrangements, which can result in embedded triplicated segments, segments of non-duplicated sequence within the duplicated region, and inversion of the duplicated sequence. These complex rearrangements may occur via Fork Stalling and Template Switching or, more generally, microhomology-mediated break-induced replication (FoSTeS/MMBIR), a replication-based mechanism that involves stalled replication forks switching templates through complementary template microhomology.³⁹ Further complicating this, duplicated segments of the Xq28 region have been found in tandem on the X-chromosome (Xq-Xp rearrangements), on the Y-chromosome (Xq-Yq translocations), as well as on autosomes (X-autosome translocations).⁴⁰

Despite the wide variation of *MECP2* duplication events in mechanism of origin, genetic content, and genomic location, they collectively result in a consistent clinical MDS phenotype.^{40,41} Individuals with MDS typically have severe developmental delay, early infantile hypotonia with progressive acquired spasticity, feeding difficulties, and recurrent respiratory infections.^{41,42} While developmental delay and hypotonia are commonly observed in neurodevelopmental disorders, the predisposition to infection distinguishes MDS. Infections range from otitis media to pneumonia, pyelonephritis, and sepsis.⁴¹ While incompletely understood, gastroesophageal reflux and immune dysfunction may contribute to infection risk, due to aspiration and immunoglobulin deficiency, respectively.^{43,44} As individuals with MDS age, over half will meet formal criteria for autism spectrum disorder (ASD) with gaze avoidance, impaired social interactions, and stereotypical movements.^{45,46} Nearly all individuals have limited or absent spoken language.⁴⁷ Seizures are also present at least half of individuals, although further research is needed to definitively determine the overall incidence and prevalence of seizures in MDS. This is particularly important because seizure onset and progression in MDS leads to marked decline in affected individuals with associated loss of skills and intellectual regression.⁴² Overall, MDS is a severely debilitating disorder that requires lifelong specialized care. Often, recurrent infections coupled with refractory epilepsy and neurological deterioration lead to early death.⁴⁷

While individual *MECP2* duplication events are rare, MDS accounts for as much as 1% of unexplained X-linked intellectual disability.⁴⁸ Most *MECP2* duplications are transmitted to male children from a carrier mother.³⁵ Carrier mothers typically exhibit extreme (>90%) skewing with preference for the X-chromosome bearing one copy of *MECP2* and are asymptomatic or exhibit neuropsychiatric symptoms. Girls who inherit a *MECP2* duplication and do not have extreme skewing can manifest variable degrees of MDS-like symptoms, ranging from mild cognitive disability to severe disease similar to that observed in males.^{49,50} *MECP2* duplication can also occur de novo in males and females. In this case, males and females are affected similarly because the *MECP2* duplication is often expressed on an autosome.^{40,47}

Due to the rarity of MDS, clinical characterization and genotype-phenotype correlations in MDS are limited. However, work by Peters et al showed that larger duplication size is correlated with increased severity across total clinical severity and motor behavioral assessment inventory scores.⁵¹ Additionally, some evidence suggests that duplication of neighboring genes contributes to additional clinical phenotypes. For example, Filamin A (*FLNA*) gene duplication has been proposed to contribute to intestinal and bladder dysfunction and development of distinct facial features amongst individuals with MDS.⁵² Furthermore, other genes that can be duplicated with *MECP2* have been associated

independently with disease: GDP dissociation inhibitor 1 (*GDII*, cognition), Ras-related protein Rab-39B (*RAB39B*, intellectual disability), and SRSF protein kinase 3 (*SRPK3*, muscle degeneration).^{53–55}

DNA Methylation and Methyl-CpG-Binding Protein 2 (MeCP2)

DNA methylation is an epigenetic mark defined by the addition of a single methyl (-CH₃) functional group onto the 5-position of DNA base cytosine (5-methyl-cytosine, 5mC). DNA methylation is integral for many cellular processes in the developing and developed organism, including cellular differentiation, genomic imprinting, X-chromosome inactivation (XCI), and silencing of repetitive DNA.^{56,57} Approximately 1% of all DNA bases and 70–80% of all CpG dinucleotides are symmetrically methylated in somatic cells of the vertebrate genome.⁵⁸ DNA methylation occurs at high levels in constitutive heterochromatin, where MeCP2 is also densely localized.⁵⁹ Other regions of the genome are methylated as well, including transcription start sites (TSS), gene bodies, enhancer elements, insulator elements, and intergenic regions.⁵⁷ DNA methylation functions differently at each of these regions, making for a complex system of epigenetic regulation. For example, the TSS of most active genes contain unmethylated CpG islands (CGIs); however, promoters of inactive genes often contain methylated CGIs, and these are associated with long-term stability of a repressed transcriptional state (eg, imprinted genes, genes on the inactive X-chromosome).⁶⁰ In contrast, gene body methylation is not associated with repression and is instead positively correlated with gene expression.⁶¹ These examples illustrate the context-dependency of DNA methylation.

In addition to CpG methylation, non-CG methylation (mCH, where H=adenine, cytosine, or thymine) occurs in an asymmetric manner throughout the genome, particularly in the developed brain of mice and humans.^{62,63} mCH increases in brain tissue of mice and humans during early postnatal development, most rapidly during the period of synaptogenesis (2–4 weeks of age in mice; 2 years of age in humans).^{64–66} Intriguingly, MeCP2 levels also increase during this period of development.⁶⁷ mCH levels reach a maximum of ~1.5% of CH genome positions and is predominantly present in the mCA context.^{62,64} In another level of complexity, methylated cytosines can also be modified by ten-eleven translocation (TET) dioxygenase enzymes that are crucial for DNA demethylation.^{68,69} During the demethylation process, intermediate hydroxymethylated cytosine residues (5hmC) are generated and retain the capacity to interact with DNA methylation reader proteins.^{70,71} Like non-CG methylation, 5hmC residues accumulate in the brain during development and in a cell type-specific manner. 5hmC is enriched in gene bodies of active genes and is thought to “functionally demethylate” these regions (generate lower occupancy of MeCP2 via decreasing high-affinity mCG availability) while retaining capacity to interact with DNA methylation readers to mediate chromatin organization.^{72,73}

One of these DNA methylation readers is MeCP2, discovered and cloned in 1992.⁷⁴ A chromatin-associated protein, MeCP2 binds symmetrically methylated cytosines in a CpG context via a methyl-binding domain (MBD, amino acids 78–162).⁷⁵ The MBD spans an 85-amino acid sequence at the N-terminal part of MeCP2 and has a DNA binding footprint of approximately 12 nucleotides (Figure 2). In addition to symmetric CpG methylation, MeCP2 can bind to methylated cytosines in a wider variety of non-CpG contexts when methylated in an asymmetric manner.^{76–78} MeCP2 has particular affinity for mCA amongst mCH contexts, especially in a CAC sequence.^{76,78} Structural work of the MBD shows that MeCP2 likely binds as a monomer to methylated DNA, recognizing the hydration in the major groove of methylated DNA.^{79,80}

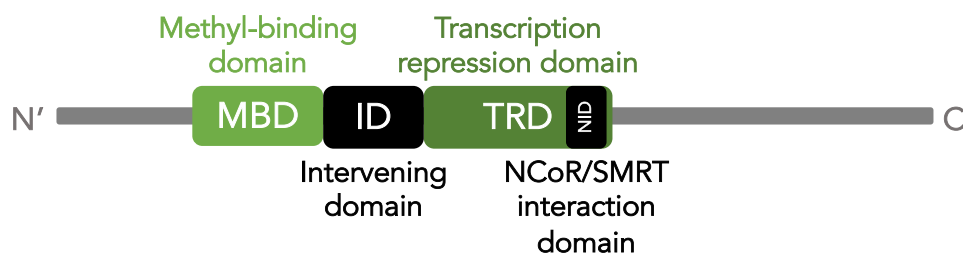


Figure 2 Schematic of MeCP2 functional domains. Schematic illustration of MeCP2 protein from the N-terminus (right) to C-terminus (left). The two main protein domains are indicated in shades of green: methyl-binding domain (MBD) and transcription repression domain (TRD). The location of the NCoR/SMRT interaction domain (NID) within the TRD is indicated in black. The intervening domain (ID) between the MBD and TRD is also indicated in black.

Once bound to DNA, MeCP2 interacts with the transcriptional repressors and activators to modulate transcription. In a well-characterized interaction, MeCP2 binds to the transcriptional co-repressor nuclear receptor co-repressor/silencing mediator of retinoid and thyroid hormone receptors (NCoR/SMRT) through its 29-amino acid NCoR interaction domain (NID, amino acids 285–313) (Figure 2).^{81–83} However, MeCP2 has also been reported to interact with a variety of other binding partners, including transcriptional co-activators, other transcriptional co-repressors, chromatin remodelers, chromatin modifiers, and splicing factors.^{82,84–94} While MeCP2 has been shown to act as a transcriptional repressor in an in vitro context,⁸¹ transcriptomics of mice with MeCP2 deficiency or MeCP2 overexpression have shown that not all differentially expressed genes are upregulated in MeCP2 deficient mice or downregulated in MeCP2 overexpression mice, as would be expected for a pure transcriptional repressor.^{77,94,95} Though it is not clear if these transcriptional changes are direct or indirect effects of MeCP2 dosage, further evidence of a complex transcriptional regulatory role for MeCP2 is provided by the finding that MeCP2 loss globally reduces (instead of elevates) transcription and translation in human embryonic stem cell (ESC) derived neurons.⁹⁶

A large portion, ~60%, of the MeCP2 sequence is disordered in solution, making structural analysis challenging.^{97,98} However, in addition to the MBD and NID, MeCP2 contains three AT-hook-like domains. AT-hooks are short DNA-binding motifs that interact with the minor groove of AT-rich DNA with the consensus sequence RGRP.⁹⁹ The three AT-hook-like domains in MeCP2 occur in 1) the interdomain (ID) between the MBD and NID at amino acids 184–195, 2) the transcription repression domain (TRD) at amino acids 264–273, and 3) the C-terminus of the protein at amino acids 341–364 (Figure 2). The second of these AT-hook-like domains has been shown to alter chromatin structure when MeCP2 is bound, suggesting that these regions can stabilize or modulate chromatin structure around MeCP2 binding sites.^{100,101}

MeCP2 is expressed predominantly in the central and peripheral nervous systems (CNS, PNS), particularly in postmitotic neurons and less so in glia.¹⁰² MeCP2 is expressed to a lesser extent in the periphery, where it is thought to help maintain muscle function required for exercise and bone integrity.¹⁰³ As a nuclear protein, MeCP2 is localized to neuronal nuclei at levels approaching that of histone octamers.¹⁰⁴ With such high levels of protein, MeCP2 shows widespread binding throughout the genome and tracks the densities of mCG, mCH, and hmC.^{77,104,105} Predominant binding sites occur in intergenic and intronic regions of the genome, but MeCP2 can also interact with elements such as promoters, enhancers, and gene bodies.¹⁰⁶ The expanse of MeCP2's DNA footprint suggests that it functions in a highly context-dependent manner. As an example of this, MeCP2 decreases the rate of transcriptional initiation of highly methylated long genes via interaction of gene body MeCP2 with the TSS.¹⁰⁷ Further, at enhancers containing a high density of mCG and mCA, MeCP2 represses enhancer activity.¹⁰⁸ These functions are likely mediated by the repressive action of MeCP2 in a context-dependent manner. Adding to the complexity of MeCP2 function, several phosphorylation sites (S308, S421) are activity-dependent and may provide an additional level of transcriptional control. While MeCP2 S308 phosphorylation reduces interaction with NCoR/SMRT, MeCP2 S421 phosphorylation permits brain-derived neurotrophic factor (*Bdnf*) transcription, dendritic growth, and spine maturation.^{109,110} Interestingly, MeCP2 has also been found to alter chromatin structure and promote chromatin compaction, introducing the notion that MeCP2 functions outside of transcriptional repression.^{111,112}

MECP2 and Disease-Causing Mutations

The *MECP2* gene contains four exons from which two transcript isoforms are expressed, MeCP2_e1 and MeCP2_e2.^{113,114} The MeCP2_e1 transcript isoform excludes exon 2 via alternative splicing and is translated from a start codon in exon 1, producing a slightly longer 498 amino acid protein with a unique 21 amino acid N-terminus in humans.^{113,114} The MeCP2_e2 transcript isoform contains all four exons and has a translation start codon in exon 2, producing a 486 amino acid protein with a unique 9 amino acid N-terminus in humans.¹¹⁵ MeCP2_e1 is more highly expressed in adult brain, while MeCP2_e2 is more highly expressed in peripheral tissues.¹¹⁶

More than 95% of individuals with typical RTT and approximately 75% of individuals with atypical RTT have a mutation in the *MECP2* gene.^{19,20} Over 200 different *MECP2* mutations have been documented as causative of RTT (RettBase: <http://mecp2.chw.edu.au>). However, some mutations are more commonly observed than others. Namely, there are 8 major point mutations that account for over 60% of all typical RTT cases: R106W, R133C, T158M, R168X, R255X, R270X, R294X, and R306C (Figure 3).¹⁹ Additionally, there is a subset of C-terminal mutations, predominately

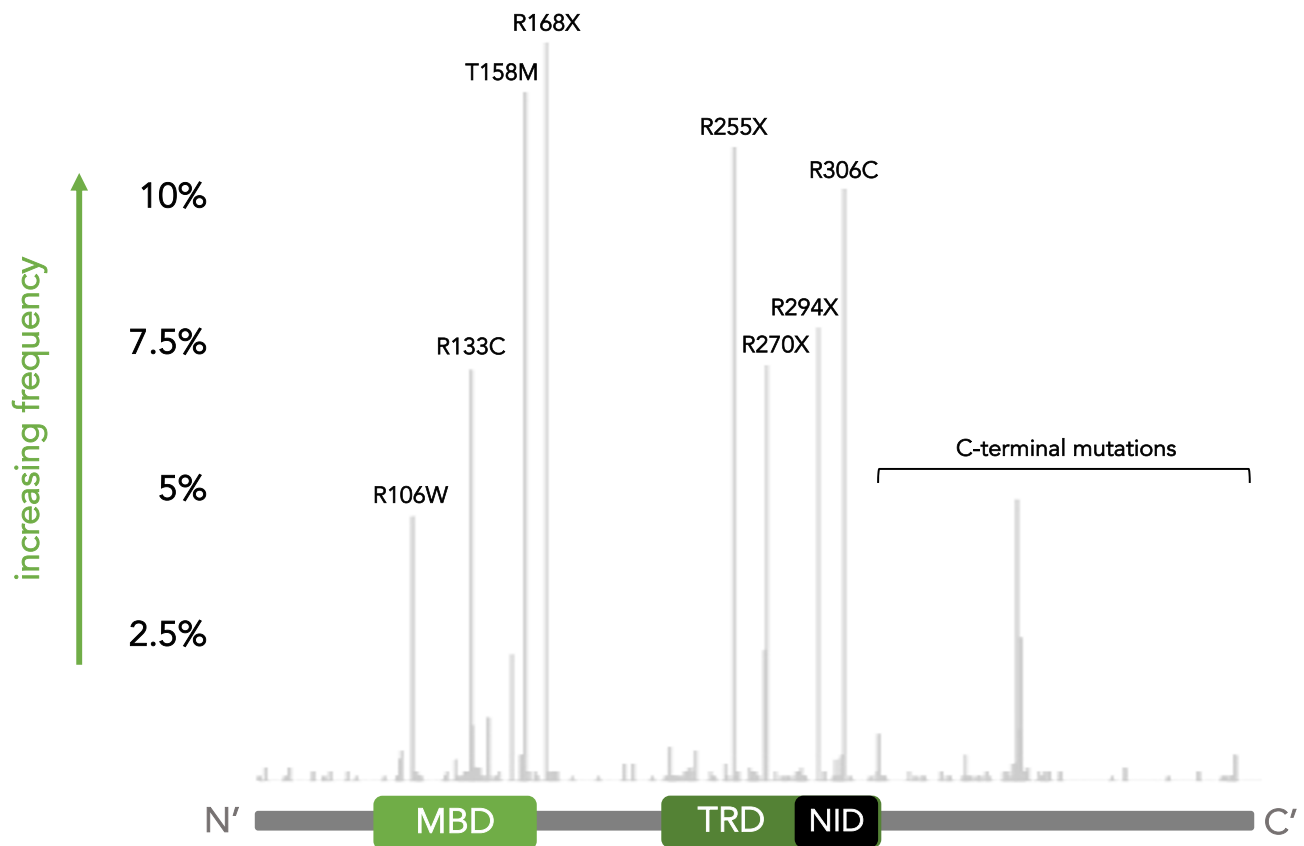


Figure 3 Common *MECP2* mutations in Rett syndrome. Frequency map of *MECP2* mutations identified in girls with RTT from the NIH Natural History Study of Rett Syndrome and Related Disorders. Mutation frequency is depicted on the y-axis while the start of the mutation along the protein sequence is indicated on the x-axis. A schematic of the *MeCP2* protein domains is provided for reference. The 8 common point mutations and set of C-terminal mutations are indicated.

small deletions, that accounts for approximately 5–10% of typical RTT cases. Less common *MECP2* mutations include those in exon 1, short deletions, large deletions, insertions, mutations at splice sites, and other point mutations.²⁰ Apart from RTT, *MECP2* mutations have also rarely been associated with other neurodevelopmental disorders including ASD,^{117–120} Angelman syndrome,^{121,122} and X-linked intellectual disability.¹²³

While most *MECP2* mutations that cause RTT are in exons 3–4 and affect both transcript isoforms, rare mutations in exon 1 of *MECP2* have been reported, as mentioned above.^{124,125} These are predominantly early frameshift mutations predicted to cause nonsense-mediated decay of the *MeCP2_e1* transcript. To date, no mutations that affect only exon 2 (and therefore only *MeCP2_e2*) have been identified. Some have proposed that the reason for this may be that *MeCP2_e1* can functionally compensate for loss of *MeCP2_e2*, but not vice versa. However, one study in mice showed that transgenic expression of *MeCP2_e2* was able to rescue of phenotypes of *Mecp2* null mice, demonstrating that work clarifying the functions of *MeCP2* isoforms in humans is needed.¹²⁶

Genotype-Phenotype Correlations in RTT

A high degree of variability in clinical severity is observed within RTT, across clinical features such as language and motor function.¹⁹ There are several possible contributors to this variability, including XCI status, *MECP2* mutation type, genetic modifiers of *MECP2* mutations, and environment. The first, XCI, occurs in a random manner as one X-chromosome is silenced in each cell during early female embryogenesis.¹²⁷ XCI status dictates relative expression of mutant to non-mutant *MeCP2* across cell and tissue types in girls. Higher preference for the non-mutant *MECP2* allele is thought to contribute to lower clinical severity.^{16,22,128} The second, *MECP2* mutation type, influences the degree to which *MeCP2* function is preserved. Mutations that are partial loss-of-function (eg, R133C, R294X) tend to yield a milder clinical course than mutations that are complete loss-of-function (eg, R255X, large deletions).¹⁹ The third,

genetic modifiers of *MECP2* mutations, may alter clinical phenotypes via direct effects on *MECP2* or indirect effects on MeCP2's function.¹²⁹ Finally, behavioral interventions such as environmental enrichment have the potential to reduce functional deficits in RTT.^{130,131}

Though it cannot explain the full complement of clinical variability, *MECP2* mutation type has emerged as a significant source for both typical and atypical RTT.^{19,20} Generally, genotype–phenotype correlations map onto both typical and atypical RTT; however, the phenotypes tend to be more highly skewed in atypical RTT.²⁰ For example, complete loss-of-function mutations such as R255X and large deletions are associated with higher clinical severity in both typical and atypical RTT, but the degree of increase in severity is greater in atypical than typical RTT (and vice versa for partial loss-of-function mutations associated with lower clinical severity). Additionally, these correlations appear to affect the entire disease course, as individuals with mild *MECP2* mutations tend to have a later diagnosis and less severe disease as children (and vice versa). Despite this difference in baseline severity, clinical severity does increase with age across most mutations. The largest contributors to clinical severity are hand use and onset of stereotypies, with smaller contributions from growth, motor dysfunction, and communication dysfunction. Ultimately, these genotype–phenotype correlations denote a pattern, but individuals with RTT may not have disease that exactly ascribes to this pattern. Other factors, including XCI, genetic modifiers, and environment, can also alter disease course. Intriguingly, individuals have been identified with RTT-causing mutations in *MECP2* and random XCI who have neurodevelopmental problems but do not have the characteristic RTT features (ie, regression) and overall clinical severity of people with RTT, pointing to such other genetic factors influencing the ultimate phenotypical manifestation of alteration in MeCP2 function.¹³²

MECP2 Mutations Associated with Mild Disease in RTT

MECP2 mutations associated with mild disease include R133C, R294X, and C-terminal mutations. The R133C mutation has been shown to partially disrupt binding to methylated DNA, particularly to hydroxymethylated cytosines, leading to partial loss-of-function of MeCP2.^{72,105,133} In contrast, the later R294X and C-terminal mutations do not disrupt the MBD and instead disrupt the NCoR/SMRT interaction domain (NID) sequence (R294X) or the 3' end of the transcript (C-terminal mutations). Recent work has shed light on the partial loss-of-function mechanism of R294X; however, the pathogenic mechanism of C-terminal mutations remains largely unknown.

The R294X mutation, one of the 8 common point mutations, occurs in approximately 6% of individuals with typical RTT and approximately 4% of individuals with atypical RTT.^{19,20} R294X is an arginine-to-stop mutation that occurs within the NID sequence. It is predicted to retain methylated DNA binding capacity and interrupt binding to the transcriptional co-repressor complex NCoR/SMRT. Recent mouse modeling work has shown that male hemizygous *Mecp2*^{R294X/Y} (R294X) mice exhibit RTT-like phenotypes with delayed onset and increased lifespan compared to mouse models of other *MECP2* mutations.¹³⁴ These mild RTT-like phenotypes are associated with production of stable truncated MeCP2, unlike other models of nonsense *MECP2* mutations. Collectively, this work suggests that the R294X mutation acts through partial loss-of-function to cause RTT. Molecular work confirmed that the R294X truncation product binds to chromatin; however, interestingly, the truncation product bears higher chromatin binding affinity compared to wild type (WT) MeCP2.¹³⁴

C-terminal domain (CTD) mutations are a class of *MECP2* mutations grouped by genomic location. They occur in approximately 9% of individuals with typical RTT and approximately 17% of individuals with atypical RTT.²⁰ CTD mutations span a region of more than 500 bp and are highly variable, with over 100 different C-terminal *MECP2* mutations reported (RettBase: <http://mecp2.chw.edu.au>). However, a hotspot of small deletion-frameshift mutations starting between c.1157–1164 comprises about 60% of all CTD mutations. Two of these hotspot mutations have been modelled in mice: c.1157_1197del41 and c.1164_1207del44.¹³⁵ Mice with the c.1157_1197del41 mutation displayed severe RTT-like phenotypes and reduced lifespan; correspondingly, mice expressed very low levels of truncated MeCP2. However, mice with the c.1164_1207del44 mutation did not display severe RTT-like phenotypes and had no difference in lifespan compared to WT mice. Interestingly, when the mouse c.1164_1207del44 *Mecp2* allele was “humanized” with several added point mutations around the mutation to more closely match the human c.1164_1207del44 *MECP2* allele, truncated protein levels were significantly reduced in a cellular model. This work suggested that human-specific

sequences around the CTD mutation hotspot might impact protein stability. Other studies have implicated the C-terminus in facilitating chromatin binding and in directing chromatin architecture, suggesting that CTD mutations might impact function as well as stability.^{97,136} Still, a clear picture of how specific CTD mutations affect MeCP2 function is needed.

Disease Modeling of RTT

While several animal models of RTT have been developed, including mice, zebrafish, and monkeys, this review will focus on mouse models given their widespread use and utility in modeling mutation-specific aspects of disease.^{137,138} The first mouse models of RTT were developed in the early 2000s, from the labs of Adrian Bird and Rudolf Jaenisch.^{139,140} Both models involved deletion of exons shared by the two isoforms of *Mecp2* (Bird null: exons 3–4; Jaenisch null: exon 3). Global deficiency of MeCP2 led to a constellation of RTT-like phenotypes that exhibited sex-specific onset. Hemizygous male *Mecp2*^{−/Y} mice were significantly underweight from 4 weeks of age (on the C57BL/6 background) and developed stiff gait, decreased activity, and tremor between 3 and 8 weeks of age.^{139,140} *Mecp2*^{−/Y} mice also developed hindlimb claspings and irregular breathing, with early death at approximately 10 weeks of age. In contrast, heterozygous female *Mecp2*[±] mice displayed a similar set of RTT-like phenotypes, including stiff gait, decreased activity, hindlimb claspings, and irregular breathing, beginning much later, at 3–4 months of age.^{139,140} The phenotypes exhibited by mice with global MeCP2 deficiency recapitulate features of RTT in humans, permitting use of this system for disease modelling.

The predominance of neurological features in RTT suggested that the central nervous system may be the predominant site of MeCP2 dysfunction. To test this, both the Bird and Jaenisch labs crossed mice expressing a loxP-flanked *Mecp2* allele with Nestin-Cre driver mice. This cross results in deficiency of MeCP2 in the CNS and PNS, including neuronal and glial precursors. Nestin-Cre conditional mutant mice were indistinguishable from global knockout (KO) mice. While unexpected due to the broad expression of MeCP2 throughout the body, this result indicated that phenotypes of *Mecp2*^{−/Y} and *Mecp2*[±] mice are attributable to loss of MeCP2 in the nervous system.^{139,140} Furthermore, the Jaenisch lab crossed mice expressing a loxP-flanked *Mecp2* allele with CamK-Cre93 driver mice to yield MeCP2 deficiency in postnatal forebrain, hippocampus, and brainstem neurons. These CamK-Cre93 conditional mutant mice displayed typical development until 3 months, at which time they began to show similar RTT-like phenotypes of stiff gait and decreased activity.¹³⁹ This result suggested that RTT-like phenotypes are attributable to MeCP2 deficiency in neurons rather than glia. Consistently, follow-up work showed that astrocyte-specific reactivation of *Mecp2* in globally MeCP2 deficient mice partially rescued behavioral abnormalities and had very minimal effect on lifespan,¹⁴¹ while microglia-specific reactivation of *Mecp2* in globally MeCP2 deficient mice did not rescue behavioral abnormalities.^{142,143}

The Jaenisch lab's work suggested that MeCP2 deficiency in post-mitotic neurons is sufficient to cause disease. This was confirmed and extended by experiments that induced MeCP2 deficiency when mice reached maturity. Specifically, a tamoxifen-inducible *Mecp2* knockout strategy was applied to mice aged 60 days or older, eliminating MeCP2 only during adulthood.¹⁴⁴ These mice exhibited RTT-like phenotypes similar to germline *Mecp2* knockout mice, including hypoactivity, abnormal gait, hindlimb claspings, and premature death with similar median time to death after dosing (~10–12 weeks). This result demonstrated that MeCP2 expression is required continuously in the developed brain and argued that, though the onset of RTT correlates with neurodevelopment, the disease may not be attributable to disruption of the developing brain.

Given these findings, the field raised the reverse question of whether reactivation of *Mecp2* after symptom onset could provide therapeutic benefit. This was shown in a tamoxifen-inducible Cre recombination experiment, whereby a loxP-flanked stop sequence is excised upon administration of tamoxifen.¹⁴⁵ Reactivation of *Mecp2* after symptom onset in hemizygous male *Mecp2* mutant mice rescued phenotypic score, improved motor behavior and breathing phenotypes, and preserved survival up to 30 weeks of age.^{145,146} Reactivation of *Mecp2* after symptom onset (~30 weeks of age) in heterozygous female *Mecp2* mutant mice similarly significantly rescued phenotypic score, representing observation of activity, gait, hindlimb claspings, tremor, breathing, and coat condition.¹⁴⁵ Together, these experiments provided early hope for the potential of disease-modifying therapeutics, through reversal of MeCP2 loss after onset of symptoms and diagnosis in people.

Since then, numerous studies have used complex mouse genetics to achieve cell-type specific loss of MeCP2 in WT mice or reactivation of *Mecp2* in global knockout mice, to determine the cell types, brain regions, and circuits involved in RTT pathogenesis. Selective reduction of MeCP2 has been behaviorally characterized in multiple different brain regions, including postnatal forebrain,^{139,147} basal ganglia,¹⁴⁸ hypothalamus,¹⁴⁹ and cerebellum,¹⁵⁰ as well as in specific cell types, including glutamatergic neurons,¹⁵¹ GABAergic neurons,¹⁵² dopaminergic and noradrenergic neurons,¹⁵³ serotonergic neurons,¹⁵³ parvalbumin-expressing interneurons,¹⁵⁴ and somatostatin-expressing interneurons.¹⁵⁴ Though the details of behavioral consequences of region- and cell type-specific MeCP2 loss are beyond the scope of this review, collectively this work demonstrates that MeCP2 function is critical across a variety of brain regions that, together, contribute to the complex behaviors observed in RTT.

Additionally, extensive work has gone into the development of mouse models harboring common *MECP2* mutations to understand their pathophysiological mechanism. To date, mouse models of all 8 common *MECP2* point mutations – R106W, R133C, T158M, R168X, R255X, R270X, R294X, and R306C – have been generated (Table 1).^{82,133,134,155–161} These mutations fall into two categories – missense (R106W, R133C, T158M, R306C) and nonsense/truncating (R168X, R255X, R270X, R294X) mutations. Each of the missense mutations yield a protein product in mice at varying levels compared to full-length WT MeCP2. In comparison, amongst the nonsense mutations, R168X and R255X produce no detectable protein product while R270X and R294X mutations yield produce stable truncation products in mice. However, R270X truncation product was not detected in a human brain lysate from a heterozygous female individual with RTT, suggesting that features of the R270X mouse model may not reflect human pathophysiology.¹⁶⁰ Despite differences in protein production amongst the 8 common point *MECP2* mutations, they each cause RTT via complete or partial loss-of-function. In the latter case, mutations disrupting the MBD are predicted to decrease binding to methylated DNA, while mutations disrupting the NID are predicted to decrease interaction with transcriptional co-repressors. Furthermore, different mutations within the MBD differentially affect binding to methylated DNA.¹⁶² For example, the R106W mutation nearly abolishes methylated DNA binding capacity, while R133C retains partial binding capacity. Apart from the 8 common point mutations, an additional 2 C-terminal mutations have been modelled in mice – c.1157_1197del41 (p.L386HfsX5) and c.1164_1207del44 (p.P389X) (Table 1).¹³⁵ L386HfsX5 mice display RTT-like phenotypes and decreased survival, consistent with other models of *MECP2* mutation. However, P389X mice did not display RTT-like behavioral phenotypes and had survival comparable to WT mice up to 1 year. As discussed in the previous section, differences in the MeCP2 C-terminus between mice and humans may explain this discrepancy; however, more work is needed to understand the mechanism underlying C-terminal *MECP2* mutation.

With the knowledge that *MECP2* loss-of-function predominantly affects neurons in the brain, the field moved to determine synaptic effects of MeCP2 loss. Basal synaptic transmission was first evaluated by input-output (I/O) curves, which correlate presynaptic volleys as input (representing depolarization of presynaptic terminals) with the slope of evoked field excitatory postsynaptic potentials as output (field EPSPs). At Schaffer collateral-CA1 synapses, male *Mecp2*^{+/Y} and female *Mecp2*^{+/±} mice have I/O ratios unchanged from WT, indicating similar basal synaptic transmission.^{145,163} MeCP2 loss does, however, affect presynaptic function. One metric of this is paired-pulse facilitation (PPF), a form of short-term plasticity. PPF is measured by delivering two stimuli within a short millisecond interstimulus interval and measuring field EPSP responses. Typically, the second recorded field EPSP is enhanced due to elevated presynaptic calcium concentration following the first stimulus, leading to a greater release of synaptic vesicles following the second stimulus. Pre-symptomatic *Mecp2* mutant mice do not display differences in PPF compared to WT littermates; however, symptomatic *Mecp2* mutant mice display decreased PPF, suggesting increased release probability.^{163–165} This PPF phenotype may be caused by direct effects of MeCP2 protein level on calcium concentration in the presynaptic terminal or indirect alterations of presynaptic proteins involved in neurotransmitter release, that take effect only during postnatal development.¹⁶⁶

Long-term potentiation (LTP) and long-term depression (LTD) are forms of long-term synaptic plasticity in which different frequency stimulations cause a persistent increase or decrease in synaptic strength. Male hemizygous *Mecp2*^{+/Y} and female heterozygous *Mecp2*^{+/±} mice both show attenuated LTP and LTD in the CA1 region of the hippocampus.^{145,163,164} Of note, and consistent with the LTP phenotype, *Mecp2*^{+/Y} mice express lower levels of N-methyl-D-aspartate (NMDA) receptor subunit NR2A and higher levels of subunit NR2B compared to WT.¹⁶³ The

Table 1 Mouse Models of *MECP2* Mutation and Duplication

Line	Mutation	Description	Tags	Reference(s)
<i>Mecp2</i> ^{tm1.1Bird}	Knockout	Cre-mediated excision of <i>Mecp2</i> exons 3 and 4	None	[140,219]
<i>Mecp2</i> ^{tm1.1Jae}	Knockout	Cre-mediated excision of <i>Mecp2</i> exon 3	None	[139]
<i>Mecp2</i> ^{tm4.1Joez}	R106W	Introduction of exon 3 containing R106W mutation and C-terminal Tavi tag	Tavi (C-terminal)	[157]
Tg(<i>MECP2</i> *R111G/ EGFP)1Hzo	R111G	Introduction of human <i>MECP2</i> gene with R111G mutation onto an autosome	EGFP (C-terminal)	[155]
<i>Mecp2</i> ^{tm6.1Bird}	R133C	Introduction of exon 4 containing R133C mutation and C-terminal EGFP tag	EGFP (C-terminal)	[133]
<i>Mecp2</i> ^{tm1.1Vnar}	A140V	Introduction of exon 4 containing A140V mutation	None	[156]
<i>Mecp2</i> ^{tm1.1Joez}	T158A	Introduction of exon 4 containing T158A mutation	None	[158]
<i>Mecp2</i> ^{tm4.1Bird}	T158M	Introduction of exon 4 containing T158M mutation and C-terminal EGFP tag	EGFP (C-terminal)	[133]
<i>Mecp2</i> ^{tm3.1Joez}	T158M	Introduction of exon 4 containing T158M mutation and C-terminal Tavi tag	Tavi (C-terminal)	[157]
<i>Mecp2</i> ^{tm1.1Jtc}	R168X	Introduction of exon 4 containing R168X mutation	None	[220]
<i>Mecp2</i> ^{tm1.1Hupp}	R168X	Introduction of exon 4 containing R168X mutation	None	[159]
<i>Mecp2</i> ^{tm1.1Irsf}	R255X	Introduction of exon 4 containing R255X mutation	None	[160]
Tg(<i>MECP2</i> *R270X/ GFP)AHzo	R270X	Introduction of exon 4 containing R270X mutation and C-terminal GFP tag	GFP (C-terminal)	[100]
Tg(<i>MECP2</i> *G273X/ GFP)AHzo	G273X	Introduction of exon 4 containing G273X mutation and C-terminal GFP tag	GFP (C-terminal)	[100]
<i>Mecp2</i> ^{R294X}	R294X	CRISPR/Cas9-mediated engineering of the R294X mutation	None	[134]
<i>Mecp2</i> ^{tm2.1Meg}	R306C	Introduction of exon 4 containing R306C mutation	None	[82]
<i>Mecp2</i> ^{tm5.1Bird}	R306C	Introduction of exon 4 containing R306C mutation and C-terminal EGFP tag	EGFP (C-terminal)	[133]
Tg (<i>MECP2</i> *R306C/ EGFP)1Hzo	R306C	Introduction of human <i>MECP2</i> gene with R306C mutation and a C-terminal EGFP tag onto an autosome	EGFP (C-terminal)	[155]
<i>Mecp2</i> ^{tm1Hzo}	T308X	Introduction of exon 4 containing T308X mutation	None	[221]
<i>Mecp2</i> ^{L386HfsX5}	L386HfsX5	CRISPR/Cas9-mediated engineering of the L386HfsX5 mutation	None	[135]
<i>Mecp2</i> ^{P389X}	P389X	Introduction of exon 4 containing P389X mutation	None	[135]
<i>Mapt</i> ^{tm1 (Mecp2)Jae}	<i>Mecp2</i> duplication	Introduction of mouse <i>Mecp2</i> cDNA sequence into exon 1 of the tau gene, in-frame with the endogenous tau start codon	None	[174,175]
Tg(<i>MECP2</i>)1Hzo	<i>MECP2</i> duplication	Introduction of human <i>MECP2</i> gene sequence onto an autosome	None	[36]

NR2B subunit is expressed predominantly at immature synapses, and the NR2A subunit is more efficient than NR2B in generating NMDA receptor-dependent LTP at adult CA1 synapses.¹⁶⁷ A final form of plasticity affected by MeCP2 loss is homeostatic plasticity, the ability of neurons to restore their activity to a setpoint following changes to network activity.

One specific form of homeostatic plasticity is synaptic scaling, characterized by cell-wide changes in synaptic strength following neuronal activity. MeCP2 deficiency prevents an activity-dependent decrease in the α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor subunit GluR2 expression and synaptic scaling in hippocampal cultures,¹⁶⁸ as well as synaptic scaling in the neocortex after sensory deprivation.¹⁶⁹

Loss of *MECP2* expression is also associated with changes to synaptic structure. Mouse and human neurons deficient in MeCP2 have smaller neuronal cell size and increased density of neurons.^{96,139,170,171} Additionally, post-mortem brain tissue and mouse neurons deficient in MeCP2 show low dendritic spine density and decreased dendritic branching complexity.^{102,170,172,173} These results argue that MeCP2 is involved in the structural maturation of neurons, including dendritic spine development and dendritic arborization.

Disease Modeling of MDS

With the successful generation of mouse models of RTT, two mouse models of MDS were subsequently developed. The first, Tg(*MECP2*), was created by the Zoghbi lab via the introduction of the human *MECP2* genomic sequence (including the genomic DNA containing the promoter and regulatory sequences) into an autosome, effectively globally duplicating protein levels throughout the life of the animal (Table 1).³⁶ Tg(*MECP2*) mice demonstrate typical development until approximately 10–12 weeks of age, at which time they show forepaw claspings, enhanced motor learning, and enhanced fear response in a contextual fear conditioning paradigm. As they age, these Tg(*MECP2*) mice develop seizures, hypoactivity, and spasticity with early death. Importantly, analysis of several transgenic lines with differing MeCP2 protein levels found that increasing levels of MeCP2 protein correlated with increased severity of MDS-like phenotypes, reinforcing the notion that *MECP2* gene dosage drives MDS. A second mouse model of MDS, *Mapt*^{tm1(Mecp2)}, was created by the Jaenisch lab via the introduction of the mouse *Mecp2* coding sequencing in-frame into the first exon of the *Tau* gene, effectively globally duplicating the gene in post-mitotic neurons (Table 1).¹⁷⁴ This tau-MeCP2 fusion protein contains the first 31 amino acids of Tau followed by the MeCP2 E2 protein and, when combined with endogenous mouse MeCP2, yields approximately twice the typical levels of MeCP2 in the brain.^{174,175} The *Mapt*^{tm1(Mecp2)} mice display motor dysfunction, increased anxiety-like behavior, and enhanced fear response in contextual and cued conditioning paradigms.

Both Tg(*MECP2*) and *Mapt*^{tm1(Mecp2)} MDS mouse models have behavioral phenotypes redolent of MDS features, including deficits in motor coordination and increased anxiety-like behavior. However, there are differences between the two; for example, the *Mapt*^{tm1(Mecp2)} mice do not display decreased lifespan while the Tg(*MECP2*) mice do display decreased lifespan. Discrepancies such as this may be attributed to differences in transcriptional control of the transgene (ie, the native *MECP2* human promoter and regulatory sequences vs those of mouse *Tau*) or differences in function of the protein (ie, human MeCP2 E1 and E2 vs tau-MeCP2-E2 fusion). Complicating this picture, the Tg(*MECP2*) model of MDS has been reported to show mouse strain-specific phenotypes, indicating that background affects phenotypic expression. To circumvent this, several studies have used F1 hybrid mice with success.^{155,176,177} Due to these complicating issues, the contributions of specific cell types and brain regions to MDS-like phenotypes have not been as thoroughly dissected as in RTT. However, one study showed reversal of social recognition deficits in MDS model mice via normalization of MeCP2 levels in the medial prefrontal cortex.¹⁷⁸ Additionally, elevated anxiety-like behavior and decreased social approach in MDS model mice were reversed by genetic reduction of corticotropin-releasing hormone (CRH) or opioid receptor mu (μ) 1 (OPRM1), respectively.¹⁷⁷

Apart from MDS-like behavior, mouse models have provided insight into the synaptic underpinnings of the disease. Electrophysiological analysis of MDS mouse model hippocampal brain slices showed impairments in synaptic plasticity. These experiments found normal I/O curves, indicating that Schaffer collateral input onto CA1 neurons is intact.¹⁷⁵ However, PPF, a form of short-term synaptic plasticity, was enhanced in hippocampal slices of both Tg(*MECP2*) and *Mapt*^{tm1(Mecp2)} mice, suggesting a presynaptic effect of MeCP2 overexpression leading to a decrease in release probability.^{36,175} Consistently, some cognitive and motor behavioral phenotypes and synaptic dysfunction can be normalized by treatment with a non-competitive GABA_A receptor antagonist, picrotoxin.¹⁷⁹ Additionally, MDS model mice show differences in LTP in the hippocampus.^{36,175} While hippocampal LTP was enhanced in Tg(*MECP2*) mice, hippocampal LTP was attenuated in *Mapt*^{tm1(Mecp2)} mice; these differences may be

due to pattern of MeCP2 expression and require further study. Spontaneous neurotransmission was evaluated alongside evoked neurotransmission in the *Mapt^{tm1(Mecp2)}* mice. Recordings from cultured hippocampal neurons found increased resting excitatory drive, with enhanced mini-excitatory postsynaptic currents (mEPSCs) and no changes to mini-inhibitory postsynaptic currents (mIPSCs).¹⁷⁵ Collectively, these studies of neurotransmission suggest that MeCP2 overexpression causes alterations to excitatory neurotransmission in the hippocampus, with increased resting excitatory drive.

While the molecular mechanism for these electrophysiological alterations is not well understood, MeCP2 overexpression has been associated with alterations to structural features of synapses. For example, in mouse hippocampal CA1, MeCP2 overexpression leads to increased glutamatergic synaptic density during early postnatal development.¹⁸⁰ Consistently, cortical neurons derived from several different *MECP2* duplication induced pluripotent stem cell (iPSC) lines show increased synaptogenesis and dendritic complexity.¹⁸¹ However, overexpression of WT *MECP2* in rat hippocampal pyramidal slices leads to a transient decrease in the density of spines, specifically of mature spines, without affecting dendritic complexity or length.¹⁷² These studies suggested that the structural effects of MeCP2 overexpression are model- and timepoint-dependent. Follow-up work used the Tg(*MECP2*) mouse model to study L5 pyramidal neurons in the barrel cortex (region of somatosensory cortex that receives input from whiskers via the thalamus) in vivo.¹⁸² In this model, dendritic spine density was initially increased until 12 weeks of age, after which point MDS model animals showed decreased dendritic spine density compared to WT littermates. Interestingly, spontaneous spine turnover remains increased in aged MDS model animals, with higher spine gain and loss rates biased towards spine loss.

In addition to baseline differences in spine density, the structural plasticity of spines is also affected by MeCP2 overexpression. In WT mice, rotarod motor training induces an increase in bouton elimination rate in the motor cortex.^{183,184} MeCP2-overexpressing mice do not exhibit this increase in bouton elimination, and instead show excessive formation and stabilization of dendritic spine clusters, consistent with the behavioral phenotype of motor overperformance. These molecular and behavioral changes are reversible by pharmacological inhibition of ERK, suggesting that Ras-ERK signaling hyperactivity contributes to these phenotypes in MDS model animals.¹⁸⁵ Collectively, this work supports the notion that excessive MeCP2 contributes to inappropriate consolidation of clustered synaptic connections during motor learning, in addition to having baseline effects on spine density.

Therapeutic Development for RTT and MDS

Prior to the systematic evaluation of animal models of RTT, treatment trials using folate and betaine to increase DNA methylation were undertaken, but this treatment did not result in clinical improvement in people with RTT.¹⁸⁶ The reversibility of RTT-like phenotypes in mouse models after symptom onset has provided hope that disease-modifying treatment could be developed for individuals diagnosed with RTT. Rescue experiments have shown that elevation of MeCP2 levels in symptomatic mice significantly improves, but does not fully rescue, overall RTT-like phenotypes, survival, and respiratory and motor function.^{145,146} The lack of complete rescue may be due to incomplete restoration of MeCP2 levels across the brain, but further work is needed to determine this. Regardless, a cautious but hopeful outlook for RTT therapy has spurred preclinical work and clinical trials in this space.

One of the first targets of RTT therapeutic development was BDNF, an important mediator of neuronal and synaptic maturation. In heterozygous female mouse models of RTT and in humans with RTT, *BDNF* transcript is downregulated with loss of functional MeCP2.¹⁸⁷ Further, overexpression of *BDNF* in *Mecp2* null male mice extends survival, rescues specific motor deficits, and reverses electrophysiological abnormalities.¹⁸⁸ Therapeutic efforts were directed towards increasing BDNF levels as an indirect method of restoring MeCP2 function. One of these methods was treatment with ampakines, drugs that enhance glutamatergic AMPA receptor activity to upregulate endogenous BDNF. Chronic treatment of hemizygous male *Mecp2^{-Y}* mice with the ampakine CX546 was able to restore normal breathing rate and minute volume/weight.¹⁸⁹ Another drug shown to increase endogenous BDNF is fingolimod, a sphingosine-1 phosphate receptor agonist that crosses the blood brain barrier, and administration of fingolimod to hemizygous male *Mecp2^{-Y}* mice normalized rotarod motor performance and extended lifespan by approximately 1 month.¹⁹⁰ A clinical trial assessing the safety and efficacy of oral fingolimod in children older than 6 years found that the drug is safe but found no evidence of an effect on clinical, laboratory, or imaging measures of disease (FINGORETT; NCT02061137).¹⁹¹

Unfortunately, the blood brain barrier penetrance of BDNF is low, making its application as a therapeutic challenging. Insulin-like growth factor 1 (IGF1), however, confers similar effects on neuronal survival and maturation through overlapping signaling pathways and crosses the blood brain barrier, especially through its metabolite glycine-proline-glutamate (GPE).^{192–194} Treatment of hemizygous male *Mecp2*^{−/Y} mice with GPE showed distinct benefits after initiating dosing from a young age (P15–P18).¹⁹⁵ Specifically, GPE promoted survival, improved structural brain abnormalities (eg, brain weight, spine density), and improved behavioral and physiological abnormalities (eg, breathing irregularity, activity). Additionally, treatment of adult female heterozygous *Mecp2*[±] mice with GPE stabilized cortical plasticity to WT levels.¹⁹⁵ This encouraging data provided the impetus for further preclinical studies of IGF1, GPE, and GPE analogues like trofinetide in RTT. Trofinetide, the proline-methylated analogue of GPE, harbors an improved pharmacokinetic profile compared to GPE and a Phase II clinical trial in individuals with RTT showed that oral trofinetide is generally safe and well tolerated (NCT01703533).¹⁹⁶ Promisingly, trofinetide at 200 mg/kg twice daily in children/adolescents with RTT improves core features across multiple disease domains as assessed by clinicians and caregivers (NCT02715115).¹⁹⁷ A recently completed Phase III trial of trofinetide in children and adolescents with RTT (NCT04181723) reported success in meeting the primary clinical efficacy outcomes (<https://acadia.com/media/news-releases/acadia-pharmaceuticals-announces-positive-top-line-results-from-the-pivotal-phase-3-lavender-trial-of-trofinetide-in-rett-syndrome/>).

Another modulator of the BDNF signaling pathway is the NMDA receptor antagonist ketamine. Previous work in mouse models has shown that ketamine can act through NMDA receptor antagonism to release a translational block of *Bdnf*.¹⁹⁸ Acute systemic treatment with ketamine in female heterozygous *Mecp2*[±] mice normalizes pre-pulse inhibition of acoustic startle, and chronic treatment of *Mecp2*^{−/Y} mice improves locomotor activity, breathing, and survival.^{199,200} A Phase II clinical trial of oral ketamine in RTT is ongoing (NCT03633058).

A final modulator of BDNF signaling is glatiramer acetate, a collection of artificial polypeptides currently used for treatment of multiple sclerosis in the United States.²⁰¹ Though its mechanism is incompletely understood, it is thought to activate peripheral T-cells that cross the blood–brain barrier and stimulate secretion of neurotrophic factors such as BDNF in the brain.^{202,203} A Phase II clinical trial of glatiramer acetate in girls with RTT showed improvement in clinical features such as gait velocity, memory, and breath-holding.²⁰⁴ However, multiple severe post-injection adverse reactions were later observed in an open-label clinical trial in girls with RTT, raising significant safety concerns with glatiramer acetate in this population.²⁰⁵ Apart from modulation of BDNF signaling, therapeutic efforts for RTT have also targeted other downstream mediators of MeCP2 function, such as neurotransmitter systems (eg, GABA, dopamine, serotonin) with variable success in mouse models.²⁰⁶

Direct methods to restore MeCP2 function aimed to elevate functional MeCP2 protein. These include 1) gene therapy, 2) X-chromosome reactivation, 3) genome editing, and 4) RNA editing strategies. First, gene therapy employs viral delivery of the *MECP2* gene to permit increased protein expression. Numerous studies have evaluated the safety and efficacy of modelled gene therapy in hemizygous *Mecp2*^{−/Y} mice and have demonstrated rescue of survival, body weight, and select behavioral phenotypes.^{207–212} However, only one study thus far has evaluated the safety and efficacy of gene therapy in heterozygous *Mecp2*^{+/-} mice, where authors demonstrated that systemic viral delivery in adulthood rescued gross phenotypic progression as well as motor and cognitive behavioral phenotypes.²⁰⁷ Collectively these studies have also identified potential barriers to the use of gene therapy in people, most prominently hepatotoxicity occurring with systemic delivery.^{209,212} Second, X-chromosome reactivation aims to pharmacologically de-repress *MECP2* transcription from the inactive X-chromosome in female neurons.²¹³ Third, genome editing aims to correct the mutant *MECP2* allele to via approaches such as CRISPR/Cas9 (clustered regularly interspaced short palindromic sequences).²¹⁴ Finally, RNA editing employs adenosine deaminase enzymes to program site-specific adenine-to-inosine editing of RNAs and correction of G > A mutations in *Mecp2* at the transcript level.^{215,216}

In contrast to those for RTT, current efforts to develop therapeutics for MDS have focused on reducing the levels of MeCP2. One approach has been through antisense oligonucleotides (ASOs), which are short, synthetic single strands of chemically modified DNA designed to alter mRNA expression and subsequently protein level.²¹⁷ A human-specific *MECP2*-ASO has been shown to reduce MeCP2 levels in brain and rescue behavioral and EEG phenotypes in mice with one human copy of *MECP2*.¹⁷⁶ This work showed that the benefits from ASO therapy were apparent approximately 4

Table 2 Similarities and Differences Between RTT and MDS

	RTT (MeCP2 Loss of Function)	MDS (MeCP2 Gain of Function)
Genetic etiology	<i>MECP2</i> mutations (predominantly de novo in sperm)	Non-recurrent <i>MECP2</i> duplications (predominantly inherited from carrier mother or de novo)
Incidence in males vs females	Females > males	Males > females
Onset of disease	Typically in early childhood	Typically in infancy
Shared clinical features	<ul style="list-style-type: none"> • Seizures • Limited or absent speech • Gait abnormalities • Autistic features • GI issues 	
Disease-specific clinical features	<ul style="list-style-type: none"> • Developmental regression • Breathing abnormalities 	<ul style="list-style-type: none"> • Early-onset hypotonia • Severe intellectual disability • Recurrent respiratory infections • Characteristic facial features
Shared synaptic features	<ul style="list-style-type: none"> • Attenuated hippocampal LTP 	
Disease-specific synaptic features	<ul style="list-style-type: none"> • Decreased spontaneous excitatory transmission • Decreased PPF short-term plasticity • Decreased dendritic spine density 	<ul style="list-style-type: none"> • Increased spontaneous excitatory transmission • Increased PPF short-term plasticity • Increased dendritic spine density
Potential therapeutic approaches	<ul style="list-style-type: none"> • Elevating neurotrophic factor levels (ampakines, fingolimod, trofine-tide, glatiramer acetate) • Ketamine • Gene therapy • Read-through compounds for nonsense mutations • X-chromosome reactivation • Genome editing • RNA editing 	<ul style="list-style-type: none"> • <i>MECP2</i>-ASOs

weeks later than benefits from genetic rescue, likely due to gradual reduction of MeCP2 levels. More recent work has shown benefit of a human-specific *MECP2*-ASO in mice harboring two copies of the human *MECP2* allele.²¹⁸ This experiment importantly introduced the potential to titrate *MECP2*-ASO and determine the optimal dosage. Promisingly, no toxicity associated with sub-therapeutic MeCP2 levels was observed across the tested ASO doses. This study did find that molecular changes in response to therapy precede behavioral changes by almost 2 months, suggesting that molecular information could be used as an early biomarker for dosing guidance.

Conclusion

Rett syndrome (RTT) and *MECP2* duplication syndrome (MDS) are related neurodevelopmental disorders that each result from errant MeCP2 function – loss and gain, respectively. This review has highlighted the genetic origin, clinical characteristics, cellular features, and therapeutic approaches under development for both RTT and MDS, summarized in Table 2. While these disorders stem from opposite effects on MeCP2 function, they share some clinical and cellular features. For example, both individuals with RTT and MDS can have seizures, impaired speech, gait abnormalities, and GI issues. However, there are RTT-specific features (eg, developmental regression, breathing abnormalities) and MDS-specific features (eg, infantile hypotonia, recurrent respiratory infections) as well. Furthermore, MeCP2 loss and gain of function have largely opposite

synaptic-level effects, though there is evidence that both RTT and MDS model mice exhibit attenuated LTP in hippocampus, suggesting that MeCP2 loss and elevation differentially affect hippocampal circuitry. This review has also described a set of therapeutic approaches under development for RTT and MDS. Perhaps most currently promising among those for RTT is trofinetide, which recently completed a Phase III clinical trial (LAVENDER; NCT04181723) with reportedly encouraging results. Though therapeutic development for MDS is still in the preclinical stage, *MECP2*-ASO therapy provides a potentially titratable, disease-modifying approach to reducing MeCP2 protein levels. Collectively, these efforts provide hope for the development of efficacious therapies for *MECP2*-related neurodevelopmental disorders RTT and MDS in the coming years.

Abbreviations

AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; ASD, autism spectrum disorder; ASO, antisense oligonucleotide; BDNF, brain-derived neurotrophic factor; BRM, BRAHMA; CGI, CpG island; CNS, central nervous system; CRH, corticotropin-releasing hormone; CRISPR, clustered regularly interspaced short palindromic repeats; CTD, C-terminal domain; DNMT, DNA methyltransferase; EPSC, excitatory postsynaptic current; EPSP, excitatory postsynaptic potential; ESC, embryonic stem cell; FLNA, Filamin A; FoSTeS, fork stalling and template switching; GABA, γ -aminobutyric acid; GDI1, GDP dissociation inhibitor 1; GFP, green fluorescent protein; GPE, glycine-proline-glutamate; HP1, heterochromatin protein 1; ID, interdomain; I/O, input-output; IGF1, insulin-like growth factor 1; iPSC, induced pluripotent stem cell; IPSC, inhibitory postsynaptic current; KO, knockout; L1CAM, L1 cell adhesion molecule; LCR, low copy repeat; LOF, loss-of-function; LTD, long-term depression; LTP, long-term potentiation; LUHMES, Lund human mesencephalic; MBD, methyl-binding domain; MDS, *MECP2* duplication syndrome; MeCP2, methyl-CpG-binding protein 2; MMBIR, microhomology-mediated break-induced replication; NCoR, nuclear receptor co-repressor; NHEJ, non-homologous end joining; NID, NCoR/SMRT interaction domain; NMDA, N-methyl-D-aspartate; OPRM1, opioid receptor mu (μ) 1; PHF14, PHD finger protein 14; PNS, peripheral nervous system; PPF, paired-pulse facilitation; RAB39B, Ras-related protein Rab-39B; RNA, ribonucleic acid; RTT, Rett syndrome; SMRT, silencing mediator of retinoid and thyroid hormone receptors; SRPK3, SRSF protein kinase 3; TET, ten-eleven translocation; TRD, transcription repression domain; TSS, transcription start site; WT, wild type; YBX1, Y-box binding protein 1; YY1, Yin Yang 1; XCI, X-chromosome inactivation.

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