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Leveraging the genetic basis of Rett syndrome to ascertain pathophysiology

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Abstract

Mutations in the methyl-CpG binding protein 2 (*MECP2*) gene cause Rett syndrome (RTT), a progressive X-linked neurological disorder characterized by loss of developmental milestones, intellectual disability and breathing abnormality. Despite being a monogenic disorder, the pathogenic mechanisms by which mutations in MeCP2 impair neuronal function and underlie the RTT symptoms have been challenging to elucidate. The seemingly simple genetic root and the availability of genetic data from RTT patients have led to the generation and characterization of a series of mouse models recapitulating RTT-associated genetic mutations. This review focuses on the studies of RTT mouse models and describe newly obtained pathogenic insights from these studies. We also highlight the potential of studying pathophysiology using genetics-based modeling approaches in rodents and suggest a future direction to tackle the pathophysiology of intellectual disability with known or complex genetic causes.

Keywords

Rett syndrome; MeCP2; Genetics; Mouse models; Pathophysiology

1. Introduction: Rett syndrome

Rett syndrome (RTT) was first described by an Austrian pediatrician Dr. Andreas Rett when he happened to observe girls sitting in his waiting room with relentless hand wringing symptoms (Rett, 1966). However, this syndrome was not well recognized until 1983, when Dr. Bengt Hagberg and colleagues reported 33 cases, all of which exhibited similar clinical symptoms, such as hand winging (Hagberg, Aicardi, Dias, & Ramos, 1983). The specific clinical features that were described, combined with the identification of additional patients worldwide, established RTT as a unique clinical syndrome. However, the cause of the disorder was not known until 1999, when Dr. Huda Zoghbi and colleagues discovered the

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Compliance with ethics guidelines

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genetic linkage of RTT to the methyl-CpG binding protein 2 (*MECP2*) gene on the X-chromosome (Amir et al., 1999). Since then, both basic and clinical studies of MeCP2 and RTT have exploded with an increasing number of publications coming online on a daily basis, owing largely to the relentless advocacy of research from patient organizations and the financial sponsorship from NIH.

RTT currently represents one of the leading causes of intellectual disability among young girls, occurring in 1 out of ~10,000 births (Cuddapah et al., 2014; Neul et al., 2010). Individuals with RTT typically appear asymptomatic for the first 6–18 months of life. After this early stage of relatively normal development, patients begin to exhibit developmental delays, including a failure to meet expected developmental milestones, deceleration in head growth, and reduced muscle tone (Chahrour & Zoghbi, 2007; Hagberg et al., 1983). This is followed by a phase of developmental regression in which patients lose acquired language and motor skills, such as the ability to speak words and purposeful use of hands. Additional characteristic clinical features arise during this regression period, including the “hand-wringing” stereotypies, impaired motor coordination, and intellectual disability. RTT patients also display severe autonomic dysfunction that is characterized by irregular respiration, such as breath holding, hyperventilation, and apneas (Chahrour & Zoghbi, 2007; Ramirez, Ward, & Neul, 2013). Notably, many RTT patients show intense eye-gaze, indicative of their social awareness, but social and language communication are severely impaired (Kaufmann et al., 2012). A large proportion of RTT patients also exhibits seizures throughout life time and gradual loss of mobility which mimicks Parkinsonian-like features to a large extent (Chahrour & Zoghbi, 2007).

2. Introduction: MeCP2

More than 95% of RTT cases are genetically linked to mutations in the *MECP2* gene, including missense, nonsense, deletions, insertions and duplications across the *MECP2* locus. Notably, 58% of RTT cases have 1 of 8 frequent mutations at the CpG hotspots where deamination of methylated cytosines frequently occurs. Among these, MeCP2 R106W, R133C, and T158M/A are missense mutations located in the methyl-CpG binding domain (MBD) of MeCP2 and have been shown to impair MeCP2 binding to methylated DNA *in vitro* and *in vivo* (Ballestar, Yusufzai, & Wolffe, 2000; Brown et al., 2016; Ghosh, Horowitz-Scherer, Nikitina, Gierasch, & Woodcock, 2008; Goffin et al., 2011; Ho et al., 2008; Johnson et al., 2017; Lamonica et al., 2017). R306C is another missense mutation that resides within the transcriptional repression domain (TRD) of MeCP2 and has been shown to disrupt the interaction between MeCP2 and the Nuclear Receptor Corepressor Complex (NCoR/SMRT) (Brown et al., 2016; Lyst et al., 2013). The remaining four most frequent mutations are nonsense mutations, with R168X in the 3'-end of MBD, R255X and R270X overlapping with the nuclear localization sequence (NLS), and R294X disrupting the TRD (Baker et al., 2013).

MeCP2 is a founding member of the MBD family of proteins that show binding affinity for methylated DNA and protein-protein interactions with various histone deacetylase (HDAC)-containing co-repressor complexes, such as NCoR-SMRT and Sin3A-HDAC1/2 complexes (Lewis et al., 1992; Lyst et al., 2013; Nan et al., 1998; Nan, Campoy, & Bird, 1997; Nan,

Tate, Li, & Bird, 1996). Therefore, MeCP2 has been traditionally thought as a transcriptional repressor. However, transcriptional de-repression in *Mecp2*-null cells has been difficult to convey in numerous studies (Tudor, Akbarian, Chen, & Jaenisch, 2002). In addition to the two principal domains of MBD and TRD that are critical for MeCP2 function as highlighted by the clustering of RTT-associated point mutations, MeCP2 also carries a long C-terminal Domain (CTD) that may play a role in modulating chromatin structure (Baker et al., 2013). Notably, a recent study reported that missense mutations in the CTD of MeCP2 strongly or partially destabilize MeCP2 protein and in some cases interfere with co-repressor recruitment, supporting a proposed critical role of MeCP2 to bring co-repressor complexes to chromatin (Guy et al., 2018).

Aside from the well conserved and highly structured MBD domain, more than 60% of MeCP2 protein is, interestingly, intrinsically disordered and does not adopt strong secondary protein structure, although the protein becomes more structured upon binding to methylated DNA (Adams, McBryant, Wade, Woodcock, & Hansen, 2007). This unusual property is consistent with a recent finding that MeCP2 modulates gene transcription in a context-specific manner (Johnson et al., 2017). Thus, MeCP2 may exhibit different functionality depending on the local chromatin context in the genome.

MeCP2 protein is widely expressed in multiple tissues and organs, but is expressed the highest in post-mitotic neurons of the mammalian brain (Shahbazian, Antalffy, Armstrong, & Zoghbi, 2002). Neuronal MeCP2 is relatively low at birth, but increases with age as neurons mature (Alvarez-Saavedra, Saez, Kang, Zoghbi, & Young, 2007; Chen et al., 2015; Shahbazian et al., 2002; Skene et al., 2010). Biochemical quantification of MeCP2 abundance in the adult mouse brain showed that each post-mitotic neuronal nucleus contains about 16 million molecules of MeCP2, in contrast to 2 million molecules of MeCP2 in glial nuclei and 500,000 molecules of MeCP2 in liver hepatocyte nuclei (Skene et al., 2010). This suggests that post-mitotic neurons contain 1 molecule of MeCP2 for every 2 nucleosomes, enough to potentially saturate all methyl-CpG sites throughout genome. This observation is consistent with MeCP2 chromatin immunoprecipitation followed by sequencing (ChIP-seq) findings that it occupies chromatin broadly throughout the genome (Skene et al., 2010). Additional studies reported that the high expression of MeCP2 protein in the brain is indeed tightly regulated, as too much (Collins et al., 2004; del Gaudio et al., 2006; Friez et al., 2006; Liu et al., 2016; Meins et al., 2005) or too little (Samaco et al., 2008) MeCP2 protein results in similar neurological phenotypes in both human patients and transgenic mouse models.

3. Genetic basis of Rett syndrome

From the genetic point of view, the majority of *MECP2* mutations occurs *de novo* and arises spontaneously in the paternal germ line. Thus, most mutations will be inherited by female offspring, resulting in heterozygote, as *MECP2* is an X-linked gene. Random X-chromosome inactivation (XCI), on the other hand, leads to mosaic expression of MeCP2 mutant and wild-type in females. Therefore, depending on the percentage of somatic cells expressing the wild-type or mutant *MECP2* allele, clinical features will vary from individual to individual. This is indeed the case. RTT has been known as a spectrum disorder. In a few

cases, XCI can be non-random, which skews expression towards the wild-type or mutant allele and leads to much milder (akin to a mild learning disability) or more severe clinical symptoms, respectively (Weaving et al., 2003). Familial cases of RTT, as well as RTT cases among males, are very rare. In cases of male patients with inherited *MECP2* mutations, they typically present with neonatal encephalopathy and rarely survive beyond two years of age (Meloni et al., 2000; Neul et al., 2010; Zeev et al., 2002).

Therefore, genotype-phenotype correlations between mutation type, mutation severity and specific RTT clinical outcomes in patients remain poor, largely due to random XCI. Furthermore, current XCI analysis is limited to peripheral tissues, which may not reflect *bona fide* XCI in the brain (Archer et al., 2007; Cuddapah et al., 2014; Temudo et al., 2011; Wu et al., 2014). Hemizygous male mice lacking MeCP2, on the other hand, can live up to 2–3 months, and thus have provided an experimental system to dissect the molecular functions of MeCP2. Notably, by tagging MeCP2 with a Cre-dependent biotinylation approach, a recent study has overcome the cellular mosaicism associated with MeCP2 expression in genetically-relevant heterozygous females, opening up an opportunity to dissect the pathogenesis of RTT in female mouse models (Johnson et al., 2017). Despite of the mosaic expression patterns of MeCP2 in heterozygous females, the clear genetic linkage and the availability of specific mutation information have led to the development of a series of mouse models, of which a great deal of information on the function of MeCP2 and the pathogenic mechanisms of RTT have now been obtained (Table 1).

4. Knockout studies of MeCP2 and Rett syndrome

Knockout mouse models of RTT were first generated by either deleting *Mecp2* exon 3, resulting in a truncated protein missing largely the MBD domain (Chen, Akbarian, Tudor, & Jaenisch, 2001), or exon 3 and a part of exon 4, leading to the ablation of MeCP2 protein production (Guy, Hendrich, Holmes, Martin, & Bird, 2001) (Table 1). Notably, in both studies, mice have lost the MBD domain completely, resulting in a failure of MeCP2 binding to methylated DNA *in vivo*. *Mecp2*-null male mice display behavioral phenotypes that began around 4–6 weeks of age, including hypoactivity, abnormal gait, hindlimb claspings, irregular breathing, and premature lethality around 2–3 months of age (Chen et al., 2001; Guy et al., 2001). In addition to the relatively normal early development prior to symptom onset, *Mecp2*-null mice also displayed reduced body size and brain size. The reduced brain size is associated with increased neuronal cell packing without gross changes in brain anatomy and morphology, suggesting that RTT is not a product of neurodegeneration (Chen et al., 2001; Guy et al., 2001). The absence of neurodegeneration was also observed in post mortem RTT brain tissues (Armstrong, 2005). Importantly, female mice with heterozygous loss of MeCP2 display a delayed onset of RTT-like symptoms starting at 3–4 months of age. The extent of behavioral phenotypes in these females resemble those shown in hemizygous males, but on the mild degree overall. Female heterozygotes survive into adulthood and are fertile (Chen et al., 2001; Guy et al., 2001).

The manifestation of RTT-like behavioral phenotypes in *Mecp2*-null mice supports a causal role of MeCP2 mutations in the pathogenesis of RTT. Given that MeCP2 is a widely expressed protein across many different tissues and organs, a key question has been to

identify the pathogenic origin of RTT through genetically mediated conditional knockout studies. Of note, conditional removal of MeCP2 from the brain using a Nestin-Cre driver, which depletes MeCP2 expression from most post-mitotic neurons and glia in the CNS, recapitulates the phenotypes observed in constitutive *Mecp2*-null mice (Chen et al., 2001; Guy et al., 2001), supporting that RTT phenotypes are the result of MeCP2 dysfunction in the CNS. To further dissect RTT phenotypes in detail and determine the contribution of particular neuronal cell types or brain regions, additional conditional knockout studies were carried out using cell type or brain region-specific Cre mouse lines. These studies revealed that specific behavioral phenotypes are tightly coupled to the loss of MeCP2 in certain cell types in the brain. For example, ablation of MeCP2 expression from Sim1-expressing neurons primarily in the hypothalamus led to alteration of stress response and feeding behaviors in Sim1-Cre conditional knockout mice (Fyffe et al., 2008). Deletion of *Mecp2* primarily from the striatum recapitulated hypo-locomotion and motor coordination deficits (Su, Kao, Huang, & Liao, 2015). Notably, loss of MeCP2 function from all GABAergic neurons in the CNS mimicked most RTT-like phenotypes, including repetitive behaviors, learning and memory deficits, and abnormalities in social interactions and breathing (Chao et al., 2010). In contrast, loss of MeCP2 function from forebrain GABAergic neurons results in milder RTT-like phenotypes, likely because neuronal circuits in the hindbrain are not affected in these conditional knockout mice (Chao et al., 2010; Goffin, Brodtkin, Blendy, Siegel, & Zhou, 2014; Su et al., 2015). Accordingly, knocking out MeCP2 expression selectively in the hindbrain using HoxB1-cre significantly impairs autonomic functions and results in early lethality (Ward et al., 2011). In comparison, loss of MeCP2 function from forebrain excitatory neurons results in deficits in cognitive behaviors, characteristic of those neurons mediating higher order cognitive function (Gemelli et al., 2006; Goffin et al., 2014). Of note, selective ablation of MeCP2 expression from individual interneurons, such as PV-expressing and SST-expressing interneurons, have also been conducted and revealed a critical role for MeCP2 in the context of neural circuit integrity and seizure-related occurrence (Goffin et al., 2014; Ito-Ishida, Ure, Chen, Swann, & Zoghbi, 2015). Together, these studies not only confirmed the genetic causality of MeCP2 mutations to the etiology of RTT, but also revealed an imperative role for MeCP2 in different brain regions and different cell types. However, how MeCP2 dysfunction in individual cell types contributes to RTT-like phenotypes in heterozygous female models remains to be determined.

5. Reactivation studies of MeCP2 and Rett syndrome

While germ line deletion of *Mecp2* leads to the development of RTT-like behavioral phenotypes, the timing of MeCP2 necessity in RTT pathogenesis was not known until a few years ago when several conditional knockout studies were carried out in a temporal manner. These studies found that postnatal deletion of the *Mecp2* gene in late juvenile or adult mice result in similar exhibition of RTT-like symptoms and early lethality seen with germ line deletion of *Mecp2* gene (Cheval et al., 2012; McGraw, Samaco, & Zoghbi, 2011; Nguyen et al., 2012). At the cellular level, neuronal cell packing and dendritic morphological defects were observed as well, similar to those in *Mecp2*-null mice (Nguyen et al., 2012). Selective proteins related to synapse function, such as AMPA receptors, NMDA receptors, and the synaptic vesicle proteins Vglut and Synapsin, were found to be altered by MeCP2 loss in

those mice (Nguyen et al., 2012). Interestingly, the decrease in synaptic protein expression were not reflected at the mRNA levels of those genes, suggesting that an indirect mechanism, such as reduced transcription of ribosomal RNAs, deficits in protein translation, or increased synapse turn over, may mediate the alteration of synaptic protein levels (Li et al., 2013; Yazdani et al., 2012). Together, these studies support that MeCP2 is required to maintain neuronal function throughout life.

Conversely, ground-breaking studies have investigated the extent to which RTT-like phenotypes are reversible upon restoration of MeCP2 expression in *Mecp2*-null mice at adult ages after the expression of RTT-like behavioral phenotypes. By removing an engineered flnull Stop cassette from the *Mecp2* gene locus, thus reactivating MeCP2 expression from the endogenous *Mecp2* gene, or from a floxed Stop-*Mecp2* trans-gene in combination with various Cre lines, two studies reported that post-natal restoration of MeCP2 expression in previously *Mecp2*-null male and female mice is sufficient to restore brain size and body weight (Giacometti, Luikenhuis, Beard, & Jaenisch, 2007; Guy, Gan, Selfridge, Cobb, & Bird, 2007). The post-natal restoration of MeCP2 expression also rescues electrophysiological deficits in long-term potentiation, a form of synaptic plasticity (Guy et al., 2007). Lifespan is noticeably extended when *Mecp2* expression is restored in all post-mitotic neurons in the CNS. However, lifespan for CaMKII-Cre mice, where MeCP2 restoration is restricted to forebrain neurons, was only extended by ~4 weeks, supporting that proper MeCP2 expression in hindbrain post-mitotic neurons, such as HoxB1 + neurons in the brainstem, is critical for lifespan (Giacometti et al., 2007; Ward et al., 2011).

Notably, despite that *Mecp2* expression is low in glia and is not necessary for initiating the development of RTT-like phenotypes, conditional restoration of MeCP2 expression in glial cells is sufficient to significantly improve locomotion, reduce anxiety levels, restore respiratory abnormalities, and prolong the lifespan of *Mecp2*-null animals (Lioy et al., 2011). This study suggests that non-cell autonomous effects may also play a role in RTT pathogenesis, and that the secreted trophic support provided by glia in the CNS may be a viable target for RTT therapeutics. Furthermore, an AAV-mediated or genetically controlled expression of a *Mecp2* mini gene, including just the MBD and co-repressor binding domains of MeCP2, is found to be sufficient to prolong the lifespan of *Mecp2*-null mice, highlighting a critical role for MeCP2 to recruit NCoR/SMRT corepressor complex to chromatin (Tillotson et al., 2017). Together, these studies lend a vital support that RTT is a reversible disorder and translational research should emphasize therapeutic avenues towards restoring MeCP2 function.

6. Knockin studies of MeCP2 and Rett syndrome

Given the difficulty in establishing genotype-phenotype correlations among clinically variable human RTT female patients (Cuddapah et al., 2014), the development of mouse models bearing RTT-associated missense and non-sense mutations has expanded our understanding of MeCP2 function and the impact of individual pathogenic mutations (Goffin & Zhou, 2012). In recent years, numerous knockin mouse models have been developed and these mouse models faithfully recapitulate key features of RTT, including the degree of

phenotypic severity observed in human RTT patients, particularly in hemizygous males (Table 1).

According RTT genetic database, 3 out of the top 8 most frequent RTT mutations are missense mutations in the MBD domain of MeCP2. *In vitro* biochemistry studies have known that these mutations impair the binding of MeCP2 to methylated DNA in a variable degree. For example, MeCP2 R133C binding to methylated CpGs is indistinguishable from wild-type MeCP2, whereas MeCP2 T158M retains partial affinity, and MeCP2 R106W completely abolishes interactions between the MBD and methylated CpGs (Ballestar et al., 2000; Brown et al., 2016; Ghosh et al., 2008; Ho et al., 2008). To date, MeCP2 R111G, R133C, T158A, T158M and R106W knockin mutations have been modeled in mice (Brown et al., 2016; Goffin & Zhou, 2012; Lamonica et al., 2017). Mice bearing MeCP2 R133C mutation are noticeably less severe compared to those carrying T158M/A mutations, as assessed by the timing of symptom onset (R133C: 42 weeks, T158M: 13 weeks, T158A: 16 weeks). The differences in lifespan correlate with the expected impairments in binding to methylated DNA, suggesting that the severity of mutations in the MBD is dictated by the degree of impairment to methylated DNA binding. Importantly, the degree of DNA binding impairment was recently found to be coupled with proteasome-mediated protein instability, and elevation of MeCP2 protein expression even with T158M point mutation results in amelioration of RTT-like phenotypes in both male and female mouse models of RTT (Lamonica et al., 2017). This is further supported by the generation of MeCP2 R111G and R106W mutant mice, which appear phenotypically similar to *Mecp2*-null mice in terms of symptoms and lifespan of about 10 weeks, consistent with the complete abolishment of binding of MeCP2 to methylated DNA and significant loss of MeCP2 protein expression (Free et al., 2001; Heckman, Chahrour, & Zoghbi, 2014; Johnson et al., 2017; Johnson et al., 2017; Kudo et al., 2003).

Mice bearing RTT-associated point mutations in the TRD of MeCP2 show phenotypic variability consistent with the extent of TRD function being retained. Mice with R168X and R255X nonsense mutations are predicted to truncate MeCP2 prior to its NLS, and thus should not be present in the nucleus. Indeed, either full length or partial MeCP2 protein products were not detected in these mice, likely due to nonsense mediated mRNA decay (NMD) (Lawson-Yuen et al., 2007; Pitcher et al., 2015; Wegener et al., 2014). As a result, both R168X and R255X male mice appear phenotypically similar to *Mecp2*-null male mice and display premature lethality, respiratory abnormalities, and impairments in motor function, effectively making these mutations null alleles that are consistent with the clinical severity observed in human RTT patients bearing these two nonsense mutations.

In contrast, among three independent lines of mice bearing R306C mutations (Brown et al., 2016; Heckman et al., 2014; Lyst et al., 2013), retention of the full MBD results in relatively mild disease severity and longer lifespan when compared to *Mecp2*-null mice, consistent with the mild clinical severity of R306C-bearing human patients. However, R306C mice continue to display deficits in locomotion, motor coordination, and learning and memory, similar to other *Mecp2*-mutant mice (Brown et al., 2016; Heckman et al., 2014; Lyst et al., 2013). Interestingly, two of the three R306C lines display a median lifespan of ~18 weeks (Heckman et al., 2014; Lyst et al., 2013), whereas the third displays a median lifespan of

~30 weeks (Brown et al., 2016), highlighting the variability associated with differences in genetic background, gene targeting strategies, or C-terminal tags. Using these mice, the partial loss-of-function phenotypes associated with the R306C mutation was linked to a disruption in protein interaction with the NCoRSMRT co-repressor complex, resulting in a failure to recruit NCoRSMRT complex to chromatin and mediate transcriptional repression (Lyst et al., 2013). Thus, these mutant mice provided critical molecular insights into the molecular function of MeCP2, highlighting a key role in co-repressor recruitment. The relatively mild phenotypic severity among R306C mice and human patients suggests that loss of transcriptional repression by NCoR-SMRT may be necessary, but not sufficient, to mediate the full severity of RTT phenotypes.

In this respect, nonsense mutations in the R270X and G273X mice are particularly interesting. Boys with the MeCP2 R270X mutation exhibit severe symptoms typical of *Mecp2* loss of function in human male patients, including neonatal encephalopathy and death before 2 years of age (Kankirawatana et al., 2006; Venancio, Santos, Pereira, Maciel, & Saraiva, 2007). However, boys with the MeCP2 G273X mutation live significantly longer with a relatively mild display of neuropsychiatric symptoms (Ravn, Nielsen, Uldall, Hansen, & Schwartz, 2003). The strong phenotypic differences associated with the difference in three amino acids is recapitulated in MeCP2 R270X and G273X mutant mice, which exhibit median lifespans of ~12 and 27 weeks, respectively (Baker et al., 2013). Further molecular investigations using these mice found that although both mutations abolished transcriptional repressor activity, the R270X mutation disrupted an AT-hook domain that severely impairs the recruitment of nucleosome remodeler ATRX. In contrast, this AT-hook domain was maintained with the G273X mutation, which exhibited more moderate impairments for the same features (Baker et al., 2013). Thus, molecular differences in the extent of MeCP2 function to modulate chromatin structure and mediate transcriptional repression likely underlie the differences in severity between these two mutations in both mice and humans.

Remarkably, a recent study carried out a comprehensive site-by-site comparisons of knockin mice bearing MeCP2 T158M and R106W missense mutations specifically at the transcriptome level (Johnson et al., 2017). They found that both mutations impair gene expression and exhibit similar molecular features among upregulated genes, including those in male and female *Mecp2* mutant neurons that encode inhibitory neuron-enriched transcription factors and chromatin remodelers. Many of these genes are functionally associated with cellular respiration and energy metabolism, which could provide a transcriptional basis for clinical features related to mitochondrial and metabolic disorders. Because T158M and R106W mutations both impair MeCP2 binding to chromatin, the misexpression of transcriptional regulators may contribute to shared etiology in T158M and R106W mice. Upon comparison, the R106W mutation affects a larger number of genes that tend to be more highly expressed and preferentially downregulated relative to the T158M mutation, regardless of cell type. This correlates with the greater impairment of MeCP2 R106W binding to chromatin in previous studies. Of note, downregulated genes are associated with synaptic morphology and function. Because R106W mice also display reduced lifespan compared to T158M mice, it is possible that reductions in long gene transcription may act as modifiers in specific neuronal cell types, worsening subsets of RTT-like phenotypes (Johnson et al., 2017). Consistently, RTT patients with mutations that

preserve MeCP2 binding do exhibit milder clinical features than patients for whom binding is disrupted (Cuddapah et al., 2014). Additional transcriptional assessments of milder mutations that better preserve MeCP2 binding to chromatin, similar to what is described in this study, are thus necessary to further standardize and refine genotype-phenotype correlations at the transcriptional level.

Interestingly, using genetically mosaic RTT female mice bearing T158M or R106W mutations, Johnson and colleagues further found functional differences demarcating upregulated and downregulated genes are conserved in both male and female *Mecp2* mutant neurons, suggesting that these distinct transcriptional changes may be a cell autonomous consequence of MeCP2 dysfunction (Johnson et al., 2017). However, RTT is primarily a heterozygous female disorder. Findings from hemizygous male mouse models may have limited translatability in pre-clinical studies. Thus, additional experiments are needed to further assess cell and non-cell autonomous contributions to RTT at the transcriptional level using females that encompass a wide range of X-inactivation ratios across multiple ages, cell types, and *Mecp2* mutations. Notably, this study found wild-type neurons expressing a normal copy of MeCP2 are still susceptible to indirect gene expression changes. These non-cell autonomous changes include activity-dependent genes that are stereotypically upregulated by neuronal depolarization, modulate synaptic plasticity at the transcriptional level, and effectively reduce neuronal responsiveness to subsequent stimuli (Johnson et al., 2017). These changes are thus consistent with the altered synaptic morphology exhibited by both wild-type and mutant-expressing neurons of heterozygous *Mecp2*-null female mice, including reduced dendritic branching and synaptic spine density (Belichenko et al., 2009). The selective upregulation of these genes in wild-type and mutant-expressing neurons of R106W mice may be a response to abnormal electrophysiological activity among severely affected mosaic neurons, in contrast to T158M mice that exhibit few non-cell autonomous changes (Johnson et al., 2017). Accordingly, electrophysiological abnormalities observed in phenotypically severe *Mecp2*-null mice might not fully represent the milder spectrum of RTT-associated mutations. A continued examination of knockin mouse models bearing different RTT mutations, such as mutations leading to mild RTT symptoms and mutations resulting in severe symptoms, particularly in heterozygous females, is imperative to reveal pathogenic insights and establish appropriate mouse models for preclinical trials.

7. Conclusions

In summary, genetic studies using various mouse models have not only established the causality of mutations in *MECP2* to the etiology of RTT, but also revealed molecular and cellular insights into the biological functions of MeCP2 and the pathogenesis of RTT. Intriguingly, these modeling studies found MeCP2 is not only required for the active and dynamic maintenance of post-mitotic neuronal function, the absence of MeCP2 at early developmental stages is not a pre-requisite for manifesting RTT-like phenotypes, but also re-activation or re-expression of MeCP2 after symptomatic onset is sufficient to reverse RTT-like phenotypes, supporting that RTT is rather a neural maintenance disorder and a curable condition. Similar genetic modeling studies shall be conducted to other ID-related disorders and determining the extent of phenotypic reversibility will significantly influence the development of therapeutic strategies.

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References

- Adams VH, McBryant SJ, Wade PA, Woodcock CL, & Hansen JC (2007). Intrinsic disorder and autonomous domain function in the multifunctional nuclear protein, MeCP2. *Journal of Biological Chemistry*, 282, 15057–15064. [PubMed: 17371874]
- Alvarez-Saavedra M, Saez MA, Kang D, Zoghbi HY, & Young JI (2007). Cell-specific expression of wild-type MeCP2 in mouse models of Rett syndrome yields insight about pathogenesis. *Human Molecular Genetics*, 16, 2315–2325. [PubMed: 17635839]
- Amir RE, Van den Veyver IB, Wan M, Tran CQ, Francke U, & Zoghbi HY (1999). Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nature Genetics*, 23, 185–188. [PubMed: 10508514]
- Archer H, Evans J, Leonard H, Colvin L, Ravine D, Christodoulou J, ... de Klerk N. (2007). Correlation between clinical severity in patients with Rett syndrome with a p. R168X or p.T158M MECP2 mutation, and the direction and degree of skewing of X-chromosome inactivation. *Journal of Medical Genetics*, 44, 148–152. [PubMed: 16905679]
- Armstrong DD (2005). Neuropathology of Rett syndrome. *Journal of Child Neurology*, 20, 747–753. [PubMed: 16225830]
- Baker SA, Chen L, Wilkins AD, Yu P, Lichtarge O, & Zoghbi HY (2013). An AT-hook domain in MeCP2 determines the clinical course of Rett syndrome and related disorders. *Cell*, 152, 984–996. [PubMed: 23452848]
- Ballestar E, Yusufzai TM, & Wolffe AP (2000). Effects of Rett syndrome mutations of the methyl-CpG binding domain of the transcriptional repressor MeCP2 on selectivity for association with methylated DNA. *Biochemistry*, 39, 7100–7106. [PubMed: 10852707]
- Belichenko PV, Wright EE, Belichenko NP, Masliah E, Li HH, Mobley WC, & Francke U (2009). Widespread changes in dendritic and axonal morphology in Mecp2-mutant mouse models of Rett syndrome: Evidence for disruption of neuronal networks. *Journal of Comparative Neurology*, 514, 240–258. [PubMed: 19296534]
- Brown K, Selfridge J, Lagger S, Connelly J, De Sousa D, Kerr A, ... Bird A (2016). The molecular basis of variable phenotypic severity among common missense mutations causing Rett syndrome. *Human Molecular Genetics*, 25, 558–570. [PubMed: 26647311]
- Chahrour M, & Zoghbi HY (2007). The story of Rett syndrome: From clinic to neurobiology. *Neuron*, 56, 422–437. [PubMed: 17988628]
- Chao H-T, Chen H, Samaco RC, Xue M, Chahrour M, Yoo J, ... Ekker M (2010). Dysfunction in GABA signalling mediates autism-like stereotypies and Rett syndrome phenotypes. *Nature*, 468, 263–269. [PubMed: 21068835]
- Chen RZ, Akbarian S, Tudor M, & Jaenisch R (2001). Deficiency of methyl-CpG binding protein-2 in CNS neurons results in a Rett-like phenotype in mice. *Nature Genetics*, 27, 327–331. [PubMed: 11242118]
- Chen L, Chen K, Lavery LA, Baker SA, Shaw CA, Li W, & Zoghbi HY (2015). MeCP2 binds to non-CG methylated DNA as neurons mature, influencing transcription and the timing of onset for Rett syndrome. *Proceedings of the National Academy of Sciences of the United States of America*, 112, 5509–5514. [PubMed: 25870282]
- Cheval H, Guy J, Merusi C, De Sousa D, Selfridge J, & Bird A (2012). Postnatal inactivation reveals enhanced requirement for MeCP2 at distinct age windows. *Human Molecular Genetics*, 21, 3806–3814. [PubMed: 22653753]
- Cohen S, Gabel HW, Hemberg M, Hutchinson AN, Sadacca LA, Ebert DH, ... Wetsel WC (2011). Genome-wide activity-dependent MeCP2 phosphorylation regulates nervous system development and function. *Neuron*, 72, 72–85. [PubMed: 21982370]

- Collins AL, Levenson JM, Vilaythong AP, Richman R, Armstrong DL, Noebels JL, ... Zoghbi HY (2004). Mild overexpression of MeCP2 causes a progressive neurological disorder in mice. *Human Molecular Genetics*, 13, 2679–2689. [PubMed: 15351775]
- Cuddapah VA, Pillai RB, Shekar KV, Lane JB, Motil KJ, Skinner SA, ... Percy AK (2014). Methyl-CpG-binding protein 2 (MECP2) mutation type is associated with disease severity in Rett syndrome. *Journal of Medical Genetics*, 51, 152–158. [PubMed: 24399845]
- del Gaudio D, Fang P, Scaglia F, Ward PA, Craigen WJ, Glaze DG, ... Berry SA (2006). Increased MECP2 gene copy number as the result of genomic duplication in neurodevelopmentally delayed males. *Genetics in Medicine*, 8, 784–792. [PubMed: 17172942]
- Free A, Wakefield RI, Smith BO, Dryden DT, Barlow PN, & Bird AP (2001). DNA recognition by the methyl-CpG binding domain of MeCP2. *Journal of Biological Chemistry*, 276, 3353–3360. [PubMed: 11035019]
- Friez MJ, Jones JR, Clarkson K, Lubs H, Abuelo D, Bier J-AB, ... Schwartz CE (2006). Recurrent infections, hypotonia, and mental retardation caused by duplication of MECP2 and adjacent region in Xq28. *Pediatrics*, 118, e1687–e1695. [PubMed: 17088400]
- Fyffe SL, Neul JL, Samaco RC, Chao H-T, Ben-Shachar S, Moretti P, ... Zoghbi HY (2008). Deletion of *Mecp2* in *Sim1*-expressing neurons reveals a critical role for MeCP2 in feeding behavior, aggression, and the response to stress. *Neuron*, 59, 947–958. [PubMed: 18817733]
- Gemelli T, Berton O, Nelson ED, Perrotti LI, Jaenisch R, & Monteggia LM (2006). Postnatal loss of methyl-CpG binding protein 2 in the forebrain is sufficient to mediate behavioral aspects of Rett syndrome in mice. *Biological Psychiatry*, 59, 468–476. [PubMed: 16199017]
- Ghosh RP, Horowitz-Scherer RA, Nikitina T, Gierasch LM, & Woodcock CL (2008). Rett syndrome-causing mutations in human MeCP2 result in diverse structural changes that impact folding and DNA interactions. *Journal of Biological Chemistry*, 283, 20523–20534. [PubMed: 18499664]
- Giacometti E, Luikenhuis S, Beard C, & Jaenisch R (2007). Partial rescue of MeCP2 deficiency by postnatal activation of MeCP2. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 1931–1936. [PubMed: 17267601]
- Goffin D, Allen M, Zhang L, Amorim M, Wang ITJ, Reyes A-RS, ... Blendy JA (2011). Rett syndrome mutation MeCP2 T158A disrupts DNA binding, protein stability and ERP responses. *Nature Neuroscience*, 15, 274–283. [PubMed: 22119903]
- Goffin D, Brodtkin ES, Blendy JA, Siegel SJ, & Zhou Z (2014). Cellular origins of auditory event-related potential deficits in Rett syndrome. *Nature Neuroscience*, 17, 804–806. [PubMed: 24777420]
- Goffin D, & Zhou ZJ (2012). The neural circuit basis of Rett syndrome. *Frontiers in Biology*, 7, 428–435.
- Guy J, Alexander-Howden B, FitzPatrick L, DeSousa D, Koerner MV, Selfridge J, & Bird A (2018). A mutation-led search for novel functional domains in MeCP2. *Human Molecular Genetics*, 27, 2531–2545. [PubMed: 29718204]
- Guy J, Gan J, Selfridge J, Cobb S, & Bird A (2007). Reversal of neurological defects in a mouse model of Rett syndrome. *Science (New York, NY)*, 315, 1143–1147.
- Guy J, Hendrich B, Holmes M, Martin JE, & Bird A (2001). A mouse *Mecp2*-null mutation causes neurological symptoms that mimic Rett syndrome. *Nature Genetics*, 27, 322–326. [PubMed: 11242117]
- Hagberg B, Aicardi J, Dias K, & Ramos O (1983). A progressive syndrome of autism, dementia, ataxia, and loss of purposeful hand use in girls: Rett's syndrome: Report of 35 cases. *Annals of Neurology*, 14, 471–479. [PubMed: 6638958]
- Heckman LD, Chahrour MH, & Zoghbi HY (2014). Rett-causing mutations reveal two domains critical for MeCP2 function and for toxicity in MECP2 duplication syndrome mice. *eLife*, 3.
- Ho KL, McNae IW, Schmiedeberg L, Klose RJ, Bird AP, & Walkinshaw MD (2008). MeCP2 binding to DNA depends upon hydration at methyl-CpG. *Molecular Cell*, 29, 525–531. [PubMed: 18313390]
- Ito-Ishida A, Ure K, Chen H, Swann JW, & Zoghbi HY (2015). Loss of MeCP2 in parvalbumin- and somatostatin-expressing neurons in mice leads to distinct Rett syndrome-like phenotypes. *Neuron*, 88, 651–658. [PubMed: 26590342]

- Jentarra GM, Olfers SL, Rice SG, Srivastava N, Homanics GE, Blue M, ... Narayanan V (2010). Abnormalities of cell packing density and dendritic complexity in the MeCP2 A140V mouse model of Rett syndrome/X-linked mental retardation. *BMC Neuroscience*, 11, 19. [PubMed: 20163734]
- Johnson BS, Zhao Y-T, Fasolino M, Lamonica JM, Kim YJ, Georgakilas G, ... Vahedi G (2017). Biotin tagging of MeCP2 in mice reveals contextual insights into the Rett syndrome transcriptome. *Nature Medicine*, 23, 1203–1214.
- Kankirawatana P, Leonard H, Ellaway C, Scurlock J, Mansour A, Makris CM, ... Fabian V (2006). Early progressive encephalopathy in boys and MECP2 mutations. *Neurology*, 67, 164–166. [PubMed: 16832102]
- Kaufmann WE, Tierney E, Rohde CA, Suarez-Pedraza MC, Clarke MA, Salorio CF, ... Naidu S (2012). Social impairments in Rett syndrome: Characteristics and relationship with clinical severity. *Journal of Intellectual Disability Research*, 56, 233–247. [PubMed: 21385260]
- Kerr B, Alvarez-Saavedra M, Saez MA, Saona A, & Young JI (2008). Defective body-weight regulation, motor control and abnormal social interactions in Mecp2 hypomorphic mice. *Human Molecular Genetics*, 17, 1707–1717. [PubMed: 18321865]
- Kudo S, Nomura Y, Segawa M, Fujita N, Nakao M, Schanen C, & Tamura M (2003). Heterogeneity in residual function of MeCP2 carrying missense mutations in the methyl CpG binding domain. *Journal of Medical Genetics*, 40, 487–493. [PubMed: 12843318]
- Lamonica JM, Kwon DY, Goffin D, Fenik P, Johnson BS, Cui Y, ... Zhou Z (2017). Elevating expression of MeCP2 T158M rescues DNA binding and Rett syndrome-like phenotypes. *The Journal of Clinical Investigation*, 127, 1889–1904. [PubMed: 28394263]
- Lawson-Yuen A, Liu D, Han L, Jiang ZI, Tsai GE, Basu AC, ... Coyle JT (2007). Ube3a mRNA and protein expression are not decreased in Mecp2(R168X) mutant mice. *Brain Research*, 1180, 1–6. [PubMed: 17936729]
- Lewis JD, Meehan RR, Henzel WJ, Maurer-Fogy I, Jeppesen P, Klein F, & Bird A (1992). Purification, sequence, and cellular localization of a novel chromosomal protein that binds to methylated DNA. *Cell*, 69, 905–914. [PubMed: 1606614]
- Li Y, Wang H, Muffat J, Cheng AW, Orlando DA, Loven J, ... Hockemeyer D (2013). Global transcriptional and translational repression in human-embryonic-stem-cell-derived Rett syndrome neurons. *Cell Stem Cell*, 13, 446–458. [PubMed: 24094325]
- Li H, Zhong X, Chau KF, Williams EC, & Chang Q (2011 7 17). Loss of activity-induced phosphorylation of MeCP2 enhances synaptogenesis, LTP and spatial memory. *Nature Neuroscience*, 14(8), 1001–1008. [PubMed: 21765426]
- Lioy DT, Garg SK, Monaghan CE, Raber J, Foust KD, Kaspar BK, ... Mandel G (2011). A role for glia in the progression of Rett's syndrome. *Nature*, 475, 497–500. [PubMed: 21716289]
- Liu Z, Li X, Zhang J-T, Cai Y-J, Cheng T-L, Cheng C, ... Bian WJ (2016). Autism-like behaviours and germline transmission in transgenic monkeys over-expressing MeCP2. *Nature*, 530, 98–102. [PubMed: 26808898]
- Lyst MJ, Ekiert R, Ebert DH, Merusi C, Nowak J, Selfridge J, ... Rappaport J (2013). Rett syndrome mutations abolish the interaction of MeCP2 with the NCoR/SMRT co-repressor. *Nature Neuroscience*, 16, 898–902. [PubMed: 23770565]
- McGraw CM, Samaco RC, & Zoghbi HY (2011). Adult neural function requires MeCP2. *Science (New York, NY)*, 333, 186.
- Meins M, Lehmann J, Gerresheim F, Herchenbach J, Hagedorn M, Hameister K, & Epplen JT (2005). Submicroscopic duplication in Xq28 causes increased expression of the MECP2 gene in a boy with severe mental retardation and features of Rett syndrome. *Journal of Medical Genetics*, 42, e12. [PubMed: 15689435]
- Meloni I, Bruttini M, Longo I, Mari F, Rizzolio F, D'Adamo P, ... Renieri A (2000). A mutation in the rett syndrome gene, MECP2, causes X-linked mental retardation and progressive spasticity in males. *American Journal of Human Genetics*, 67, 982–985. [PubMed: 10986043]
- Nan X, Campoy FJ, & Bird A (1997). MeCP2 is a transcriptional repressor with abundant binding sites in genomic chromatin. *Cell*, 88, 471–481. [PubMed: 9038338]

- Nan X, Ng HH, Johnson CA, Laherty CD, Turner BM, Eisenman RN, & Bird A (1998). Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. *Nature*, 393, 386–389. [PubMed: 9620804]
- Nan X, Tate P, Li E, & Bird A (1996). DNA methylation specifies chromosomal localization of MeCP2. *Molecular and Cellular Biology*, 16, 414–421. [PubMed: 8524323]
- Neul JL, Kaufmann WE, Glaze DG, Christodoulou J, Clarke AJ, Bahi-Buisson N, & Renieri A (2010). Rett syndrome: Revised diagnostic criteria and nomenclature. *Annals of Neurology*, 68, 944–950. [PubMed: 21154482]
- Nguyen MVC, Du F, Felice CA, Shan X, Nigam A, Mandel G, ... Ballas N (2012). MeCP2 is critical for maintaining mature neuronal networks and global brain anatomy during late stages of postnatal brain development and in the mature adult brain. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 32, 10021–10034. [PubMed: 22815516]
- Pitcher MR, Herrera JA, Buffington SA, Kochukov MY, Merritt JK, Fisher AR, ... Neul JL (2015). Rett syndrome like phenotypes in the R255X Mecp2 mutant mouse are rescued by MECP2 transgene. *Human Molecular Genetics*, 24, 2662–2672. [PubMed: 25634563]
- Ramirez J-M, Ward CS, & Neul JL (2013). Breathing challenges in Rett syndrome: Lessons learned from humans and animal models. *Respiratory Physiology & Neurobiology*, 189, 280–287. [PubMed: 23816600]
- Ravn K, Nielsen JB, Uldall P, Hansen FJ, & Schwartz M (2003). No correlation between phenotype and genotype in boys with a truncating MECP2 mutation. *Journal of Medical Genetics*, 40, e5. [PubMed: 12525553]
- Rett A (1966). On a unusual brain atrophy syndrome in hyperammonemia in childhood. *Wiener Medizinische Wochenschrift*, 116, 723–726. [PubMed: 5300597]
- Samaco RC, Fryer JD, Ren J, Fyffe S, Chao H-T, Sun Y, ... Neul JL (2008). A partial loss of function allele of methyl-CpG-binding protein 2 predicts a human neurodevelopmental syndrome. *Human Molecular Genetics*, 17, 1718–1727. [PubMed: 18321864]
- Shahbazian MD, Antalffy B, Armstrong DL, & Zoghbi HY (2002). Insight into Rett syndrome: MeCP2 levels display tissue- and cell-specific differences and correlate with neuronal maturation. *Human Molecular Genetics*, 11, 115–124. [PubMed: 11809720]
- Skene PJ, Illingworth RS, Webb S, Kerr ARW, James KD, Turner DJ, ... Bird AP (2010). Neuronal MeCP2 is expressed at near histone-octamer levels and globally alters the chromatin state. *Molecular Cell*, 37, 457–468. [PubMed: 20188665]
- Su S-H, Kao F-C, Huang Y-B, & Liao W (2015). MeCP2 in the rostral striatum maintains local dopamine content critical for psychomotor control. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 35, 6209–6220. [PubMed: 25878291]
- Tao J, Hu K, Chang Q, Wu H, Sherman NE, Martinowich K, ... Sun YE (2009). Phosphorylation of MeCP2 at Serine 80 regulates its chromatin association and neurological function. *Proceedings of the National Academy of Sciences of the United States of America*, 106, 4882–4887. [PubMed: 19225110]
- Temudo T, Santos M, Ramos E, Dias K, Vieira JP, Moreira A, ... Barbot C (2011). Rett syndrome with and without detected MECP2 mutations: An attempt to redefine phenotypes. *Brain and Development*, 33, 69–76. [PubMed: 20116947]
- Tillotson R, Selfridge J, Koerner MV, Gadalla KKE, Guy J, De Sousa D, ... Bird A (2017). Radically truncated MeCP2 rescues Rett syndrome-like neurological defects. *Nature*, 550, 398–401. [PubMed: 29019980]
- Tudor M, Akbarian S, Chen RZ, & Jaenisch R (2002). Transcriptional profiling of a mouse model for Rett syndrome reveals subtle transcriptional changes in the brain. *Proceedings of the National Academy of Sciences of the United States of America*, 99, 15536–15541. [PubMed: 12432090]
- Venancio M, Santos M, Pereira SA, Maciel P, & Saraiva JM (2007). An explanation for another familial case of Rett syndrome: Maternal germline mosaicism. *European Journal of Human Genetics*, 15, 902–904. [PubMed: 17440498]
- Ward CS, Arvide EM, Huang T-W, Yoo J, Noebels JL, & Neul JL (2011). MeCP2 is critical within HoxB1-derived tissues of mice for normal lifespan. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 31, 10359–10370.

- Weaving LS, Williamson SL, Bennetts B, Davis M, Ellaway CJ, Leonard H, ... Christodoulou J (2003). Effects of MECP2 mutation type, location and X-inactivation in modulating Rett syndrome phenotype. *American Journal of Medical Genetics Part A*, 118A, 103–114. [PubMed: 12655490]
- Wegener E, Brendel C, Fischer A, Hulsmann S, Gartner J, & Huppke P (2014). Characterization of the MeCP2R168X knockin mouse model for Rett syndrome. *PloS One*, 9, e115444. [PubMed: 25541993]
- Wu H, Luo J, Yu H, Rattner A, Mo A, Wang Y, ... Nathans J (2014). Cellular resolution maps of X chromosome inactivation: Implications for neural development, function, and disease. *Neuron*, 81, 103–119. [PubMed: 24411735]
- Yazdani M, Deogracias R, Guy J, Poot RA, Bird A, & Barde Y-A (2012). Disease modeling using embryonic stem cells: MeCP2 regulates nuclear size and RNA synthesis in neurons. *Stem Cells*, 30, 2128–2139. [PubMed: 22865604]
- Zeev BB, Yaron Y, Schanen NC, Wolf H, Brandt N, Ginot N, ... Orr-Urtreger A (2002). Rett syndrome: Clinical manifestations in males with MECP2 mutations. *Journal of Child Neurology*, 17, 20–24. [PubMed: 11913564]

Table 1

List of MeCP2 mouse models made to date.

	Mutation	Protein product	Reference
Mecp2 null mice	Exon 3 deletion	Lacks MBD domain (fragment detected)	Chen et al. (2001)
	Exon 3&4 deletion	No Protein detected	Guy et al. (2001)
	R106W-Tavi	Impaired MBD, TRD preserved	Johnson et al. (2017)
MeCP2 RTT mutation (Targeted <i>Mecp2</i> gene locus)	R133C-GFP	Impaired MBD, TRD preserved	Brown et al. (2016)
	T158A	Impaired MBD, TRD preserved	Goffin et al. (2011)
	T158M-GFP	Impaired MBD, TRD preserved	Brown et al. (2016)
	T158M-Tavi	Impaired MBD, TRD preserved	Johnson et al. (2017)
	R168X	C-terminal truncation, retains MBD, lacks TRD	Lawson-Yuen et al. (2007)
	P225R	Protein level reduced to ~22%	Guy et al. (2018)
	R255X	C-terminal truncation, retains MBD, lacks TRD	Pitcher et al. (2015)
	R306C-GFP	Preserved MBD, TRD impaired	Brown et al. (2016)
	R306C	Preserved MBD, TRD impaired	Lyst et al. (2013)
	P322L	Protein level reduced to ~3%	Guy et al. (2018)
MeCP2 RTT Mutations (Human PAC/BAC, transgenic alleles expressed in <i>Mecp2</i> -null background)	R111G-EGFP	Impaired MBD, TRD preserved	Heckman et al. (2014)
	R270X	C-terminal truncation, disrupts AT-hook	Baker et al. (2013)
	G273X	C-terminal truncation, preserves AT-hook	Baker et al. (2013)
	R306C-EGFP	Preserved MBD, TRD impaired	Heckman et al. (2014)
MeCP2 Non-RTT Mutations	floxed <i>Mecp2</i>	Hypomorphic expression MeCP2 (~50% reduction)	Guy et al. (2001), Kerr, Alvarez-Saavedra, Saez, Saona, and Young (2008), Samaco et al. (2008)
	A140V	Preserved MBD and TRD	Jentara et al. (2010)
	C-terminal truncation	Truncation after amino acid 308, Preserved MBD and TRD	Shahbazian et al. (2002)
	S80A	Preserved MBD and TRD	Tao et al. (2009)
<i>MECP2</i> transgenic	S421A	Preserved MBD and TRD	Cohen et al. (2011)
	S421A; S424A	Preserved MBD and TRD	Li, Zhong, Chau, Williams, and Chang (2011)
	Human <i>MECP2</i> BAC Transgene	WT protein, ~1 fold expression	Collins et al. (2004)