

Advances in Molecular Mechanisms and Therapies for Rett Syndrome: From MECP2 Dysfunction to Small-Molecule Interventions

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Abstract. Rett syndrome (RTT) is a rare and severe neurodevelopmental disorder primarily affecting females, caused by pathogenic mutations in the MECP2 gene, which disrupts neuronal transcriptional regulation and synaptic function. Understanding the molecular basis of MECP2 dysfunction has catalyzed the development of targeted therapies aiming to restore neuronal homeostasis. This paper integrates current knowledge of MECP2 structure, function and mutation-driven pathology, highlighting how disruptions in its methyl-CpG-binding, transcriptional repression, and C-terminal domains lead to widespread neuronal deficits. This study summarizes emerging therapeutic strategies, focusing on small-molecule approaches including protein stabilization, readthrough compounds, synaptic and neurotrophic pathway modulation and epigenetic regulation. Notably, trofinetide, the first FDA-approved therapy, underscores the potential of translational progress while revealing limitations in delivery and efficacy. This paper further discusses cutting-edge drug discovery pipelines leveraging iPSC-derived neurons and organoids, and explores future solutions such as nanocarriers, PROTACs and precision medicine approaches. By bridging molecular mechanisms to therapeutic innovation, this review underscores the promise of rationally designed treatments to move beyond symptomatic management toward disease modification in RTT.

1 Introduction

Rett syndrome (RTT) is a rare, devastating neurodevelopmental disorder primarily affecting girls, following a seemingly normal early infancy and characterized by loss of motor and language skills, stereotyped hand movements, seizures, autonomic dysfunction, and cognitive impairment [1]. At the molecular level, pathogenic variants in the MECP2 gene impair its epigenetic regulatory functions—disrupting chromatin architecture and gene expression programs essential for neuronal maturation and network stability [2–4]. In recent years, mechanistic advances have included multi-omics analyses—spanning transcriptomic, proteomic, and epigenomic studies in patient-derived neurons and mouse models—that link MECP2 dysfunction to synaptic deficits, mitochondrial dysregulation, and alterations in inflammatory pathways [2]. On the translational front, trofinetide (Daybue™) became the first U.S. FDA-approved therapy for RTT in March 2023, with phase 3 trial data revealing statistically significant improvements in caregiver behavioral assessments (RSBQ) and clinician-rated global impression scores (CGI-I), although gastrointestinal adverse events like diarrhea and vomiting were common [5,6]. In China, large-scale studies have delineated the MECP2 mutation spectrum, showing a predominance of exon 4 variants and uncovering novel mutations, thereby supporting

genotype–phenotype mapping in domestic cohorts [7]. Nonetheless, gaps persist—nationwide epidemiological coverage remains incomplete, access to genetic diagnostics and specialized care varies regionally, and interventional clinical research trails behind international efforts. Accordingly, this review has two main objectives: first, to integrate mechanistic insights—from chromatin dysregulation and transcriptomic disruption to synaptic dysfunction—into a unified disease model; and second, to align potential therapeutic strategies—including proteostasis modulation, readthrough agents, epigenetic targeting, and receptor-based approaches—with considerations of CNS delivery, tolerability, and female mosaicism, thereby forging a translational roadmap toward disease-modifying interventions in RTT.

2 Molecular mechanism of MECP2 dysfunction and Rett syndrome

MECP2 dysfunction in Rett syndrome is best understood through its structural domains and how mutations compromise neuronal function [2]. The protein has three main domains: the methyl-CpG-binding domain (MBD), transcriptional repression domain (TRD), and C-terminal domain (CTD) [2]. The MBD binds methylated DNA, interpreting epigenetic marks to regulate transcription; mutations here disrupt

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DNA binding and cause widespread transcriptional dysregulation [2,4]. The TRD recruits co-repressors like SIN3A and HDACs to silence genes and maintain chromatin structure; loss-of-function mutations destabilize gene repression, upsetting transcriptional balance critical for synaptic and neuronal homeostasis [3]. The CTD supports structural stability and protein interactions, and mutations can impair integration into regulatory complexes [2]. Together, these domains allow MECP2 to act not as a simple on-off switch but as a fine-tuner of thousands of neuronal genes, especially those involved in synaptic plasticity and connectivity [2,4].

Disruption of MECP2 profoundly affects neuronal development and synaptic architecture. Neurons rely on tightly regulated transcriptional programs for dendritic branching, axonal guidance, and synaptic maturation [2]. MECP2 deficiency reduces dendritic complexity and spine density, limiting functional network formation, as seen in patient-derived iPSC neurons and *Mecp2*-null mice [8,9]. Synaptic transmission, particularly excitatory signaling, is also impaired, with decreased postsynaptic currents and altered glutamatergic receptor expression, shifting the cortical excitation-inhibition balance [9]. Additionally, MECP2 loss deregulates hundreds of genes, creating a transcriptional storm in activity-dependent pathways controlling synaptic vesicle release, neurotrophic signaling, and ion channel function [2,4]. This combination of morphological and transcriptional defects undermines neuronal responsiveness, plasticity, and learning.

The diversity of MECP2 mutations further shapes Rett pathology. Missense mutations, often in the MBD, can partially or fully disrupt DNA binding, producing variable severity [2,10]. Nonsense mutations create premature stop codons and truncated proteins degraded by nonsense-mediated decay, while frameshift mutations produce misfolded or unstable proteins that may act dominantly negative [2]. MECP2 is dosage-sensitive; even minor destabilization disrupts chromatin and neuronal function [2]. Animal studies show partial MECP2 activity results in milder phenotypes, whereas complete loss causes severe deficits and early lethality [2,9,11]. These genotype-phenotype correlations inform therapeutic strategies: stabilizing residual MECP2, enhancing wild-type expression, or modulating downstream targets may restore sufficient function [2,12]. Overall, MECP2's domains, transcriptional roles, and mutation spectrum underpin Rett syndrome's molecular pathology and highlight the need for targeted intervention.

3 Treatment strategies for Rett syndrome

One of the most actively pursued areas in Rett syndrome therapy revolves around identifying druggable strategies that either restore MECP2 function directly or compensate for its absence by targeting downstream pathways, and within this context, several complementary approaches have emerged, ranging from

protein stabilization to epigenetic modulation. Protein stabilization strategies have attracted significant interest because many MECP2 mutations, particularly missense or frameshift variants, destabilize the protein's tertiary structure and accelerate degradation through cellular proteostasis systems [2]. By targeting these degradation pathways, researchers aim to prolong the half-life of mutant MECP2 and thereby recover partial function. Proteostasis regulators, for example, can alter the balance between protein folding and degradation by modulating the ubiquitin-proteasome system or autophagy machinery [2]. Small molecules such as proteasome inhibitors or modulators of molecular chaperones have shown preliminary promise in cell-based models of Rett syndrome. Similarly, histone deacetylase (HDAC) inhibitors have a dual effect: not only do they relieve transcriptional repression at MECP2-dependent promoters by enhancing histone acetylation, but they can also indirectly improve protein stability by influencing cellular stress responses and protein-folding networks. Chaperone inducers such as HSP90 modulators, meanwhile, provide another avenue to stabilize mutant MECP2 by facilitating correct folding, thereby rescuing some degree of functional protein even in the presence of pathogenic mutations [2].

Another promising line of therapy lies in the development of readthrough compounds, which are specifically designed to overcome nonsense mutations that introduce premature stop codons in the MECP2 gene. These nonsense mutations are particularly devastating because they truncate the protein and often trigger nonsense-mediated decay, eliminating MECP2 from the cell entirely [10,13]. Small molecules such as aminoglycoside antibiotics (e.g., gentamicin) and next-generation analogs (e.g., ataluren, also known as PTC124) can induce the ribosome to "ignore" premature stop codons during translation, allowing the synthesis of a full-length protein [13]. Though the efficiency of this process is variable and context-dependent, even low levels of full-length MECP2 generated by readthrough have the potential to produce significant clinical benefit given the protein's dosage sensitivity. Preclinical studies in animal models and patient-derived neuronal cultures have shown encouraging signs of restored MECP2 function and downstream gene regulation when treated with these compounds [13]. However, challenges remain in optimizing the specificity of readthrough without inducing global translational errors or toxicity, and clinical trials are needed to evaluate whether these benefits translate into functional recovery in patients. Nevertheless, readthrough therapies represent one of the few strategies that directly target the genetic root of Rett syndrome, particularly for patients with nonsense mutations, and thus continue to be a high priority for translational research.

Given the multifaceted role of MECP2 in regulating neuronal transcriptional homeostasis, another major therapeutic approach is to modulate the downstream pathways most affected by its loss. Among these, the brain-derived neurotrophic factor (BDNF) pathway has received the most attention because MECP2 directly regulates activity-dependent BDNF expression, and

insufficient BDNF levels are linked to impaired synaptic maturation, plasticity, and dendritic growth in Rett syndrome [4,12]. Therapeutic strategies aimed at elevating BDNF levels, either through recombinant protein delivery, gene therapy vectors, or small molecules that enhance BDNF transcription, have shown promise in animal models, with evidence of improved motor function, breathing irregularities, and survival [12]. Similarly, the insulin-like growth factor-1 (IGF-1) pathway has emerged as a critical target, as IGF-1 promotes synaptic maturation and neuroprotection. Administration of recombinant IGF-1 or IGF-1 analogs has been tested in clinical trials, showing safety and modest improvement in some Rett patients, though results remain mixed and require larger studies [14]. Other downstream targets, such as glutamatergic receptor modulation and GABAergic signaling pathways, are also being explored as adjunctive strategies to correct the excitatory-inhibitory imbalance characteristic of MECP2 dysfunction. These pathway modulation approaches may not cure Rett syndrome by restoring MECP2 itself, but they provide a valuable therapeutic angle by ameliorating the neurodevelopmental deficits downstream of MECP2 loss, potentially offering symptomatic relief and functional improvement [12].

Finally, epigenetic modulation has emerged as both a conceptually elegant and mechanistically relevant therapeutic avenue, given that MECP2 itself functions as an epigenetic regulator of gene expression. Compounds that target histone acetylation, DNA methylation, or chromatin remodeling could theoretically restore transcriptional equilibrium in neurons lacking functional MECP2 [2,12]. For example, HDAC inhibitors not only stabilize proteins but also directly increase histone acetylation, thereby loosening chromatin and compensating for the excessive repression seen in MECP2-deficient cells. Other small molecules, such as bromodomain and extra-terminal domain (BET) inhibitors, are being investigated for their ability to fine-tune transcriptional output and restore proper gene expression dynamics. DNA methylation modifiers, though more challenging due to the risk of broad off-target effects, represent another conceptual pathway, particularly in selectively reactivating silenced alleles of MECP2 or normalizing aberrant methylation signatures in downstream genes. Moreover, chromatin remodelers that influence nucleosome positioning and higher-order chromatin architecture could play a role in re-establishing transcriptional balance in MECP2-null neurons. While these approaches remain largely preclinical, they reflect a broader recognition that Rett syndrome arises not solely from the absence of one protein but from a global disturbance of transcriptional and epigenetic networks, making epigenetic modulators particularly attractive as systems-level interventions [2,12].

4 Development and challenges of small-molecule drugs for Rett syndrome

The development of small molecules for Rett syndrome has become a critical translational focus, as such compounds offer the possibility of non-invasive, scalable therapies compared to gene replacement or cell-based interventions. Several promising candidates have advanced through preclinical and even clinical testing, each reflecting different strategies to compensate for MECP2 loss or to modulate downstream pathways. Among the most advanced is Trofinetide (NNZ-2566), a synthetic analog of the IGF-1 tripeptide glycine-proline-glutamate (GPE). Trofinetide was designed to harness IGF-1's neurotrophic and synaptogenic properties while improving pharmacokinetics for human use. Preclinical studies demonstrated that Trofinetide improved synaptic morphology, reduced neuroinflammation, and normalized neurotransmitter signaling in *Mecp2*-null mice, leading to increased survival and behavioral improvements. These encouraging findings justified clinical trials, where Trofinetide recently became the first FDA-approved drug for Rett syndrome, marking a historic milestone in translating basic discoveries into therapeutic benefit [5,6,12].

Other molecules under investigation include adenosine A2A receptor antagonists, which have potential in Rett syndrome due to effects on synaptic plasticity, dopaminergic signaling, and neuroinflammation, and small-molecule partial agonists of the TrkB receptor (e.g., LM22A-4) aimed at restoring synaptic growth and activity-dependent neuronal maturation; in mouse models, such approaches have shown improvements in respiratory stability, motor coordination, and dendritic spine density [12]. Together, these candidates illustrate the range of small molecule strategies being pursued, from mimicking growth factor signaling to modulating neurotransmitter and neurotrophic receptor pathways.

Identifying such candidates relies on robust drug discovery pipelines, which increasingly combine high-throughput screening (HTS), *in silico* docking, and phenotypic assays. HTS platforms allow thousands of compounds to be rapidly tested for their effects on MECP2-dependent pathways or neuronal phenotypes, often using fluorescent or luminescent readouts to detect changes in gene expression, synaptic activity, or cell survival. *In silico* docking complements these experimental approaches by predicting how small molecules interact with protein targets implicated in Rett syndrome, such as TrkB or HDACs, thereby prioritizing candidates before costly wet-lab screening. Beyond these target-centric methods, phenotypic screening in induced pluripotent stem cell (iPSC)-derived neurons and brain organoids has become a powerful tool. Patient-derived iPSCs differentiated into excitatory and inhibitory neurons provide a human-relevant platform to evaluate candidate compounds, capturing complex disease phenotypes such as impaired dendritic arborization, synaptic dysfunction, and altered calcium signaling. Brain organoids, which recapitulate aspects of cortical architecture, further enable screening for compounds that restore network activity and cellular diversity disrupted by MECP2 mutations. Together, these screening strategies form a multi-layered

framework that integrates computational predictions, biochemical assays, and human disease models to accelerate small molecule discovery [8,12].

Validation of lead compounds requires rigorous preclinical testing in both in vitro and in vivo models. iPSC-derived neuronal cultures allow assessment of molecular mechanisms, including rescue of BDNF expression and normalization of MECP2-related transcriptional signatures, while electrophysiology and calcium imaging measure synaptic function and excitability. In vivo, *Mecp2*-null and heterozygous mice provide whole-organism evaluation of efficacy and safety, exhibiting hallmark Rett features such as motor deficits, breathing abnormalities, and reduced survival. Compounds acting on neurotrophic pathways have improved lifespan, synaptic plasticity, and motor or respiratory phenotypes in these models, demonstrating translational potential and guiding dosing, pharmacokinetics, and toxicity profiling [9,11,12].

Despite these advances, CNS delivery remains a major challenge due to the blood-brain barrier, which limits penetration and can actively efflux drugs. Strategies to overcome this include optimizing molecular properties, using nanoparticle or liposomal carriers, and developing brain-activated prodrugs. Ensuring tissue specificity is critical, as broad activation of TrkB or IGF-1 pathways may cause off-target effects in the CNS or periphery, including seizures, mood disturbances, or neurotoxicity [12,14,15]. Female mosaicism in MECP2 expression further complicates efficacy predictions, underscoring the need for individualized strategies. Overcoming these delivery and specificity barriers is essential to maximize the therapeutic potential of candidates like Trofinetide while minimizing risk [5,6,12].

5 Challenges and future directions in the treatment of Rett syndrome

Translating small-molecule and genetic discoveries in Rett syndrome into effective therapies faces major challenges. Current compounds often lack specificity, risking off-target effects that disrupt synaptic homeostasis or peripheral tissues. Toxicity and short half-lives limit efficacy, necessitating frequent dosing. Even Trofinetide, the first FDA-approved therapy, requires multiple daily doses and can cause gastrointestinal side effects [5,6]. These limitations highlight the need for precise, durable, and patient-tailored strategies beyond symptomatic relief.

To address these issues, novel approaches are emerging. PROTACs (proteolysis-targeting chimeras) enable selective degradation or stabilization of proteins, including MECP2, reducing off-target toxicity while enhancing efficacy. Dual-target drugs simultaneously modulate complementary pathways such as synaptic plasticity and neuroinflammation for broader benefit. Nanocarrier systems—including lipid nanoparticles, polymers, and exosomes—improve CNS delivery, extend half-life, and facilitate precision delivery of small molecules or nucleic acid therapeutics like ASOs or CRISPR constructs [12,15].

Advanced molecular tools further expand therapeutic options. CRISPRa/i can fine-tune MECP2 or downstream targets, avoiding risks of overexpression, while ASOs can correct splicing, stabilize transcripts, or suppress toxic isoforms, building on successes in spinal muscular atrophy [12,15]. Combination therapies integrating neurotrophic, anti-inflammatory, and epigenetic agents offer synergistic correction of complex neuronal deficits.

Future strategies must integrate drug design with patient stratification and trial optimization. Biomarkers such as EEG patterns, neurofilament light chain, and transcriptomic signatures will monitor efficacy. Stratifying patients by mutation type, disease severity, and residual MECP2 activity can improve outcomes and tailor therapies to specific subgroups [1,12,15]. By combining advanced molecular tools, smarter delivery systems, and individualized treatment plans, the field aims not just to manage symptoms but to modify the course of Rett syndrome itself.

6 Conclusion

This paper set out to explore the molecular underpinnings of MECP2 dysfunction in Rett syndrome and to evaluate the current and emerging small-molecule strategies aimed at addressing its complex pathophysiology. Taken together, the evidence illustrates that RTT is not merely the consequence of a single gene defect but rather the product of widespread transcriptional, synaptic, and epigenetic disturbances that compromise neuronal development and connectivity. Mechanistic insights into MECP2's structural domains, gene-regulatory roles, and mutation spectrum have clarified how its loss destabilizes chromatin homeostasis, synaptic signaling, and plasticity. These mechanistic findings directly inform therapeutic avenues, from protein stabilization and readthrough approaches to pathway modulation and epigenetic tuning, demonstrating the diverse ways in which pharmacological interventions can restore at least partial functionality. Clinically, the approval of trofinetide represents a landmark in translating decades of preclinical progress into tangible patient benefit, while parallel advances in compounds targeting BDNF, IGF-1, and neurotransmitter receptors underscore the momentum in drug discovery. The significance of this study lies in its attempt to bridge basic molecular understanding with pragmatic therapeutic strategies, emphasizing that aligning drug design with disease biology is essential for genuine progress in RTT. By situating small-molecule development within both international and Chinese research contexts, the review also highlights how global and regional infrastructures can be leveraged for trial readiness, patient stratification, and long-term treatment access. In this sense, the work not only consolidates mechanistic knowledge but also provides a roadmap for rational drug development, aiming to move the field closer to disease modification rather than symptomatic relief. Nonetheless, limitations remain evident. Small molecules often face challenges of CNS penetration,

specificity, and durability, while female mosaicism complicates predictions of treatment efficacy. Many strategies that appear promising in cell and animal models may fail to achieve sufficient safety or functional rescue in patients. Moreover, the rarity of RTT and the heterogeneity of MECP2 mutations impose constraints on large-scale clinical trials and biomarker validation. Looking ahead, future progress will likely depend on combining small-molecule strategies with advanced molecular tools such as gene editing, RNA modulation, or nanocarrier-based delivery systems. Equally important will be integrating robust biomarkers and personalized treatment paradigms that align therapeutic mechanisms with patient-specific biology. By building on mechanistic insight, embracing precision approaches, and fostering global collaboration, the field has the potential to transform RTT treatment from incremental symptom management toward meaningful, disease-modifying therapies.

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