REVIEW

Mechanisms and therapeutic challenges in autism spectrum disorders: insights from Rett syndrome

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Purpose of review
A major challenge for understanding ~~the~~ neurodevelopmental disorders, including autism spectrum disorders (ASDs), is to advance the findings from gene discovery to an exposition of neurobiological mechanisms that underlie these disorders and subsequently translate this knowledge into mechanism-based therapeutics. A promising way to proceed is revealed by the recent studies of rare subsets of ASDs. In this review, we summarize the latest advances in the mechanisms and emerging therapeutics for a rare single-gene ASD, Rett syndrome.

Recent findings
Rett syndrome is caused by mutations in the gene coding for methyl CpG-binding protein 2 (MeCP2). Although MeCP2 has diverse functions, examination of MeCP2 mutant mice suggests the hypothesis that MeCP2 deficiency leads to aberrant maturation and maintenance of synapses and circuits in multiple brain systems. Some of the deficits arise from alterations in specific intracellular pathways such as the PI3K/Akt signaling pathway. These abnormalities can be at least partially rescued in MeCP2 mutant mice by treatment with ~~the~~ therapeutic agents.

Summary
Mechanism-based therapeutics are emerging for single-gene neurodevelopmental disorders such as Rett syndrome. Given the complexity of MeCP2 function, future directions include combination therapeutics that target multiple molecules and pathways. Such approaches will likely be applicable to other ASDs as well.

Keywords
molecular signaling pathways, monogenic disorders, neurodevelopmental disorders, pharmacological therapeutics

INTRODUCTION

Autism spectrum disorders (ASDs) are a group of neurodevelopmental disorders that share common core symptoms of deficits in language and communication, impaired social interactions, and stereotypic or repetitive behaviors. ASDs are characterized by tremendous heterogeneity in their clinical diagnosis and pathophysiological mechanisms. Although all ASD diagnoses share the core symptoms above, individuals with ASD show great differences in severity across these domains and may also suffer from secondary symptoms such as various degrees of cognitive impairment that are specific for each disorder. Similarly, ASD ~~cause is~~ diverse and existing evidence point~~s~~ to a complex genetic basis.

Both 'rare' and 'common' genetic variants are considered to be risk factors for ASD. Large copy number variants (CNVs) have been recently implicated in subsets of ASDs [1], with specific recurrent CNVs observed across various phenotypes. With few exceptions, CNVs affect several genes and are extremely rare for any given locus. However, many neurodevelopmental disorders with overlapping diagnosis to classic autism, often accompanied by cognitive impairment, are caused by single-gene mutations. Although monogenic disorders account for a small percentage of the total cases diagnosed with autism (Table 1), they might be very informative about the role of common pathogenic pathways affected by the single-gene changes, and hence could be of importance for the discovery of potential

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KEY POINTS

- Latest advances in therapeutics for Rett syndrome.
- Latest considerations of MeCP2 mechanisms, emphasizing its interactions with microRNAs.
- Necessity of new pharmacological strategies that combine analysis of molecular pathways with suitable drugs to enable therapeutic effects.

therapeutic targets (reviewed in [2]). What the vast majority of single-gene ASDs have in common is that the affected gene product is involved in one or more signaling pathways and therefore exerts influence over discrete effectors. In many instances, however, it may be difficult to pin down the defective gene product to a specific symptom and unlikely that a single targeted molecule would restore regular function in the organism. Crucially, single-gene mutations provide accurate mouse models of ASD, which are central for assaying molecular, circuit, and behavioral functions and for developing therapeutics.

Because ASDs are considered to be neurodevelopmental disorders, an important variable to be considered in the development of therapeutics is the time point of intervention. Several kinds of evidence point to the possibility that although early intervention for ASD is likely to have the most impact, therapeutics may still be effective at a range of ages. The development of brain modules, circuits, and synapses, particularly those mediating complex behaviors, has multiple and diverse time courses of maturation and plasticity. Furthermore, the view of ASDs as purely 'developmental' disorders is being challenged by the findings that the gene products and molecules affected by the specific disorders are required for appropriate function well past ~~the~~ early development and even throughout life, ~~for~~ deletion of relevant genes in adulthood still leads to the expression of the phenotype [3,4]. The goal of mechanism-based therapeutics is to take advantage of the growing knowledge of development and plasticity of circuits and synapses, and the function and impact of disease genes, in order to intervene at appropriate processes and levels.

We will focus in this review on Rett syndrome, a monogenic X-linked neurodevelopmental syndrome with significant phenotypic overlap with ASD. Rett patients, who are almost always women, develop normally until 6–18 months of age, but suffer from a progressive loss of developmental milestones similar to that observed in regressive forms of autism, such as cognitive and communication deficits, anxiety, irritability, and repetitive behaviors. In addition, the syndrome is characterized by severe motor, sensory, and autonomic nervous system disturbances, such as irregular breathing and heart rate. Other symptoms include seizures, growth failure, and gastrointestinal problems.

MECP2 AS A HIGH-LEVEL GENOMEWIDE REGULATOR

About 90% of Rett syndrome cases are caused by mutations in the gene methyl CpG-binding protein 2 (*MECP2*) [5]. MeCP2 is a highly conserved basic nuclear protein with very diverse functions (Fig. 1a). It was initially found to be associated with methylated DNA, binding to methyl CpG islands especially if AT-rich sequences are adjacent to them [6]. AQ2
A transcriptional repressor domain associated with the Sin3A–HDAC1–HDAC2 co-repressor complex that blocked mRNA transcription was also identified, so MeCP2 was originally thought to be solely a chromatin-silencing regulator [7]. However, recent evidence has shown that it plays a far more complex role as it might also act as a RNA-splicing modulator and transcriptional activator by binding to ~~CREB~~ AQ3 [8,9]. This would explain the fact that transcriptional profiling does not show dramatic changes ~~of~~ gene expression in the brain of mutant MeCP2 mice as expected from a putative ~~genomewide~~

Table 1. Some rare monogenic forms of ASD and their associated gene functions AQ6

Name	Gene	Gene function
Fragile X syndrome	FMR1	Translational control
Rett syndrome	MECP2	Transcriptional regulator
Tuberous sclerosis	TSC 1/2	Akt/mTOR signaling
Neurofibromatosis	NF1	MAPK signaling
Hamartoma tumor syndrome ~~(Bannayan–Riley–Ruvalcaba syndrome)~~	PTEN	Akt/mTOR signaling ~~(PI3K inhibitor)~~

ASD, autism spectrum disorder.

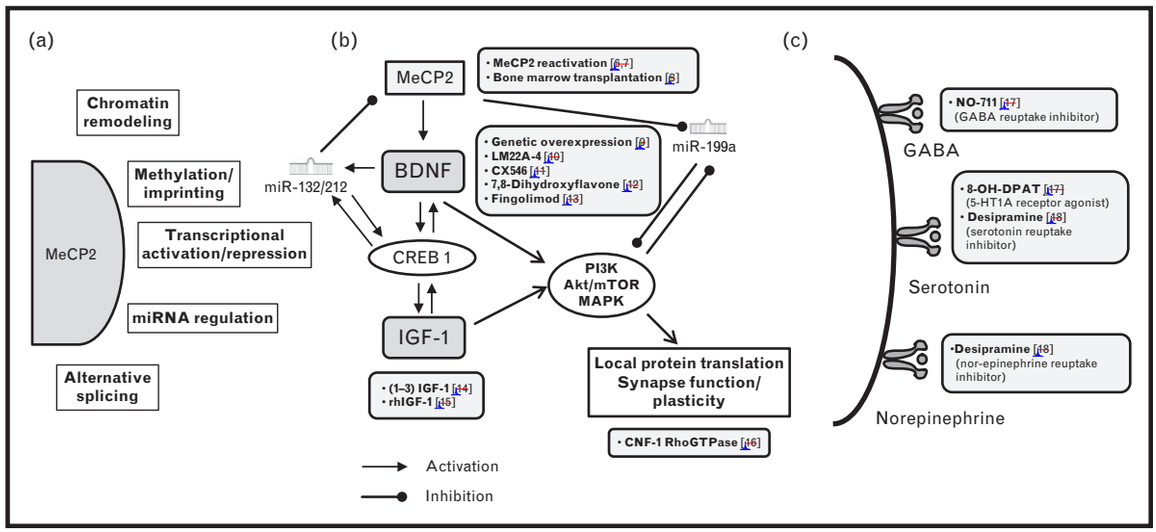

FIGURE 1. MeCP2-mediated molecular mechanisms that provide opportunities for therapeutics. (a) Schematic of different mechanisms by which MeCP2 can affect gene expression. (b) Schematic showing how MeCP2 regulates protein-coding genes and miRNAs, so as to influence local translation and synaptic development, plasticity, and function. Also shown are attempted targets and molecules for therapeutic interventions. MeCP2 is known to increase the transcription of BDNF which directly increases PI3K/Akt/MAPK signaling, or affect plasticity-related miR-132, which in turn can target MeCP2 expression, thus forming a regulatory loop. In addition, BDNF can activate CREB, which can bind to IGF-1 promoter and regulate transcription. IGF-1 is another positive regulator of the signaling pathways implicated in translation and synaptic plasticity. Akt/MAPK and mTOR are further involved in a double-negative inhibitory loop with miR-199a, a miRNA inhibited by MeCP2. (c) Therapies aimed at restoring proper synaptic function by neurotransmitter rebalancing. BDNF, brain-derived neurotrophic factor; IGF-1, insulin-like growth factor 1; MeCP2, methyl CpG-binding protein 2.

repressor [9,10], and that increased expression levels of MeCP2 are equally detrimental [11]. The role of MeCP2 in epigenetic regulation is expanded further by the finding that MeCP2 can globally alter the state of chromatin condensation [12].

Furthermore, MeCP2 is able to activate retrotransposon element transcription in neurons [13] and promote gene imprinting [14], thus inserting additional layers of complexity to the role of MeCP2 in brain function [15]. In addition, MeCP2 has been recently shown to regulate the expression of microRNAs (miRNAs) important for brain development and plasticity [16–18]. One specific family of MeCP2-regulated miRNAs is miR-132/miR-212 which is known to affect experience-dependent cortical plasticity [19,20]. Intriguingly, miR-132/miR-212, brain-derived neurotrophic factor (BDNF), and CREB signaling (Fig. 1b) are involved in a positive feed-forward loop [16,21,22]. CREB-binding elements exist in the promoter of IGF-1 which in turn can also activate CREB-mediated BDNF expression [22], thus forming a complex regulatory system representative of the bi-directional interactions between MeCP2 and a subset of its targets. Both BDNF and IGF-1 can directly activate PI3K/Akt/mTOR signaling. In addition, MeCP2 knockout mice also express high levels of the miR-199 family [17,18], that is involved in a mutually inhibitory loop with the PI3K/Akt/mTOR pathway [23,24], which as discussed below is known to be affected in the disease (Fig. 1b). Therefore, MeCP2 is able to modify a subset of PI3K/Akt regulating microRNAs, thus controlling indirectly a large array of molecules responsible for local protein synthesis and synaptic plasticity (Fig. 1). Future work is needed to elucidate whether this proposed mechanism is of importance for Rett syndrome and whether it could provide insights for novel therapeutic options. Moreover, MeCP2 targets can in turn modify other microRNAs, so that we cannot rule out the possibility of MeCP2 further controlling a larger array of molecules. Lastly, MeCP2 mediates the activity-dependent derepression of miR-184 in cortical neurons [25]. Notably, this activity-dependent dissociation of MeCP2 from miR-184 promoter occurs only in the paternal chromosome, thus linking MeCP2 to imprinting of small noncoding RNAs.

MeCP2 is ubiquitously expressed, but higher protein levels are found in the brain [7]. Within the brain, neurons were the first cell population where MeCP2 was detected, but several recent studies have revealed the presence and functional role of MeCP2 in astrocytes [26–28], microglia [29], and neuronal subsets, including inhibitory

interneurons [30]. Interestingly, MeCP2 has been shown to bind to the promoter of somatostatin (SST) and positively regulate its expression together with CREB [18]. It would be therefore worthwhile to study the effects of specific inhibitory cell-type-specific deletions of MeCP2, including SST. In addition to its spatial expression pattern, MeCP2 has a temporal expression regulation in the brain that directly correlates with postnatal neuron and circuit development, peaking postnatally, but remaining high into adulthood. This suggests that MeCP2 is involved in the maturation of existing neurons, rather than in just the differentiation and development of neuronal precursors or neurons [12,31,32], and also that its expression is required throughout life as recent evidence has shown [4].

FUNCTIONAL MECHANISMS OF MECP2 AND THE CHALLENGES FOR THERAPEUTICS

As mentioned above, MeCP2 expression peaks during early postnatal development in mice and humans [33], a period when maturation and refinement of synapses and circuits takes place in the cortex. MeCP2 mutant mice have immature excitatory synapses in the cortex and hippocampus [34–36]; furthermore, visual cortical circuits show prolonged plasticity into adulthood, indicating that synapses and circuits fail to mature [34]. The nature and extent of the influence of MeCP2 on synaptic maturity and plasticity are still being investigated. So far, no major circuit connectivity changes have been consistently detected in human Rett syndrome brains or in mouse models [37,38]. This, in conjunction with the reversibility of some of the symptoms in mouse models [34,39,40,41▪,42,43], might imply that the deficits are at the microcircuit level, involving synaptic transmission and synaptic structural reorganization. In fact, ~~the key~~ therapies tested to ameliorate Rett syndrome revolve around the restoration of synapse and circuit function with drugs that affect the molecular mechanisms of synaptic maturation [34,41▪].

BDNF is one of the first recognized direct targets of MeCP2 [44]. MeCP2 control over BDNF expression has proven to be complex, implicating different binding sites in the BDNF gene and partners [45], but there is convincing evidence that the level of BDNF is downregulated in MeCP2 mutant mice in different brain regions [9,44] and its re-expression is beneficial [44]. Not surprisingly, BDNF was one of the first target candidates for a potential therapy. Unfortunately, its therapeutic effects are compromised by its limited capacity to cross the blood–brain barrier, which has prompted the use of BDNF receptor (TrkB) agonists [41▪,46] or sphingosine-1 phosphate receptor modulator (Fingolimod), which increase BDNF levels *in vivo* and the activation of its downstream signaling pathway MAPK [47▪].

Another strategy to address this problem is to use insulin-like growth factor 1 (IGF-1) and its tri-peptide form ([1–3]IGF-1) [34,48]. Both IGF-1 and BDNF supplementation therapies are based on their effects on neuronal developmental, synaptic maturation, and plasticity exerted through two key cell-signaling pathways, PI3K/Akt and MAPK [49,50]. These signaling pathways are known to be disrupted in MeCP2 mutant mice [41▪,51▪], and have been directly involved in the regulation of protein translation and neural function by promoting the synthesis of postsynaptic proteins such as PSD-95 [52]. Although there is not a complete picture of how MeCP2 can control the molecular and cellular changes that translate into deficits in synaptic maturation and circuit connectivity, the PI3K/Akt and MAPK pathways should be considered as important players through their control over dendritic and spine genesis, and local protein synthesis [53,54]. In line with the importance of therapies aimed to favor a dynamic and appropriate microcircuit establishment, Rho-GTPases, a family of proteins involved in actin assembly and remodeling, are another potential candidate for therapeutic action in Rett syndrome [55▪]. This superfamily of proteins is known to specifically control neurite outgrowth and differentiation, spine genesis, and synapse development (reviewed in [56]).

Rett syndrome has a high prevalence of autonomic dysfunction with a remarkable respiratory and cardiac endophenotype. The control of respiration requires a concerted interplay between several neurotransmitters across distinct brainstem nuclei to maintain a normal breathing pattern. Rett syndrome has been characterized by excessive excitatory activity in expiratory neurons and deficits in medulla of nor-epinephrine and serotonin, which contribute to breathing regularity [57,58]. Several therapies have been designed to improve this imbalance (Fig. 1c) ~~variously~~ by increasing GABA availability through GABA reuptake inhibitors, increasing expiratory neuron inhibition by serotonin agonists, or directly increasing norepinephrine and serotonin concentrations with the serotonin norepinephrine reuptake inhibitor Desipramine [59,60]. In addition, a recent study suggests a potential therapy to mitigate arrhythmic cardiac function expressed as prolonged QT intervals, which is another aspect of Rett Syndrome. The sodium-channel-blocking antiseizure drug phenytoin reduces this interval as well as the general occurrence of arrhythmias [61▪].

Other therapies that have proven to be partially effective in ameliorating some of deficits in the mutant mouse models and patients include supplementing with L-carnitine, an important antioxidant that improves mitochondrial function [62,63]. Oxidative stress and mitochondrial dysfunction have been recently reported to be increased in the hippocampal cells of MeCP2 mutant mice [64▪,reviewed in 65]. However, the most impressive rescue of the phenotype of MeCP2 mutant mice was recently observed following bone marrow transplant from wildtype to mutant mice, which resulted in the engraftment of wildtype microglia into the brain of mutant mice [66▪▪]. Nevertheless, the need of exposure to high doses of radiation so as to disrupt hematopoiesis in the mutant mice and to permeate the blood–brain barrier, perhaps, limits the potential applications of this therapeutic option to treat Rett syndrome patients.

CONCLUSION

MeCP2 has complex functions and mechanisms of action. The transcriptional modulatory polyvalence of MeCP2, its heterogeneity of spatial and temporal expression, and its genomewide binding interactions make it unlikely that a single-target therapeutic agent will suffice to completely restore physiological functions in Rett syndrome patients. It is, therefore, crucial to identify combinatorial therapeutic approaches and test them in mouse models of the disorder, so that multiple molecular players and pathways affected in Rett syndrome are simultaneously restored, thus increasing the chances of a strong therapeutic response.

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Conflicts of interest

No conflicts of interest.

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REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ▪ of special interest
- ▪▪ of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

1. Pinto D, Pagnamenta AT, Klei L, *et al.* Functional impact of global rare copy number variation in autism spectrum disorders. Nature 2010; 466:368–372.
AQ4 2. Banerjee A, Castro J, Sur M. Rett syn[illegible]: genes, synapses, circuits, and therapeutics. Front Psychiatry 2012; [illegible]
AQ5 3. McGraw CM, Samaco RC, Zoghbi HY. Adult neural function requires MeCP2. Science 2011; 333:186.
4. Nguyen MV, Du F, Felice CA, *et al.* 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. J Neurosci 2012; 32:10021–10034.
5. Amir RE, Van den Veyver IB, Wan M, *et al.* Rett syndrome is caused by mutations in X-linked MeCP2, encoding methyl-CPG-binding protein 2. Nat Genet 1999; 23:185–188.
6. Klose RJ, Sarraf SA, Schmiedeberg L, *et al.* DNA binding selectivity of MeCP2 due to a requirement for A/T sequences adjacent to methyl-CPG. Mol Cell 2005; 19:667–678.
7. Kishi N, Macklis JD. Dissecting MeCP2 function in the central nervous system. J Child Neurol 2005; 20:753–759.
8. Young JI, Hong EP, Castle JC, *et al.* Regulation of rna splicing by the methylation-dependent transcriptional repressor methyl-CPG binding protein 2. Proc Natl Acad Sci USA 2005; 102:17551–17558.
9. Chahrour M, Jung SY, Shaw C, *et al.* MeCP2, a key contributor to neurological disease, activates and represses transcription. Science 2008; 320:1224–1229.
10. Tudor M, Akbarian S, Chen RZ, Jaenisch R. Transcriptional profiling of a mouse model for Rett syndrome reveals subtle transcriptional changes in the brain. Proc Natl Acad Sci USA 2002; 99:15536–15541.
11. Na ES, Nelson ED, Adachi M, *et al.* A mouse model for MeCP2 duplication syndrome: MeCP2 overexpression impairs learning and memory and synaptic transmission. J Neurosci 2012; 32:3109–3117.
12. Skene PJ, Illingworth RS, Webb S, *et al.* Neuronal MeCP2 is expressed at near histone-octamer levels and globally alters the chromatin state. Mol Cell 2010; 37:457–468.
13. Muotri AR, Marchetto MC, Coufal NG, *et al.* L1 retrotransposition in neurons is modulated by MeCP2. Nature 2010; 468:443–446.
14. LaSalle JM. The odyssey of MeCP2 and parental imprinting. Epigenetics 2007; 2:5–10.
15. De Leon-Guerrero SD, Pedraza-Alva G, Perez-Martinez L. In sickness and in health: the role of methyl-CPG binding protein 2 in the central nervous system. Eur J Neurosci 2011; 33:1563–1574.
16. Klein ME, Lioy DT, Ma L, *et al.* Homeostatic regulation of MeCP2 expression by a CREB-induced microRNA. Nat Neurosci 2007; 10:1513–1514.
17. Wu H, Tao J, Chen PJ, *et al.* Genome-wide analysis reveals methyl-CPG-binding protein 2-dependent regulation of microRNAs in a mouse model of Rett syndrome. Proc Natl Acad Sci USA 2010; 107:18161–18166.
18. Urdinguio RG, Fernandez AF, Lopez-Nieva P, *et al.* Disrupted microRNA expression caused by MeCP2 loss in a mouse model of Rett syndrome. Epigenetics 2010; 5:656–663.
19. Mellios N, Sugihara H, Castro J, *et al.* miR-132, an experience-dependent microRNA, is essential for visual cortex plasticity. Nat Neurosci 2011; 14:1240–1242.
20. Tognini P, Putignano E, Coatti A, Pizzorusso T. Experience-dependent expression of miR-132 regulates ocular dominance plasticity. Nat Neurosci 2011; 14:1237–1239.
21. Im HI, Hollander JA, Bali P, Kenny PJ. MeCP2 controls BDNF expression and cocaine intake through homeostatic interactions with microRNA-212. Nat Neurosci 2010; 13:1120–1127.
22. Lonze BE, Ginty DD. Function and regulation of CREB family transcription factors in the nervous system. Neuron 2002; 35:605–623.
23. Sayed D, Abdellatif M. Akt-ing via microRNA. Cell Cycle 2010; 9:3213–3217.
24. Fornari F, Milazzo M, Chieco P, Gramantieri L. miR-199a-3p regulates mTOR and c-Met to influence the doxorubicin sensitivity of human hepatocarcinoma cells. Cancer Res 2010; 70:5184–5193.
25. Nomura T, Kimura M, Horii T, *et al.* MeCP2-dependent repression of an imprinted miR-184 released by depolarization. Hum Mol Genet 2008; 17:1192–1199.
26. Ballas N, Lioy DT, Grunseich C, Mandel G. Noncell autonomous influence of MeCP2-deficient glia on neuronal dendritic morphology. Nat Neurosci 2009; 12:311–317.
27. Maezawa I, Swanberg S, Harvey D, *et al.* Rett syndrome astrocytes are abnormal and spread MeCP2 deficiency through gap junctions. J Neurosci 2009; 29:5051–5061.
28. Lioy DT, Garg SK, Monaghan CE, *et al.* A role for glia in the progression of Rett's syndrome. Nature 2011; 475:497–500.
29. Maezawa I, Jin LW. Rett syndrome microglia damage dendrites and synapses by the elevated release of glutamate. J Neurosci 2010; 30:5346–5356.
30. Chao HT, Chen H, Samaco RC, *et al.* Dysfunction in GABA signalling mediates autism-like stereotypies and Rett syndrome phenotypes. Nature 2010; 468:263–269.
31. Samaco RC, Nagarajan RP, Braunschweig D, LaSalle JM. Multiple pathways regulate MeCP2 expression in normal brain development and exhibit defects in autism-spectrum disorders. Hum Mol Genet 2004; 13:629–639.
32. Kishi N, Macklis JD. MeCP2 is progressively expressed in postmigratory neurons and is involved in neuronal maturation rather than cell fate decisions. MolCell Neurosci 2004; 27:306–321.
33. Shahbazian MD, Antalffy B, Armstrong DL, Zoghbi HY. Insight into Rett syndrome: MeCP2 levels display tissue- and cell-specific differences and correlate with neuronal maturation. Hum Mol Genet 2002; 11:115–124.

34. Tropea D, Giacometti E, Wilson NR, *et al.* Partial reversal of Rett syndrome-like symptoms in MeCP2 mutant mice. Proc Natl Acad Sci USA 2009; 106:2029–2034.
35. Chao HT, Zoghbi HY, Rosenmund C. MeCP2 controls excitatory synaptic strength by regulating glutamatergic synapse number. Neuron 2007; 56: 58–65.
36. Dani VS, Nelson SB. Intact long-term potentiation but reduced connectivity between neocortical layer 5 pyramidal neurons in a mouse model of Rett syndrome. J Neurosci 2009; 29:11263–11270.
37. Armstrong DD. Neuropathology of Rett syndrome. J Child Neurol 2005; 20:747–753.
38. Belichenko PV, Wright EE, Belichenko NP, *et al.* Widespread changes in dendritic and axonal morphology in MeCP2-mutant mouse models of Rett syndrome: Evidence for disruption of neuronal networks. J Comp Neurol 2009; 514:240–258.
39. Guy J, Gan J, Selfridge J, *et al.* Reversal of neurological defects in a mouse model of Rett syndrome. Science 2007; 315:1143–1147.
40. Giacometti E, Luikenhuis S, Beard C, Jaenisch R. Partial rescue of MeCP2 deficiency by postnatal activation of MeCP2. Proc Natl Acad Sci USA 2007; 104:1931–1936.
41. ■ Schmid DA, Yang T, Ogier M, *et al.* A TrkB small molecule partial agonist rescues TrkB phosphorylation deficits and improves respiratory function in a mouse model of Rett syndrome. J Neurosci 2012; 32:1803–1810.
A TrkB small-molecule agonist improves molecular and respiratory abnormalities in a mouse model of Rett syndrome and highlights the TrkB BDNF receptor as a good therapeutic candidate. Analysis of signaling pathways reveals complex region-specific activity modifications of MAPK and Akt.
42. Ogier M, Wang H, Hong E, *et al.* Brain-derived neurotrophic factor expression and respiratory function improve after ampakine treatment in a mouse model of Rett syndrome. J Neurosci 2007; 27:10912–10917.
43. Kline DD, Ogier M, Kunze DL, Katz DM. Exogenous brain-derived neurotrophic factor rescues synaptic dysfunction in MeCP2-null mice. J Neurosci 2010; 30:5303–5310.
44. Chang Q, Khare G, Dani V, *et al.* The disease progression of MeCP2 mutant mice is affected by the level of BDNF expression. Neuron 2006; 49:341–348.
45. Abuhatzira L, Makedonski K, Kaufman Y, *et al.* MeCP2 deficiency in the brain decreases BDNF levels by REST/CoREST-mediated repression and increases TrkB production. Epigenetics 2007; 2:214–222.
46. Johnson RA, Lam M, Punzo AM, *et al.* 7,8-Dihydroxyflavone exhibits therapeutic efficacy in a mouse model of Rett syndrome. J Appl Physiol 2012; 112:704–710.
47. ■ Deogracias R, Yazdani M, Dekkers MP, *et al.* Fingolimod, a sphingosine-1 phosphate receptor modulator, increases BDNF levels and improves symptoms of a mouse model of Rett syndrome. Proc Natl Acad Sci USA 2012; 109:14230–14235.
Fingolimod increases the levels of BDNF and MAPK in a mouse model of Rett syndrome, improving lifespan and motor skills. This is an approved drug for multiple sclerosis and also crosses the blood–brain barrier.
48. Castro J, Garcia R, Kwok S, *et al.* Functional recovery with recombinant human IGF-1 treatment in a mouse model of Rett syndrome. New Orleans: Society for Neuroscience; 2012.
49. Yoshii A, Constantine-Paton M. Postsynaptic BDNF-TrkB signaling in synapse maturation, plasticity, and disease. Dev Neurobiol 2010; 70:304–322.
50. Deak F, Sonntag WE. Aging, synaptic dysfunction, and insulin-like growth factor (IGF)-1. J Gerontol A Biol Sci Med Sci 2012; 67:611–625.
51. ■ Ricciardi S, Boggio EM, Grosso S, *et al.* Reduced Akt/mTOR signaling and protein synthesis dysregulation in a Rett syndrome animal model. Hum Mol Genet 2011; 20:1182–1196.
This study demonstrates the direct relationship between signaling pathways and development of symptoms in a mouse model of Rett syndrome.
52. Yoshii A, Constantine-Paton M. BDNF induces transport of PSD-95 to dendrites through PI3K–Akt signaling after NMDA receptor activation. Nat Neurosci 2007; 10:702–711.
53. Kumar V, Zhang MX, Swank MW, *et al.* Regulation of dendritic morphogenesis by Ras–PI3K–Akt–mTOR and Ras–MAPK signaling pathways. J Neurosci 2005; 25:11288–11299.
54. Cuesto G, Enriquez-Barreto L, Carames C, *et al.* Phosphoinositide-3-kinase activation controls synaptogenesis and spinogenesis in hippocampal neurons. J Neurosci 2011; 31:2721–2733.
55. ■ De Filippis B, Fabbri A, Simone D, *et al.* Modulation of RhoGTPases improves the behavioral phenotype and reverses astrocytic deficits in a mouse model of Rett syndrome. Neuropsychopharmacology 2012; 37: 1152–1163.
The first study to describe the therapeutic role of RhoGTPase activator delivered by the intracerebroventricular injection in a mouse model of Rett syndrome.
56. Tolias KF, Duman JG, Um K. Control of synapse development and plasticity by Rho GTPase regulatory proteins. Prog Neurobiol 2011; 94:133–148.
57. Viemari JC, Roux JC, Tryba AK, *et al.* MeCP2 deficiency disrupts norepinephrine and respiratory systems in mice. J Neurosci 2005; 25: 11521–11530.
58. Medrihan L, Tantalaki E, Aramuni G, *et al.* Early defects of GABAergic synapses in the brain stem of a MeCP2 mouse model of Rett syndrome. J Neurophysiol 2008; 99:112–121.
59. Abdala AP, Dutschmann M, Bissonnette JM, Paton JF. Correction of respiratory disorders in a mouse model of Rett syndrome. Proc Natl Acad Sci USA 2010; 107:18208–18213.
60. Roux JC, Dura E, Moncla A, *et al.* Treatment with desipramine improves breathing and survival in a mouse model for Rett syndrome. Eur J Neurosci 2007; 25:1915–1922.
61. ■ McCauley MD, Wang T, Mike E, *et al.* Pathogenesis of lethal cardiac arrhythmias in MeCP2 mutant mice: Implication for therapy in Rett syndrome. Sci Transl Med 2011; 3:113ra125.
By brain-specific removal of MeCP2 in mice, the authors show that prolonged QT intervals and potentially other cardiac endophenotypes in Rett syndrome are centrally generated. Mutant cardiomyocytes have altered electrical properties probably caused as an indirect response to the alterations of the nervous system control of the heart. Treatment with phenytoin, a sodium channel blocker, shortens the QT intervals.
62. Schaevitz LR, D'Iddio E, Iannoni R, *et al.* Acetyl-L-carnitine improves behavioral and dendritic morphology abnormalities in a mouse model of Rett syndrome. 849.05, Society for Neuroscience Meeting; 2011.
63. Ellaway CJ, Peat J, Williams K, *et al.* Medium-term open label trial of L-carnitine in Rett syndrome. Brain Dev 2001; 23 (Suppl. 1):S85–S89.
64. ■ Grosser E, Hirt U, Janc OA, *et al.* Oxidative burden and mitochondrial dysfunction in a mouse model of Rett syndrome. Neurobiol Dis 2012; 48:102–114.
The authors show for the first time that hippocampal slices of MeCP2 mutant male mice present an increased cytosolic oxidative state and more intense responses when an oxidative challenge is induced.
65. De Felice C, Signorini C, Leoncini S, *et al.* The role of oxidative stress in Rett syndrome: an overview. Ann N Y Acad Sci 2012; 1259:121–135.
66. ■■ Derecki NC, Cronk JC, Lu Z, *et al.* Wild-type microglia arrest pathology in a mouse model of Rett syndrome. Nature 2012; 484:105–109.
By grafting wildtype bone marrow into two different MeCP2 knockout models for Rett syndrome, the study shows that disease progression can be significantly arrested. To date, this therapy has reported the highest improvement in the phenotype of MeCP2-deficient mice.

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<AQ5>	Please check whether the single page detail provided in Refs. [3,61] is correct. If not, please provide the page range detail.	Ref 3: Is correct, one-page brevia comment Ref 6: Is correct
<AQ6>	Please check and confirm the table caption.	See edits