Advances in understanding of Rett syndrome and *MECP2* duplication syndrome: prospects for future therapies

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The X-linked gene encoding *MECP2* is involved in two severe and complex neurodevelopmental disorders. Loss of function of the MeCP2 protein underlies Rett syndrome, whereas duplications of the *MECP2* locus cause *MECP2* duplication syndrome. Research on the mechanisms by which MeCP2 exerts effects on gene expression in neurons, studies of animal models bearing different disease-causing mutations, and more in-depth observations of clinical presentations have clarified some issues even as they have raised further questions. Yet there is enough evidence so far to suggest possible approaches to therapy for these two diseases that could go beyond attempting to address specific signs and symptoms (of which there are many) and instead target the pathophysiology underlying *MECP2* disorders. Further work could bring antisense oligonucleotides, deep brain stimulation, and gene therapy into the clinic within the next decade or so.

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Introduction

It has been more than 50 years since Andreas Rett first described the unusual clinical entity that came to be known as Rett syndrome (Online Mendelian Inheritance in Man number 312750), and 20 years since the discovery that Rett syndrome is caused by mutations in X-linked *MECP2*.[1] Intensive research of all types, from molecular studies of MeCP2–DNA interactions to clinical studies delineating the spectrum of *MECP2*-related disorders—which now also includes *MECP2* duplication syndrome—have given rise to a complex picture of MeCP2 function. As its name suggests, the MeCP2 protein binds methylated DNA to influence or coordinate the expression of thousands of genes.[2] MeCP2 itself is ubiquitously expressed but reaches its greatest abundance in the brain: there might be as many as one molecule of MeCP2 for every two nucleosomes in the nucleus of mature neurons.[3] This abundance, plus the status of MeCP2 as an unstructured protein that can adopt multiple functional conformations, has made its molecular activities difficult to delineate: it seems to both repress and activate transcription, interact with the RNA splicing machinery, interact with the microRNA machinery, and have special functions in maintaining neuronal synapses. There is an ongoing debate about the molecular functions of MeCP2.[4–7]

In this Review, we focus on the manifestations of *MECP2*-related disorders and their neural underpinnings, and highlight studies that may lead the search for viable therapies in useful new directions. We are particularly interested in the insights provided by these studies that are beginning to answer some long-standing questions. Are children affected by Rett syndrome completely healthy before the regression phase? Why do Rett syndrome and *MECP2* duplication syndrome seem to share several clinical features? Is it useful to target specific molecules to treat a disease that involves transcriptional alterations in thousands of genes? Is gene therapy a realistic goal? What treatments might help affected children now, while we wait?

When does Rett syndrome actually begin?

Classic Rett syndrome, in contrast to mild or severe forms of the disease, has been conceptualised as evolving over four stages (table 1; panel).[8,10] In stage one, developmental maturation stalls somewhere between 6 months and 18 months, after a period of so-called normal development. About half of children with Rett syndrome develop microcephaly or show a deceleration of head growth during this period. The second stage, which can occur between the age of 1 year and 4 years, is marked by regression (loss of acquired skills over weeks or months). The child's purposeful hand movements are replaced by movements such as clapping or wringing—99·5% of girls in the Rett syndrome Natural History Study[11] had hand stereotypies—and social withdrawal that often leads to a diagnosis of autism. This stage also sees the development of ataxia (if the child is ambulatory) and apraxia. Autonomic dysfunction leads to respiratory dysrhythmias such as the involuntary holding of breath, hyperventilation, and apnoea. Sleep disturbances are common, as are cold hands and feet, and many children develop a tremor, daytime bruxism, spells of inappropriate laughing, or periods of inconsolable crying or screaming that can go on for weeks or months. The third stage begins between the ages of 2 and 10 years and brings the deterioration to a plateau: there is nominal recovery or stabilisation of motor functions, less irritability, and a greater inclination to interact, but many children also develop seizures at this stage. Stage four, or late motor deterioration, begins when a child is older than 10 years and can last for decades. This stage is characterised by parkinsonism (rigidity and bradykinesia in addition to the aforementioned tremor), joint contractures, scoliosis, muscle weakness, and osteoporosis, although comprehension, communication, and hand skills might improve somewhat and seizures can diminish. Life expectancy has been extended with better supportive care (eg, surgical correction of scoliosis can relieve postural pressure on respiratory movements), and women with Rett syndrome often survive past their 40s or 50s. Cardiorespiratory issues are the most common causes of death.[12]

	Percentage of individuals with Rett syndrome with these features
Main criteria	
A period of regression (age range 1–4 years) followed by recovery or stabilisation (2–10 years)	96–99%
Partial or complete loss of acquired purposeful hand skills	87–100%
Partial or complete loss of acquired language skills	87–99%
Gait abnormalities (dyspraxic or absent)	45–99%
Stereotypical hand movements, typically centred in the midline (wringing or squeezing, clapping, flapping, tapping; mouthing, washing, or rubbing)	68–100%
Supportive criteria	
Breathing dysrhythmias when awake	54–99%
Bruxism when awake	62%
Impaired sleep	45–80%
Abnormal muscle tone	..
Peripheral vasomotor disturbances (small, cold hands and feet)	75–99%
Scoliosis or kyphosis	≥52%
Growth retardation or deceleration of head growth	54–80%
Inappropriate laughing or screaming spells	30%
Diminished response to pain	..
Intense eye communication (so-called eye pointing)	88%

Where available, the percentage of individuals with classic Rett syndrome with each feature are shown.[8,9] The sometimes wide range of percentages is mostly because of different cohorts and evaluations at different disease stages; these percentages are offered only to give a general guide to the relative likelihood.

Table 1: **Main and supportive criteria for diagnosing Rett syndrome**

Animal models replicate this pattern to a remarkable degree: male *Mecp2* knockout mice appear grossly normal until 3–5 weeks of age, when they develop an ataxic gait, rigidity, hypoactivity, irregular breathing, nervousness, tremor, and premature death by 10–12 weeks.[13,14] Heterozygous female mice develop a more variable phenotype about 1 month later, depending on genetic background, pattern of X chromosome inactivation (XCI), and how the pups are nurtured.[15] Remarkably, turning off *Mecp2* in juvenile mice (5–10 weeks old), leads to the same symptoms within 3 months,[16,17] showing that MeCP2 has a crucial function in neuronal maintenance. There seems to be an even more crucial period for MeCP2 function at 15 weeks: one study[18] found that silencing *Mecp2* in 15-week-old adult male mice caused them to die within days, rather than weeks or months. At this age, the murine brain is reaching full maturation; at 10 and 15 weeks the brain size and dendritic complexity increase substantially, and synaptic proteins are upregulated. Intriguingly, the amounts of MeCP2 protein do not continue to rise during this transitional period, suggesting that some perhaps as-yet-undefined function of MeCP2, such as regulating synaptic protein expression, becomes crucial at this stage. Additional work in animal models supports the notion that MeCP2 is required for experience-dependent plasticity and activity-dependent gene transcription—namely, all forms of learning—but the precise mechanisms have yet to be established.[19–24]

Both the diagnostic criteria for classic Rett syndrome and the many animal studies that rely on male mice have a similar drawback: by prioritising homogeneity (for clinical studies[25]) and reproducibility (for animal studies), they might not reflect the extreme variability of phenotypes that can be caused by *MECP2* mutations.[26,27] Mutations in *MECP2* can cause anything from a severe disease to a phenotype so mild it hardly looks like Rett syndrome. The phenotype depends on the specific mutation and its combination with XCI, which can be skewed favourably toward the wild-type allele. Unfavourable XCI has not been reported in female patients with Rett syndrome or *MECP2* duplication syndrome, outside of X-autosome translocation (Zoghbi HY, unpublished). A genotype–phenotype study in boys with *MECP2* mutations showed a range of severity from fatal neonatal encephalopathy to psychiatric abnormalities (ie, schizophrenia or bipolar disorder), depending on the type and position of the mutation.[27] Accordingly, we propose that psychiatric symptoms co-occurring with a tremor or developmental disability in boys should prompt testing for *MECP2* mutations.

Moreover, as is evident from boys with severe inactivating mutations who show neonatal encephalopathy, the period of early development is not invariably healthy. The caveat here is that male patients, unlike female patients, are MeCP2-deficient in every cell, rather than mosaic for MeCP2 deficiency. Early difficulties with feeding and the delayed acquisition of motor skills, such as sitting independently and learning to crawl, have been well documented in girls who were later diagnosed with Rett syndrome, but such signs are non-specific and can occur even in healthy children. There is a growing literature on the topic of pre-regression signs,[28] but in light of the difficulty of achieving rigorous clinical studies of children prior to diagnosis with a rare disease, animal models might provide a better means of examining the early postnatal period. *Mecp2*-null mice do show subtle behavioural, physiological, and neuro-morphological alterations from birth,[28] such as differences in ultrasonic vocalisations, but whether the mosaic loss of MeCP2 function that causes classic Rett syndrome produces reliable early signs has not been established yet.

Improving the differential diagnosis of Rett syndrome

Delving into the factors that influence diagnosis, a longitudinal referral to treatment study[29] showed several interesting observations. The average age at which Rett syndrome is diagnosed has been steadily decreasing and now hovers around a median age of 30–36 months for classic Rett syndrome, and 3–6 years for atypical forms of the disease. Most diagnoses are made by specialists (developmental paediatricians, neurologists, and geneticists) rather than paediatricians; access to specialists and a higher socioeconomic status correlated with an earlier diagnosis, but so did specific disease features. Children tend to be diagnosed earlier if they are delayed in pulling

to stand, walking, or finger feeding; but surprisingly, children who developed and then lost more advanced skills, such as pincer grasping, actually took longer to diagnose. Children with a healthy head size also took more time to diagnose because of a persistent but erroneous belief that microcephaly is a necessary feature of Rett syndrome. Paradoxically, children who displayed early microcephaly also had a delayed diagnosis, indicating that the presence of this feature did not raise suspicion of Rett syndrome, although it should. General paediatricians were most likely to diagnose Rett syndrome if the child could no longer be soothed by the parent or respond to simple commands, but were less likely to diagnose Rett syndrome in the presence of unusual stereotypies or a loss of ability to respond to more complex commands. Midline hand stereotypies, which are pathognomonic for Rett syndrome, were often present for more than 1 year before the child was diagnosed. The hallmark features of Rett syndrome (regression and hand stereotypies) are therefore not triggering clinical suspicion as they should.

The confusion with autism, especially during stage two, is an understandable one, especially since children with autism often develop hand stereotypies, but videotape analysis of 20 children with Rett syndrome and 20 children[30] with severe autism diagnosed on the basis of Diagnostic and Statistical Manual of Mental Disorders III-R criteria discerned several distinctions between the stereotypies in these groups. All hand movements in patients with Rett syndrome were in the midline; hand washing or wringing was observed only in those with Rett syndrome; whereas only children with autism made repetitive motions involving objects. The hand movements were continuous in children with Rett syndrome, but intermittent in those with autism. Although this cohort was small, the observations were largely borne out by the much larger Natural History Study[11] of almost 1000 girls, in which three-quarters showed midline positioning, for example.

Currently, a diagnosis of Rett syndrome is made if the child has classic clinical features (table 1) plus a molecular confirmation of a *MECP2* variant. In the approximately 6% of girls with typical features of Rett syndrome and no known pathogenic variant, Rett syndrome is diagnosed if the patient meets all four main criteria (table 1) and deficits are not due to brain injury secondary to trauma, neurometabolic disease, or infection. There are several other genes whose mutation causes features that overlap with Rett syndrome,[31–35] but careful clinical assessments have revealed differentiating characteristics. Children with mutations in *CDKL5*, for example, tend to have an onset of seizures before 12 months of age, whereas children with mutations in the *FOXG1* tend to have so-called congenital phenotypes and structural brain changes; both groups tend to have more severe motor and verbal disabilities.[34] Nevertheless, molecular diagnosis is the only way to reliably distinguish Rett syndrome from other neurodevelopmental disorders.

Panel: A portrait of classic Rett syndrome

An 18-month-old girl was referred to a developmental paediatrician by her primary care physician because of concerns about global developmental delays and a possible loss in language and social interaction skills. Her birth history was unexceptional, except her mother noted that she moved less in utero than her older sister did. The parents noted slightly delayed milestones compared with her older sister, but she was meeting all the expected milestones by 12 months—ie, she was sitting up at 7 months, pulling to a stand at 10–11 months, and saying a few words by 12 months. At around 14 months, the parents noticed the girl was not bearing weight on her left leg and had developed a shoulder-shrug tic. At 15 months, she stopped waving goodbye and stopped using the three words and phrases she knew well: "Gahsius" (for gracias), "tat-oo" (for thank you), and "E-I-E-I-O" (from the children's song).

She had a leg radiograph and was evaluated by physical therapists at 15 months, but there were no specific musculoskeletal abnormalities. She received physical therapy for gross motor delays and fine motor delays. The audiometry evaluation was normal, but she required speech therapy for her language delays. Some of her developmental skills improved with physical therapy, such as standing up to cruise with handholding.

At 17 months she began drooling excessively. At 18 months, she still put everything she grabbed in her mouth and constantly ground her teeth. On examination, her head was noted to occasionally drop forwards as if she was unable to control it.

She was seen in a genetics clinic at 25 months, when she was noted to have tremor of both hands and sometimes the head. Her examination also noted a head circumference between the 25th and 50th percentile, facial asymmetry, and some gait ataxia. Multiple clinicians from developmental, neurological, and genetic perspectives suspected Angelman syndrome. However, brain MRI, karyotype analysis, chromosomal microarray, fragile X testing, urine organic acid, serum amino acid, long chain fatty acid, and Angelman sequencing results were all normal (these tests were done during a time when whole exome sequencing was not yet financially and clinically feasible). Rett syndrome sequencing showed R168X because of a nucleotide change of 502C→T in one copy of the *MECP2* gene.

With continued physical, occupational, and speech therapy, she was able to walk up steps with assistance by 48 months, and was able to use her hands to feed herself, and make choices using her eye gaze and two or three meaningful words. She showed continual midline repetitive hand movements and hand-in-mouth behaviours. She walked on her toes and had increased tone at the left ankle. Her feet were cold to the touch, and at times purple.

Just before her 6th birthday, she had her first seizure, which was characterised as tonic activity of the left side of the body with a right central parietal electrographic correlate on EEG. She also developed generalised tonic-clonic seizures shortly thereafter. She was started on levetiracetam (25 mg/kg per day).

Now 16 years old, the girl is on levetiracetam (90 mg/kg per day) monotherapy for seizures, has a T4-L4 posterior spinal fusion for scoliosis of 64 degrees, does not express words, walks on her toes, and uses night time ankle-foot orthosis and periodic botox injections for spasticity. She has received physical therapy nearly every week for the past 15 years.

Clinical features of *MECP2* duplication syndrome and areas of overlap with Rett syndrome

Duplications (or, more rarely, triplications) spanning *MECP2* in Xq28 cause a progressive neurodevelopmental disorder that affects about 1% of male patients with an X-linked intellectual disability.[36] Intriguingly, *MECP2* duplication syndrome shares some features with Rett

	Current treatment options	Treatments under investigation
Epilepsy (present in 80% of patients with Rett syndrome, 50% of patients with *MECP2* duplication syndrome)	Valproate, lamotrigine, carbamazepine, ketogenic diet[43,44] (many Rett syndrome and *MECP2* duplication syndrome seizures are refractory to treatment)	Triheptanoin (estimated enrolment 12 participants, 1–4 mg/kg bodyweight for 4 months; NCT02696044),[45] ketamine (estimated enrolment 48 participants, 0·75–4·50 mg/kg twice a day *vs* placebo for 5 days; NCT03633058);[46] studies were done only in patients with Rett syndrome
Difficulty initiating and maintaining sleep (prevalent in Rett syndrome)	Sleep hygiene: alter environment, sleep schedule, sleep routines, physiological factors such as meals, caffeine, and exercise;[47] melatonin, clonidine, trazadone	None known
Respiratory dysrthymias due to aberrant aminergic neuronal pathways in the medulla (prevalent in Rett syndrome)	None known	Sarizotan (studied only in Rett syndrome; estimated enrolment 129 participants, 2–10 mg twice a day *vs* placebo for 6 months; completed, results awaited; NCT02790034)
Sialorrhea (hypersalivation; present in both)	None known	Ultrasound-guided salivary gland botulinum toxin injection (small efficacious study in Rett syndrome only, expecting a larger long-term study)[49]
Learning impairment (present in both)	None known	Forniceal deep brain stimulation efficacious in a preclinical study in a mouse model of Rett syndrome;[50] although invasive, this stimulation is generally well-tolerated, safe, and modifiable
Bone health (fragility or fractures and scoliosis are major causes of morbidity;[51] prevalent in Rett syndrome)	Bisphosphonates[52] increase intentional ambulation; surgical spinal fusion extends life expectancy[53] (in Rett syndrome only)	None known
Immunodeficiency (in *MECP2* duplication syndrome only)	Pneumococcal immunisation	Long-term prophylactic antibiotics might be worth the risk of potential bacterial resistance to prevent major pulmonary infections[54]

Table 2: **Current and experimental treatments for the isolated features of Rett syndrome and MECP2 duplication syndrome**

syndrome: hypotonia shifting to progressive spasticity, motor and cognitive delays, sparse speech, seizures, stereotypies, ataxic gait, regression (loss of gross motor skills), sleep disturbances, bruxism, screaming or laughing spells, and small, cold feet.[36,37] The most obvious differences are that children with *MECP2* duplication syndrome are much more likely to be male and have recurrent pulmonary infections; many are hospitalised with pneumonia multiple times in the first few years of life.[36–38] Immunodeficiency seems to be more severe in male than female patients.[37] Comparing the *MECP2* duplication syndrome and Rett syndrome phenotypes, *MECP2* duplication syndrome rarely causes breathing dysrhythmias during wakeful periods; hand stereotypies in *MECP2* duplication syndrome develop around the age of 3 years, and do not necessarily interfere with purposeful hand use, whereas in Rett syndrome they replace purposeful hand movements around 2 years of age.[38] *MECP2* duplication syndrome is also associated with facial dysmorphias, whereas Rett syndrome is not.[38] Regression or loss of speech and motor skills tends to occur later, at approximately 6 years old, and more gradually in *MECP2* duplication syndrome than in Rett syndrome, although for two of nine male patients in the case cohort study[38] who lost hand function, the decline was abrupt. Roughly a third of this cohort had hearing loss,[38] which is slightly more common than has been reported in Rett syndrome.[39] Approximately a quarter of patients with *MECP2* duplication syndrome die before the age of 25 years, often from respiratory infections; some children have died of (or with) severe pulmonary hypertension as early as 11 months old, although death in infancy is more strongly associated with the triplication of the *MECP2* locus.[36,38] Approximately 60% of children with *MECP2* duplication syndrome develop seizures,[37,38] which tend to be refractory to medication; of these children, approximately half meet the criteria for Lennox-Gastaut syndrome,[40] and the onset of regression coincides with the development of epilepsy.[40] It is important to note, however, that seizure onset in *MECP2* duplication syndrome tends to be much later than in Rett syndrome, with a median age of 8 years.[37]

Although few clinical differences have been noted between male and female children with *MECP2* duplication syndrome, skewed XCI can prevent the duplication from being fully penetrant in women and girls. Thus, in contrast to Rett syndrome, which is nearly always sporadic, *MECP2* duplication syndrome is typically inherited from mothers with favourable XCI. Note that carrier mothers with favourably skewed XCI might show psychiatric symptoms, such as generalised anxiety, depression, and compulsions, that precede the birth of their affected children.[41,42] These mothers also seem to have greater difficulties conceiving or more difficult pregnancies, or both.[37] Therefore, we propose that mothers of children with *MECP2* duplication syndrome—or of boys with the much rarer loss-of-function mutations in *MECP2*—should be tested and genetic counselling offered to the whole family. A history of unexplained neurological disorders and the premature death of male relatives of a woman planning to have a child also warrants testing.

	Preclinical outcomes	Clinical outcomes
BDNF augmenters		
Fingolimod	Increases BDNF concentration, locomotor activity, and striatal size in a mouse model of Rett syndrome[55]	Trial completed, no results published yet (0·25 mg *vs* 0·5 mg; primary outcome measure, BDNF concentrations in the blood and cerebrospinal fluid before and during treatment measured for 12 months, n=6; NCT02061137)
IGF-1 augmenters		
Clenbuterol (β2-adrenergic agonist)	Increases endogenous IGF-1, extends survival in mice with Rett syndrome[56]	No trial yet
Mecasermin (human recombinant IGF-1)	Improves breathing abnormalities, reduces anxiety in mice with Rett syndrome[57]	Safe in girls, might ameliorate breathing abnormalities (12 girls with *MECP2* mutations, nine with Rett syndrome; NCT01253317),[58] might have no clinical effect (crossover trial, n=30; NCT01777542)[59]
Trofinetide (terminal tripeptide of IGF-1)	Trofinetide improves locomotor function, breathing, heart rate, and lifespan of *Mecp2* null mice[60]	Safe in girls, some efficacy shown in early small studies (n=56, randomly assigned in dose-escalation trial);[61,62] ongoing efficacy (NCT04181723) and safety tolerability (NCT04279314) studies
GABAergic modulators		
Picrotoxin ($GABA_A$ antagonist)	Improves motor coordination and episodic memory in mouse model of *MECP2* duplication syndrome[63]	No trial yet
Glutamatergic modulators		
Ketamine (NMDA receptor antagonist)	Improves cortical function and extends life span of mice with Rett syndrome[46]	Clinical trial ongoing (estimated enrolment 48 participants; NCT03633058)
Dextromethorphan (NMDA receptor antagonist)	No animal studies	Dose-dependent improvement in seizures, receptive language, and behavioural hyperactivity (n=35, open-label trial, no placebo group; NCT00593957)[64]
σ-1 agonist		
Anavex 2-73 (blarcamesine)	Female heterozygous Rett syndrome mice have improved motor coordination, balance, acoustic and visual responses, and respiration[65]	Multiple clinical studies ongoing (NCT04304482; NCT03941444; NCT03758924)
Aminergic agents		
Levodopa/dopa-decarboxylase (dopaminergic)	Improves mobility and breathing in Rett syndrome mouse model[66]	No trial yet
Sarizotan (serotonin 1A receptor agonist/D_2 partial agonist)	Corrects irregular breathing and apnoea in mouse model of Rett syndrome[67]	Sarizotan (estimated enrolment 129 participants, 2–10 mg per day *vs* placebo; completed, results awaited; NCT02790034)
Metabolic interventions		
Triheptanoin	Improves motor function, longevity and biochemical markers in a mouse model of Rett syndrome[45]	Clinical trial ongoing (estimated enrolment 12 participants, single group; NCT02696044)
Vatiquinone	Improves mitochondrial function	Phase 2 study completed, no published results (24 enrolled participants, EPI-743 15 mg/kg per day *vs* placebo; NCT01822249)
Neuronal stimulation		
Deep brain stimulation (forniceal)	Improves spatial learning and memory in mice with Rett syndrome[50]	No trial yet
Genetic manipulation		
Gene replacement (in Rett syndrome)	Reverses molecular and behavioural deficits of Rett syndrome[68]	No trial yet
Antisense oligonucleotides to suppress Mecp2 levels (in *MECP2* duplication syndrome)	Reverses molecular and behavioral deficits of *MECP2* duplication syndrome[69]	Clinical readiness trials ongoing
CRISPR-Cas9 genetic engineering (in Rett syndrome)	Repair of mutation in human pluripotent stem cells[70]	No trial yet

Table 3: **Therapeutic interventions that seek to address underlying defects in Rett syndrome and MECP2 duplication syndrome**

Approaches to therapy in Rett syndrome and *MECP2* duplication syndrome

Over the past few decades, basic research discoveries have enabled the development of dozens of potential therapies, which have been or are being tested in clinical trials. Therapeutic approaches for Rett syndrome fall into three general categories: symptom treatment (table 2), pharmacological modulators of downstream MeCP2 targets (table 3), and genetic interventions (still experimental; table 3). In this section we present several discoveries that are likely to guide future approaches.

MeCP2 neurobiology in Rett syndrome: from gene targets to neural circuits

MeCP2 is expressed throughout the body, but to date the only aspects of Rett syndrome that are attributable to peripheral tissue are exercise, fatigue, and bone defects.[71] The majority of the Rett syndrome phenotype arises from

the loss of MeCP2 function in the CNS, as shown by mice with the deletion of *Mecp2* in specific neuronal types or brain regions.[72,73] Whatever the tissue, MeCP2 clearly regulates gene expression, but exactly how it does so has yet to be elucidated.[4–7]

One of the reasons it has been difficult to decipher MeCP2's molecular effects is that the alterations in gene expression after its loss of function are mostly subtle. Furthermore, only a handful of direct targets have been identified: not only does the widespread binding pattern of MeCP2 make it extremely hard to pinpoint which loci are directly regulated, but the loss of MeCP2 compromises neuronal function,[72,73] leading to secondary gene expression changes that can mask the primary changes. Another challenge is neuronal heterogeneity, which occurs at two levels. First, the brain comprises numerous cell types with widely different functions, which could dilute the results of transcriptomic profiling done on whole-brain extracts. Second, even discrete brain regions will be genetically heterogeneous (in women and girls, at least) because of XCI: one neuron will express the wild-type X chromosome whereas another of the same type will express the mutant chromosome. Two studies[74,75] have sought to meet this heterogeneity head on. One group developed single-nucleus RNA sequencing to enable the comparison of gene expression between mutant and wild-type cells within the same individual.[74] Another group tagged MeCP2 with biotin in several different mouse lines bearing different mutations causing Rett syndrome, to separately examine inhibitory and excitatory cortical neurons.[75] This group found that different *Mecp2* mutations exert different effects on gene expression in different cell types, which suggests that downstream molecular therapies would have to be targeted to each different mutation type—a daunting prospect.

However, the investigation of one potentially promising approach was prompted by the recognition that, even though the transcription of thousands of genes is influenced in opposite directions in the two syndromes—ie, genes that are up-regulated in Rett syndrome are down-regulated in *MECP2* duplication syndrome, and vice versa—their phenotypes have a striking amount of overlap. Hypothesising that the same neural circuits are being disrupted, even if by opposing mechanisms, a study[76] investigated the hippocampal circuit in both Rett syndrome and *MECP2* duplication syndrome mouse models to better understand their defects in learning and memory. This study found that Rett syndrome and *MECP2* duplication syndrome model mice show an abnormally high degree of synchrony in the firing activity of hippocampal neurons and cannot restore circuit homoeostasis in response to perturbations of excitatory-inhibitory balance. Furthermore, this study showed that the baseline hypersynchrony is because of MeCP2 dysregulation in excitatory neurons, whereas hypersynchronisation in response to perturbations is because of MeCP2 dysregulation in inhibitory neurons. Lastly, the authors showed that deep brain stimulation corrects these circuit dysfunctions, which might explain why deep brain stimulation rescued hippocampal memory in female mice with Rett syndrome.[50] It is remarkable that deep brain stimulation was able to exert benefits despite substantial cognitive deficits; future work will need to explore the potential use of other deep brain stimulation targets for other features of Rett syndrome and even for other intellectual disabilities. Stimulating neural circuits might be a more promising route to treating complex disorders than trying to address molecular defects one pathway at a time.

That said, transcriptomic studies have yielded several genes that are prominently downregulated by the loss of MeCP2 function in the context of Rett syndrome. Two of these—BDNF and IGF-1, which both mediate neural plasticity—might yet hold therapeutic promise despite disappointing results from early clinical trials (table 3).

BDNF is one of the best-studied factors involved in the progression of the Rett syndrome phenotype in mouse models. The conditional deletion of *Bdnf* in *Mecp2* knockout mice accelerates the onset of symptoms, whereas *Bdnf* overexpression in the *Mecp2* null mice leads to some improvements.[77] This partial rescue was puzzling, but it has been hypothesised that the general upregulation of BDNF did not target the right neural circuits,[78] specifically excitatory glutamatergic neurons.[79] Building on the earlier discovery that axonal transport is disrupted in Rett syndrome neurons,[80] one study[78] stimulated BDNF transport in corticostriatal neurons—first, in an elegant microfluidic network-on-a-chip, then in mice—by increasing the phosphorylation of huntingtin, the protein that is responsible for BDNF vesicular transport. The study found that simply promoting BDNF transport, without increasing BDNF production, was sufficient to restore synaptic connectivity in the corticostriatal network and to improve the phenotype and survival of *Mecp2* knockout mice. One prospective open-label trial in ten girls with Rett syndrome found that glatiramer acetate, which stimulates BDNF secretion in the brain, improved gait velocity, memory, and respiration, and decreased epileptiform discharges,[81] but another study found that three (21%) of 14 patients developed a severe reaction to the glatiramer acetate injections, possibly because of autonomic dysfunction or having a more severe phenotype.[82] Larger scale and placebo-controlled studies are warranted, perhaps combining compounds that stimulate BDNF secretion with those that facilitate its axonal transport.

IGF-1 therapy is another approach that showed promising results in male *Mecp2* null mice: human recombinant IGF-1 (mecasermin) restores synaptic spine density and synaptic transmission, and functionally improves locomotor activity, autonomic responses, and lifespan.[57] Early clinical studies in 12 girls with Rett syndrome showed that mecasermin is safe, although it did not show any clinical improvement in 30 girls with Rett syndrome.[58,59]

Nevertheless, trofinetide, the terminal tripeptide of IGF-1, has a proven safety profile[61] and shows efficacy in improving some Rett syndrome features, such as repetitive face movements, night-time behaviours, and mood, in young girls (5–15 years).[62]

Another study investigated the use of the cannabidiol analogue cannabidivarin on normalising downstream genetic targets. The non-psychoactive cannabinoid reversed memory deficits in mice, along with upregulating BDNF, IGF-1, and cannabinoid receptor expression.[83] A clinical trial (NCT03848832) is investigating the efficacy of cannabidiol oral solution in individuals with Rett syndrome, which might not only reduce seizure frequency[84] but also promote neuronal homoeostasis, leading to other phenotypic improvements.

Gene therapy and gene editing

In theory, the best treatment for Rett syndrome would be to replace the mutant *MECP2* gene with a wild-type version. A study[85] provided proof of principle in 2007, by engineering a mouse model in which an inactivated *Mecp2* allele could be reversibly activated. Male mice born with the gene turned off developed the features of Rett syndrome, but when the gene was turned on, the mice recovered. A subsequent study[68] showed that systemic delivery (by tail vein injection) of adeno-associated virus bearing *Mecp2* largely rescues both male and female mice. Notably, the study found that intracranial injection into the striatum, thalamus, and deep cerebellar nuclei improved some symptoms in another group of mice but also caused them to develop parkinsonism, possibly because of the local overexpression of MeCP2 at the injection sites. One consideration for gene therapy, then, is the need for better vectors that enable even distribution throughout the brain.

The more important problem, however, concerns the dose. Girls with Rett syndrome are not null for *MECP2*, and half of their cells have a normal allele. Because introducing another copy of the gene could raise the amount of MeCP2 to the point of creating features of *MECP2* duplication syndrome, the aim would have to be to deliver just enough MeCP2 (possibly 50% of normal amounts) to rescue the null cells but not enough to cause dysfunction in the wild-type cells. Given the constraints of adeno-associated virus vectors, shortening the coding sequence would allow more room for regulatory sequences that might enable better control of gene expression. This might indeed be possible: a study[86] used a severely truncated MeCP2 protein encompassing two key functional domains (the methyl-binding and transcriptional repression domains) in null male mice to see whether that was sufficient to rescue disease features. Although there was benefit, the effect on symptoms was incomplete and the adeno-associated virus-treated mice died prematurely (although this might have been because of failure to attain adequate amounts of MeCP2). It would be important to do this study in female mice with Rett syndrome and compare the truncated and full-length proteins directly, controlling for MeCP2 amounts, before drawing firm conclusions.

Given the challenge of maintaining proper quantities of MeCP2, site-directed repair might be a better alternative.[87] CRISPR-Cas9 has been proven to be able to edit genes in the neurons of adult living animals,[88,89] and one study repaired experimentally designed *MECP2*^{R270X} human-induced pluripotent stem cells in vitro.[70] There have not yet been mouse studies investigating the efficacy of CRISPR-Cas9 in reversing the Rett syndrome phenotype. One study did, however, harness an enzyme (adenosine deaminase acting on RNA) to specifically convert mRNA A→G in targeted sites on the transcript in a mouse model of G→A mutations.[90] Although G→A mutations account for less than 2% of cases of Rett syndrome (RettBASE), several of the more common C→T mutations create TGA codons (approximately 20% of Rett syndrome cases) that are also theoretically amenable to RNA editing.

For the **RettBASE resource** see http://mecp2.chw.edu.au

The permanence of changes evoked by gene therapy or editases warrants caution.[91] For example, given that restoring MeCP2 function would stimulate neuronal growth and increase brain size, genetic treatments would best be started early (ie, before the skull sutures close). Further, peripheral delivery, which is less invasive, will be a challenge because of the blood–brain barrier.[92] Such considerations add to the challenges directly confronting gene therapy as a technology.

Antisense oligonucleotides to reduce MeCP2 in *MECP2* duplication syndrome

In principle, *MECP2* duplication syndrome should be easier to treat than Rett syndrome, because it should simply be a matter of reducing the amount of the wild-type MeCP2 protein back to a healthy range. Antisense oligonucleotides have been used to silence the extra allele at the mRNA level, both in *MECP2* duplication syndrome mice engineered to bear human *MEPC2* alleles and in human *MECP2* duplication syndrome lymphoid cells.[69] The administration of the antisense oligonucleotides in adult symptomatic mice reversed molecular, behavioural, and synaptic defects.[69] Treatment initiated even in mice that were 7–9 months old stopped their seizures and normalised their EEG.[69] Whereas these results show promise for antisense oligonucleotide therapy, the challenge again is getting the quantity right (too low, and one risks causing Rett syndrome). Fortunately, antisense oligonucleotide effects typically wear off after approximately 12 weeks, so overcorrection could be reversed with an antisense for the antisense oligonucleotides itself, but molecular biomarkers that register early changes in MeCP2 expression are needed to avoid overcorrection. Clinical readiness studies are underway to identify such molecules as a first step to prepare for future clinical trials.

Searching for an alternative approach to reducing MeCP2 protein expression, a forward genetic screen identified

druggable kinases and phosphatases that destabilise, and thereby reduce, the amount of MeCP2.[93] The pharmacological inhibition of protein phosphatase 2A with fostriecin reduced MeCP2 in the CNS and rescued motor abnormalities in mice with *MECP2* duplication syndrome, providing proof of concept that inhibiting positive MeCP2 regulators might be useful for *MECP2* duplication syndrome. This approach has the advantage of being able to fine-tune dosing so that MeCP2 amounts do not drop below the threshold that would induce symptoms of deficiency—ie, Rett syndrome. It does, however, have the limitation of exerting more modest effects than antisense oligonucleotides.

Conclusions and future directions

The field is undertaking every conceivable approach to develop treatments, from deep brain stimulation to gene therapy, antisense oligonucleotides, and pharmacologically targeting neurotrophic pathways or influencing MeCP2 protein stability. A number of basic science discoveries have already led to clinical trials, with some modest successes (table 2, table 3). Yet there are still major unmet needs that we believe are crucial for translating all this progress into viable therapies.

First and foremost it is necessary to have a set of sensitive, specific, and non-invasive biomarkers to track safety and treatment response. Because over-correcting, either by reducing MeCP2 amounts too much in *MECP2* duplication syndrome or increasing them too much in Rett syndrome, is deleterious, biomarkers that can predict the dose needed by each individual patient are needed to achieve close to wild-type function of MeCP2. This will require, among other things, a much better understanding of the effect of specific mutations, some of which seem to primarily destabilise the protein and reduce its expression. For example, a common mutation that spares the methyl-binding domain and transcriptional repression domains but produces a premature stop codon (pLeu386Hisfs*5) reduced MeCP2 amounts by only 16%, yet this decrease is sufficient to cause Rett syndrome.[94] Second, because both Rett syndrome and *MECP2* duplication syndrome have a complex clinical presentation that moves through different stages over time and varies considerably from one individual to the next, we need outcome measures that reflect changes at several levels (molecular, physiological, and behavioural) so that it is possible to develop a composite profile for each individual. Different biomarkers could vary according to both the disease stage and the vagaries of the XCI pattern of a particular patient or the presence of a yet-unidentified genetic or environmental factor that influences disease progression. Moreover, the timing of responses in these different domains is likely to be uncoupled, such that a molecular response might be apparent long before any behavioural change.

Regardless of the treatment approach, an earlier diagnosis is going to be essential to improve the success of any therapy for Rett syndrome or *MECP2* duplication syndrome. The reversal of long-standing cognitive, motor, and behavioural deficits will take concerted rehabilitation efforts, even if the therapy evokes the desired response at the molecular level. We must therefore continue to develop better interventions in the realm of physical, speech, and occupational therapies to address the complex needs of affected children and adults. Nevertheless, the astonishing amount of progress that has taken place in the short time that the genetic causes of Rett syndrome and *MECP2* duplication syndrome have been known gives reason to hope that the lives of individuals and families afflicted with these diseases will be improved within our lifetime.

Search strategy and selection criteria

We searched PubMed and MEDLINE for articles published in English from Jan 1, 2015, to Feb 20, 2020, with the search terms "MECP2", "Rett Syndrome", "MECP2 duplication syndrome", "X chromosome inactivation", "epigenetics", "DNA methylation", "hand stereotypies", "neurogenesis", "triheptanoin", "IGF1", "trofinetide", "sarizotan", "CRISPR-Cas9", "BDNF", "IGF-1", and "deep brain stimulation". We generated the final reference list on the basis of topics that fit the scope of this Review.

Contributors

AJS and HYZ generated the outline of the Review. AJS wrote the first draft and prepared the tables. AJS, VLB, and HYZ revised the manuscript and approved the final version.

Declaration of interests

HYZ serves on the board of directors of Regeneron, and collaborates with Ionis in the setting of *MECP2* Duplication and with Union Chimique Belge in the setting of Parkinson's disease and Alzheimer's disease. AJS and VLB declare no competing interests.

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