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UPDATE

Towards a better diagnosis and treatment of Rett syndrome: a model synaptic disorder

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With the recent 50th anniversary of the first publication on Rett syndrome, and the almost 20 years since the first report on the link between Rett syndrome and *MECP2* mutations, it is important to reflect on the tremendous advances in our understanding and their implications for the diagnosis and treatment of this neurodevelopmental disorder. Rett syndrome features an interesting challenge for biologists and clinicians, as the disorder lies at the intersection of molecular mechanisms of epigenetic regulation and neurophysiological alterations in synapses and circuits that together contribute to severe pathophysiological endophenotypes. Genetic, clinical, and neurobiological evidences support the notion that Rett syndrome is primarily a synaptic disorder, and a disease model for both intellectual disability and autism spectrum disorder. This review examines major developments in both recent neurobiological and preclinical findings of Rett syndrome, and to what extent they are beginning to impact our understanding and management of the disorder. It also discusses potential applications of knowledge on synaptic plasticity abnormalities in Rett syndrome to its diagnosis and treatment.

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Introduction

Rett syndrome (OMIM 312750) is a severe and progressive neurodevelopmental disorder characterized by a wide range of neurologic and behavioural features. With an incidence of 1:10 000–15 000, Rett syndrome is the second most common cause of severe intellectual disability in females and, during its period of developmental regression, a substantial proportion of affected individuals meet diagnostic criteria for autism spectrum disorder (ASD) (Chahrour and

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Zoghbi, 2007; Guy *et al.*, 2011; Percy, 2011; Banerjee *et al.*, 2012; Katz *et al.*, 2016; Leonard *et al.*, 2016). Rett syndrome results in >90% cases from sporadic pathogenic mutations in the X-linked methyl-CpG-binding protein 2 gene (*MECP2*, human; *Mecp2*, mouse; located at Xq28). Other genes, most notably *CDKL5* and *FOXG1*, have been associated with atypical and rarely classic forms of Rett syndrome (Neul *et al.*, 2010; Sajan *et al.*, 2017). While Rett syndrome has not been included as a separate entity within the framework of ASD in the Diagnostic and Statistical Manual of Mental Disorders – Fifth Edition (DSM-5) (American Psychiatric Association, 2013), discoveries following the identification of the link between Rett syndrome and *MECP2* mutations have contributed towards deepening our understanding of the genetic and pathophysiological mechanisms of Rett syndrome and of the neurobiology of ASD and neurodevelopmental disorders in general. In this context, Rett syndrome has become one of the best disease models of abnormal synaptic plasticity. Thus, half a century after the first publication on Rett syndrome, and almost 20 years since the first report on *MECP2* mutations in Rett syndrome, it gives us an opportunity to both look back at the long and winding road the field has travelled, lessons that have been learned, and how they have impacted our understanding and management of the disorder. We also discuss how our knowledge on synaptic plasticity abnormalities in Rett syndrome could improve diagnosis and treatment of the disorder.

Clinical features and genetics of Rett syndrome: a dynamic disorder associated with *MECP2* mutations

Rett syndrome was originally described in German in 1966 by the Viennese paediatrician Andreas Rett (Rett, 1966, 2016). This initial description of 22 female children, which included the core features of the disorder mentioned below, was expanded in 1983 in an article in *Annals of Neurology* by the Swedish child neurologist Bengt Hagberg. This study, which reported 35 cases, established the first link between Rett syndrome and autistic features (Hagberg *et al.*, 1983). Four clinical manifestations constitute the core diagnostic features of Rett syndrome: loss of expressive language; loss of fine motor (i.e. hand) skills; impairment in ambulation; and presence of hand stereotypic movements. These characteristic features, and others more variable in frequency and severity, emerge at different times during the dynamic course of Rett syndrome. After a relatively normal early postnatal period, head growth deceleration and global cognitive and motor delay become apparent and, typically between 1.5 and 3 years, variable loss of spoken language and hands skills develop. Sometimes, ambulation also regresses. Developmental regression is the distinctive diagnostic feature of Rett syndrome; although recovery of function is common, it is usually partial (Neul *et al.*, 2010). Following loss of skills, other clinical manifestations emerge. They include, among others, breathing and other autonomic abnormalities, seizures, scoliosis, and abnormal muscle tone (that constitute supportive criteria for individuals with two or three core features, which are labelled as 'atypical') (Neul *et al.*, 2010). Gastrointestinal and bone density anomalies, both related directly or indirectly to MECP2 deficiency, in conjunction with the abovementioned orthopaedic problems make Rett syndrome a multi-system disorder that requires multi-disciplinary management. The complex evolution of Rett syndrome continues after the relatively stable ('pseudo-stationary') post-regression period, when most of the associated features become evident, and extends into adolescence and adulthood, which are characterized by progressive decline, particularly in the motor domain (i.e. parkinsonian features could also be present at later stages). Behavioural problems can be obvious at different periods in the evolution of Rett syndrome (Buchanan *et al.*, 2018). Autistic features are severe in a subset of individuals with Rett syndrome (~20–50%) almost exclusively during the regression period, with long-standing deficits in social communication and interaction skills in only 10–15% of affected females (Neul *et al.*, 2014; Percy and Glaze, 2017). The complex clinical course of Rett syndrome has been described in different ways, including the four stages proposed by Hagberg (2002), which suggest a continuous disruption in brain development and function. This profile, in conjunction with data discussed in the following sections, suggests a disruption in synaptic function.

From the aetiological viewpoint, a major breakthrough was the report in 1999 by Huda Zoghbi's laboratory of the association between mutations in *MECP2*, a gene located at the Xq28 site, and most cases of Rett syndrome (Amir *et al.*, 1999). Development of mouse models of Rett syndrome followed quickly, first from the laboratories of Adrian Bird and Rudolf Jaenisch (Chen *et al.*, 2001; Guy *et al.*, 2001), opening a floodgate of studies using these preclinical animal models. More recently, patient-derived induced pluripotent stem cell (iPSC) models of human neurons with and without mutations in *MECP2* have provided powerful systems for discovering Rett syndrome mechanisms and potential therapeutics (Marchetto *et al.*, 2010; Li *et al.*, 2013; Mellios *et al.*, 2017).

Rett syndrome is associated with more than 500 pathogenic or likely pathogenic *MECP2* mutations involving mainly exons 3 and 4 and the C-terminal region (Gold *et al.*, 2018). However, eight point *MECP2* mutations, four missense and four nonsense (R106W, R133C, T158M, R168X, R255X, R270X, R294X, and R306C); C-terminal deletions; and exonic deletions account for the vast majority of cases (Neul *et al.*, 2008; Glaze *et al.*, 2010). Mapping mutations in the crystal structure of the protein reveal that most of these mutations are located in three key domains: a methyl-binding domain (MBD), a transcriptional-repression domain (TRD), and nuclear localization signal (NLS) domain (Gold *et al.*, 2018).

While genotype–phenotype correlations are limited, some general profiles are recognized: a group with milder presentation and more protracted course, and a more severe group, including nonsense mutations affecting the transcriptional repression domain of MECP2 (Cuddapah *et al.*, 2014). Intermediate severity *MECP2* mutations have also been reported (Archer *et al.*, 2007), while ~5% of classical and 25% of variant Rett syndrome patients do not have mutations in the *MECP2* (Neul *et al.*, 2014; Gold *et al.*, 2018).

Molecular function of MECP2: regulation of transcription

The fundamental mechanisms of MECP2 function have long been known, yet understanding phenotypic symptoms of MECP2 abnormality is complicated by its complex targets and by pleiotropic effects in target genes and signalling pathways (Ip *et al.*, 2018). However, several recent studies have substantially clarified the mode of action of MECP2 and the effects of *MECP2* mutations. MECP2 was originally identified based on its ability to bind methylated DNA, and was hence considered an epigenetic transcriptional repressor (Lewis *et al.*, 1992; Lyst and Bird, 2015). It was subsequently described as a transcriptional activator or repressor, depending on the molecular context (Chahrour *et al.*, 2008). Functionally, it is now clear that activity-dependent phosphorylation in specific residues of MECP2 mediates its interaction with several important transcriptional co-repressors including the NCoR/SMRT complex (Ebert *et al.*, 2013). While several missense mutations in the MBD of MECP2 are associated with Rett syndrome and variable clinical severity, missense mutations (e.g. R306C) distal to the TRD, in the NCoR interaction domain (NID), abolish the interaction of MECP2 with the NCoR/SMRT co-repressor (Lyst *et al.*, 2013), also causing a disruption in MECP2 function and Rett syndrome with a relatively milder phenotype (Cuddapah *et al.*, 2014). The role of MECP2 in regulating BDNF levels, a major synaptic modulator and one of the first identified targets of MECP2, is an example of the complex mechanisms underlying synaptic activity and transcription. Adequate BDNF levels seem to be defined by brain regional, temporal, and functional state requirements. Whether the latter are reflected by BDNF plasma measurements is still unclear but worth exploring in the context of the development of new treatments (Li and Pozzo-Miller, 2014). During early life experiences, DNA methyltransferase (DNMT3A) transiently binds across transcribed regions of lowly expressed genes, specifying methylated CA (mCA) patterns on which MECP2 can further fine-tune long-lasting cell type-specific transcription via its repression effects (Stroud *et al.*, 2017). Consistent with this pattern, MECP2 represses expression, especially of long mCA-enriched genes (Gabel *et al.*, 2015). The effects of MECP2 seem to be genome-wide and gene body-dependent, rather than promoter/enhancer-specific, and subtle, but correlate well with DNA methylation density, affecting several brain-specific long genes (Kinde *et al.*, 2016; Lagger *et al.*, 2017; Stroud *et al.*, 2017). A recent study reinforces the importance of the MBD and NID by showing that introduction of a truncated protein containing only the MBD and NID domains into MECP2-deficient mice reverses neurological symptoms (Tillotson *et al.*, 2017). Although research on nonsense (truncating) *MECP2* mutations has been more limited, it is critical in the context of the study by Tillotson *et al.* (2017). It is also important because mutations involving the TRD, particularly, the nuclear localization signal within this domain, are associated with the most severe Rett syndrome phenotypes (Cuddapah *et al.*, 2014).

At the same time, several lines of evidence suggest additional mechanisms of MECP2 function. MECP2 is known to interact with 5-hydroxymethylcytosine (5hmC)-bearing DNA (Mellén *et al.*, 2012) and can bind to hmCG, albeit with a lower affinity than hmCA (Kinde *et al.*, 2016). 5hMC is associated with functional demethylation of expressed genes (Mellén *et al.*, 2017), potentially enabling MECP2 to achieve de-repression of transcription. Cell type-specific biotin tagging of MECP2, combined with knock-in mice carrying Rett syndrome-associated mutations (R106W and T158M), reveals gene expression changes specific to each mutation and to excitatory or inhibitory cell types, including reductions in the transcription of long genes and post-transcriptional compensation at the cellular level (Johnson *et al.*, 2017). Furthermore, MECP2 can bind to RNA and regulate alternative splicing and miRNA processing (Young *et al.*, 2005). Importantly, early deficits in neuronal differentiation and migration in MECP2 mutant mice and in cerebral organoids derived from Rett syndrome patients arise from upregulation of miR-199 and miR-214, and their effects on extracellular signal-regulated kinase (ERK/MAPK) and protein kinase B (PKB/AKT) signalling (Mellios *et al.*, 2017). MECP2 deficiency also misregulates miR-15a, affecting brain derived neurotrophic factor (BDNF) expression and dendritic morphogenesis (Gao *et al.*, 2015).

Neurobiology of Rett syndrome: a disorder of plasticity at synapses and circuits

Since the early post-mortem characterizations of brain abnormalities in Rett syndrome, later expanded by *in vivo* neuroimaging, there has been strong evidence for a generalized dendritic and synaptic disorder (Kaufmann and Moser, 2000; Kaufmann *et al.*, 2016). This work has been greatly enhanced by the availability of mouse models of Rett syndrome. Indeed, studies of synapses and neuronal subtypes were the initial focus for assessments of synaptic function in *Mecp2* knockout mice (Banerjee *et al.*, 2012; Zoghbi and Bear, 2012; Sahin and Sur, 2015). Global brain-wide activity mapping using immediate early gene c-Fos expression has revealed distinct functional deficits

including decreased activity levels in key nodes of the default-mode network in forebrain and midbrain areas and hyperexcitability in hindbrain areas (Kron *et al.*, 2012). Thus, the effects of *Mecp2* deletion need to be examined keeping in mind spatio-temporal variations in development and function of different brain regions (Katz *et al.*, 2016). Furthermore, the balance and spatiotemporal relationship between excitation and inhibition appear to be an important facet in Rett syndrome, instead of deficits of either excitation or inhibition alone.

The effects of MECP2 deficiency on both excitation and inhibition can be found on at least three different levels. The first level is anatomical, where increased perisomatic GABAergic terminals (Durand *et al.*, 2012; Krishnan *et al.*, 2015; but see He *et al.*, 2014) and decreased excitatory projections (Sceniak *et al.*, 2016, but see Li *et al.*, 2016) have been found in the cortex and hippocampus. Second, at the level of specific synapses: while the potency of inhibitory synapses in the cerebral cortex of *Mecp2* knockout mice appear not to be changed (Dani *et al.*, 2005; Nelson *et al.*, 2006; Wood *et al.*, 2009), or decreased (Calfa *et al.*, 2015) as measured with the amplitude of miniature inhibitory postsynaptic currents (mIPSC), there is no clear result on excitatory synapses. Increases (Calfa *et al.*, 2015), decreases (Dani *et al.*, 2005; Chao *et al.*, 2007; Wood *et al.*, 2009) or no change (Nelson *et al.*, 2006) have all been reported for excitatory miniature excitatory postsynaptic currents (mEPSC). A reduction of sensory-evoked feedforward excitatory drive has generally been reported in *Mecp2* knockout mice, and this reduction has also been shown to affect several neuronal subclasses including parvalbumin (parvalbumin-positive) expressing inhibitory interneurons (Banerjee *et al.*, 2016). Brainstem circuits, on the other hand, shows reduced GABAergic inhibition and hyperexcitable expiratory neurons resulting in autonomic disturbances such as apnoeas and respiratory irregularities (Abdala *et al.*, 2016). Altered cellular excitability and deficits in synaptic plasticity can, in turn, significantly affect higher-order processes such as impaired spatial and contextual memory formation (Kee *et al.*, 2018). Third, the efficacy of synapses is affected by the internal status of the postsynaptic target neuron. At least one such mechanism in *Mecp2* knockout mice and patients with Rett syndrome has been identified: decreased cross-membrane chloride gradient resulting from reduced potassium chloride co-transporter 2 (KCC2) expression alters the $GABA_AR$ reversal potential and reduces the potency and efficacy of GABAergic inhibition (Duarte *et al.*, 2013; Banerjee *et al.*, 2016).

Alterations in excitation-inhibition (E/I) balance have been shown to have consequences for cortical plasticity in neural circuits. Consistent with a reduction in inhibition received by pyramidal neurons, hence controlling the strength and spatial range of immature inhibition (Lo *et al.*, 2017), monocular deprivation induced ocular dominance plasticity has been found to extend into adulthood in *Mecp2* heterozygous female mice (Tropea *et al.*, 2009; Castro *et al.*, 2014). Parvalbumin-specific deletion in mice leads to immature adult visual cortical plasticity (Banerjee *et al.*, 2016), which is rescued by enhancing inhibition via intracerebral infusion of the $GABA_A$ receptor agonist diazepam (He *et al.*, 2014). Similarly, pup gathering behaviour and associated auditory cortical plasticity are also impaired in *Mecp2* heterozygous female mice (Krishnan *et al.*, 2017). The direction of changes in E/I balance favours excitation: intracellular measurements in cortical neurons reveal that whereas inhibition and excitation are both reduced in *Mecp2* knockout mice, inhibition is reduced to a greater degree, thus enhancing the E/I ratio (Banerjee *et al.*, 2016). Indeed, patients with Rett syndrome (Jian *et al.*, 2007; Glaze *et al.*, 2010; Cardoza *et al.*, 2011) and *Mecp2* knockout mice have reduced threshold and increased propensity for seizures, along with altered network oscillations (Calfa *et al.*, 2011; McLeod *et al.*, 2013; Goffin *et al.*, 2014; Zhang *et al.*, 2014). While some of the changes in *Mecp2* knockout mice can be explained as results of homeostatic maladaptive compensation (Nelson and Valakh, 2015), deleting *Mecp2* from parvalbumin-positive neurons alone causes reduction of inhibitory as well as excitatory drive to visual cortex neurons (Banerjee *et al.*, 2016), similar to brain-wide deletion of *Mecp2*, indicating that reduced inhibition is almost certainly a primary effect of MECP2 deficiency in sensory cortices (*cf.* Robertson and Baron-Cohen, 2017).

One important way the field has advanced is in going beyond simple synaptic measurements in *ex vivo* brain slices and probing alterations in circuits in intact animals *in vivo*. Some of these mechanisms can be used as a new biological variable to study pathophysiological mechanisms contributing to the maladaptive processes in Rett syndrome. In these studies, sensory modalities have served as a window to provide novel and fundamental insights into biological processes of pathophysiology of Rett syndrome (Robertson and Baron-Cohen, 2017). Deficits in maturation of cell type-specific inhibition have emerged as an important facet of the circuit defects in Rett syndrome. Loss of *Mecp2* from forebrain GABAergic neurons recapitulates diverse and prominent features of Rett syndrome (Chao *et al.*, 2010). Interestingly, GABAergic neuron-specific conditional knockout leads to a wide range of symptoms similar to global-knockout of *Mecp2* (Ure *et al.*, 2016), whereas excitatory neuron-specific conditional deletion leads to milder, and specific symptoms, namely, stereotypies and social anxiety (Meng *et al.*, 2016). Furthermore, interneuron-specific re-expression of *Mecp2* has been shown to ameliorate some of the deficits seen in Rett syndrome (Goffin *et al.*, 2014; Ure *et al.*, 2016). Deconstructing MECP2 deficit in specific GABAergic neuronal populations using cell-specific manipulations resulted in parsing out these non-overlapping neurological features in parvalbumin-positive or somatostatin expressing interneurons (Ito-Ishida *et al.*, 2015). Behaviourally, parvalbumin-specific conditional *Mecp2* knockout mice show deficits in sensory, social, and motor dysfunction, whereas somatostatin

conditional knockout mice show seizure and stereotyped behaviour (Ito-Ishida *et al.*, 2015).

Using cell-specific conditional mutant Rett syndrome mouse models, sensitive assays have been developed to study neural signatures of sensory perception. Deletion of *Mecp2* from a small population of parvalbumin-positive and not somatostatin-positive neurons alone recapitulate effects of global *Mecp2* deletion on pyramidal neurons, indicating parvalbumin-positive neurons play a crucial role in propagating the effect of MECP2 deficit in a non-cell autonomous manner to a distributed circuit affecting cortical coding in mouse primary visual cortex (V1). Deleting *Mecp2* globally or from parvalbumin-positive neurons causes a significant increase in response variability, reduced signal-to-noise ratio, and a reduction in response reliability to natural visual scene, significantly altering network correlation structure in V1 (Banerjee *et al.*, 2016). Interestingly, loss- and gain-of-function mutations in *Mecp2* are thought to underlie two distinct neurological syndromes yet both have strikingly similar phenotypic features in animal models (Lombardi *et al.*, 2015), and Rett syndrome and MECP2 duplication syndrome share many neurobehavioural features (Neul *et al.*, 2010; Van Esch, 2012). However, limited attempts have been made so far to understand the exact nature of underlying mechanisms leading to these changes (Lim *et al.*, 2017). Altered visual processing has also been found in a mouse model of *MECP2* duplication syndrome (MECP2 Tg1) showing higher visual acuity and contrast sensitivity at 8 and 14 weeks (Zhang *et al.*, 2017). *Mecp2* overexpressing mice show abnormally elevated synchrony in the firing rate of hippocampal CA1 pyramidal neurons, an impaired homeostatic response to perturbations of E/I balance, and decreased excitatory input onto inhibitory neurons (Lu *et al.*, 2016). Mice overexpressing *Mecp2* also demonstrate altered structural plasticity, including altered stability of dendritic spine clusters and enhanced motor learning (Jiang *et al.*, 2013; Ash *et al.*, 2018).

Although the data reviewed here emphasize the individual neuron and circuit bases of MECP2 deficiency and Rett syndrome, contributions from astrocytes and, perhaps also, microglia should not be overlooked. Their role in regulating glutamate levels and other aspects of synaptic function has already been postulated as a mechanism underlying Rett syndrome (Kaufmann *et al.*, 2016). The ability to gain insight into glial abnormalities through *in vivo* neuroimaging (Horská *et al.*, 2009) also make glia an important target for the development of synaptic biomarkers in Rett syndrome.

Despite our detailed understanding of synaptic alterations in Rett syndrome, many questions still remain about abnormalities in synaptic plasticity in Rett syndrome and MECP2 deficiency. Do the initial synaptic deficits become less modifiable or enhanced by inefficient compensatory processes? What is the timeline of these processes in humans with MECP2 deficiency? Additionally, is it possible to improve E/I imbalances of different nature in different brain regions (i.e. cortical hypoexcitability, brainstem hyperexcitability) using the same therapeutic strategy?

Management of Rett syndrome: current practices and development of new treatments

Considering the multi-systemic involvement in Rett syndrome, a coordinated multidisciplinary approach to medical care and management is the preferred option. At an early age, shortly after the diagnosis, intensive early intervention as applied to other neurodevelopmental disorders is recommended (Warren *et al.*, 2011). Recently, this approach has received empirical support from a recent environmental enrichment trial consisting of motor learning and exercise, combined with social, cognitive and sensory enrichment. The 6-month study on 12 females with Rett syndrome, under age 6, resulted in improvements in gross motor function and increased plasma BDNF levels but no changes in growth, sleep, or behaviour (Downs *et al.*, 2018). Currently, there are no specific treatments for Rett syndrome; therefore, medical management is symptomatic. In addition to traditional drug treatments [e.g. antiepileptic drugs for seizures, selective serotonin reuptake inhibitors (SSRIs) for anxiety], preventive approaches that include nutritional management, with emphasis on caloric intake and vitamin D levels, prevention of gastrointestinal and orthopaedic complications, and personalized rehabilitation therapies, are becoming standards of care. For details on management, see corresponding chapters in Kaufmann *et al.* (2017).

The last few years have been a very active period for clinical trials in Rett syndrome. In contrast with previous studies, this new era attempts to take advantage of the neurobiology of the disorder. Specifically, most trials have been based on proof-of-principle research in *Mecp2*-deficient mice. A few studies have focused on a specific neurotransmitter or circuit [preliminary positive results in preliminary study of dextromethorphan, an NMDA receptor antagonist, mainly for cognition and seizures (Smith-Hicks *et al.*, 2017); negative outcome of desipramine, a noradrenaline reuptake inhibitor, for breathing abnormalities (Mancini *et al.*, 2018); ongoing study of sarizotan, a serotonin 1A receptor agonist, for breath holding (NCT02790034)]. Completed trials have targeted more general mechanisms, specifically IGF-1 function, and they have included both full-length IGF-1 (i.e. mecasermin) and a modified IGF-1 active N-terminus peptide (i.e. trofinetide) (Khwaja *et al.*, 2014; Glaze *et al.*, 2017; Neuren Pharmaceuticals, 2017; O'Leary *et al.*, 2018). While the outcome of the mecasermin trials has been mixed, suggesting selective (e.g. communication) or not long-lasting effects, the two phase 2 trofinetide trials have been labelled as positive. The paediatric study, which showed more robust outcomes, demonstrated global but variable improvements that were more prominent in the behavioural

domain. Other planned trials, to begin during the next year, will test new classes of drugs with presumed generalized neural function. These drugs with the potential of, at least, optimizing function and facilitating intrinsic compensatory mechanisms include a sigma 1 receptor agonist, a modulator of mitochondrial function and neuronal homeostasis (Anavex Life Sciences Corp., 2018); and cannabidiol (CBD), a compound isolated from cannabis with broad neurobehavioural effects but without psychoactive action (GW Pharmaceuticals, 2018). The next trofinetide trial, the first ever phase 3 (pivotal) study looking for a disorder-specific drug approval in Rett syndrome, will also begin in the next year (Acadia Pharmaceuticals and Neuren Pharmaceuticals, 2018).

Completing this general overview, it is important to note the first gene (*MECP2*) therapy trial in Rett syndrome (Rett Syndrome Research Trust, 2018) was modelled after the successful (i.e. improved survival and motor function) single dose intravenous adeno-associated virus serotype 9 delivery of complementary DNA (encoding the SMN protein) in spinal muscular atrophy type 1 (Mendell *et al.*, 2017). Despite these exciting developments, the Rett syndrome field is experiencing the same challenges as other neurodevelopmental disorders pursuing neurobiologically-based treatments: inadequate outcome measures and markers of response (Katz *et al.*, 2016; Kaufmann *et al.*, 2016; Budimirovic *et al.*, 2017). To this critical shortcoming must be added the need for innovative study designs that can monitor safety and efficacy in dynamic ways at early stages of drug development. This is particularly important because preclinical work with mouse and other experimental models has been, in general, unable to point out specific symptoms and signs of Rett syndrome and related disorders to be improved by a particular drug (Kaufmann *et al.*, 2016; Berry-Kravis *et al.*, 2018). The heterogeneity of Rett syndrome, manifested as variable severity of common symptoms, leads to variable positive responses (e.g. in some individuals greater decrease in disruptive behaviours; in others, greater improvement in non-verbal communication), as demonstrated in the recent mecasermin and trofinetide trials discussed above. How to capture these beneficial effects at every step of the drug development pathway is a major concern in Rett syndrome trials. (For details about clinical trials in Rett syndrome, including outcome measure and study design issues, see Katz *et al.*, 2016; Kaufmann *et al.*, 2016.)

Disrupted synaptic plasticity: implications for diagnosis and treatment of Rett syndrome

The preceding sections have summarized evidence supporting the notion that Rett syndrome is a disorder of synaptic function and, more specifically, of synaptic plasticity. Many specific issues, including to what extent maladaptive synaptic responses could exacerbate or perpetuate initial deficits, remain unclear. More important is the extent to which data from animal studies with MECP2 deficiency could be extended to individuals with Rett syndrome and to which particular stage of the disease or clinical manifestation. Despite all these uncertainties, approaching Rett syndrome as a synaptic plasticity disorder may have practical implications.

To date the diagnosis of Rett syndrome is still clinical and based predominantly on the history of developmental regression, with the associated challenge of caregivers' recollections. Presence of *MECP2* mutations is supportive, but not confirmatory because of the limited genotype–phenotype correlations in Rett syndrome (Neul *et al.*, 2014). Females with *MECP2* mutations may present with a wide range of phenotypes other than Rett syndrome, without necessarily loss of skills (Neul *et al.*, 2010). Early neurophysiological studies indicate that visual evoked potential (N1-P1) amplitude in Rett syndrome is decreased in individuals during the post-regression period and suggest that this change evolves during regression (LeBlanc *et al.*, 2015). Although electrophysiological markers of brain abnormalities prior to or during regression could be more informative and lead to early interventions, these data show the promise of synaptic biomarkers as complementary diagnostic tools. Other examples of potential biomarkers of disease stage or progression are the levels of *N*-acetylaspartate (NAA) and myoinositol, magnetic resonance spectroscopy neuronal and glial markers, respectively. While the former decreases with respect to control levels in Rett syndrome beginning at age 5–6 years, the latter shows a continuous increase from early childhood (Horská *et al.*, 2009). Thus, NAA levels could become a reliable marker of the pseudo-stationary post-regression period, characterized by appearance of seizures and autonomic disturbances. On the other hand, myoinositol levels would reflect the degree of glial abnormalities associated with synaptic disruption. Biomarkers of disease progression may have prognostic relevance and assist in deciding on more aggressive interventions.

The natural history of Rett syndrome, in conjunction with our knowledge on synaptic abnormalities, indicate that age, and more importantly disease stage, are key factors when designing drug trials. The recently reported early intervention trial (Downs *et al.*, 2018) suggests that intense and prolonged sensory-motor stimulation can improve motor function, when instituted shortly after diagnosis and up to the early post-regression period. These data are in correspondence with the timing of spontaneous but partial recovery of spoken language and hand skills observed in most patients with Rett syndrome (Hagberg, 2002). Whether brain responsiveness to stimulation extends beyond this stage in Rett syndrome is unknown; however, these data raise the possibility of enhancing or complementing drug effects by combining drug administration with sensory-motor stimulation. Although most clinical trials aim at testing drugs as early as possible in the evolution of Rett syndrome, safety and logistical considerations

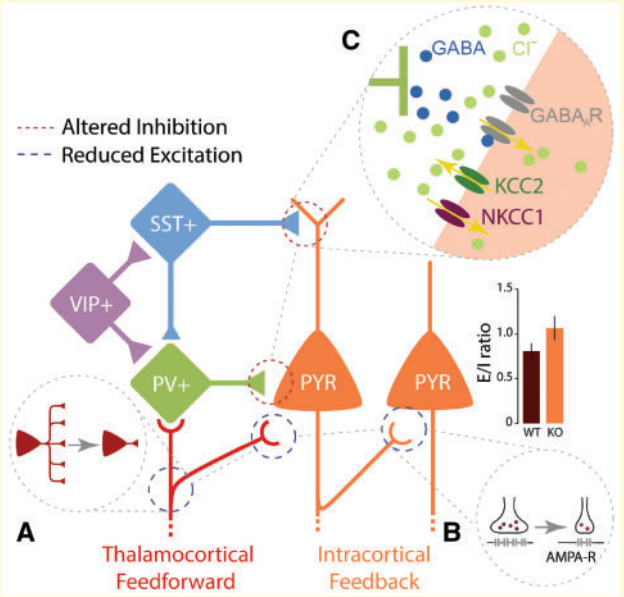

Figure 1 Altered synapse-specific computation in Rett syndrome. While different synapses in distinct parts of the brain are differentially modulated upon loss of MECP2, three essential ideas capture synaptic-specific changes that alter excitatory and inhibitory (E/I) balance and increase E/I ratio in animal models of Rett syndrome. (**A**) A significant reduction of thalamocortical feedforward excitatory drive to supragranular excitatory pyramidal neurons (PYR) and inhibitory parvalbumin (PV+) interneurons in the cortex has been found (Banerjee *et al.*, 2016). (**B**) Weakening of excitatory drive could be resulted from a long-term depression (LTD) like mechanism at glutamatergic synapses (thalamocortical as well as intracortical), upon a downregulation of AMPA-type glutamate receptors (AMPA-R). A third mechanism (**C**) is a reduction of inhibitory currents can be due to altered GABAA receptor (GABAAR) mediated reversal potential in excitatory neurons. This is caused by a developmental delay in the maturation of GABAergic inhibition and a reduction in chloride exporter KCC2 expression (Banerjee *et al.*, 2016). SST = somatostatin; VIP = vasoactive intestinal polypeptide.

determine that some of them will only enrol older children, teens, or adults. Since severity of symptoms, including breath-holding, seizures, and motor dysfunction, is particularly high in late childhood and adolescence (Tarquinio *et al.*, 2017, 2018), combination of drugs with non-pharmacological therapies or other interventions for overcoming decreased or aberrant plasticity seems to be compelling. The experience with the $GABA_B$ agonist arbaclofen in fragile-X syndrome suggests age-dependent efficacy with greater efficacy in childhood than at later ages (Berry-Kravis *et al.*, 2017). While using different designs, the paediatric and adolescence/adult trofinetide trials in Rett syndrome suggest similar differences.

Important seminal work in Rett syndrome animal models demonstrating the possibility to achieve prolonged survival and reversibility of disease phenotypes with gene reinstatement, even into adulthood, seemingly makes Rett syndrome one of the more tractable neurodevelopmental disorders as far as potential for disease modification and improvement (Guy *et al.*, 2007; Gadalla *et al.*, 2017). Viral gene therapy approaches can potentially rescue a radically truncated version of MECP2 that has recently been shown to be able to reverse neurological symptoms (Tillotson *et al.*, 2017). The planned gene delivery trial, the first attempt at correcting the primary defect in Rett syndrome, raises the key question of lower age limit for major reversal or prevention of the phenotype. Taking into consideration that in the USA, the median age of diagnosis is 2.7 years for classic Rett syndrome and 3.8 years for atypical Rett syndrome, would newborn *MECP2* mutation screening be necessary for successful corrective interventions? Alternatively, will it be possible to reverse most abnormal synaptic plasticity already established by the time of diagnosis? A final consideration about the 'double jeopardy' effect of MECP2 deficit (Kaufmann *et al.*, 2005) is that, even if abnormal developmental plasticity is not ameliorated, the need of MECP2 for adult learning and memory indicates that it is never too late for attempting to improve neurological function in Rett syndrome.

Conclusion

It has been more than 50 years since the discovery of Rett syndrome, yet the current standard of care for patients remains limited to supportive and symptomatic therapies. Drug treatment consists mainly of off-label prescriptions due to the lack of approved medications for the disorder. However, with the current collaborative efforts of academic investigators, patient organizations and advocacy groups, and industry partners, the future of clinical trials in Rett syndrome looks promising, trending towards probing disease-modifying therapies and rational, innovative clinical trial designs. The availability of multiple treatment options is encouraging since, most likely, not all individuals with Rett syndrome will respond to the same degree to different drugs. Our review also argues for an essential role of MECP2 in the development and maintenance of neuronal circuits, and its specific role in distinct cellular subtypes. Understanding this neurobiology is crucial in enhancing the effectiveness of animal models in designing robust and reproducible preclinical translational studies. Taking into consideration synaptic abnormalities in Rett syndrome is also key for the design and interpretation of drug and non-pharmacological trials, as well as for the development of strong clinical biomarkers.

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Competing interests

In 2018, W.E.K. was a consultant to Anavex, AveXis, EryDel, GW Pharmaceuticals, Neuren/Acadia, Newron Pharmaceuticals, Ovid, Stalicla, and Zynerba. As of January 1, 2019, W.E.K. is the Chief Medical Officer of Anavex Life Sciences Corp.

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