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Primer

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Rett syndrome

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Abstract

Rett syndrome (RTT) is a severe, progressive, neurodevelopmental disorder, which affects predominantly females. In most cases, RTT is associated with pathogenic variants in *MECP2*. MeCP2, the protein product of *MECP2*, is known to regulate gene expression and is highly expressed in the brain. RTT is characterized by developmental regression of spoken language and hand use that, with hand stereotypies and impaired ambulation, constitute the four core diagnostic features. Affected individuals may present multiple other neurological impairments and comorbidities, such as seizures, breathing irregularities, anxiety and constipation. Studies employing neuroimaging, neuropathology, neurochemistry and animal models show reductions in brain size and global decreases in neuronal size, as well as alterations in multiple neurotransmitter systems. Management of RTT is mainly focused on preventing the progression of symptoms, currently improved by guidelines based on natural history studies. Animal and cellular models of MeCP2 deficiency have helped in understanding the pathophysiology of RTT and guided the development of trofinetide, an IGF1-related compound, which is an approved drug for RTT, as well as of other drugs and gene therapies currently under investigation.

Sections

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Introduction

Rett syndrome (RTT; OMIM identifier #312750) is a severe neurodevelopmental disorder that predominantly affects girls, constituting one of the most common genetic causes of intellectual disability in females[1–3]. RTT is a dynamic condition characterized by initial variable developmental delay followed by regression of spoken language and hand function (typically beginning in the second year of life and ending before 3 years of age) and subsequent manifestation and progression of a wide range of neurological symptoms including distinctive hand stereotypies[4] (Fig. 1). Most individuals with RTT (>96%)[5] carry one of >300 loss-of-function (LOF) de novo variants in *MECP2*, located on the X chromosome (Xq28; chrX:154,021,573–154,097,717 (GRCh38/hg38)), which encodes methyl-CpG-binding protein 2 (MeCP2)[6]. Nonetheless, eight 'hotspot' pathogenic variants constitute >60% of documented cases[5]. These hotspot variants involve C-to-T transitions at CpG sites and when methylated, are prone to spontaneous deamination and conversion to thymine. A small proportion of individuals with RTT harbour variants in other genes or have no known genetic contribution[4,7,8]. Moreover, known RTT-causing *MECP2* variants are also found in individuals with other clinical presentations[9]. Owing to this lack of correlation between *MECP2* variants and manifestation of RTT symptoms, the diagnosis of RTT remains clinical. Boys harbouring common pathogenic *MECP2* variants usually have shortened life expectancy, rarely reaching adulthood; however, most girls survive into adulthood, with ~70% reaching their 50s[10,11].

Although ubiquitous, MeCP2 is highly expressed in the brain and is required for normal nervous system development and function[12]. The main role of MeCP2 is transcriptional repression, by linking methylated DNA to deacetylated histones, although other gene regulatory functions have been postulated[12–17]. The best-characterized consequence of MeCP2 deficiency is alterations in neuronal function via disruptions in their synaptic connections; however, MeCP2 also has a key role in astrocyte function[18,19].

Until the 2010s, management of RTT predominantly involved addressing symptomatic concerns[20,21]. Increased knowledge regarding the natural history of RTT has allowed the development of practices focused on anticipating and ameliorating neurological manifestations (for example, seizures) and systemic manifestations (for example, osteoporosis)[22–27]. This approach includes the entire range of rehabilitation modalities and off-label use of multiple drugs. Although no cure exists for RTT, FDA approval of trofinetide, an IGF1-related compound, for the treatment of multiple clinical manifestations of the disorder has provided encouragement for the further development of pharmacological and genetic treatments[28].

In this Primer, we summarize current knowledge of the epidemiology, pathophysiology, diagnosis, clinical presentation and evolution, and management of RTT. In addition, we discuss the quality of life of affected individuals and caregivers and outline the outstanding research questions aimed at improving diagnosis, knowledge on genetics and neurobiology, and development of new treatments.

Epidemiology

RTT is a rare genetic disorder, defined as a disorder occurring in <1 in 200,000 individuals in the USA or <1 in 2,000 individuals in the European Union[29]. As the disorder was originally described in girls and the initial diagnostic criteria specifically excluded boys, the epidemiology of RTT reflects paediatric female-focused studies[30] with no available data on males with *MECP2* variants. Epidemiological data on RTT come from a combination of sources, including national or regional populations, schools and a variety of registries. Differences in period of study, age range, diagnostic criteria, genetic testing, clinical awareness and health-care systems have contributed to variable estimates of incidence and prevalence in surveys conducted mainly in high-income countries. Hence, regional differences in the incidence or prevalence of RTT cannot be simply attributed to ethnic differences. A meta-analysis integrating data from Europe, Australia and China showed a prevalence range of 5–10 per 100,000 females[29], which is in line with the single USA registry-based study showing a prevalence of 4.4 per 100,000 females[31]. Current data on incidence are lacking and the prevalence varies depending on the country, with estimates of 1.09 per 10,000 females (<12 years of age) in Australia[1,32], 0.586 per 10,000 female live births in Serbia[33] and 0.43 to 0.71 per 10,000 females in France[34]. Life expectancy in girls with RTT has increased over the years, with survival into the sixth decade being currently common (~70% of cases in the USA), a progress attributed in part to a decrease in extreme frailty[10,35,36]. Cardiorespiratory involvement is the leading cause of death, with sudden death accounting for 20–30% of deaths[10]. As delineation of the clinical presentation in boys with *MECP2* variants is ongoing, estimates of life expectancy are not possible and, therefore, unavailable. Nonetheless, as more boys with less severe phenotypes are being identified, survival beyond early postnatal life seems to be common[11].

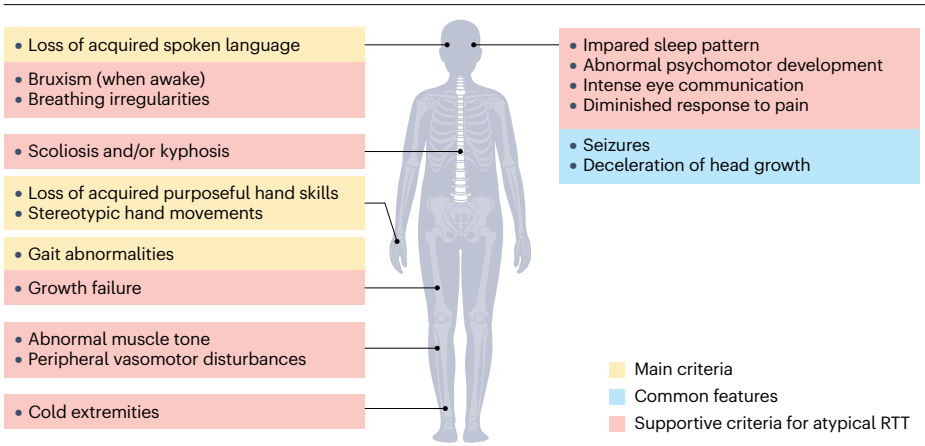

Fig. 1 | Clinical features of RTT. Core diagnostic features of Rett syndrome (RTT) as well as some other common clinical manifestations.

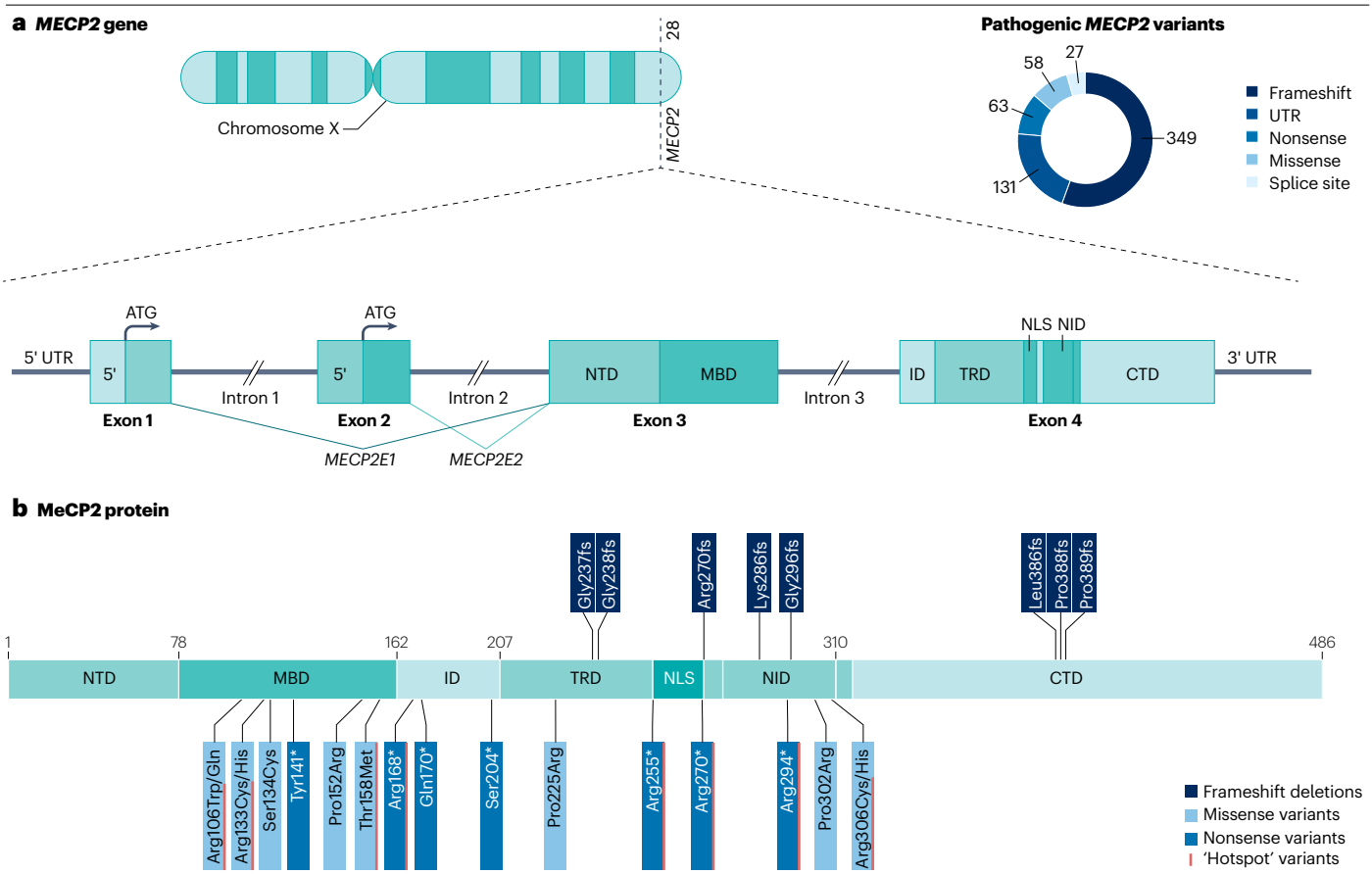

Fig. 2 | Human *MECP2* gene, MeCP2 protein, and common variants in Rett syndrome. a, Structure of the *MECP2* gene including the MeCP2 protein domains. The two mRNA isoforms, *MECP2E1* and *MECP2E2*, code for two protein isoforms, MeCP2E1 (498 amino acids) and MeCP2E2 (486 amino acids), which differ in their amino termini due to alternative translation start sites and selective inclusion of exons 1 or 2 in the *MECP2* transcript. **b**, Structure of the MeCP2 protein and location of common missense and nonsense pathogenic variants (according to the NM_004992.4 isoform), which cluster in the methyl-CpG-binding domain (MBD), transcription repressor domain (TRD), and the NCoR-SMRT interaction domain (NID). The carboxy-terminal domain (CTD) is the location for common deletions. Structures and variants are traditionally referred to and called by their location within the sequence of *MECP2E2*/MeCP2E2, the slightly shorter transcript/protein produced from translation initiating at exon 2. ID, intermediate domain; NLS, nuclear localization signal; NTD. N-terminal domain; UTR, untranslated region.

Mechanisms/pathophysiology

In this section, we review the genetic basis of RTT and the molecular and cellular consequences of MeCP2 deficit in the central nervous system (CNS) and other body systems as a foundation for understanding the clinical manifestations and evolution of the disorder. The MeCP2 neurobiology covered here also constitutes the basis for ongoing drug and gene therapy development programmes.

Genetics

MECP2 is alternatively spliced, generating two isoforms *MECP2E1* (previously referred to as MECP2B or MECP2α) and *MECP2E2* (previously referred to as MeCP2A or MECP2β). The MeCP2E1 isoform (NM_001110792.2), which uses the translation start site (ATG) in exon 1, and comprises exons 1, 3 and 4, is the predominant isoform in the CNS[37,38], whereas the MeCP2E2 isoform (NM_004992.4) uses a translation start site in exon 2 and comprises exons 2, 3 and 4. Both isoforms share the methyl-CpG-binding domain (MBD), the transcription repression domain (TRD), the NCoR/SMRT interaction domain (NID) and the carboxy-terminal domain (Fig. 2). MeCP2 is ubiquitously expressed in human tissues with particularly high protein levels observed in the brain. Within the brain, neurons contain the highest levels of MeCP2, with decreased expression levels observed in astrocytes and oligodendrocytes[39].

Variants in *MECP2*, first reported in 1999, have been associated with the common sporadic cases (de novo variants) and rare familial cases of RTT[6]. Studies have identified >300 LOF *MECP2* variants in individuals with RTT, including missense, nonsense, frameshift and in-frame insertions or deletions, as well as large deletions spanning whole exons or even the entire gene[40]. In addition, genome sequencing studies have also revealed complex chromosomal rearrangements[41,42]. Despite the wide range of variants associated with RTT, eight recurrent 'hotspot' variants (encoding, according to the most widely used Human Genome Variation Society nomenclature, the amino acid substitutions p.Arg106Trp (R106W), p.Arg133Cys (R133C), p.Thr158Met (T158M),

p.Arg168* (R168X), p.Arg255* (R255X), p.Arg270* (R270X), p.Arg294* (R294X) and p.Arg306Cys (R306X)) constitute >60% of documented cases of RTT[5]. Profiles of clinical features and severity have been consistently associated with these and other common *MECP2* variants in RTT[5]. Variants p.Arg106Trp, p.Arg168*, and the two involving the nuclear localization signal p.Arg255* and p.Arg270*, are associated with the most severe clinical manifestations, whereas individuals with p.Arg133Cys, p.Arg294* and p.Arg306Cys variants tend to have mild RTT; and p.Thr158Met is associated with intermediate severity[5,43,44]. Large deletions including substantial portions of exons 3 and 4 are also associated with severe symptoms; conversely, smaller C-terminal truncations (deletions and/or insertions) are associated with milder symptoms[5,43,44]. Although increasing age is associated with increased severity, relatively independent of the *MECP2* variant[44], a few studies have shown that other factors may influence clinical severity. These factors include skewed pattern of X chromosome inactivation[45], although inactivation patterns in blood may not correlate with those in brain and/or clinical presentation[46,47], and polymorphisms in *BDNF*[48,49], a known modifier of neuropsychiatric manifestations[50]. Furthermore, other genetic and environmental factors, including therapeutic interventions, may also influence clinical outcome in RTT as illustrated by the increase in the life expectancy over the last few decades[10,35,36].

MeCP2 biology

MeCP2, the protein encoded by *MECP2*, was first identified through its binding to methylated DNA[51,52]. MeCP2 was long considered a 'reader' of DNA methylation marks. MeCP2 binds methyl CG dinucleotides as well as non-canonical methyl CAC sites that are enriched in neurons and laid down during development[53]. As DNA methylation is typically linked to gene silencing, a long-held view was that MeCP2 is important in transcription repression, by recruiting histone deacetylases (including HDAC3), through its TRD, following its binding to methylated DNA (Fig. 3). Over time, studies have ascribed alternative and overlapping functions to MeCP2, including gene activation and regulation of chromatin architecture, splicing and microRNA processing, mediated via interactions with diverse binding partners (reviewed in ref. 54). Contradictory to such a multifunctional role, missense variants that cause RTT cluster discretely in the MBD and in the NID (Fig. 2) – two key domains essential for linking methylated DNA with the NCoR repressor complex[52]. NCoR and SMRT were originally identified as co-repressors mediating repression by nuclear hormone receptors, through the formation of a complex with histone deacetylases (Fig. 3). This evidence supports a simple model of gene expression regulation through the tethering of repressor proteins to methylated sites in the genome[55]. However, the relatively mild phenotype of variants involving the NID emphasize the complexity of MeCP2 function and the possibility that it extends beyond linking NCoR to DNA.

Disruption of MeCP2 function and/or expression levels alters the overall cellular levels of many gene products with the most reproducibly described targets being *BDNF* and *IGF1* (refs. 56–60). However, effects at the individual gene level are typically small and do not point to the existence of a single pathogenic pathway[61,62]. Studies attempting to understand downstream pathways have demonstrated disruption in various systems including synaptic function, protein synthesis, mitochondrial function and oxidative stress, and alterations in various signalling and homeostatic pathways (for example, mTOR-AKT pathway), as well as in energy and lipid metabolism (reviewed in ref. 63). The most consistent lipid abnormalities found in individuals with RTT are increases in levels of total cholesterol and LDL, with some studies also finding increased triglycerides or decreased levels of HDL[64–67]. Although data suggest that the link between *MECP2* LOF and signalling and metabolic abnormalities is deficient transcriptional repression[68], mechanisms have not been fully elucidated. Single-cell transcriptomic data from post-mortem brain tissue obtained from individuals with RTT show differential perturbations in expression in different cell types with increased levels of gene body methylation, further supporting the role of MeCP2 as a DNA methylation-dependent transcriptional regulator[69]. The relative importance of these effects on cellular dysfunction may depend on the type and state of the cell. Moreover, subtle but generalized gene dysregulation is likely to lead to a cumulative or cascade effect on cell function.

MECP2 is a dosage-sensitive gene and the level of MeCP2 within a given cell type is believed to be crucial for adequate cellular homeostasis. Both LOF and overexpression of MeCP2 have neurological consequences, resulting in RTT or *MECP2* duplication syndrome, respectively[6,70]. This gene dosage sensitivity, including variability in expression shown across cell types[71] has created a challenge for therapies aimed at augmenting MeCP2 levels through gene therapy and related approaches.

MeCP2 and Rett syndrome neurobiology

As the majority of individuals with RTT have a LOF *MECP2* variant, RTT neurobiology has been delineated on the basis of animal and cellular models of MeCP2 deficiency and a small set of human CNS post-mortem data (Box 1). Neuropathological, neurochemical and neuroimaging studies indicate that RTT is a widespread, multiregion and multineurotransmitter system disorder characterized by volumetric reduction, from cellular to brain regional levels, and atypical astrocytes, in the absence of features of neurodegeneration (reviewed in refs. 72–74) (Fig. 4). These features are supported by data from RTT models; *Mecp2* deletion in experimental rodents and primates confirms that the dominant RTT-like phenotypes result specifically from MeCP2 deficiency in CNS cells[75–77]. MeCP2 is especially abundant in the brain relative to other tissues and selective silencing of *Mecp2* outside the brain in mice leads to minimal phenotypes including exercise fatigue and bone abnormalities[78]. The latter phenotype is consistent with scoliosis and early osteoporosis and fractures, which are common in RTT[24,79]. Within the CNS, multiple studies have shown that selective deletion of *Mecp2* in different brain areas and neuronal subtypes results in specific phenotypes associated with dysfunction of the affected region or the local circuit (reviewed in ref. 80). Neural activity-dependent MeCP2 phosphorylation seems to influence its transcriptional regulatory role and dendritic development[81,82]; nonetheless, its impact on RTT phenotypes is unclear. *Mecp2* is expressed at much lower levels in astrocytes than in neurons; nonetheless, astrocyte-specific *Mecp2* deletion leads to non-cell-autonomous consequences on neuronal and synaptic function[18], effects which can be reversed by its re-expression in symptomatic *Mecp2*-mutant mice[83]. Neuropathological and magnetic resonance spectroscopy studies in individuals with RTT show evidence of increased number of astrocytes and increased expression of astrocytic markers (for example, GFAP and *myo*-inositol)[84–86]. MeCP2-deficient mice also showed alterations in astrocytic markers, although contrary to those observed in individuals with RTT (for example, a decrease in *myo*-inositol in mice but an increase in individuals with RTT)[87]. Altered function of microglial cells, in particular increased release of glutamate by *Mecp2*-null microglia[88], may contribute to RTT pathophysiology; however, their impact is still poorly understood as most reports are from in vitro preparations of dissociated cells in culture[89]. Moreover,

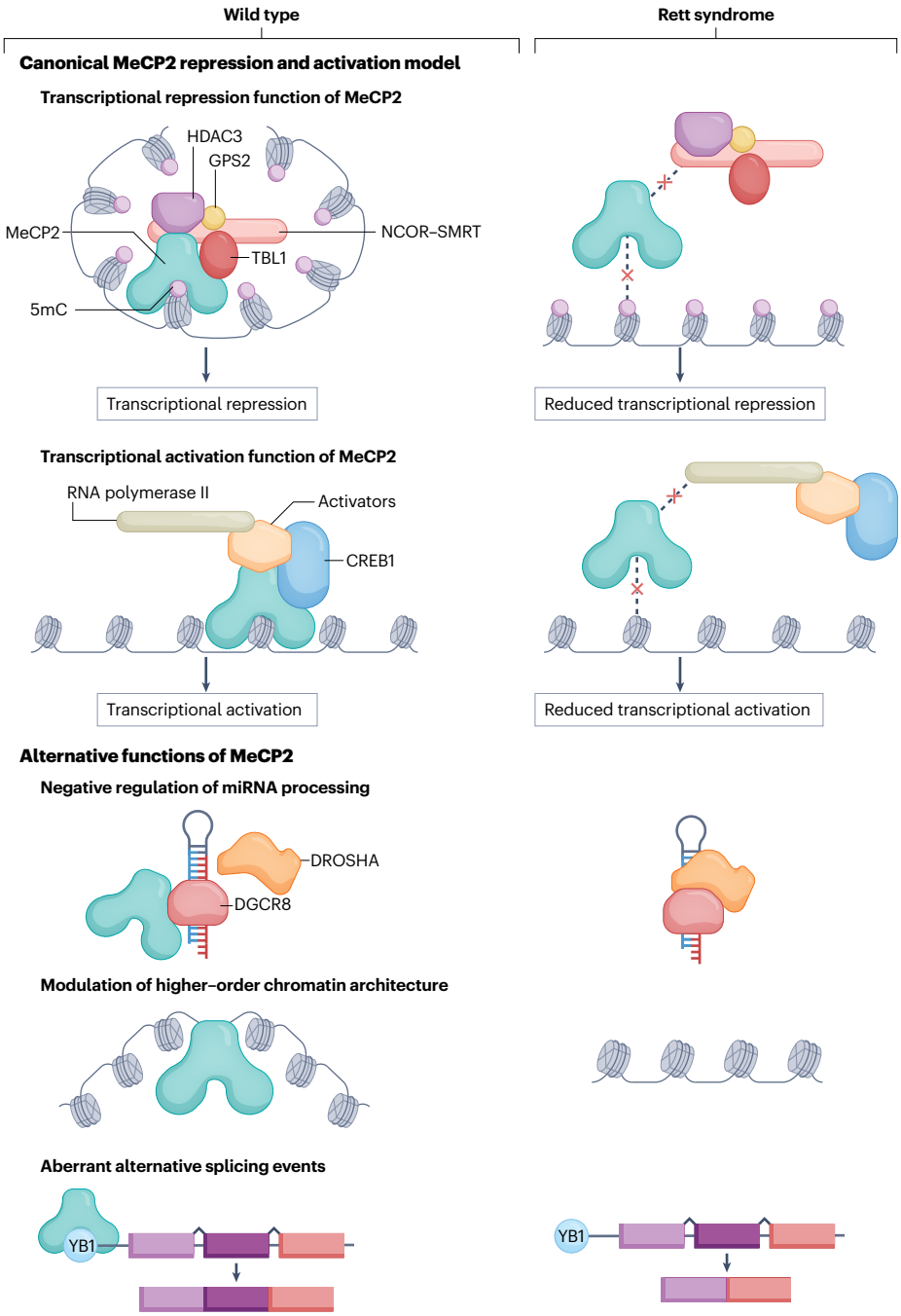

Fig. 3 | MeCP2 molecular function. MeCP2 binds to both methylated and unmethylated DNA. Under typical conditions (wild type), MeCP2's binding to 5mC sites leads to the recruitment of co-factors involved in transcriptional repression (histone deacetylase 3 (HDAC3), transducin β-like protein 1 (TBL1), nuclear receptor co-repressor-silencing mediator of retinoic acid and thyroid hormone receptor (NCoR-SMRT) and G protein pathway suppressor (GPS2)). This binding results in a closed chromatin configuration. By contrast, the partial or complete inability of MeCP2 to recruit co-repressors in Rett syndrome leads to reduced transcriptional repression and an open chromatin configuration. In addition, alternative functions of MeCP2 have been described including transcriptional activation, microRNA (miRNA) processing, chromatin architecture and alternative splicing.

early attempts to restore wild-type microglia as a therapeutic strategy by bone marrow transplants[90] were not reproducible and selective expression of *Mecp2* in microglia did not rescue RTT-like phenotypes in mice[91].

Neuronal and synaptic alterations. Consistent with pathological findings from human post-mortem material, *Mecp2* LOF variants in rodent models are associated with structural and functional consequences at the neuronal and synaptic levels (reviewed in ref. 80). These abnormalities cascade into severe phenotypes at the organismal level over the course of the developmental maturation of neuronal circuits and their experience-dependent refinement. An overall reduction in brain volume is almost universally observed in individuals with RTT, with selective decrease in the dorsal parietal cortex on MRI as a distinctive feature of the disorder and reduction in the anterior frontal lobe as a correlate of clinical severity[92]. Diffusion tensor imaging has shown involvement of multiple fibre tracts, with reductions in superior longitudinal fasciculus' fractional anisotropy correlating with degree

of verbal impairment[93]. The prototypical features of RTT neuropathology, including smaller brains, smaller neurons, higher neuronal packing density, shorter dendrites with fewer branches and atypical dendritic spines, confirmed and expanded by in vivo neuroimaging and neurochemical post-mortem studies (reviewed in refs. 72,74), have all been observed in rodent models (reviewed in ref. 94) (Fig. 4). These morphological alterations result in dysfunction at the neuronal intrinsic, synaptic and circuit levels, but with major regional specificity derived from the different developmental trajectories of excitatory neurons, inhibitory neurons and synapse maturation, as well as circuit formation and their experience-dependent refinement. Functional consequences include, in general, altered excitatory and inhibitory synaptic transmission, as well as disturbed Hebbian and homeostatic long-term synaptic plasticity[95].

Whilst the neurodevelopmental consequences of MeCP2 deficiency during prenatal and postnatal development are well established, there is an enduring role for MeCP2 in the nervous system throughout life. Importantly, postnatal reinstatement of *Mecp2* expression in juvenile and adult mice can rescue a range of functional, electrophysiological, behavioural and morphological phenotypes[96–98]. In related but orthogonal studies, silencing of the *Mecp2* gene in juvenile or adult mice results in the emergence of a similar constellation of overt RTT-like phenotypes observed in the germ-line mutants[99]. As such, MeCP2 is considered to be important for the maintenance of a healthy neuronal phenotype rather than having a specific neurodevelopmental role. This concept has important implications in suggesting that RTT is likely to be amenable to gene restoration therapies.

Box 1 | Animal and cellular models of RTT

Although genetic models such as non-human primates, zebrafish and rats have been developed, the majority of the work on the consequences of *Mecp2* deletion or the expression of *Mecp2* harbouring Rett syndrome (RTT)-associated variants has relied on genetically modified mice (reviewed in ref. 12). Male hemizygous *Mecp2*-mutant mice, which lack MeCP2 in all cells, show the strongest phenotypes and represent a valuable system for understanding the principles of MeCP2 biology, and for developing and evaluating therapeutic strategies[198]. By contrast, female heterozygous *Mecp2*-mutant mice undergo X chromosome inactivation, where ~50% of cells are not affected due to expression of the wild-type rather than the mutant allele, and show milder phenotypes with later symptom onset than male hemizygous *Mecp2*-mutant mice but enable the study of cell-autonomous and non-cell-autonomous consequences of MeCP2 deficiency pertinent to females with RTT. Overall, deletion of *Mecp2* or a knock-in of human RTT-causing genetic variants in mice results in a constellation of severe neurological phenotypes relevant to individuals with RTT, including motor and sensory impairments, breathing irregularities and cognitive dysfunction[75,76,199,200]. These features can represent useful outcome measures of disease progression in therapeutic development programmes (reviewed in ref. 94). Even though animal models have greatly contributed to a better understanding of disease mechanisms, humanized 2D and 3D models of RTT using human embryonic stem cells, and patient-derived or genetically-modified human induced pluripotent stem cell models have been deployed as a complementary approach to existing disease models, offering a more human-centred approach (reviewed in refs. 201,202). 2D differentiated neuronal cultures and glia cell cultures and 3D complex organoid models recapitulate many cellular RTT features such as smaller soma size, reduced dendritic branching, spine density and axonal arborization, as well as synaptic dysfunction and excitatory–inhibitory imbalance and non-cell-autonomous effects but have not yet contributed any novel findings.

Alterations in neurotransmitters. *Mecp2* is expressed broadly across the nervous system and deficiency of MeCP2 has been shown to affect all neurotransmitter systems and brain areas investigated in animal models and in human brain and CSF samples. Early focus on aminergic systems was based on behavioural alterations observed in individuals with RTT[74], whilst individual monoamine systems have been linked to specific disease domains including breathing (noradrenaline) and motor function (dopamine). Efforts to investigate this experimentally by deleting *Mecp2* in specific cell populations have confirmed that MeCP2 deficiency is associated with decreased levels of brain noradrenaline, dopamine and serotonin, their metabolites, as well as their neurotransmitter receptor proteins[100,101]. Human and animal studies have also shown consistent alterations in cholinergic signalling in the brain, including reduced levels of cholinergic markers such as choline acetyltransferase activity and receptor expression[102]. Selective deletion of *Mecp2* in cholinergic neurons produces specific impairments in cholinergic signalling and in recognition memory[103]. Indeed, all cell type-specific and brain region-specific knockout studies including monoaminergic neurons[101], cholinergic neurons[103], the hypothalamus[104], cerebellum[105], striatum[106], basal ganglia and forebrain[107], and spinal and sensory neurons[108], have produced a range of specific observable RTT-like phenotypes. However, the most profound and comprehensive RTT-like phenotypes are observed in glutamatergic cell-specific or GABAergic cell-specific knockouts of MeCP2. Indeed, adult mice lacking MeCP2 in glutamatergic and GABAergic neurons demonstrate impaired motor coordination, learning and memory deficits, atypical electroencephalogram hyperexcitability, severe respiratory dysrhythmias, obesity and premature death[109,110]. Furthermore, restoration of *Mecp2* expression in GABAergic cells is sufficient to rescue many core disease phenotypes (for example, ataxia, apraxia) in mice[111]. These findings are consistent with amino acid neurotransmission being the most ubiquitous synaptic signalling in the brain, with glutamatergic and GABAergic circuits found across all brain regions. The consequences of MeCP2 deficiency have also been studied most intensively at GABAergic and glutamatergic synapses (reviewed in ref. 74). Similar to that reported for other neurotransmitter systems in vivo, MeCP2-deficient GABAergic neurons show reduced neurotransmitter release, and decreased biosynthesis markers (*Gad1*, *Gad2*) and GABA levels[109]. Atypical patterns of glutamate receptor (for example, NMDAR) density have also been shown in a small study in individuals with RTT, supported by a larger study using MeCP2-deficient mice[112,113]. Thus, alterations in glutamatergic and GABAergic signalling probably result in the brain region-specific excitation-inhibition imbalance observed in the MeCP2-deficient brain, in both individuals with RTT and animal models[72].

Overall circuit dysfunction is a key driver of disease pathophysiology in RTT, probably owing to a combination of factors including altered synaptic transmission and plasticity coupled with changes in

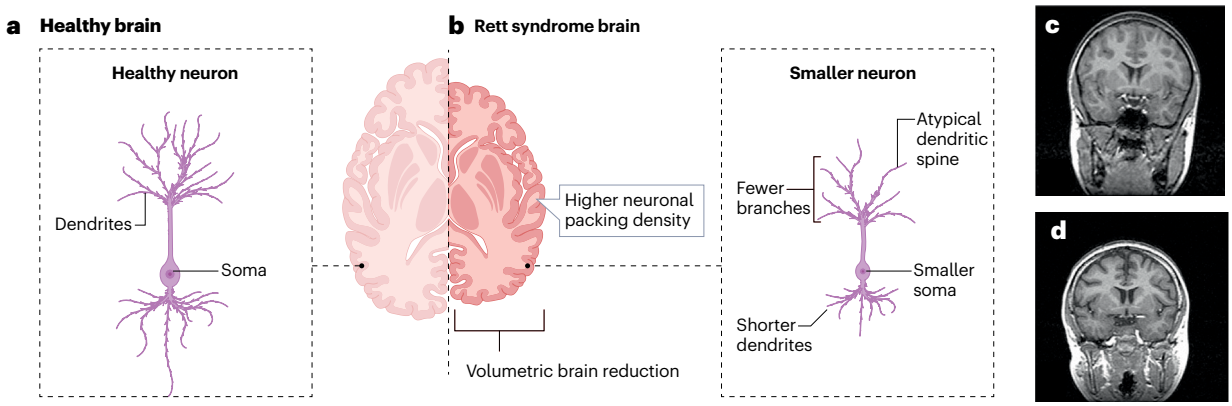

Fig. 4 | Neuroanatomical features of Rett syndrome. Rett syndrome is characterized by widespread neuronal alterations associated with cellular, regional and whole-brain volumetric reductions. Neurons have smaller cell bodies and dendrites with sparse or atypical dendritic spines, which is manifested at the regional level as increased neuronal packing density (parts **a** and **b**). MRI T1-weighted coronal images at the level of the anterior commissure in a healthy brain (part **c**) and in a brain from an individual with Rett syndrome (part **d**) illustrate the marked volumetric reduction seen in the brain of individuals with Rett syndrome (part **d**). Adapted with permission from ref. 195, Elsevier.

intrinsic properties and neuromodulation, as well as structural changes at the cellular and circuit levels. The relative contribution of these factors in different brain regions and long-range pathways have been challenging to untangle and remain to be fully understood. Altogether, neurobiological data support the notion that RTT is a dynamic disorder of synaptic development and maintenance.

Diagnosis

Diagnosis of RTT is based on the 2010 consensus criteria, a revision of previous guidelines, which require the presence of developmental regression following an apparently normal early postnatal period (that is, suggestions of abnormality are often overlooked or extremely subtle)[4,26,30,114] (Box 2 and Fig. 1).

Most individuals diagnosed with RTT (~85%) fulfil criteria for classic RTT[25]. The presence of *MECP2* pathogenic variants is supportive but not confirmatory of RTT, as variants in the gene are identified in >96% of individuals with classic RTT but only in ~75% of those with atypical RTT[5]. Considering the high association between RTT core features and *MECP2* variants and their prognostic and therapeutic implications, genetic testing is routinely conducted when RTT diagnosis is suspected. Genetic testing may include panels covering other neurodevelopmental disorder-related genes, whole-exome or whole-genome screens. To date, only clinical presentation and evolution of clinical manifestations constitute the diagnostic bases for RTT, as no laboratory or imaging tests (frequently performed as part of developmental delay diagnostic work-up) are informative in this regard[4]. However, *MECP2* pathogenic variants are also found in individuals who do not meet the clinical criteria for RTT[9,115] (Box 3). RTT is commonly diagnosed during the second year of life, with reported median ages of 2.7 years and 3.8 years for classic and atypical presentations, respectively[116]. Additionally, multiple reports have described individuals who display some core or supportive diagnostic features of RTT but have variants in other genes associated with neurodevelopmental disorders[117]. For example, individuals with conditions such as Phelan–McDermid syndrome (22q13.3) and Pitt–Hopkins syndrome (*TCF4*) show a clinical overlap with RTT[8,118–121]. Although these individuals have features associated with RTT, they should be characterized and managed based on their distinct genetic variations.

Some early features in RTT can facilitate the diagnosis whereas others make it more difficult (Box 2 and Fig. 1). Among the former are 'too good' behaviour, where infants tend to eat and sleep but do not cry or fuss. Head growth may also decelerate as early as 30–45 days of life, contributing to diagnostic suspicion[122]. In addition, developmental delay and plateauing, noticeable after 6 months of age[25], may obscure the loss of communication and hand skills. Children with RTT typically rarely develop crawling, and delays in pulling to stand and cruising along furniture are common[25]. During the period of regression, usually between 18–30 months of age, individuals may be too quiet, less interactive or even reject attempts to be held if upset, and other autism-like features may become prominent[115,123,124]. At about the same time, or certainly close to this regression phase, hand stereotypies appear[27]. Each individual with RTT develops a unique pattern of hand stereotypies, rather than the same patterns of these repetitive movements developing uniformly across the entire group[27]. Most individuals enter the regression period after their first birthday, with 90% or more regressing by 30 months[116]. Around this time and extending through the next 1 or 2 years, emergence from the regression period may be noted by improvements in attention and interaction, disappearance of autistic features and presentation of typical manifestations of RTT, such as seizures and breathing abnormalities. Seizures and irregular breathing are uncommon before 2 years of age, aiding in differentiating RTT from Angelman syndrome, one of several conditions that display RTT-like features in which early seizures are more common than in RTT[125,126]. Conditions that have features similar to those in RTT and are associated with variants in >70 genes have been termed RTT spectrum disorders[8,119–121,127].

RTT and *MECP2* variants in males

Although RTT was initially believed to only affect girls and women, studies have identified boys with pathogenic variants of *MECP2* who present with a wide range of neurological symptoms[11]. As *MECP2* is located on the X chromosome, males present a disorder that seems markedly different from that in females[11]. Literature review identified

Box 2 | Revised diagnostic criteria for RTT

Consider Rett syndrome (RTT) diagnosis when postnatal deceleration of head growth is observed.

Required for typical or classic RTT
- A period of regression followed by recovery or stabilization[a]
- All main criteria and all exclusion criteria
- Supportive criteria are not required, although often present in typical RTT

Required for atypical or variant RTT
- A period of regression followed by recovery or stabilization[a]
- At least two out of the four main criteria
- Five out of 11 supportive criteria

Main criteria
- Partial or complete loss of acquired purposeful hand skills
- Partial or complete loss of acquired spoken language[b]
- Gait abnormalities: impaired (dyspraxic) or absence of ability
- Stereotypic hand movements such as hand wringing or squeezing, clapping or tapping, mouthing and washing or rubbing automatisms

Exclusion criteria for typical RTT
- Brain injury secondary to trauma (perinatally or postnatally), neurometabolic disease or severe infection that causes neurological problems[c]
- Grossly abnormal psychomotor development in the first 6 months of life[d]

Supportive criteria for atypical RTT[e]
- Breathing disturbances when awake
- Bruxism when awake
- Impaired sleep pattern
- Abnormal muscle tone
- Peripheral vasomotor disturbances
- Scoliosis or kyphosis
- Growth retardation
- Small cold hands and feet
- Inappropriate laughing or screaming spells
- Diminished response to pain
- Intense eye communication — 'eye pointing'

Adapted with permission from ref. 4, Wiley. [a]Because *MECP2* variants are now identified in some individuals prior to any clear evidence of regression, the diagnosis of 'possible' RTT should be given to those individuals under 3 years old who have not lost any skills but otherwise have clinical features suggestive of RTT. These individuals should be reassessed every 6–12 months for evidence of regression. If regression manifests, the diagnosis should then be changed to definite RTT. However, if the child does not show any evidence of regression by 5 years, the diagnosis of RTT should be questioned. [b]Loss of acquired language is based on best acquired spoken language skill, not strictly on the acquisition of distinct words or higher language skills. Thus, an individual who had learned to babble but then loses this ability is considered to have a loss of acquired language. [c]There should be clear evidence (neurological or ophthalmological examination and MRI/CT) that the presumed insult directly resulted in neurological dysfunction. [d]Grossly abnormal to the point that typical milestones (acquiring head control, swallowing, developing social smile) are not met. Mild generalized hypotonia or other previously reported subtle developmental alterations during the first six months of life are common in RTT and do not constitute an exclusion criterion. [e]If an individual has or ever had a clinical feature listed, it is counted as a supportive criterion. Many of these features have an age dependency, manifesting and becoming more predominant at certain ages. Therefore, the diagnosis of atypical RTT may be easier in older individuals than in younger. In the case of a younger individual (under 5 years old) who has a period of regression and two or more main criteria but does not fulfil the requirement for 5 of 11 supportive criteria, the diagnosis of 'probably atypical RTT' may be given. Individuals who fall into this category should be reassessed as they age and the diagnosis revised accordingly.

at least 100 males reported with *MECP2* variants with variable clinical difficulties ranging from milder impairment than in females with typical RTT to neonatal encephalopathy with limited life expectancy, through a clinical presentation with features of RTT termed male *MECP2* encephalopathy[11,128–135]. This variability emphasizes the challenges in diagnosing and surveying males with *MECP2* variants, and increasing access to *MECP2* testing and genomic sequencing are crucial to identify these cases.

Screening

Screening for *MECP2* variants although theoretically possible has not been pursued clinically owing to the absence of an effective therapy. With the current availability of trofinetide and other pharmaceutical products and the emerging efforts in gene therapy, early detection of RTT has become more compelling. However, detecting a *MECP2* variant by a newborn screen array does not guarantee that the variant will result in RTT, as some individuals, as noted above, may have a *MECP2* variant and either be neurologically typical or have only moderate developmental delay owing to favourable levels of X chromosome inactivation. Thus, although screening would provide evidence of the population frequency of the variant, it cannot be utilized at present to determine the likelihood of an individual developing RTT because predictive criteria for *MECP2* variants are not currently available.

Prevention

At present, primary prevention is not possible as 99% of cases arise from de novo or sporadic variants, most frequently from the paternal X chromosome[136–139]. In extremely rare cases, the variant is inherited due to germline mosaicism[140] or skewed X chromosome inactivation[141] where mothers are largely unaffected and have complete or nearly complete X chromosome inactivation in which most of their cells express the typical X chromosome. This expression results in the mothers being phenotypically normal or having substantial developmental delay without other characteristic features of RTT[142,143]. Secondary prevention currently focuses on comorbidities, which is facilitated by the increasing knowledge on the natural history of the disease. Accordingly, consensus management guidelines have been developed, which are discussed in the section below.

Management

RTT is a severe and dynamic disorder with multiple neurological and systemic manifestations[4,21,63]. Hence, management of individuals with RTT is complex and requires continued monitoring over time as specific issues requiring surveillance vary with increasing age (Fig. 5). Growing knowledge on the natural history of the disorder[144] has allowed the delineation of the neurological alterations experienced by affected individuals during their lifespan as well as the timing of emergence and course of comorbidities[21,63]. In the course of time, consensus guidelines developed by international groups of clinical experts using modified Delphi procedures have incorporated further evidence into recommendations based on experiences from clinical practice. So far, management has involved prevention and amelioration of common manifestations in combination with implementation of the entire range of rehabilitation therapies and off-label use of medications[21,145,146].

Trofinetide, an IGF1-related compound, for multiple RTT symptoms is the first disorder-specific drug to be added to the therapeutic repertoire[28,147,148] (Fig. 6). The FDA approved the drug in March 2023, following clinical trials that demonstrated improvement in a wide range of signs and symptoms including hand movements or stereotypies, repetitive behaviours, vocalizations, facial expressions, eye gaze and atypical behaviours (that is, mood abnormalities and disruptive behaviour) in cohorts aged 2–20 years[28,149]. Consequently, the drug was approved for the treatment in individuals with RTT 2 years and older. Trofinetide is available as a liquid oral solution, which is administered either orally or by a gastrostomy tube. Administration via gastrojejunal tubes is possible using the gastronomy port. Most patients experience diarrhoea, which can be mitigated by a variety of published recommendations[150,151]. Weight loss is also frequent. The pivotal trofinetide trial also showed more common vomiting, fever, seizures, anxiety, decreased appetite, fatigue and nasopharyngitis among those who received the drug than among those receiving placebo[152]. No contraindications are indicated to the administration of trofinetide; however, renal function and potential drug interactions need to be taken into consideration. The specific length of administration has not been determined; nonetheless, considering the nature and severity of RTT, long-term administration is expected. The hypothesized roles of trofinetide on cell signalling and neuroinflammation (that is, reduction in pro-inflammatory cytokines) also highlight the need for a better understanding of these processes in the pathogenesis of RTT and other synaptic disorders. Although the safety and efficacy profile of trofinetide is less than ideal, its successful development as the first therapeutic agent for RTT approved by regulatory agencies is a milestone in the field and provides encouragement for future work in the development of more efficacious next-generation therapy options.

Age-based approach

Although studies have described disease stages for RTT[153], an approach based on conventional age period is currently recommended considering the broad phenotypic spectrum observed. Each period is characterized by distinctive impairments and comorbidities that require preventive and therapeutic measures[21]. For example, early childhood (up to 5 years), when regression and core features emerge; late childhood (5–12 years of age), when multiple comorbidities are identified on a background of relative neurological stability; adolescence (12–21 years), characterized by puberty-related concerns, progression of communication and motor impairments and musculoskeletal comorbidities, and the end of school attendance; and adulthood (21 years of age and older), a period in which impairments and comorbidities may continue to progress on a background of parkinsonian symptoms and social withdrawal (Fig. 5). Further details can be found in general and specific guidelines and consensus documents[21,154–158].

From the time of diagnosis, evaluations to monitor progression of the disorder and response to interventions (for example, routine health maintenance, common RTT clinical manifestations) are recommended annually or more frequently, depending on patient age and clinical presentation. Although neurological management is central to the care of individuals with RTT, the wide range of clinical manifestations mentioned above and in the following sections requires the involvement of multiple medical specialists[21]. Rehabilitation services, including physical therapy, occupational therapy, and speech therapy and/or communication devices, are also essential and must be instituted as early as possible[146,159]. Other therapies, such as supervised swimming, horseback riding and music therapy may be included based on individual needs and local availability. Implementation of some of these interventions at home as well as respite services are also important to alleviate caregiver burden. Top caregiver concerns across the disease span and age spectrum include communication, seizures, hand function, ambulation and constipation[160]. All require constant vigilance, but communication impairment is highlighted as fundamental to managing care throughout daily activities including school.

Early childhood. In early childhood, in addition to managing emerging symptoms, the focus should be on mapping growth and nutrition to ensure continued progress. As issues with feeding and digestion are lifelong, the gastrointestinal tract needs to be assessed from top to bottom, potentially including tests such as swallowing study, upper gastrointestinal imaging or endoscopy, stomach emptying study and even gallbladder imaging or endoscopy. Common clinical manifestations include chewing and swallowing problems, gastroesophageal reflux, delayed stomach emptying and constipation. Individuals with RTT show a deceleration in height, weight and head circumference growth, and therefore, these parameters should be assessed according to standard normative charts and recorded accordingly. Furthermore, RTT-specific growth charts have been developed[122,161]. Keeping up proper growth by monitoring the relationship between weight and height via BMI measurements, at least up to adulthood, is crucial. Failure to maintain adequate weight, which may become problematic

Box 3 | Other clinical presentations of *MECP2* variants

MECP2 variants have been associated with other clinical presentations, including non-syndromic autism, Angelman-like syndrome, global developmental delay with obsessive–compulsive disorder, and attention-deficit–hyperactivity disorder[9,115]. Prior to the identification of the link between *MECP2* and Rett syndrome, two specific clinical entities were recognized as having some features of the disorder: an early-onset seizure variant, first described by Hanefeld[203], and a variant characterized by very early developmental delay, described by Rolando[204]. Subsequently, these disorders were associated with genetic variants in *CDKL5* (CDKL5 deficiency disorder)[188,205] and *FOXG1* (FOXG1 deficiency)[189], respectively. Although these disorders have phenotypical overlap with Rett syndome, their distinct clinical features and disease progression indicate that they should be considered as separate disorders[206].

during the second year of life due to inadequate intake or inability to swallow food and liquids safely, could require either food thickeners or complete reliance on alternative feeding mechanisms such as a gastrostomy tube, which may also provide an alternative route for medications when oral medication is not tolerated safely. The use of gastrostomy tubes is common, involving one-third or more of the individuals with RTT[162]. At the time of insertion of the gastrostomy tube, the added procedure of fundoplication (in which the top of the stomach is

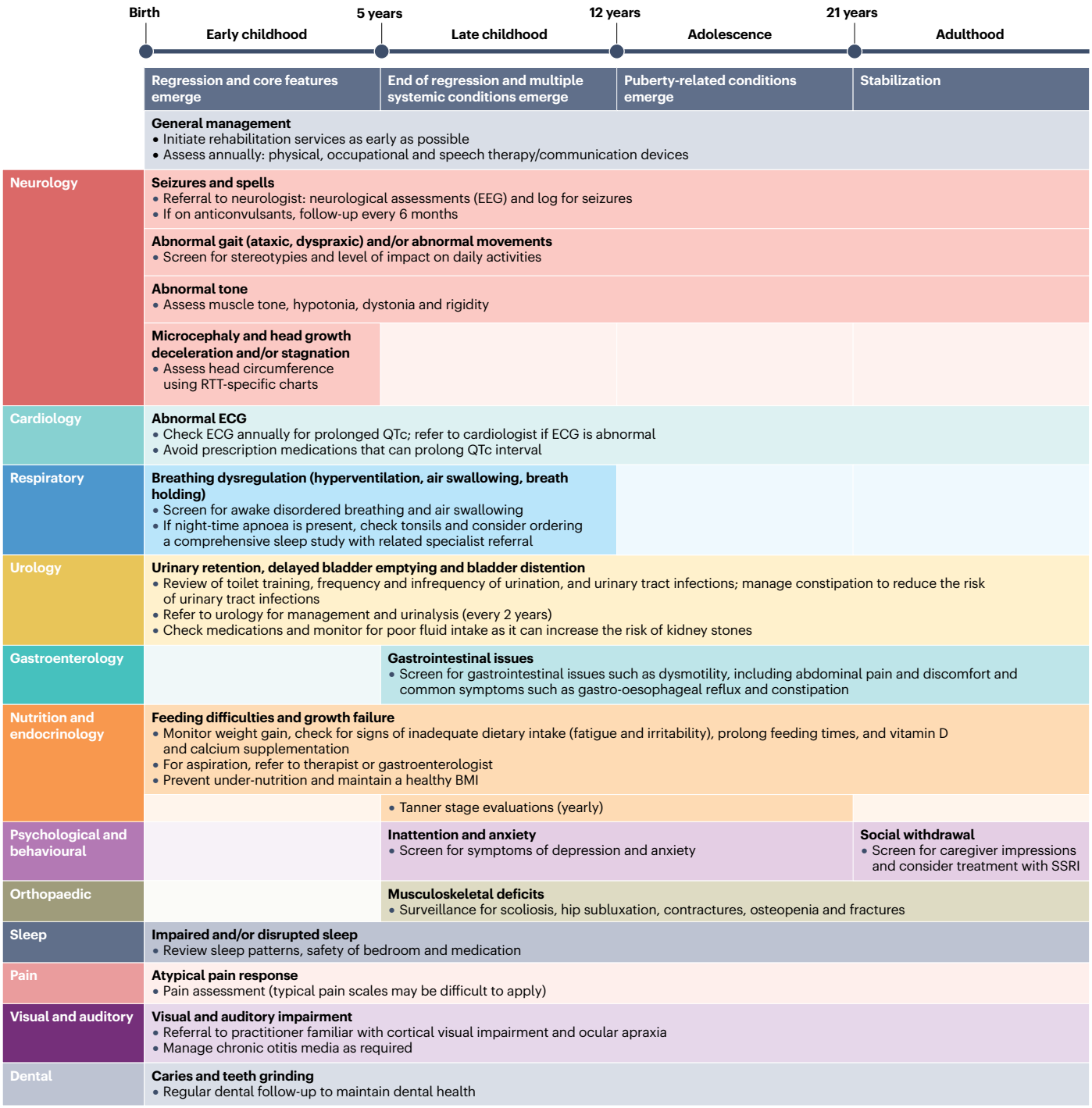

Fig. 5 | Management of RTT. The main clinical manifestations of Rett syndrome (RTT) and their management throughout life are illustrated[21]. Common concerns are organized by system or clinical discipline. ECG, electrocardiography; EEG, electroencephalography; QTc, corrected QT interval; SSRI, selective serotonin reuptake inhibitor.

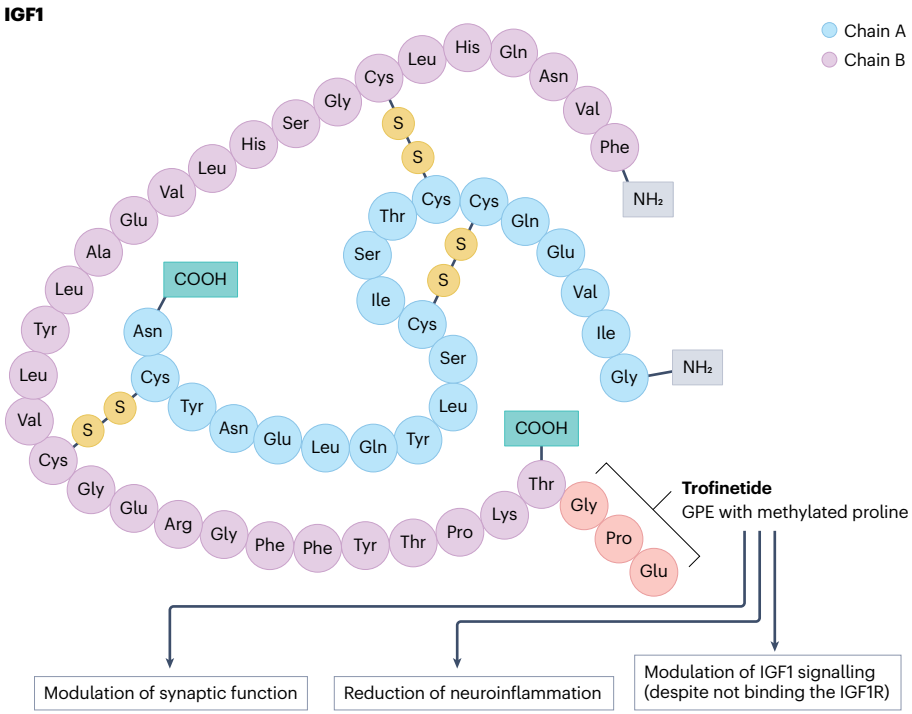

Fig. 6 | Approved treatment for Rett syndrome. Daybue (trofinetide) is the only currently approved specific treatment for Rett syndrome. Trofinetide (glycyl-L-2-methylprolyl-L-glutamic acid) is a synthetic tripeptide analogue of glypromate (GPE), the amino-terminal peptide of insulin-like growth factor 1 (IGF1) consisting of glycine (Gly), 2-methyl-L-proline (Pro), and L-glutamic acid (Glu) joined in sequence by peptide linkages. The hypothesized mechanisms of this drug are indicated, including modulation of synaptic function, reduction of neuroinflammation and modulation of IGF1 signalling.

wrapped around the bottom of the oesophagus to strengthen the lower oesophageal sphincter) is strongly recommended, as the gastrostomy tube alone does not obviate the possibility of gastroesophageal reflux. Fracture risk should be discussed, and bone density assessments, every 1–2 years, should be considered in early childhood and throughout life[157] since non-accidental fractures may be observed in up to 30% of individuals with RTT[157,163].

Late childhood and adolescence. In late childhood, in addition to concerns about nutrition and growth, seizures, breathing problems, scoliosis and behavioural issues (such as, anxiety, self-injury) may become evident. During this period and adolescence, assessing pubertal development is important. Precocious puberty is observed in as many as 25% of girls[164] and, although it may be restricted to pubic hair development (adrenarche), referral to an endocrinologist may be required. The problems described above persist in adolescence, with orthopaedic issues, especially scoliosis including joint contractures and hip displacement, becoming prominent and frequently require surgical intervention. Spine curvature may be inspected visually (in the supine position) but may also require an X-ray examination. The presence of an abnormal curvature will require at least annual follow-up and semi-annual follow-up if the curvature exceeds 20°. Surgery should be considered if the curvature exceeds 40° (refs. 79,155,165). As dystonia may occur during this period, proper orthotics and referral to physical medicine may be required for any individual with abnormal muscle tone or toe-walking[21].

Adulthood. In adulthood, a new set of issues add up to the ongoing impairments and comorbidities. In those countries where interventions are provided at school, adulthood may lead to the end of day care and some therapies. Thus, identifying appropriate services to fill this niche is crucial. In addition, deterioration in bone health may become obvious and may require assessment again by endocrinology (for example, bone density scan). Motor issues, including increasing rigidity, contractures and dystonia, tend to become more evident than in childhood or adolescence, and require increased attention of neurologists, orthopaedists and physical medicine specialists[22,166]. Social withdrawal and depression-like behaviours should also be surveyed in adults with RTT[22,166]. In many countries, as children approach adulthood, addressing the transition by developing guardianship and conservatorship documents for the parents or responsible caregivers is essential. This approach will enable proper access to services for individuals who cannot choose for themselves, as well as provide protection for their assets from governmental intervention.

General care considerations

Following a clinical diagnosis, *MECP2* testing is undertaken to provide supporting evidence. A positive gene test is found in >96% of individuals meeting the clinical criteria for classic RTT[5]. Current recommendations support the full range of genetic testing, including sequencing and other assays for detecting large deletions, at the time of the initial clinical diagnosis[21]. Following a positive result, testing of parents for carrier status is desirable. Prenatal testing is also an option, in the right clinical context. Family counselling on gene test results is essential and may require referral to genetic counselling. As with any person, individuals with RTT require regular health wellness checks, but providers should recognize the complex issues involved and provide extra time for these. Hence, identifying a health-care provider who is knowledgeable about individuals with RTT and can coordinate care and provide referrals to appropriate services according to patient needs is critical. Support for patients should include at least annual visits and any acute care assessments to encompass general health review including medications and allergies, growth evaluations, Tanner stage evaluations (a standardized

way to track the development of secondary sex characteristics during late childhood and puberty) and at least annual laboratory tests to include a complete blood count, a metabolic profile, lipid profile and vitamin D levels (which is recommended to be in the middle of the normal range (>30–40 ng/ml))[21]. Because QT interval prolongation is present in up to 20% of individuals with RTT, annual electrocardiography assessments and referral to cardiology, when abnormal, are necessary[21].

Multidisciplinary care

In addition to the specific management discussed above, other clinical manifestations present at different ages include decreased appetite, which may require referral to gastroenterology, and multiple breathing abnormalities (that is, awake breath-holding, cyanosis, hyperventilation, air swallowing and associated bloating). Notably, minimal evidence exists to support any medications for breathing irregularities[21]. Neurological referrals may be needed for a variety of issues, most prominently, the presence of seizures/epilepsy or non-epileptic paroxysmal events (referred to as Rett spells). Differentiating epileptic from non-epileptic paroxysmal episodes without the aid of an electroencephalogram is often difficult[21]. However, caregivers can assist by providing records of these events including home videos. Ultimately, diagnosis may be determined by video-electroencephalogram monitoring. Antiseizure management should be instituted based on this collective evidence and will require regular follow-up, typically every 6 months with blood test(s) depending on the type of antiseizure medication chosen. Clinical providers should also assess communication and social interaction; muscle tone, including dystonia and rigidity; hand function and stereotypies; gait, whether independent or assisted; presence of a movement disorder; and evidence of abnormal behaviours[21,132]. Frequent hand-mouthing, self-biting or recurrent hand wringing should be monitored for loss of normal skin integrity. Behavioural difficulties, which may become prominent in unfamiliar or busy or noisy environments, include anxiety and social withdrawal, as well as self-injury and aggression towards others[21,22]. Selective serotonin reuptake inhibitors and atypical neuroleptics are recommended, respectively, when behavioural therapy has failed or is unavailable[21]. Difficulties with sleep initiation and maintenance may require expert consultation. Interruption in sleep pattern may be frequent and related to hunger, gastroesophageal reflux or constipation. If snoring or common disrupted breathing during sleep are present, a sleep study may be required. In the case of restless legs, ferritin levels may need to be checked. In terms of sleep aids, melatonin for initiating and trazodone, clonidine and gabapentin (in children <6 years of age) for maintaining sleep are recommended[21,167]. Additionally, the safety of the bed and bedroom should be assessed. If the child is ambulatory and capable of wandering during the night, when the caregivers are sleeping, a sleep-safe bed or restriction from exiting the bedroom is appropriate. Other issues to consider are: recurrent urinary tract infections, due to mobility impairment; pain tolerance, which is typically high, can mask fractures or other injuries; and limited heat tolerance, even when hands and feet are cool, so that exposure to high summer temperatures should be carefully monitored.

Barriers to care

Owing to its rare occurrence, primary care providers and other health-care professionals may have substantial limitations in providing appropriate care to individuals with RTT[168]. Access to specialists and services in an adult care system unfamiliar with neurodevelopmental disorders further complicates the situation. Incorporation of paediatricians, internists or family physicians, after appropriate training, may be a solution as this problem is common in neurodevelopmental disorders and not exclusive to RTT[169,170]. Some of these limitations are addressed by the aforementioned consensus guidelines, which intend to promote the integration of care across the range of primary and subspecialty providers and offer age-based guidelines for managing a range of symptoms[21]. Although data are limited for neurodevelopmental disorders in general[171], challenges in accessing quality care for individuals with RTT seem to be greater in low-income and middle-income regions than in high-income regions[172].

Quality of life

Owing to improved advances in care, life expectancy for individuals with RTT has increased, with a large proportion of patients surviving into their 50s[10,35]. Inability to walk, number of hospitalizations, poor nutritional status and global health, and seizure severity are among the factors that greatly influence long-term morbidity and mortality. Nevertheless, as a severe and progressive neurodevelopmental disorder, quality of life is affected beyond health-related issues, since continuing functional deficits and comorbidities may lead to substantial physical, psychological, social and financial burden on affected individuals and their families. Factors with greatest influence on the quality of life of individuals with RTT include communication, ambulation and feeding skills, age at onset of hand stereotypies, severity of seizures, sleep problems and behavioural difficulties[173–175].

The severity of an affected child's physical and behavioural problems, in particular feeding difficulties, are known to impact the physical and mental well-being of caregivers[1,176–178]. Other factors playing a role in the quality of life of caregivers include their age and demands, and financial and family functioning challenges[177–180]. Mothers of individuals with RTT seem to be at an increased risk of developing anxiety and, perhaps also, depression[176,181]. Of note, caregiver mental health seems to be more affected than their physical quality of life[177]. Data on siblings of individuals with RTT are limited, with a small study showing relatively good psychological adjustment[176], whereas another report on a larger sample, evaluating the effect on siblings of children with Down syndrome, demonstrated both benefits and disadvantages for siblings in the RTT group[182]. A large international investigation on burden[183] of illness in RTT found that, in line with reports on top caregiver concerns in the US Natural History Study[160], core features and related impairments, such as decreased communication and hand use abilities, especially affect the quality of life of individuals with RTT and their caregivers[183]. Nonetheless, some clinical manifestations considered by caregivers to be mild in severity, including sleep difficulties, seizures, non-epileptic paroxysms (Rett spells), pain and behavioural abnormalities, can also have a high impact on the quality of life of affected individuals and, particularly, on their caregivers[160,183].

Although available data on quality of life provide valuable information, the lack of standardized measures appropriate for individuals with a severe neurodevelopmental disorder such as RTT, has represented a major limitation. Hence, new instruments[184] and analytical strategies[185] under development have begun to assess domains of quality of life that are important for children[186] and adults[187] with RTT. Future investigations are expected to further refine our understanding of quality of life of individuals with RTT and their families.

Outlook

Since the original description of RTT 40 years ago[3], the scientific community has seen remarkable progress in the delineation, understanding,

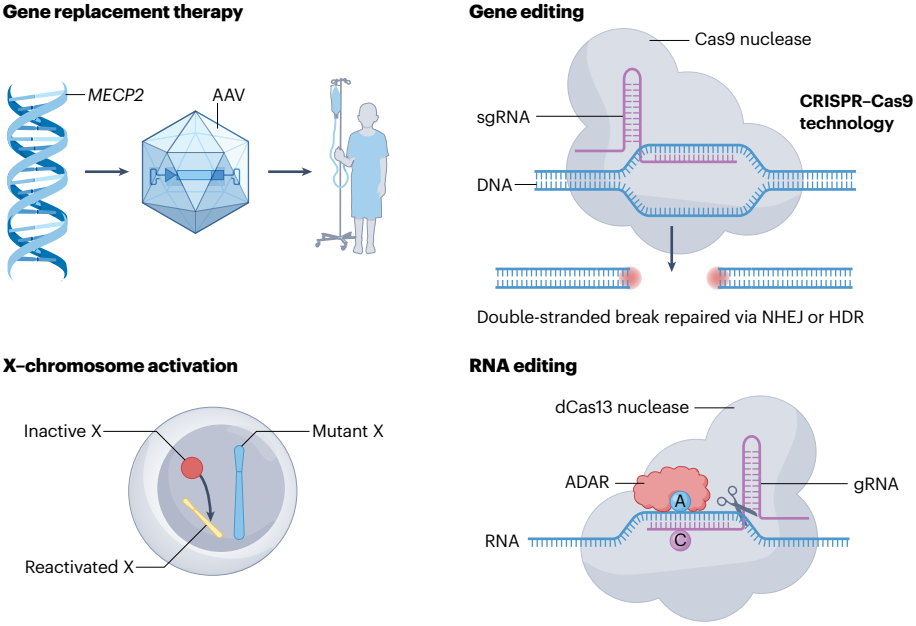

Fig. 7 | Genetic strategies for managing Rett syndrome. Genetic strategies include gene replacement therapy using adeno-associated virus (AAV) 9 delivery of *MECP2*; gene editing using CRISPR–Cas9 to edit and correct specific variants in *MECP2*; X chromosome activation to reactivate the silenced wild-type copy of *MECP2* in females; and base-editing RNA strategies to modify specific variants in *MECP2* transcripts (as reviewed in refs. 193,196,197). ADAR, adenosine deaminase acting on RNA; dCas13, catalytically dead CRISPR–Cas13; gRNA, guide RNA; HDR, homology-directed repair; NHEJ, non-homologous end joining; sgRNA, single guide RNA; X, X chromosome.

management and prognosis of the disorder. Despite such advances, many challenges remain but opportunities have also arisen.

Diagnostic and assessment challenges

As with other monogenic neurodevelopmental disorders, the clinical presentation of RTT is surprisingly variable leading to difficulties in diagnosis, and in particular, in assessing disease progression. The lack of complete correlation between the presence of *MECP2* pathogenic variants and clinical features makes it necessary to continue relying on clinical criteria for the diagnosis of RTT, which frequently results in long diagnostic lead times. The availability of genetic testing for the evaluation of children with developmental delay and/or early-onset epilepsy has led to the identification of *MECP2* variants in very young individuals, before diagnostic features of RTT such as developmental regression have emerged, which requires follow-up to confirm their association with a RTT phenotype. Novel diagnostic challenges are exemplified by genomic sequencing revealing a *MECP2* variant of unknown significance in a child displaying features of atypical or even classic RTT. The presence of *MECP2* variants in males with abnormal neurodevelopment represents a major challenge, as unlike females, the vast majority of males do not present with classic RTT. Ongoing delineation of the phenotype of males with *MECP2* variants will, ultimately, determine the suitability of implementing current RTT diagnostic criteria in this group. Another dilemma is represented by children with some diagnostic features of RTT and variants in other genes linked to other neurodevelopmental disorders. In these cases, the diagnostic classification should take into consideration the splitting of CDKL5 deficiency disorder[188] and FOXG1 deficiency[189] as disorders distinct from atypical RTT. Ultimately, additional knowledge on the features and evolution of individuals without RTT but with a *MECP2* variant or with partial RTT features will determine the genotypical and phenotypical boundaries of RTT and will, hopefully, lead to early and precise diagnoses.

Evaluation and treatment of individuals with RTT requires a multidisciplinary approach, involving virtually all clinical and rehabilitation disciplines, with experience in this rare population. Hence, access to adequate care is a constant struggle. Other factors that add complexity include the fact that care requirements are specific to each affected individual, varying over time, and that management of individuals with RTT should go beyond those that are disorder-specific (for example, screening for cancer in adults). The need for a medical system that accommodates individuals with developmental disabilities throughout their life is not a concern exclusive to RTT; however, improvements in the disorder's life expectancy underscore its importance. Continuous collection of natural history data and clinical practice experience, along with improved diagnostic rates and early diagnosis are key to overcoming most of these challenges, as well as caregiver's insights on impairments and comorbidities with the greatest effect on quality of life.

Increasing knowledge on pathophysiology

Even prior to the discovery that variants in *MECP2* result in RTT[6], brain and CSF studies in affected individuals allowed its characterization as a widespread synaptic disorder involving all major neurochemical systems. The *MECP2* era of RTT, with the availability of animal and in vitro genetic models, has refined and expanded this concept by characterizing individual neuron-level, synaptic-level and circuit-level disruptions caused by MeCP2 deficit. Demonstration of atypical long-term synaptic plasticity, manifested by cell type-specific and brain region-specific excitation–inhibition imbalances, has allowed a better understanding of the symptomatology of RTT. Importantly this neurobiological knowledge, combined with a better understanding of the role of MeCP2 in the CNS, has also allowed the development and testing of RTT-specific treatments (Fig. 7). Nonetheless, many aspects of *MECP2* expression and its regulation, particularly in the context of pathogenic variants, are still unknown and could be critical in the development of new therapies.

Development of new treatments

The use of animal models of MeCP2 deficit has been essential for the identification and testing of a broad range of potential therapeutic

targets, which include neurotransmitter systems, neuronal growth factors and related signalling pathways, and metabolic pathways. Although the outcome of any drug development programme depends on multiple factors, such as study design and quality of outcome measures, efforts in the field have led to the success of trials of trofinetide, a synthetic version of the endogenous tripeptide from the amino-terminal domain of IGF1 (refs. 28,147,148). Considering the global neuronal and synaptic disruption associated with MeCP2 deficit, approaches involving multiple neural systems and signalling pathways are in principle more efficacious than targeting a single signalling pathway or neurotransmitter system. Given the severity and clinical complexity of RTT, traditional treatment approaches (for example, targeting the glutamatergic system) will continue to be attempted.

Another major milestone in RTT therapeutics is the beginning of gene therapy testing[190–192]. Although current gene replacement trials are mainly focused on demonstrating safety in children and adults, against a backdrop of new gene therapy approvals in other devastating neurological disorders, their innovative methodologies for ensuring gene dosage balance are the result of many years of detailed characterization of *MECP2* expression[193,194]. In addition, other gene therapy strategies such as X chromosome reactivation, gene editing and mRNA editing are on the horizon and improve the prospect of modifying the course of RTT (Fig. 7). Considering the relatively late diagnosis of RTT, combination of drug and gene treatments are also a possibility for preventing progression and even reversing clinical manifestations. The development of outcome measures sensitive to changes in natural evolution, in a relatively heterogeneous disorder, will be key for accomplishing these goals. In conclusion, the state of the RTT field offers hope for making substantial improvements in the quality of life of individuals with this severe neurodevelopmental disorder and similar conditions.

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Author contributions
Introduction (W.A.G., A.K.P., J.L.N., J.K.I. and W.E.K.); Epidemiology (W.A.G., A.K.P., J.L.N. and W.E.K.); Mechanisms/pathophysiology (W.A.G, S.R.C., L.P.-M., J.L.N. and W.E.K.); Diagnosis, screening and prevention (A.K.P., J.L.N., B.B.-Z. and W.E.K.); Management (A.K.P. and W.E.K.); Quality of life (A.K.P., A.V. and W.E.K.); Outlook (A.K.P. and W.E.K.); overview of the Primer (W.E.K.).

Competing interests
A.K.P. has a consulting relationship with Acadia Pharmaceuticals, Anavex, Ionis, Neurogene, and Taysha, and has previously had a contract researcher relationship with Acadia Pharmaceuticals. J.L.N. has received research funding from the National Institutes of Health, the International Rett Syndrome Foundation and the Rett Syndrome Research Trust, and has served as investigator for clinical trials conducted by Acadia Pharmaceuticals, GW Pharmaceuticals, Neuren Pharmaceuticals and Newron. J.L.N. has also received personal consultancy for Acadia Pharmaceuticals Inc., Analysis Group, Anavex, AveXis, GW Pharmaceuticals, Hoffmann-La Roche, Ionis Pharmaceuticals, Myrtelle, Neurogene, Newron Pharmaceuticals, Signant Health and Taysha Gene Therapies, and the preparation of CME activities for the France Foundation, MedEdicus, Medscape, PeerView Institute, Medscape and TotalCME; and served on the scientific advisory board of Alcyone Lifesciences; was a scientific cofounder of LizarBio Therapeutics; and is a member of a data safety monitoring board for clinical trials conducted by Ovid Therapeutics and Ultragenix. S.R.C. is currently the Chief Scientific Officer at Neurogene Inc. He received research funding from the Rett Syndrome Research Trust, Simons Initiative for the Developing Brain, Neurogene and Rettco Inc. He has received patent royalties relating to gene therapy products being developed for Rett syndrome. B.B.-Z. received research funding for clinical trials from Ultragenix and the Rett Syndrome Research Trust. She has been a consultant to Taysha in the past and is serving as a monitor for Neurogene gene therapy programme. A.V. is a consultant for Anavex and GW Pharmaceuticals, and has conducted clinical trials with Newron Pharmaceuticals. L.P.-M. has received research funding from the National Institutes of Health, the International Rett Syndrome Foundation, and the Rett Syndrome Research Trust. W.E.K. was the Chief Scientific Officer of Anavex Life Sciences Corp. He received funding from the International Rett Syndrome Foundation, the National Institutes of Health and the Centers for Disease Control and Prevention, and he has been a consultant for Anavex, AveXis, Acadia, Compass, EryDel/Quince, Neuren Pharmaceuticals, Newron, GW Pharmaceuticals, Marinus, Biohaven, Zynerba, Ovid Therapeutics, Stalicla and Tetra. He has conducted clinical trials with Neuren and Ipsen. W.A.G sits on the Scientific Advisory Board of the International Rett Syndrome Foundation and has received funding from the Australian National Health and Medical Research Council. J.K.I. declares no competing interests.

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