RARE DISEASES

Clinical and biological progress over 50 years in Rett syndrome

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Abstract | In the 50 years since Andreas Rett first described the syndrome that came to bear his name, and is now known to be caused by a mutation in the methyl-CpG-binding protein 2 (*MECP2*) gene, a compelling blend of astute clinical observations and clinical and laboratory research has substantially enhanced our understanding of this rare disorder. Here, we document the contributions of the early pioneers in Rett syndrome (RTT) research, and describe the evolution of knowledge in terms of diagnostic criteria, clinical variation, and the interplay with other Rett-related disorders. We provide a synthesis of what is known about the neurobiology of MeCP2, considering the lessons learned from both cell and animal models, and how they might inform future clinical trials. With a focus on the core criteria, we examine the relationships between genotype and clinical severity. We review current knowledge about the many comorbidities that occur in RTT, and how genotype may modify their presentation. We also acknowledge the important drivers that are accelerating this research programme, including the roles of research infrastructure, international collaboration and advocacy groups. Finally, we highlight the major milestones since 1966, and what they mean for the day-to-day lives of individuals with RTT and their families.

In 1966, Andreas Rett first reported on a series of 22 young female patients with similar characteristics. He was initially alerted to their similarities when he observed two of the group sitting together in his waiting room, demonstrating almost identical stereotypic hand movements[1] (FIG. 1), and so the gestalt of Rett syndrome (RTT) was first recognized. RTT was initially thought to be of metabolic origin because of an apparent association with hyperammonaemia, but this idea was later discounted because of laboratory error. 17 years later, Bengt Hagberg and colleagues attributed Rett's name to the condition that they had also seen in their patients[2]. The disorder affected girls, who initially seemed to develop normally, but began to lose their previously achieved abilities — in particular, hand use and speech — at 7–18 months of age (or sometimes later, as has subsequently been shown[3]).

Our aim in this Review is to describe the 50-year journey from the recognition of RTT to the present day, a journey that has included iterations of the diagnostic criteria and growing understanding of the clinical and biological variation of the disorder. We focus particularly on the discovery that RTT is caused by a mutation on the *MECP2* gene, the burgeoning knowledge of its neurobiology, and ensuing pathways to clinical trials. We include a detailed review of the phenotype and observed relationships with genotype, and reflect on how knowledge of RTT has advanced rapidly, in part due to database infrastructure, international collaborations and strong advocacy groups.

Pivotal discoveries and advances

The original description of RTT by Hagberg and colleagues[2] (FIG. 1) was followed by an explosion of literature about the disorder, much of which was published as proceedings of early meetings held in Vienna and Baltimore.

An important outcome of the first Vienna symposium was the need for a set of clinical criteria to facilitate diagnosis (FIG. 1), and a schema of clinical characteristics with eight inclusionary and four exclusionary criteria was published soon afterwards[4]. Over the past three decades, these criteria have undergone several iterations[5–7].

The international workshop held in Baltimore was co-sponsored by a newly formed parent organization, the International Rett Syndrome Association, and was attended by over 85 health-care professionals, along with 70 girls with RTT and their families. This workshop was the beginning of a close collaboration between parents and researchers, which has contributed greatly to the rapid advancement of knowledge in this condition. A case series that emerged as a consequence was seminal in informing the medical community about the

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Key points

- In the 50 years since its description by Andreas Rett, we have witnessed an explosion of knowledge about Rett syndrome (RTT) in relation to its genetic basis and clinical characteristics, and their interrelationships
- Initially, the diagnosis of RTT was based solely on clinical criteria, but identification of its genetic cause has revolutionized this process, while presenting new challenges as we enter the era of next-generation sequencing
- Mutations in the methyl-CpG-binding protein 2 (*MECP2*) gene were found to be causative of RTT, accounting for fundamentally altered neurobiological pathways, and providing the stimulus to identify pathways that can be manipulated therapeutically
- The type of *MECP2* mutation is associated with clinical severity, and influences many aspects of the phenotype, including functional abilities, onset of scoliosis, bone health, and sleep disturbances
- Considerable progress has been made in understanding the natural history of RTT, leading to improvement in clinical management in selected areas, and changes in attitudes and allocation of health-care resources have increased life expectancy
- The advancement in knowledge about RTT has been dependent on global efforts to study this disorder, including the establishment of database infrastructures, the input of advocacy groups, and the development of international collaborations

clinical features of this disorder[8], as was the description of 19 cases in the west of Scotland[9].

A staging system, which characterized the disease profile into four distinct phases, was developed from information relating to 29 Swedish cases[10]. On the basis of citation history, this system seems to have been widely adopted but, as yet, has not been formally validated in the light of the currently available genetic knowledge and longitudinal data.

The pivotal discoveries that followed on from the original clinical revelations are outlined in FIG. 1, and their enormous significance will become clear as we follow the story of RTT — in the laboratory, in the clinic and across the world — over a further three decades.

Identifying the genetic cause of RTT

The relationship between the *MECP2* gene and RTT was discovered in Huda Zoghbi's laboratory in 1999 (REF. 11) (FIG. 1). This crucial milestone was reached as a consequence of preceding exclusion mapping studies, which had narrowed down the area of interest on chromosome Xq28 (REFS 12,13). The nuclear protein MeCP2 had hitherto been of interest largely in the field of epigenetics, and the finding that MeCP2 lay at the root of RTT resulted in a convergence of clinical, neuroscience and epigenetics researchers to begin to understand the disease process.

This momentous discovery had two immediate sequelae, the first being its impact on research. A second study from the Zoghbi laboratory identified a *MECP2* mutation in just over three-quarters of screened patients with sporadic RTT, and in two of seven familial cases[14]. Severity was scored from previous clinical observations, and mutations were categorized as either truncating or missense. Although nonrandom X-inactivation also affected the phenotype, no overall genotype–phenotype relationships were identified at this stage[14]. However, this was just the first of numerous such investigations that were conducted across the globe in the ensuing years[15–18]. One of the earliest papers identified *MECP2* mutations in 80% of typical RTT cases[18]. These included eight recurrent missense and nonsense mutations, which are now known to account for almost two-thirds of the mutations seen in RTT[19,20] (FIG. 2).

The second consequence of Zoghbi's findings was the burgeoning availability of genetic testing, at least in European countries with equitable public funding systems, and for appropriately insured patients in the USA. Sadly, however, genetic testing remains inaccessible to patients in many countries. Techniques other than direct sequencing, such as multiplex ligation-dependent probe amplification (MLPA), which is necessary for the identification of large deletions of exon 3 and 4 (REFS 21,22), have also become available. Such developments would have major implications for the subsequent identification of these mutation types.

Neurobiology of MeCP2

RTT is not considered to be a degenerative brain condition, but patients with this syndrome exhibit reductions in gross brain volume, which are associated with the presence of abnormally small, densely packed neurons with reduced dendritic complexity and synapse density[23]. The discovery, in 1999, that genetic lesions in the *MECP2* gene represent the underlying cause of RTT[11] dramatically intensified efforts to model the disorder biologically.

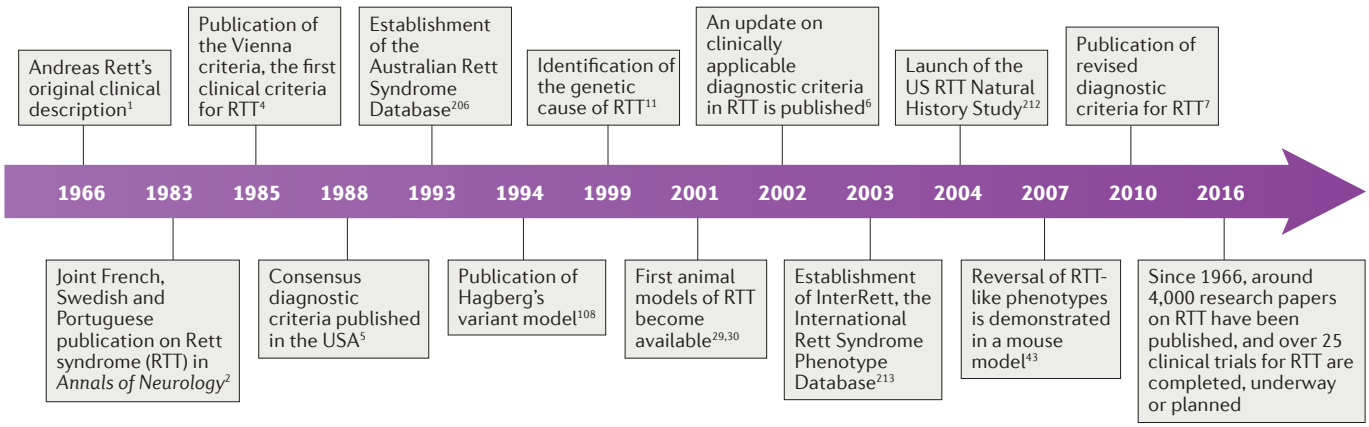


Figure 1 | **Timeline of key events and discoveries in Rett syndrome.**

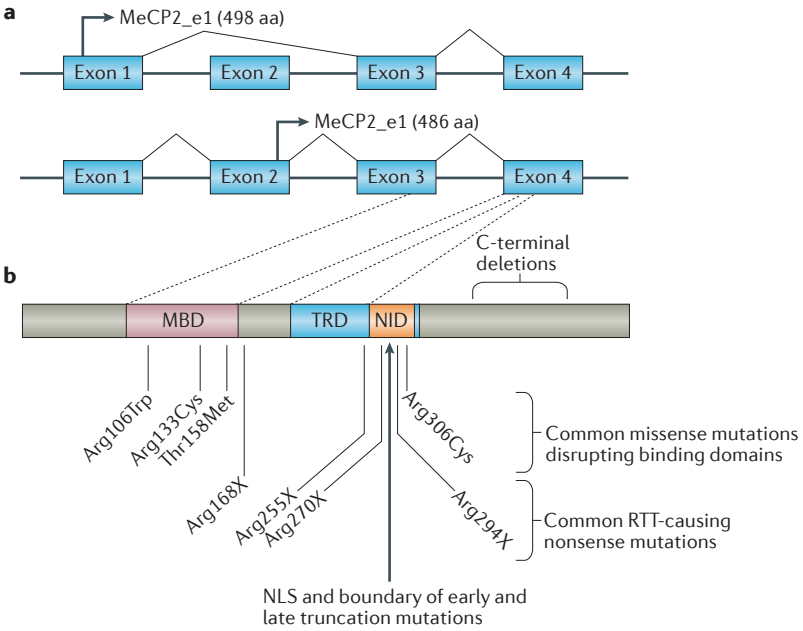


Figure 2 | **The *MECP2* gene and Rett syndrome.** The figure shows the structure of the methyl-CpG-binding protein 2 (*MECP2*) gene, and key MeCP2 protein domains implicated in Rett syndrome (RTT) pathogenesis. **a** | The two known mRNA isoforms, *MECP2_e1* and *MECP2_e2*, generate two protein isoforms, which differ only at the extreme N-termini owing to the use of alternative translation start sites (bent arrows) and selective inclusion of exon 2 in the transcript. **b** | The MeCP2 protein contains distinct functional domains that are pertinent to RTT pathology: the methyl-CpG-binding domain (MBD), the transcriptional repression domain (TRD), the NCOR–SMRT interaction domain (NID), and the nuclear localization signal (NLS). Missense mutations causing RTT predominantly cluster across the MBD and TRD/NID, whereas neutral variants tend to lie outside these domains. The locations of common RTT-causing point mutations are indicated, as is the region in which common C-terminal deletions occur.

MeCP2 is essential for normal brain function. Much of the work on MeCP2 has relied on patient-derived cells[24–28] and genetically modified mice, including *Mecp2*-knockout lines[29,30] (FIG. 1), as well as a variety of conditional knockout lines in which the gene has been deleted from specific brain regions or brain cell types[30–38], or at different stages of development[39]. This work has shown that loss of MeCP2 disrupts the given brain region or system from which it is deleted, and that localized disruption results in a subset of the commonly observed symptoms of RTT. Deletion from GABAergic circuits, which are ubiquitous across brain systems, produces a near-complete *Mecp2*-null phenotype, including motor and cognitive impairments[32]. By contrast, deletion from glutamatergic cells causes anxiety and tremor[40]. Interestingly, postnatal deletion of *Mecp2*, even within a mature nervous system, results in RTT-like phenotypes[41,42].

In mouse models, activation of a previously silenced *Mecp2* allele globally, or within GABAergic neurons, reverses many established RTT-like phenotypes, including locomotor and behavioural impairments, and promotes functional and structural plasticity within the brain[43–45] (FIG. 1). These findings suggest that many of the features that characterize an RTT-like disorder in mice are amenable to reversal, but also indicates that RTT is not a straightforward neurodevelopmental disorder, and that MeCP2 has an essential and ongoing role in the mature nervous system. These observations have important implications when considering potential therapeutic interventions. An important caveat in interpreting the mouse data is that hemizygous (*Mecp2*$^{-/y}$) null male mice are frequently used experimentally, owing to their more overt and rapidly apparent phenotypes. One should note, however, that heterozygous (*Mecp2*$^{+/-}$) female mice are the most accurate genetic representation of the majority of patients with RTT, despite the fact that they develop overt phenotypes at a much later developmental time point than do humans.

MeCP2 is especially abundant in postmitotic neurons[46,47], but is also expressed at modest levels in non-neuronal cells in the brain[48,49] and in other tissues throughout the body[50,51]. Deletion of *Mecp2* from glia in mice has relatively minor phenotypic consequences, but restoration of MeCP2 to astrocytes in an otherwise MeCP2-deficient nervous system results in partial amelioration of phenotypes, including normalization of breathing patterns, motor activities and anxiety levels[48]. As also indicated in primary culture experiments[52], MeCP2 in glial cells might contribute to certain non-cell-autonomous functions, such as supporting normal dendritic morphology through the release of trophic factors within the nervous system. However, a lack of functional MeCP2 in neurons is generally considered to be the dominant driver of RTT[53].

MeCP2 in non-neural cells. The relative importance of MeCP2 in peripheral tissues is unclear. The consequences of global MeCP2 deficiency are observed in several peripheral systems, and they include fatty liver and metabolic disease[54], lung lesions[55], cardiac effects[56,57], and aberrant bone phenotypes[58,59]. Selective deletion of *Mecp2* in hepatocytes did not recapitulate the metabolic dysfunction (insulin resistance, glucose tolerance and altered circulating fatty acids) or overt neurological effects[54] seen in knockout mice, but did recapitulate the fatty liver seen in some *Mecp2*-null lines, possibly reflecting a phenotype with a genuine peripheral origin. Evidence has also been obtained for altered bone cell regulation in MeCP2-deficient osteocytes[60], probably explaining the osteoporotic phenotypes described in RTT. By contrast, no changes have been observed in skeletal muscle following selective local *Mecp2* deletion[61].

Overall, MeCP2 depletion studies have revealed that the majority of RTT-like behavioural, sensorimotor and autonomic phenotypes are associated with MeCP2 deficiency in the brain. However, some less extreme but still clinically significant aspects of the disorder may arise independently of defects in the nervous system[51].

MECP2 mutations and protein function. The structure and function of MeCP2 protein have been reviewed in detail elsewhere[39,62]. The two known protein isoforms of MeCP2, MeCP2_e1 and MeCP2_e2, differ only at the extreme N-terminus and, despite some evidence for isoform-specific functions[63], the two forms are considered to be largely functionally equivalent[53,64], although

MeCP2_e1 is the dominant brain isoform. MeCP2 was originally discovered as a result of a biochemical screen for factors that interact with DNA, in particular, with methylated cytosines (within the context of CpG sequences)[65]. MeCP2 is a nuclear protein that tracks DNA methylation by virtue of its methyl-CpG-binding domain (MBD)[66]. Emerging evidence suggests that the MBD of MeCP2 does not interact exclusively with CpG dinucleotides, but also has an affinity for methylated CpA[67]. The MBD is also reported to interact with 5-hydroxymethylcytosine-containing DNA[68,69], and these modified DNA sequence contexts might be of special importance in the brain[70]. The importance of the MBD is highlighted by the fact that pathogenic missense mutations in this region cause reduced binding to methylated DNA[71]. Regions distinct from the MBD, including AT-hooks[72] and a basic cluster[73], have also been implicated in DNA binding. The functional importance of these regions remains to be fully established, but it is possible that, together with the MBD, they contribute to chromatin structure.

A presumed major function of MeCP2 is to regulate gene expression at either a local or a global level. DNA methylation is a modification that is linked to gene silencing, and a long-held view is that MeCP2 is important in transcriptional repression[74]. However, MeCP2 has also been linked to gene activation[75]. MeCP2 interacts with a wide range of proteins[39], including the histone deacetylase co-repressor complexes SIN3A, NCOR (nuclear receptor co-repressor) and SMRT (also known as NCOR2)[76–79]. The NCOR–SMRT interaction domain (NID) has been mapped within the wider transcriptional repression domain (TRD) of MeCP2, and a cluster of RTT-causing missense mutations, including the common Arg306Cys variant, have been shown to disrupt this interaction[71] (FIG. 2). These findings have led to the idea of a bridge model, whereby MeCP2 functions as a tether between DNA and the NCOR–SMRT complex, and missense mutations at either end of the bridge will result in RTT[71]. Recent reports suggest that MeCP2-associated transcriptional regulation is preferentially targeted to long genes, which might be important in the downstream cellular pathologies[80,81].

In addition to the repressor model of MeCP2 function, a number of alternative or overlapping functions have been ascribed, including direct roles in chromatin remodelling (compaction)[82], gene activation[75], regulation of alternative splicing[83,84], and microRNA (miRNA) processing[85]. In turn, MeCP2 function can be regulated by miRNAs[86,87] and activity-dependent phosphorylation[88,89]. The relevance of this latter mechanism to RTT is unclear, as no RTT-causing point mutations have been reported within known MeCP2 phosphorylation sites. The level of MeCP2 within a given cell type is believed to be crucial for normal cellular homeostasis, and both loss of function and overexpression have neurological consequences[53,90–92]. *MECP2* duplication syndrome, the clinical manifestation of overexpression, is more commonly reported in males than in females[91,93], and its phenotype is gradually being delineated. When modelled in mice, *MECP2* duplication syndrome, like RTT, has shown the potential for phenotypic reversal when MeCP2 levels are restored to normal[94].

Loss of MeCP2 alters the cellular levels of many gene products, but the effects at the individual gene level are typically small[75,95], and are likely to be cell-type-specific. The fact that a wide variety of genes are affected suggests that the existence of a single pathogenic pathway that can act as a focus for all therapeutic interventions is unlikely. Downstream, many cellular systems are disrupted, and there have been reports of altered synaptic function and plasticity[43,96–100], reduced protein synthesis[101], impaired mitochondrial function[102], oxidative stress[103], and alterations in various signalling and homeostatic pathways, such as the mTOR–AKT pathway[101] and energy and lipid metabolism[54]. The relative importance of these effects to cellular dysfunction may depend on the type and state of the cell.

Clinical features and diagnosis

Diagnosis of Rett syndrome and related disorders — evolution over time. Until 1999, RTT remained solely a clinical diagnosis, based initially on the Vienna criteria[4], and subsequently on modifications made by a US group[5] (FIG. 1). The modifications included a slight expansion of the exclusion criteria, and the addition of a set of supportive criteria relating to breathing dysfunction, peripheral vasomotor disturbances, seizures, scoliosis, growth retardation and small feet.

The revised diagnostic criteria were initially restricted to include only classic cases of RTT (BOX 1; see Supplementary information S1 (video)), with the intention of providing a homogenous patient population

Box 1 | **Severe Rett syndrome phenotype**

The severe Rett syndrome (RTT) phenotype is exemplified by a 12-year-old girl with the Arg270X mutation in the methyl-CpG-binding protein 2 (*MECP2*) gene (see Supplementary information S1 (video)). She learned to sit at 9 months, but did not learn to walk. She did learn to say "mum", "dad" and "nan", but she found it difficult to grasp objects. Her mother was concerned about her poor developmental progress and jerky movements, and sought specialist advice when, at 14 months, her daughter suddenly stopped using words and developed hand stereotypies. RTT was confirmed at the age of 2 years by the presence of a *MECP2* Arg270X mutation, an early diagnosis that was consistent with her severe clinical presentation (FIGS 2–5).

The girl exhibited early regression of communication skills but no apparent loss of hand function. As a young child, she could grasp a large object but could not hold it — a feature that she still demonstrates. She also illustrates many other features of RTT. Altered breathing patterns first developed at 18 months, and she still experiences daily hyperventilation and breath-holding with abdominal bloating. Epilepsy was diagnosed at the age of 4 years 8 months, although her seizures are currently well controlled. Since the age of 9 years, she has been fed via a gastrostomy tube to ensure adequate fluids and nutrition, and to protect her respiratory health. Scoliosis was diagnosed at 5 years, and she underwent spinal fusion at 9 years. She has also sustained several long bone fractures in the lower limbs.

The girl's sleep is regularly disturbed; she grinds her teeth, has a high pain tolerance and also has small and cold feet. She has experienced many episodes of bronchitis, although her respiratory health improved markedly after her spinal fusion. Unusually, she recently developed inflammatory bowel disease. There are many limitations to her health and functioning, but she also lives a full life. She loves larking around with her family — her big eyes light up — and she enjoys school, music and swimming.

Written consent for publication of this case report was obtained from a responsible relative.

for epidemiological research[104]. Subsequently, it was recommended that cases not fulfilling all the necessary criteria should be designated as atypical[105]. In Europe, the term 'variant' was used to describe a range of Rett-like phenotypes that were categorized as atypical in other regions. These phenotypes included forme fruste (BOX 2; see Supplementary information S2 (video)), congenital, infantile seizure onset[106], male, late childhood regression and preserved speech variants[107]. Subsequently, a model to categorize atypical RTT in "a girl with unspecified mental retardation, aged 10 years or more" was developed, and required the presence of three or more primary criteria and five or more supportive criteria[108] (BOX 2; FIG. 1; see Supplementary information S2 (video)). The purpose of this model was to cover the full range of clinical manifestations that are likely to be encompassed by the underlying biological disorder, which was subsequently revealed by the discovery of the true genetic cause of RTT[11] (FIG. 1).

At a meeting in Baden-Baden in 2001, the existing three sets of criteria[4,5,108] were assessed and combined to form two new versions, one for classic RTT (BOX 1; see Supplementary information S1 (video)) and one recognizing atypical RTT (BOX 2; see Supplementary information S2 (video)) as its own entity[6] (FIG. 1). The new criteria reflected some additional lessons that had been learned since the formulation of the previous criteria, such as the fact that early development was not invariably normal[109], and that head growth did not always decelerate[110].

In 2010, a further set of criteria was introduced in the hope of clarifying some of the differences in terminology between Europe and North America[7] (FIG. 1). In contrast to previous iterations, and in addition to the four core criteria relating to loss of hand skills, loss of spoken language, gait abnormality and stereotypic hand movements, a mandatory criterion of a period of regression followed by recovery or stabilization was introduced. For atypical RTT, a period of regression was also mandatory, but only two of the four criteria were required, along with at least five of eleven supportive criteria.

One may question the need for the additional regression criterion, given that regression in some patients is often described as "fleeting or unrecognized[111]," or may not yet have occurred at the time of genetic testing, which is now widely used by clinicians diagnosing RTT. Although dependence on clinical criteria without genetic confirmation is necessary in some parts of the world, in many developed countries direct sequencing is being replaced by a range of next-generation sequencing techniques, including targeted gene sequencing, whole-exome sequencing and whole-genome sequencing. Consequently, molecular testing for children with developmental problems could be undertaken at an early age before the hallmark features that characterize particular disorders have become apparent. These technological advances may eventually prove to be more efficient and cost-effective for diagnosis[112], and the RTT clinical criteria that relate to the evolution of the disorder could become redundant.

The final component of the most recent criteria[6] provides further clinical description of some of the original 'variant' forms, two of which — the early seizure onset variant now recognized as the CDKL5 disorder[113] and the congenital variant, mostly caused by mutations in *FOXG1* (REF. 114) — must now be considered only as Rett-related disorders[111]. The third atypical form, the Zappella or preserved speech variant[107], is most often associated with an Arg133Cys mutation[115] or a C-terminal deletion[116] (FIG. 2). However, by additionally describing the forme fruste, late regression and male variants, Hagberg had already provided the best delineation of the full spectrum of clinical presentations[117]. As we reflect today on these early descriptors, we can see how well they fit with our current understanding of the relationships between genotype and phenotype.

Overall severity and relationship with genotype. As early as 1987, the issue of the danger of masking the true clinical variation in RTT (BOXES 1,2; see Supplementary information S1,S2 (videos)) by the adoption of 'artificial' inclusion and exclusion criteria based on phenotype and not on cause was raised by the esteemed medical geneticist John Opitz[118]. Much later, and endorsing this concept in a different way, Hagberg acknowledged the wide clinical variation of what he called the "*MECP2*-deviant phenotypes," with a spectrum ranging from the severe newborn encephalopathy in males to the female carrier mothers[119]. We now know, as Opitz might have predicted, that much of this spectrum relates to the type of genetic mutation, with the very mild variants often represented by individuals with C-terminal deletions in *MECP2* (REFS 119–121) (BOX 2; see Supplementary information S2 (video)). Although RTT is mostly considered

Box 2 | **Mild Rett syndrome phenotype**

The mild Rett syndrome (RTT) phenotype is exemplified by a 13-year-old girl with a Pro389X mutation in the methyl-CpG-binding protein 2 (*MECP2*) gene (see Supplementary information S2 (video)). This teenage girl illustrates the mild phenotype of a C-terminal deletion, possibly further influenced by another genetic modifier.

Initially, the girl progressed well, learning to walk at 12 months, and feeding herself and using four-word sentences as a toddler. However, speech delay, poor sleep and tremulous movements, which developed when she was 2 years old, raised some concerns. The girl's mother began to suspect RTT when her daughter was 4 years old. After the girl developed epilepsy at 8 years of age, the possibility of RTT was dismissed because of her high level of gross motor skills (walking well across different terrains), good hand use and ability to maintain a conversation. In addition, her head growth had not decelerated. Her mother's continued concern led to further genetic testing at 12 years, which demonstrated a *MECP2* C-terminal deletion (Pro389X). The lateness of diagnosis was consistent with her mild clinical presentation (FIGS 3–5).

In retrospect, the girl experienced loss of finesse in her ability to turn pages when she was 2–3 years old, but showed no loss of communication skills. Her gait is mildly ataxic and she developed mild hand stereotypies at 6 years of age. She has had ongoing sleep disturbances since 3 years, her feet are cold and small, she has decreased sensitivity to pain, and displays substantial tremor, which is managed with trihexyphenidyl and a vagal nerve stimulator. Consistent with the C-terminal phenotype, her growth is good. A diagnosis for this young girl has provided important answers to her family.

Beyond RTT, the girl is deeply involved in family, school and community life. She loves fun times with her father and competes in the Special Olympics in horse riding. Her diagnosis is an important part of her life, but is also compatible with learning and participation.

Written consent for publication of this case report was obtained from a responsible relative.

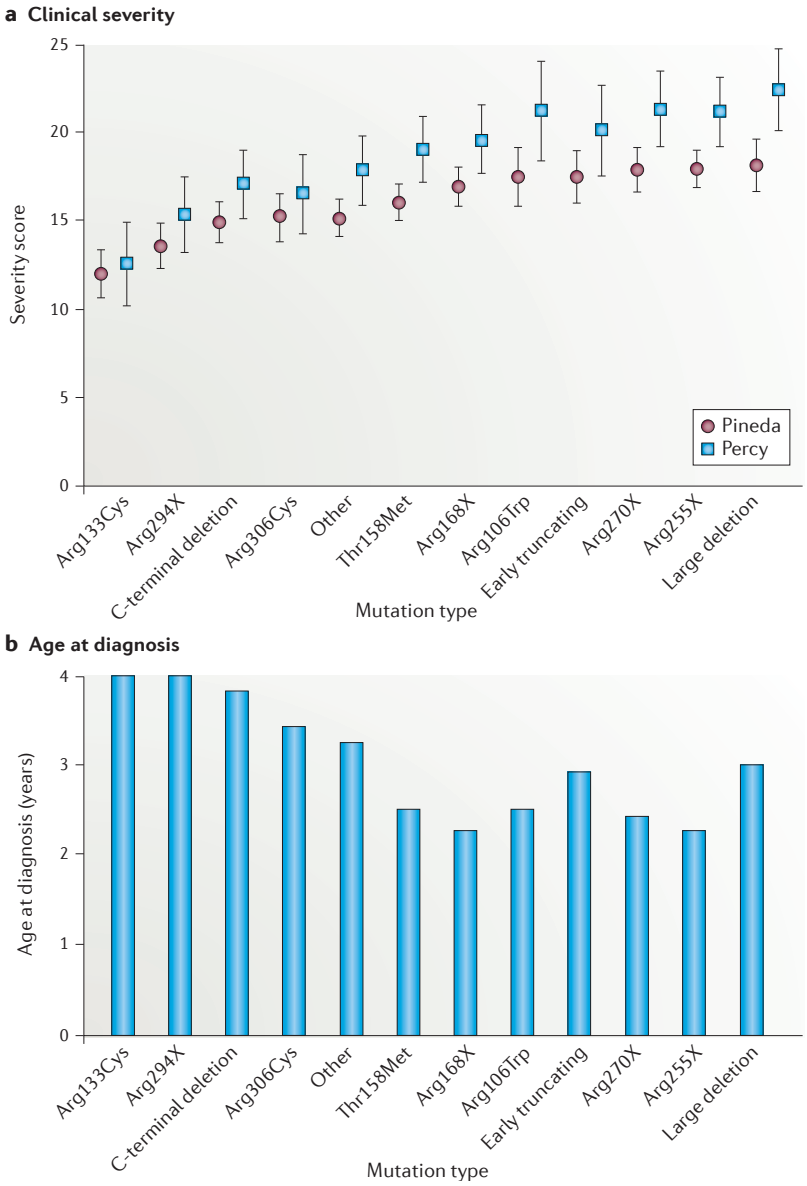


Figure 3 | **Rett syndrome severity and age at diagnosis by mutation type.** **a** | Association between clinical severity and methyl-CpG-binding protein 2 (*MECP2*) mutation type in 974 individuals with Rett syndrome (RTT) assessed via the Percy score, and 776 assessed via the Pineda score. Data points are the mean scores adjusted for age and data source, with 95% confidence intervals. Permission obtained from Macmillan Publishers Ltd © Bebbington, A. *Eur. J. Hum. Genet.* 20, 921–927 (2012)[129]. **b** | Age at diagnosis by mutation type in 1,040 individuals with RTT. Data points indicate the median age. Data from the Australian and International Rett Syndrome (InterRett) databases.

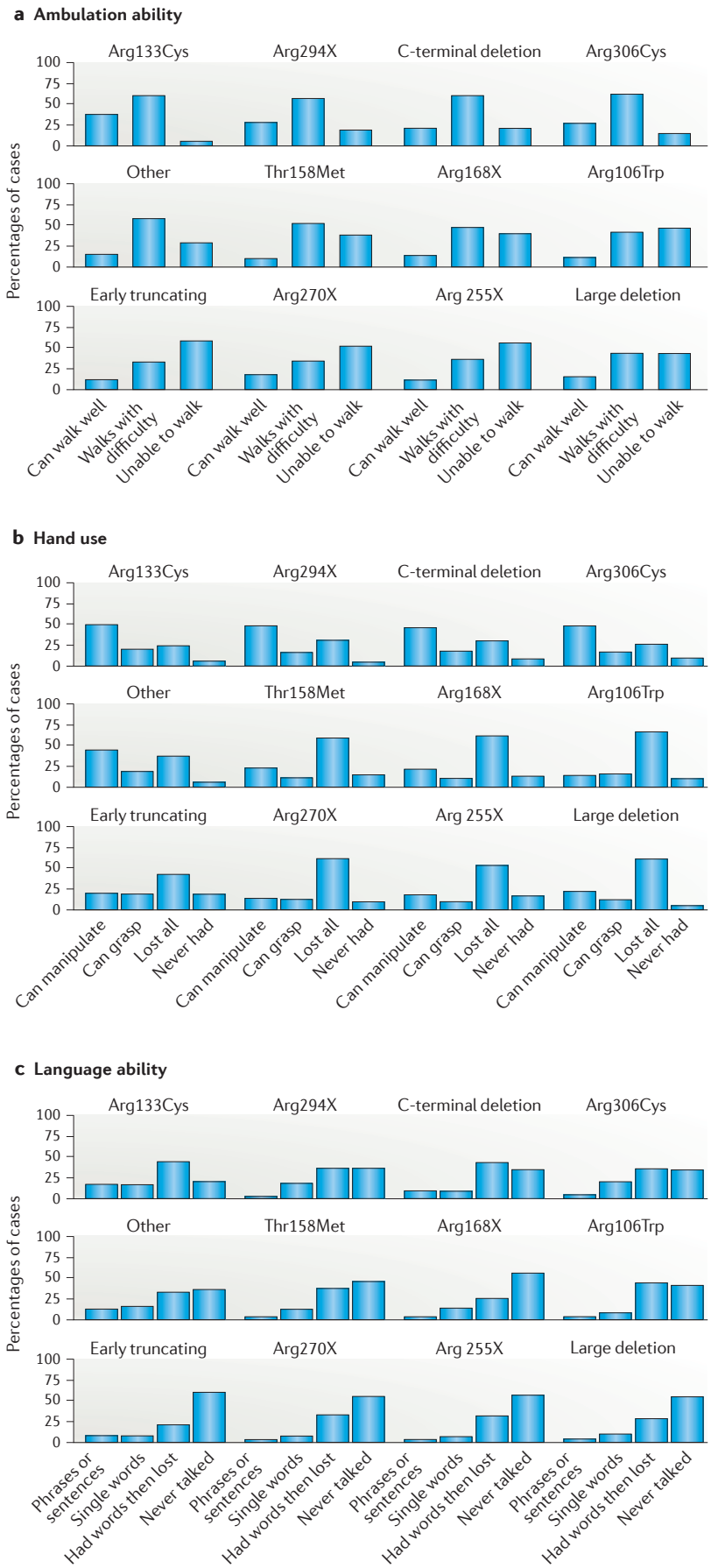
to be a clinical diagnosis, a fine line remains between the naming of such individuals as "female forme fruste Rett syndrome variants[119]" or as "people without Rett syndrome[121]."

The Australian register first provided the means to examine the spectrum of presentations in a total RTT population cohort using three previously published measures, designated as the Kerr[122], Percy[123] and Pineda[124] scores[125]. Considerable variability in the early regression period, current functioning and comorbidities, much of which was subsequently shown to relate to genotype, was demonstrated, and severity generally increased with age.

Despite numerous small studies, it took time to accumulate adequate data to provide consistency in genotype–phenotype relationships. The two most seminal studies were published within months, the first using data from InterRett[126], and the second from the US Natural History study[127]. Where comparable, the findings were broadly similar, with the most severe mutations being Arg270X, Arg255X and Arg168X, whereas Arg133Cys, Arg294X and C-terminal deletions produced less-severe phenotypes (BOXES 1,2; FIGS 2,3a; see Supplementary information S1,S2 (videos)). Overall, individuals with severe mutations were less likely to walk, retain hand use or use words, and tended to be diagnosed at an earlier age[128] (FIGS 2,3b,4). A group with large deletions, which was not included in the initial InterRett study, was subsequently described separately, thereby confirming earlier US findings[127] of phenotypic severity[129] (FIGS 2,3a). A later publication also studied the C-terminal deletions — a milder group which, due to their comparatively later loss of skills and onset of stereotypies[120], fitted with the initial 'late regression' descriptor (BOX 2; FIGS 2,3a; see Supplementary information S2 (video)). Also of interest were the better growth parameters and increased likelihood of kyphosis in individuals with C-terminal deletions[120]. Information from these[120,126,127,129] and other studies[20] is enormously useful when considering prognosis in RTT, although it is clear that genotype is but one factor, and other factors, such as X-inactivation[130], genetic modifiers[131] and, possibly, environmental factors[132], also have a role (BOX 2; see Supplementary information S2 (video)).

Variation in functional abilities. The classic signs of RTT include severe functional impairments, usually necessitating substantial support in daily life. Subtle changes in development often precede the onset of regression[109], which is characterized by either gradual or sudden loss of hand and communication skills, loss of balance, and development of hand stereotypies[7,133]. Patterns in the relationships between genotype and hand and gross motor skills have been observed[126,134,135]. Although cross-sectional studies suggest that motor function declines with increasing age, further longitudinal research is necessary to confirm or refute this idea. For example, some adults with RTT — probably those with a mutation associated with a milder phenotype — retain the capacity to walk[136,137] (FIGS 2,4a). Similarly for communication, those with milder mutations such as Arg133Cys or Arg306Cys are more likely to learn to babble or use words before regression, to regress at a later age, to retain some oral communication skills after regression, and to be diagnosed later[115,128] (FIGS 2,3b,4c).

For individuals with RTT, a fundamental goal is to build the capacity for movement and communication in everyday life, and with a deeper understanding of motor deficits, the potential role of the enriched environment[132], and technological advances in assisted communication systems, the capacity to respond



◀ Figure 4 | **Functional abilities and mutation type in Rett syndrome.** Graphs show the relationship between methyl-CpG-binding protein 2 (*MECP2*) mutation type and functional ability in individuals with Rett syndrome (RTT). Data were obtained from the International Rett Syndrome Database. **a** | Ambulation ability in 1,112 individuals with RTT. **b** | Hand use acquisition and loss in 1,097 individuals with Rett syndrome. **c** | Language ability and history in 1,046 individuals with RTT.

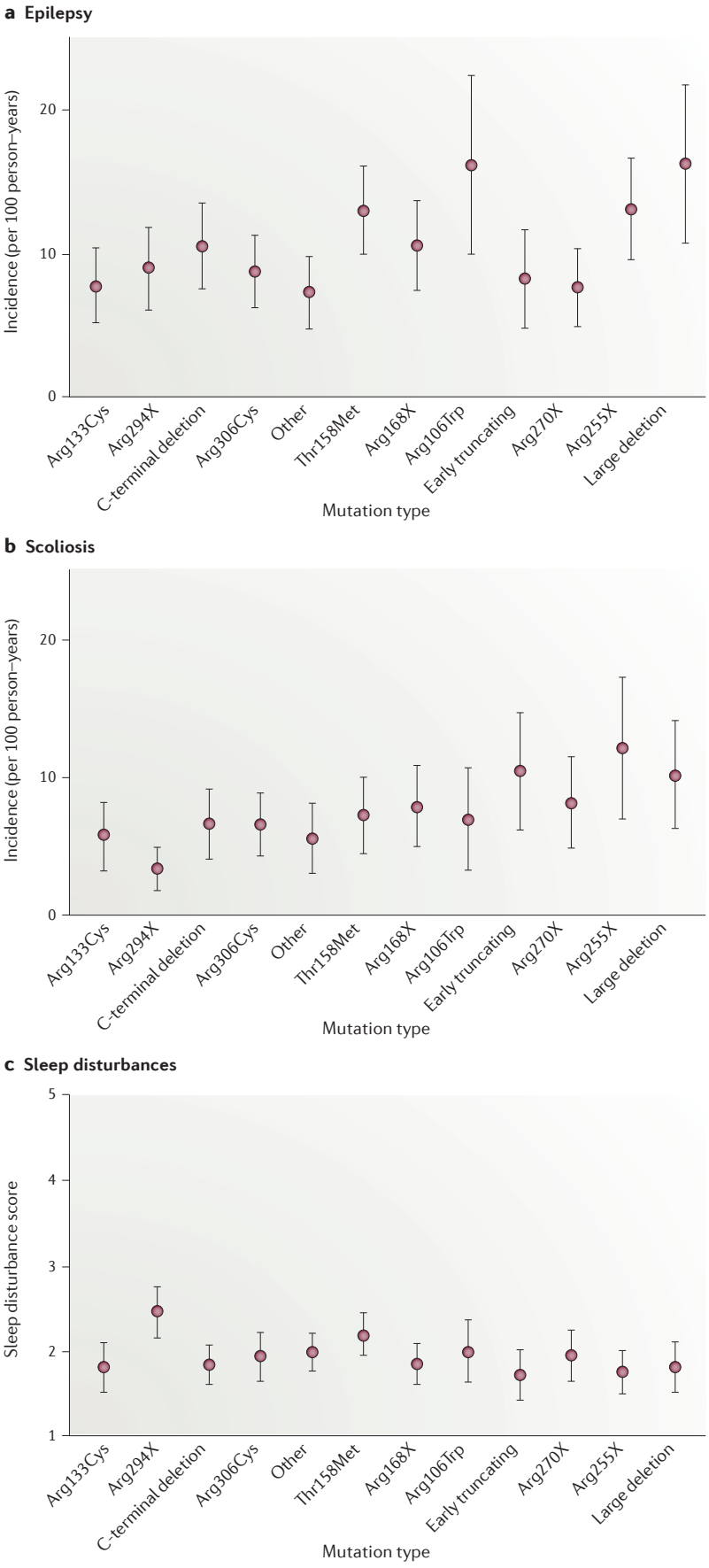
is expanding. However, no relevant studies, beyond single or small case series[138,139], have been conducted, so we do not fully understand what interventions are associated with favourable outcomes, and how treatments should be modified for variation in phenotype.

Comorbidities and their management

Epilepsy. Epilepsy is a particularly challenging comorbidity to study in RTT. Although the EEG is uniformly abnormal, typically from about 18 months of age[140], this finding does not necessarily reflect seizure activity[141]. Moreover, some seizures seen during video–EEG monitoring may not be recognized by caregivers as clinical events, and many events characterized by caregivers as seizures are not associated with EEG seizure discharges. These issues have contributed to difficulties in validating an epilepsy diagnosis and recording the seizure history for research, and probably to the comparative dearth of literature.

With these caveats in mind, a number of investigations have been undertaken to study epilepsy in RTT. Epilepsy was diagnosed in 95% of a Swedish representative series ($n = 53$), although seizure frequency declined with age[142]. In one Australian study, the prevalence of epilepsy diagnosis was 81%, with a median age of onset of 4 years[143]. In another study, seizure rates were found to be generally higher in individuals with greater clinical severity and lower in those with Arg294X or Arg255X mutations or C-terminal deletions[144]. In recent years, three substantially sized studies have reported on epilepsy in RTT[145–147]. On average, just over 60% of cases were diagnosed with epilepsy, but in a US study[145], a lower proportion had physician-verified seizures. Variations that were observed in relation to the effects of genotype (FIGS 2,5a) may have resulted from methodological differences, but in all three studies the mutation Thr158Met conferred some additional risk of epilepsy[145–147].

Growth and nutrition. Growth retardation was listed in the early versions of the supportive criteria for RTT[5], with head growth deceleration occurring first, followed later by slowing of weight and height increase, and even of hand and foot growth[148]. Although the exact underlying mechanism remains unclear[149–151], a definite relationship with genotype exists[120,150]. Growth charts have been generated using cross-sectional and longitudinal data from 816 US cases, and the growth failure was found to be especially pronounced in individuals with the more severe Thr158Met, Arg168X, Arg255X, Arg270X and large deletion mutations[152] (FIGS 2,3a).



◀ Figure 5 | **Comorbidities and mutation type in Rett syndrome. a** | Incidence of epilepsy onset by methyl-CpG-binding protein 2 (*MECP2*) mutation type in 560 individuals with Rett syndrome (RTT). Data points are the mean incidence, with 95% confidence intervals[145]. **b** | Incidence of scoliosis diagnosis by *MECP2* mutation type in 392 individuals with RTT. Data points are the mean incidence, with 95% confidence intervals[160]. **c** | Relationship between sleep disturbances (disorders of initiating and maintaining sleep, or DIMS) and *MECP2* mutation type in 325 individuals with RTT. Data points are the mean DIMS score adjusted for age, seizure frequency and mobility, with 95% confidence intervals[168].

Enteral support for individuals with RTT is common practice in developed countries. This approach is now being used in over one-quarter of cases[153], particularly those with large deletions and Arg168X mutations (FIG. 2), with apparent benefits in terms of both growth parameters and parental satisfaction[153]. A large multinational group collated existing evidence and used expert opinion to provide guidance on the assessment and management of growth and feeding problems in RTT[154]. These published guidelines, which are available in user-friendly formats for clinicians and families, represent an important step in tackling this comorbidity[154].

Autonomic dysfunction. Individuals with RTT commonly exhibit abnormal breathing patterns, which are considered to be a manifestation of autonomic dysregulation. These problems generally present as episodes of either hyperventilation or breath-holding[155,156]. Abdominal bloating, which in rare cases can lead to gastric perforation[157], is a common sequela, and may need alleviation through the release of air via a gastrostomy. Vasomotor disturbances causing cold and blue hands and feet were also identified as supportive clinical criteria for RTT[5].

Despite the intensive autonomic monitoring that is now undertaken in some European centres[155], the prevalence and natural history of these disturbances, and their potential relationships with genotype, remain unknown. The literature on autonomic disturbance in humans with RTT is currently lagging behind that in animal models[158]. This knowledge gap is worrying, given that animal studies suggest the need for pharmacological interventions, and clinical trials of compounds that aim to reduce autonomic dysfunction are imminent.

Scoliosis. With the combination of neurological impairment and altered motor skills in individuals with RTT, the development of deformity such as scoliosis can be relentless. An early case series indicated that neurological signs were often asymmetrical, with the right side being more severely affected[159], and subsequent larger studies found scoliosis to be a common deformity[160,161]. In the Australian study, 75% of girls developed scoliosis by 15 years of age, with earlier onset in those with more severe mutations, such as Arg255X or large deletions[160] (FIGS 2,5b). Scoliosis is usually progressive, particularly in children who are unable to walk, and in those with

most common mutations other than Arg306Cys[160]. The health implications can be profound, as scoliosis with a Cobb angle >70° has particularly detrimental effects on respiratory health[162].

In response to a poor evidence base, an international group developed a set of clinical guidelines for the management of scoliosis in individuals with RTT, using the available literature but also drawing heavily on the literature for neuromuscular scoliosis. The consensus was that scoliosis should be regularly monitored and spinal fusion considered when the Cobb angle is >50° (REF. 163). In a subsequent study, spinal fusion was associated with improved survival and, in individuals with early-onset scoliosis, a moderate reduction in frequency of severe respiratory tract infections[164]. This information is important for clinicians and families when weighing up the pros and cons of spinal fusion in individual girls and circumstances[165].

Sleep disturbances. Sleep disturbances have recently been considered as supportive criteria for RTT[6,7], and their burden on the affected person and their family is often considerable. An early Australian study in individuals with RTT ($n = 83$) reported poor night time sleep overall, and daytime naps that persisted with age[166]. Seizure disorders were associated with increased daytime sleep, and ability to walk was associated with less daytime sleep[166]. Further population-based research found a high prevalence of sleep problems, some of which (in particular, night laughing and screaming) decreased with age[167,168]. The highest likelihood of sleep problems was observed in individuals with a large deletion — in whom night laughing was particularly common — or with Arg294X[167,168] (FIG. 2).

A recent study, which used InterRett for ascertainment, surveyed parents or carers of 364 genetically confirmed cases aged 2–57 years[169]. Night waking was frequent and, consistent with previous research, individuals with the Arg294X mutation were most likely to have problems initiating and maintaining sleep[169] (FIGS 2,5c). Individuals with epilepsy and/or limited mobility were more likely to have excessive somnolence, also consistent with earlier findings[166].

In one small clinical trial ($n = 9$), melatonin seemed to improve total sleep time and efficiency in individuals with poor sleep quality at baseline, without any adverse effects[170]. Considering the frequency of sleep dysfunction in RTT, and its impact on the child and their family, our evidence base for management remains remarkably sparse.

Bone health. Unlike other comorbidities, adverse bone health has not been one of the supportive criteria for RTT. Susceptibility to osteopenia and fractures was first highlighted through US[171] and Australian research[172,173]. Fracture risk was four times higher in individuals with RTT than in the general female population, and was specifically increased in those with Arg168X and Arg270X mutations[173] (FIG. 2).

Several Danish[174,175], US[176,177] and Australian studies[178,179] have investigated which particular bone parameters are most adversely affected in RTT, and their potential nutritional[180] (for example, vitamin D status), environmental and genetic risk factors. Risk factors for fractures, such as genotype[173] and use of certain antiepileptic medications[181], did not always correlate exactly with those for low bone density, which also varied by outcome parameter and body site. For example, in comparison with other parameters, right femoral neck areal bone mineral density was particularly impaired with increasing age and lack of mobility[178]. A recent Danish study concluded that comparatively reduced levels of biochemical bone markers in RTT signified a low bone turnover state[182]. Cross-study comparison has been hindered by non-representative and small sample sizes, often without longitudinal collection, as well as a lack of childhood population bone parameter norms and accommodation for decreased stature and different analytical methods.

MeCP2 deficiency has been shown to alter the biomechanical integrity of bone in a mouse model[58,59], underlining the importance of understanding bone health in RTT. A set of guidelines for bone health was developed, which aimed to provide the best available evidence at the time of publication[183]. We hope that these guidelines can soon be modified with results from clinical trials assessing the effectiveness of drugs such as bisphosphonates in RTT[184].

Therapeutic strategies

The increased understanding of MeCP2 function and the availability of valid cellular and animal models has fuelled efforts to identify and develop therapeutic strategies for RTT[185–188]. These efforts include targeting of the various brain systems and downstream cellular processes that are affected in RTT, as well as approaches that target the root cause of the disorder, namely, MeCP2 dysfunction[185] (FIG. 6).

Approaches that target MeCP2 at the level of the gene or protein to restore functional MeCP2 within the nervous system are appealing, as they have the potential to produce profound amelioration or reversal of symptoms, as demonstrated by reversal studies in mice[43,44,189]. Such approaches involve molecular and genetic manipulations, ranging from gene transfer[190,191] and protein substitution to novel forms of DNA and RNA editing[192]. However, the level of MeCP2 in a given cell may be critical[193], and restoring MeCP2 function without producing overexpression-related pathology is likely to be a considerable challenge. Strategies targeting *MECP2* typically require the development of completely novel molecules, which creates substantial uncertainty in terms of adequate brain delivery, safety and ensuing regulatory hurdles. The MeCP2 protein is a macromolecule with multiple functional domains, and restoration of normal function using small-molecule drugs is not considered to be practical. However, it might be possible to develop small molecules to act at the genomic level to reactivate the *MECP2* allele on the inactive X chromosome[194], or at the level of RNA to enable read-through of nonsense mutations[195,196].

In contrast to targeting of *MECP2*, pharmacological strategies that target downstream mechanisms in the pathogenic process can make use of small molecules that

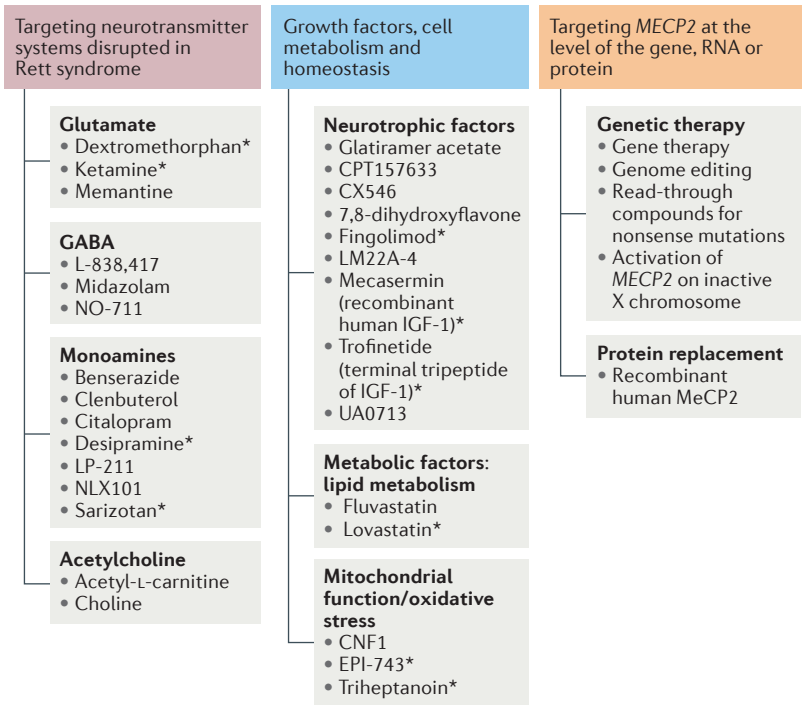


Figure 6 | **Therapeutic strategies for Rett syndrome.** Primary therapeutic strategies and compounds being investigated in preclinical animal models and in clinical trials (asterisks). IGF-1, insulin-like growth factor 1; *MECP2*, methyl-CpG-binding protein 2.

have already been developed or approved for other indications. Indeed, several drugs with proven efficacy in *Mecp2* knockout mice have proceeded to clinical trials in patients with RTT[185] (FIG. 6). However, such approaches do not address the underlying aetiology, and the lack of a dominant cellular process or pathway downstream of MeCP2 deficiency suggests that the benefits could be restricted to a subset of symptoms. The approaches that have been developed to date can be broadly divided into three categories: pharmacological agents that affect major neurotransmitter systems in the brain, most notably glutamate, GABA, acetylcholine and monoamines (FIG. 6); drugs and trophic factors that promote brain growth and development, mostly by modulating the brain-derived neurotrophic factor pathway; and drugs that modulate other cellular processes known to be perturbed in models of RTT, such as energy metabolism and protein synthesis.

Clinical trial design

Clinical trials for rare disorders present many challenges, including mutation heterogeneity, variation in disease severity, and the pool of available participants. Additional considerations include the optimal time for intervention and the nature of trial design[185]. Important starting points include high-quality natural history data, and objective and robust outcome measures. Several clinical severity scores[122,124,197] have worked well in studies of genotype–phenotype relationships[126,127], but have not necessarily proved to be optimal as outcome measures in clinical trials[198]. For example, the Motor-Behavioural Assessment (MBA), which comprises 39 items scored with a five-point scale to describe clinical severity[199], was used in one clinical trial[198]. However, this scale is poorly operationalized, with some items describing historical aspects of regression, and has never been validated. Similarly, the Rett Syndrome Behaviour Questionnaire[200] was developed for the purpose of differentiating individuals with RTT from those with other causes of intellectual disability before genetic testing became available. This questionnaire has been used successfully in genotype–phenotype studies to assess some aspects of behaviour such as mood and anxiety[137,201], but may not appropriately measure behaviour as an outcome in a clinical trial. A clear need exists for the further development of such instruments, and work is currently underway in this regard[202].

The Clinical Global Impression scales are clinician-rated, seven-point rating scales that are used to describe severity and change, and have recently been adapted to RTT for use in clinical trials[203]. This process has involved the development of seven category descriptors for the domains of communication, ambulation, hand use, use of eye contact, autonomic function, seizures, and attentiveness. Initial validation studies, including testing of responsiveness to change, are being undertaken[203]. More sensitive measures of specific domains are also becoming available. For example, the 15-item Rett Syndrome Gross Motor Scale has undergone substantial validation, suggesting capacity to demonstrate responses to interventions in the motor domain[135]. Wearable technologies have also been used for objective measurement of the patterns and regularity of respiratory and cardiac function in RTT in small observational studies[156,204], and in a recent clinical trial[198]. Thus, some progress is being made in the important area of outcome measures, but much work is still needed to ensure that future clinical trials are able to provide the necessary answers.

Global efforts to study a rare disorder

Epidemiology. The Texas registry, which used multiple sources of ascertainment monitored with capture–recapture methods, was the first population-based registry to be established for RTT[205]. This registry provided a model for the Australian Rett Syndrome Database (FIG. 1), which in 1997 reported that RTT had a cumulative incidence of 0.96 per 10,000 females by the age of 12 years[206]. Further studies in 2011 demonstrated that the cumulative incidence was increasing with age, and that the median age at diagnosis had fallen from 4.5 years before 1999 to 3.5 years after this date[207].

Infrastructures. The establishment of registries is a first step towards understanding the epidemiology, natural history and life expectancy of a rare disorder. Following Alison Kerr's use of the British Paediatric Surveillance Unit to launch the British Isles RTT Survey in 1990 (REF. 208), the Australian database (FIG. 1), established 3 years later, took advantage of the newly formed

Australian Paediatric Surveillance Unit to ascertain cases[206]. The database has now been maintained for over two decades, and each additional year of follow-up increases its value[137], providing the capacity to monitor children into adulthood and identify trajectories of functioning and comorbidities[209]. Population-based longitudinal follow-up with minimization of attrition is essential for studies of life expectancy, but is uncommon in the field of rare disorders.

Genotype–phenotype investigations are, ideally, sourced from population-based sources[210], but when mutations are rare or effect sizes are small, large sample sizes, sometimes obtained through aggregation of data from multiple sources, can provide much greater power. InterRett is one infrastructure that has served this purpose well by collecting questionnaire data internationally from both clinicians and families in over 50 countries since 2003 (REF. 211) (FIG. 1). Another such infrastructure, but based solely in the US, is the Rare Disease Consortium Research Network for RTT[145], which was initially established in 2004 by Alan Percy as a natural history study[212] (FIG. 1), and is now funded by the NIH. Although both of these data collections are likely by their nature to be highly selective, it has been possible to compare some characteristics of InterRett with an Australian population-based source[213]. The InterRett families were of a somewhat higher socioeconomic status than the Australian families, but the distributions of mutation type were broadly comparable.

The original structure of the NIH-funded study involved the collection of data from clinic visits to inform the understanding of the natural history of RTT. Currently, the main aim is to increase our knowledge of the molecular basis of RTT, and to identify treatments that could improve functioning in affected individuals. Like InterRett, the European Rett Syndrome Database Network (EuroRett) combines data from multiple sources, and to date has mainly been applied to investigations on epilepsy[147]. RettBASE, the *MECP2* Variation Database, has a different but valuable function, which is to catalogue the range of different genetic variants, both pathogenic and non-pathogenic, reported in publications and from laboratories[214].

Role of advocacy groups. Advocacy groups have played a major part in the funding of infrastructures and RTT research. The main organization, which provides support and advocacy as well as funding, was established in 1984 as the International Rett Syndrome Association (IRSA)[215]. When commenting about the achievements of this organization, its founder, Kathy Hunter, wrote that "parents soon understood the critical part they must play in making sure that funds are available for research" and "they also understand the need for them to participate vigorously in research[216]."

International collaboration — challenges and accomplishments. International collaborations are vital for rare disease research. Over the years, however, differences have emerged at the international level in the understanding of RTT, and particularly in its associated terminology. Such differences can hamper progress. One example is a simple scoring system initially proposed by a UK researcher, which has not been widely adopted in North America[122]. Another is the wide variation in autonomic monitoring and management, which is underpinned by limited evidence[155]. The Australian group has led a number of successful collaborative initiatives to develop guidelines for treatment of common RTT comorbidities. Often, in the absence of a strong evidence base, these initiatives depended on expert opinion garnered in a collegial fashion through the Delphi process[154,163,183].

Conclusions and future prospects

In terms of the clinical presentation, many components of the original model of RTT proposed by Hagberg still ring true. Over the past 50 years, life expectancy for individuals with RTT has increased dramatically, partly because of changing attitudes and allocation of resources towards the health care of those with disability.

The value of surgical treatment for scoliosis was first highlighted by Kerr *et al.*, who reported positively on family perspectives of well-being 1 year after the fusion operation[217]. The benefits of this approach were further validated in recent studies, which used population-based data[164,218]. Enteral nutrition is now commonly available, at least in developed countries, and preliminary evidence indicates a positive impact on growth[153]. The beneficial effects of these management approaches may be reflected in the 71% survival rate at 25 years, reported in an Australian population cohort in 2010 (REF. 219), compared with 21% in Rett's original cohort. Recent population data, using longitudinal follow-up over more than two decades, suggest that approximately 60% of individuals with RTT will survive to their late thirties[137]. This figure is considerably less optimistic than the estimates of 50% at 50 years from the North American Database[220] (data derived from 50% response to questionnaires administered to IRSA family members) and 75% at 45 years from a 9-year follow-up of the US Natural History sample[221]. Both of the latter samples are large but select groups, and are likely to be more economically advantaged than the general US population.

Other societal changes include our passage into the digital age: the value of connecting families affected by RTT via the Internet was first demonstrated only 12 years ago[222]. Nowadays, social media sites are often the first port of call for families with a new diagnosis. Traditionally wary of patients seeking information from non-reputable sources, clinicians now appreciate the importance of this virtual peer support, especially for geographically isolated families affected by a rare disease.

The greatest explosion of knowledge on RTT has occurred in the 16 years since the discovery of the genetic cause. During this period, US and Australian natural history studies and international databases have informed our understanding of genotype–phenotype relationships, and the comorbidities that occur in this disorder. We have learned much about the function of the MeCP2 protein, in particular, its role as a regulator

of gene expression and its interaction with other proteins. The reversal of neurological deficits in a mouse model in 2007 (REF. 43) raised hopes of a treatment that can restore MeCP2 expression in humans.

Although some progress has been made in improving clinical management, we still lack treatment options to resolve or substantially reduce the comorbidities of RTT. Many individuals — as well as their families — are adversely affected by poor sleep, a substantial proportion have refractory epilepsy, no evidence-based management options are available for autonomic breathing abnormalities, and the best methods to improve functional ability are not yet known. These are all important clinical challenges to address.

The probability of translating promising preclinical outcomes to effective clinical treatments for nervous system disorders is modest, and expectations must be tempered accordingly. However, the developing pipeline of putative therapies, and the coordinated efforts of clinicians, scientists and family organizations, together with increasing engagement of the biomedical industry, assure exciting developments ahead.

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Competing interests statement
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