

## CNS-ICNA Symposium: Neurogenetics

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# Current Status of Developmental Encephalopathies: Rett Syndrome, *MECP2* Duplication Disorder, *CDKL5* Deficiency Disorder and *FOXP1* Disorder

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## Current Status of Developmental Encephalopathies

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**Abstract.** This review describes the similarities and differences between the developmental encephalopathies including Rett syndrome (RTT), *MECP2* Duplication Disorder (MDD), *CDKL5* Deficiency Disorder (CDD), and *FOXP1* Disorder (FD). RTT and MDD represent opposite ends of deficiency and duplication of *MECP2* whereas CDD and FD were initially described as variants of RTT before their specific genetic variants were identified and they were recognized as distinct disorders. The subsequent discussions outline the clinical features, phenotype-genotype relationships, and potential for therapeutic intervention including gene replacement. Natural history studies involving each disorder are providing the essential underpinnings for developing outcome measures and biomarkers to enhance the advancement of such treatment efforts.

**Keywords:** Rett syndrome, *MECP2* Duplication Disorder, *CDKL5* Disorder, *FOXP1* Disorder

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Rett syndrome was first recognized by Andreas Rett [1], predominantly in females, more than 50 years ago and first termed Rett syndrome (RTT) in the landmark 1983 paper of Hagberg and colleagues in the *Annals of Neurology* [2], launching this disorder worldwide. Clinical and genetic studies progressed rapidly culminating in identification of pathogenic variants in the gene, Methyl-CpG-binding-protein 2 (*MECP2*) in 1999 [3]. However, variant forms of RTT had already been described by Hagberg (atypical RTT) [4], Hanefeld (early infantile seizure variant) [5], and Rolando (congenital RTT) in 1985 [6]. Shortly after the identification of variants in *MECP2*, a disorder was recognized predominantly in males associated with duplications of *MECP2*, proving to be as difficult clinically as RTT [7]. In 2003, the US Natural History Study (NHS) of RTT began enrolling individuals with both classic and atypical RTT (NCT00299312), including some individuals with *MECP2* Duplication disorder and some newly recognized individuals with mutations in *CDKL5* and *FOXP1*, heretofore regarded as those with atypical RTT as described by Hanefeld and by Rolando. The inclusion criteria for entry into the US NHS were very simple and straightforward: the individual, female or male, should meet the clinical criteria for RTT [8] and should have proof of genetic testing. Copies of such testing, whether positive or negative, were essential for enrollment. If results were unavailable, provision was made through the Greenwood Genetic Center, Greenwood, South Carolina for obtaining the specific test results. Individuals could enroll in the study if they met clinical criteria for RTT, even if they did not have a genetic variant in *MECP2*, or if they had a genetic variant in *MECP2*, even if they did not meet the clinical criteria for RTT.

In 2014, the NHS study then began enrolling individuals representing all four disorders (NCT02738281): RTT, *MECP2* Duplication Disorder (MDD), *CDKL5* Deficiency Disorder (CDD), and *FOXP1* Disorder (FD). Significant natural history data has developed across the world in each disorder, leading to their collective categorization as Developmental Encephalopathies [9]. In addition, other disorders that could fit within this rubric, such as the severe form of Epileptic-Aphasia Disorder (*GRIN2A*) or Pitt-Hopkins (*TCF4*), are beginning to emerge.

Thus, it is timely to consider the current understanding of this group of disorders, their similarities and their differentiating features. This will include the current diagnostic strategies, the array of clinical, neurophysiologic and neuroimaging features, and their developmental trajectories across the age range. With the identification of specific variants (or different duplications in the *MECP2* Duplication Disorder) in each disorder, consideration of phenotype-genotype correlations has become important in developing an understanding for the range of developmental difficulties in each. In addition, the identification of the specific genes involved has allowed important basic and translational studies to progress with an eye to treatment strategies in each. The US NHS has provided the opportunity to develop comparative analyses of these four disorders, which provide clear points of differentiation to allow for accurate diagnosis and subsequent care. Finally, effective disease-modifying agents are already undergoing initial trials and potentially disease-reversing strategies are under careful consideration. These strategies will be considered in each presentation. Originally planned to address each of these disorders individually with a concluding presentation summarizing comparative features, the program was modified to combine the description of CDD with final discussion of comparison features in these four disorders.

## 1. Statement of goals

The Developmental Encephalopathies have emerged as a relevant diagnostic group over the past decade or more. Initially described as variants of RTT, the diagnostic picture clarified rapidly in the past twenty years with identification of *MECP2* variants in RTT, duplications of the same gene in MDD, and the identification of *CDKL5* and *FOXP1* variants in the respective disorders, CDD and FD.

Current understanding now recognizes the importance of denoting these disorders as such, rather than variants of RTT. Substantial progress has occurred in the identification and diagnosis of individuals with these disorders and of developing natural history data in preparation for on-going or future clinical trials. In this symposium, each disorder will be described in detail, their core features compared, and the prospects for meaningful trials discussed.

## 2. Statement of objectives

Understanding the similarities and unique features of each disorder is critical to advance both clinical and basic research. The subsequent presentations will review the diagnostic strategies for establishing the specific diagnosis and propose proper treatment guidelines. Fundamental to successful treatment of each of these disorders is the development of effective remediation. As these disorders have different timelines of discovery and subsequent study, comparison of the disease-modifying or gene replacement clinical trial protocols will have different timelines from clinical trials currently in place to current concepts being planned.

## Competing interests

Dr. Percy has no competing interests.

## Author contributions

Dr. Percy is solely responsible for this review.

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# Therapy Development in Rett Syndrome

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Keywords: Rett syndrome, MECP2, phenotype-genotype, X-chromosome inactivation, treatment trials, gene therapy, biomarkers, outcome measures

First recognized more than 60 years ago by Andreas Rett in Austria and Bengt Hagberg in Sweden, Rett syndrome (RTT) burst on the international scene following the publication of Hagberg and colleagues in 1983 [1, 2]. Thereafter, significant clinical research commenced resulting in the identification of pathogenic variants in *MECP2* (methyl-CpG-binding protein 2) as the cause of this rare neurodevelopmental disorder [3]. This led to increased efforts to understand the natural history of RTT and the fundamental issues in brain development underlying this disorder. RTT, primarily affecting females, has an incidence of  $\sim 1 : 10,000$  female births,  $>95\%$  having mutations in the *MECP2* gene [9, 10]. Clinical criteria were elaborated first in 1985 [11], revised in 2002 [12], and revised again in 2010 to account for advances in understanding of RTT [8]. The current criteria (Table 1) represent a simplification of prior efforts as well as an effort to create more easily understandable terminology. For classic RTT, the supportive features originally included in the initial criteria are no longer required. For atypical RTT, individuals must meet 2 of 4 main criteria and 5 of 11 supportive criteria. Those with atypical RTT may have either more or less significant difficulties than those with the classic form.

In 2003, a multi-center longitudinal natural history study (NHS) was begun in the US with funding by the NIH/ORDR/NICHD, focusing on RTT and individuals with *MECP2* Duplication Syndrome (MDS). The inclusion criteria for the US NHS were outlined in the opening section of this symposium. In 2014, this study was extended to its current 14 sites across the US with emphasis expanded to add individuals with *CDKL5* Deficiency Disorder (CDD) and *FOXG1* Disorder (FD), previously considered to have more severe forms of atypical RTT. Together, these studies have enrolled more than 1700 individuals with one of these disorders including individuals with *MECP2* variants representing females who do not meet criteria for classic or atypical RTT or males who range from very severe infantile encephalopathy to those with signs of mild to moderate developmental delay.

In classic RTT, significant phenotype-genotype differences are observed across specific mutations [13, 14]. Although these group-wise differences are significant (Fig. 1), in specific individuals, differences may be less apparent. This is probably due to a number of factors including variations in X-chromosome inactivation (XCI), clonal distribution of mutant genes across brain regions, background variation in other genes, and environmental factors. Aside from differences in XCI, evidence is lacking to support these suppositions.

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Table 1  
Rett syndrome: diagnostic criteria

<i>Classic Rett Syndrome</i>	<i>Atypical Rett Syndrome</i>
<i>Main criteria</i>	<i>Main criteria</i>
Regression of development	Regression of development
Partial or complete loss of acquired purposeful hand skills	Must meet 2 of 4 main criteria stated above
Partial or complete loss of acquired spoken language	Must meet 5 of 11 supportive criteria
Gait abnormalities: Impaired or absent ability	
Stereotypic hand movements	
<i>Supportive criteria: not required</i>	
<i>Supportive Criteria</i>	
Breathing disturbances when awake	Scoliosis/kyphosis
Bruxism when awake	Small, cold hands and feet
Impaired sleep pattern	Inappropriate laughing/screaming spells
Abnormal muscle tone	Peripheral vasomotor disturbances
Diminished response to pain	Growth retardation
	Intense eye communication – eye pointing

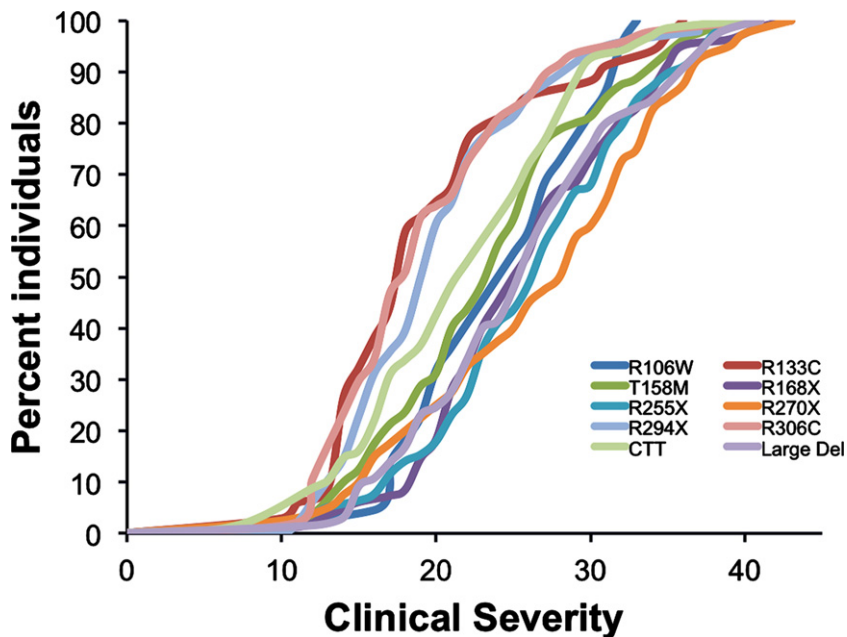


Fig. 1. Genotype-phenotype relationship.

### 1. The case for effective treatment

Mouse and rat models of RTT share many features with the human disorder despite some differences including later onset in the animal models [15-17]. In 2007, Jacky Guy and colleagues presented convincing evidence in a mouse model of RTT that inserting a normal *MECP2* gene regulated by a specific controller yielded marked improvement in both male and female mutants and markedly

prolonged survival [18]. This provided the necessary encouragement to investigate the potential for disease-modifying agents including gene replacement in RTT animal models.

## 2. Pharmaceutical treatment in RTT

The first substantive effort utilized the amino-terminal tripeptide of the insulin growth factor-1 (IGF-1) in a mouse model, showing partial reversal of specific features including prolongation of survival [19]. This led to human trial of the full length, recombinant IGF-1 (Mecasermin) in children with RTT at Boston Children's Hospital [20, 21]. While a phase I trial demonstrated safety and some evidence of efficacy, the subsequent phase II study demonstrated no clear evidence of improvement, perhaps, related to limited ability of this agent to transit the blood-brain barrier (BBB). Subsequently, Neuren Pharmaceuticals launched randomized placebo-controlled study of a modified amino-terminal tripeptide (Trofinetide), which crosses the BBB. Trofinetide was utilized in two phase II studies, one in adolescents and adults, the other in children 5-15 years [22, 23]. The trial in the older age group demonstrated good safety profiles and suggestive evidence for effectiveness in a four-week exposure. The subsequent (and somewhat longer trial) in children again demonstrated safety and tolerability and at the highest dose of 200 mg/kg showed improvements in the parent-reported Rett Syndrome Behavioral Questionnaire (RSBQ) and in two physician scales, the Clinical Global Impression – Improvement (CGI-I) and Rett Syndrome Domain Specific Concerns. Currently, Acadia Pharmaceuticals has completed a phase III, 12-week placebo-controlled trial (Lavender) followed by a 40-week open label extension (Lilac) utilizing two of the outcome measures noted above, the RSBQ and the CGI-I. Neuren Pharmaceuticals is planning a similar phase III trial in Europe for next year.

Following the demonstration that chronic administration of the N-Methyl-D-Aspartate receptor antagonist, ketamine, improved the phenotype of RTT animal mice [24], a double blind, placebo-controlled crossover trial with escalating doses is on-going in the US (NCT03633058). This is an investigator-initiated phase II trial with safety as the primary endpoint. Secondary endpoints include measurements of breathing and the same two outcome measures described above: RSBQ and CGI-I. This trial is being conducted in children ages 6-12. The first cohort (0.75 mg/kg) is completed, and the next escalating dose is ongoing.

Two additional trials are ongoing in the US. The GW Pharmaceuticals trial of Epidiolex (cannabidiol) oral solution in children with RTT is a phase III double blind, placebo-controlled study of efficacy with the same two outcome measures. Anavex Life Sciences is engaged in a phase II placebo controlled, double-blind study on older adolescents and adults studying the muscarinic and Sigma-1 receptor agonist, Anavex2-73. This agent was shown to be effective in a mouse model of RTT. The primary outcome is safety with the same two measures as secondary outcomes.

Additional trials have failed to establish efficacy. These include Sarizotan (Newron Pharmaceuticals) and glatiramer acetate.

## 3. Gene therapy in RTT

Two more recent RTT mouse model studies in female animals demonstrated that both systemic and cisternal delivery of an optimized MeCP2 transgene rescued both motor, behavioral, and cellular deficits leading to the development an AAV vector for gene therapy [25, 26]. Two companies, Taysha Gene Therapies and Neurogene, are preparing to launch early phase safety trials in optimized *MECP2* platforms.

#### 4. Current challenges for clinical trials in RTT

*Biomarkers:* Biomarkers could broaden the links to disease severity, identify treatment responders, and be early predictors of treatment responses. Current efforts include neurophysiological and metabolic biomarkers. Neurophysiological biomarkers have been examined including a project in the US NHS to evaluate visual and auditory evoked potentials [27]. Visual evoked responses in RTT mutant mice and individuals with RTT show a marked reduction in amplitudes compared to normal controls [28]. In the NHS, both parameters were markedly reduced in individuals with RTT versus typically developing children. In female RTT heterozygous mice, an age dependent effect was demonstrated as well as a strong correlation with phenotypic severity [29]. These studies need to be extended in individuals with RTT including the evaluation of power and coherence [30]. Similarly, longitudinal characterization of these features in heterozygous RTT mice should be coupled with the possible responses in mice who have received MeCP2 reconstitution.

Metabolic biomarkers have also been examined in the US NHS [31]. Significant differences were noted between normal siblings in a number of important metabolic pathways including links to changes in gut microflora, aging, and oxidative stress. While much work remains to validate these findings, additional correlations with overall severity and specific clinical issues such as epilepsy, abnormal breathing patterns, movement disorders, and others will be evaluated. As with the neurophysiologic biomarkers, examining the same metabolic parameters in heterozygous RTT mice needs to be evaluated versus disease progression and examined after gene therapy.

*Outcome Measures:* Clinician and parent-reported outcome measures are areas of importance for clinical trials. The need for reliable and valid measures is of major concern for investigators and trial developers. The currently utilized measures are the Clinical Global Impression-Improvement (CGI-I) and the Rett Syndrome Behavioral Quotient (RSBQ). Additional markers under consideration are a Revised Motor Behavioral Assessment, video assessments of motor function, breathing assessment, and adaptation of the Mullen Scales of Early Learning. Both the CGI-I and the RSBQ are currently being utilized in several human trials. The CGI scales were developed initially in RTT for the first Trofinetide trials [32]. This is a clinician's assessment of global function which is divided into two forms, Clinical Global Impression – Severity (CGI-S) and Clinical Global Impression – Improvement (CGI-I), both as 7-point Likert scales. These are both accepted by the US (FDA) and European (EMA) regulatory agencies. Specific anchors were developed for the CGI-S based on clinical features of RTT. The CGI-I does not provide details on specific domains of improvement, rather indicating graded levels of both positive and negative effects. In order to utilize these scales in a multi-site clinical trial, it is critical that all investigators are trained on these disease-specific anchors and measures of improvement and provide evidence of training currently using written vignettes. Even better rater training and reliability could be assessed utilizing video-based vignettes.

The RSBQ is a 45-item caregiver completed rating scale that was not established initially as an outcome measure. Although the RSBQ focuses on aberrant behavior, it does contain measures of other disabling features in RTT. It has undergone psychometric evaluation for factor analysis and test-retest reliability, is relatively simple to complete, and is accepted by the FDA and EMA. However, it does not cover all domains of interest equally, has a limited three-point response range (not at all, sometimes, always), and contains items that require training to explain the intent. Further, a recent publication expressed concerns about its reliability [33].

The development of a new clinician reported outcome has emerged from a psychometric analysis of the Motor Behavioral Assessment (MBA) score originally developed by Fitzgerald, Percy, and Jankovic [34, 35]. This was initially designed as a 37 item, 5-point Likert scale with a range of 0-148, higher scores representing increased severity. The scale was divided into three conceptual domains, Behavioral-Social, Orofacial-Respiratory, and Motor-Physical signs. This has been collected consis-

tently over 17 years in the US NHS. Some items were known to have marked floor or ceiling effects and they did not factor well into the three conceptual domains. It was never assessed psychometrically until last year when the cross-sectional responses from 1075 individuals were submitted to formal psychometric analysis in collaboration with Research Triangle Institute (RTI), a research institute in Durham, North Carolina [36]. Two-thirds of the sample were used as a development sample, the remaining one-third was used as a confirmatory sample. Factor analysis reduced the scale to 21 items in five factors: Motor dysfunction, Functional Skills, Social Skills, Aberrant Behavior, and Rett-specific Behavior. The total revised MBA (R-MBA) score included 21 items plus 3 additional clinically important items: stereotypical hand movements/hand mouthing, truncal rocking, and seizures. Internal consistency was reasonable for the subscales, yielding Cronbach's  $\alpha=0.55-0.8$  for the subscales and 0.78 for the total. Comparing these factors with a separate parent report of clinical domains for validity demonstrated excellent correlation. The R-MBA also showed excellent correlation with the Clinical Severity Scale (CSS), significant correlation by genotype group, and significant correlation with the CGI-S. Interestingly, the R-MBA increased overall with age, similar to the full-scale MBA. Further assessment of the R-MBA will include longitudinal assessment of NHS data, establishment of internal reliability using video-based assessment, intra- and inter-rater reliability, external validity, and formal evaluation of sensitivity and responsiveness to establish the minimally clinically important difference.

Other potential outcome measures under consideration are breathing assessments using band plethysmography, adapting the Mullen Scales of Early Learning for a standard assessment of development in RTT, and development of two video-based evaluation tools to assess hand and gait function.

## **5. Concluding remarks**

RTT is a rare genetic neurodevelopmental disorder with a variety of clinical features found in other disorders. Mouse models of RTT reproduce many of the features seen in humans and provide evidence for modification and even reversibility. Clinical trials are underway or proposed to provide disease-modifying therapies. Problems still remain in terms of improving clinical outcome measures for which the US NHS data can be critical. Biomarker development and implementation is needed both for clinical assessment and for use in clinical trials. Animal models can provide useful avenues for identifying and characterizing these.

## **Competing interests**

Dr. Neul has no competing interests.

## **Author contributions**

Dr. Neul is solely responsible for this review.

## **Conflicts of interest**

For the respective Trofinetide trials, Dr. Benke, Dr. Marsh, Dr. Neul, and Dr. Percy did not have any conflict of interest. These authors were blinded as to participant involvement, whether as placebo or pharmacologic agent and did not participate in the data analysis.



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# *MECP2* Duplication Syndrome

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**Keywords:** *MECP2* duplication disorder, males, X-chromosome inactivation, seizures, regression, clinical trials, biomarkers, outcome measures

*MECP2* Duplication Syndrome (MDS) represents the antithesis of Rett Syndrome (RTT). To understand this idea, one must examine the *MECP2* (methyl-CpG-binding protein 2) gene. *MECP2* was well-studied in cancer genetics as a methylation binding regulator prior to its identification as the principal cause of RTT in 1999 [3]. Located at Xq28, MeCP2 functions in the cell nucleus as an epigenetic gene regulator by binding to methylated DNA nucleotides. It is recognized that MeCP2 regulates genes critical for normal brain development including the maturation and maintenance of synapses. Following the identification of *MECP2* as causative in RTT, an animal model was engineered to over-express *MECP2* producing an equally devastating condition [37, 38]. Subsequently, duplications of *MECP2*, principally in males, were associated with significant neurodevelopmental delay, hypotonia, and recurrent infections [7, 39-44]. The duplication was mainly transmitted by the mother who had marked skewing of X chromosome inactivation and was regarded as cognitively normal. Evidence was presented that these carrier females do have increased evidence of anxiety and depression [45]. Thus, the Goldilocks Paradigm (Fig. 1) emerged. The under-expression of *MECP2* yields RTT, the “normal” expression is just right, and the over-expression produces MDS.

To reiterate, MDS is generally identified in males accounting for 1-2% of X-linked intellectual disability [46]. Females have been identified, but much less frequently, often as *de novo* mutations from the paternal allele or autosomal duplications. The typical clinical features of MDS include infantile hypotonia, developmental delays, epilepsy, choreiform movements, progressive spasticity, and recurrent infections. Thus, MDS is quite different from RTT and probably under-diagnosed due to relative absence of distinctive features such as deceleration in rate of head growth, regression in early childhood, or stereotypic hand movements.

Important questions regarding regression and epilepsy have been addressed in the US Natural History Study (NHS). Among 69 participants (62 males and 7 females; age range 1-28 yr.) with MDS, the most common features (Table 1) included 70% with developmental delay in the first six months, 88% with hypotonia, 81% with constipation, 77% with drooling, and 73% with vasomotor disturbances. Other features such as bruxism and sleep issues were less prominent. Regression, impacting motor skills predominantly and seen at a much older age than RTT, was typically associated with the onset of significant, often refractory, seizures. Among those with seizures, 73% had developmental regression. The Revised Motor Behavioral Assessment (R-MBA) revealed an increased severity with age (Fig. 2). When assessing the parental concerns, the most prominent were lack of effective communication, abnormal walking or balance, constipation, seizures, and ineffective chewing and swallowing. Recurrent infections were sixth most prominent (<6%). Repetitive hand movements, virtually 100%

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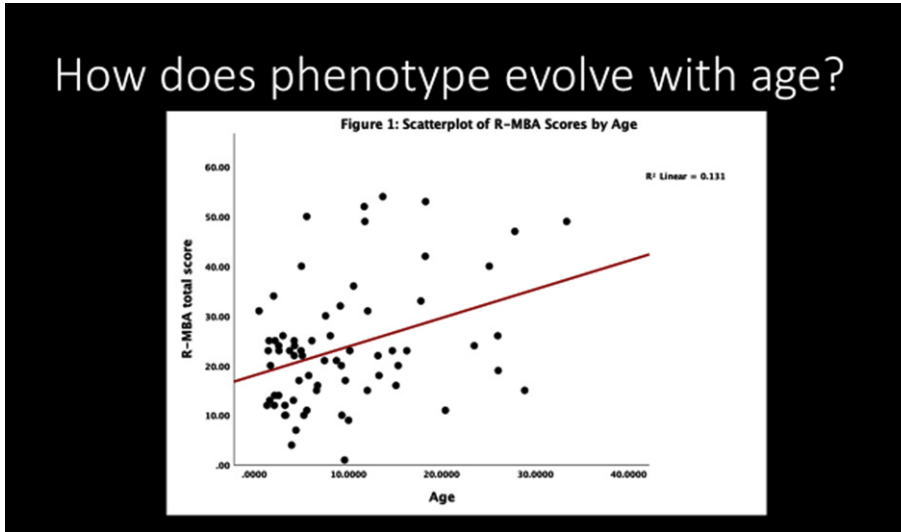


Fig. 2. Change in revised motor behavioral assessment with age.

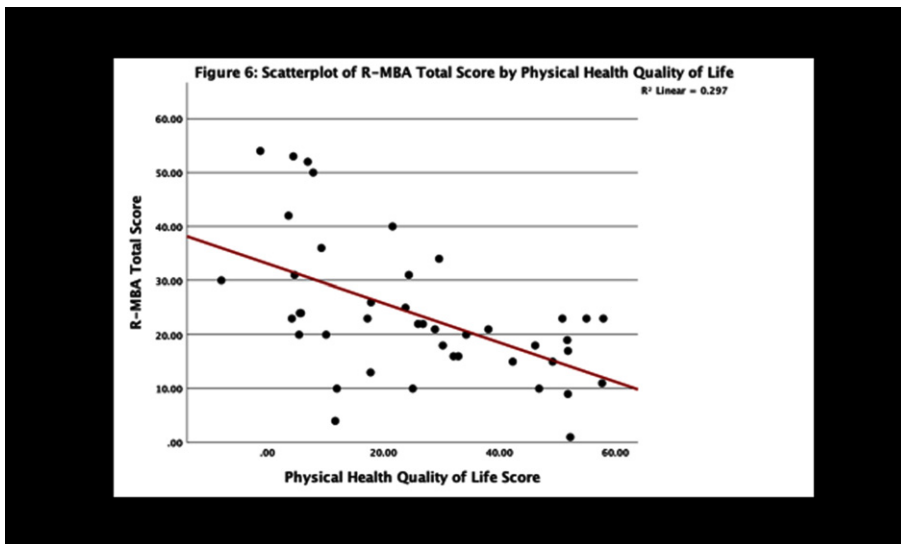


Fig. 3. Comparison of revised motor behavioral assessment with physical health quality of life.

A second question regards what factors lead to the correct diagnosis? This is coupled with a rather wide variability in phenotypic characteristics and what could account for this variability. Possible factors include duplication size, presence or absence of epilepsy, and immunologic or inflammatory markers. Comparing duplication size and composition with the Clinical Severity Score (higher scores representing greater severity) showed a close correlation overall with duplication size and composition [47], particularly those including *HCFC1*, *IRAK1*, *FLNA*, *GDI1*, and *RAB398*, in addition to *MECP2* (Fig. 4).

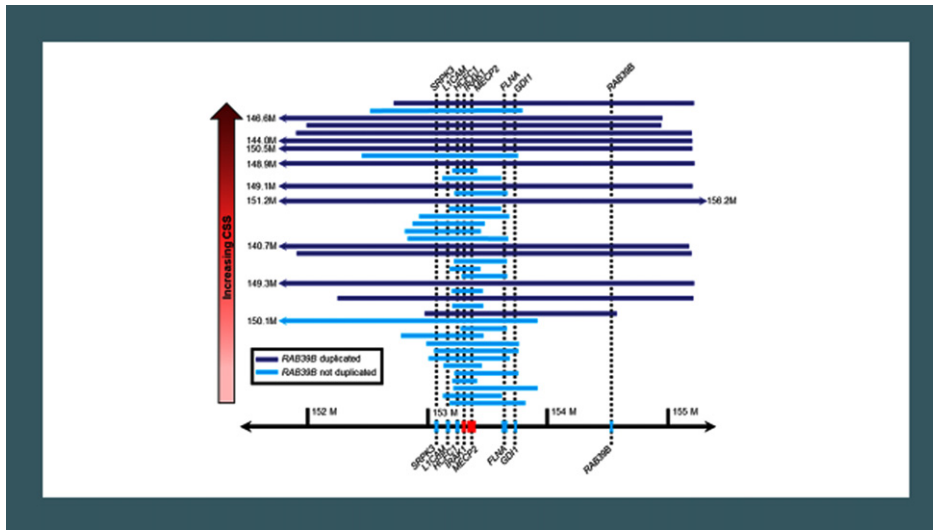


Fig. 4. Duplication size in *MECP2* duplication syndrome.

## 1. Advancing toward clinical trials

Important progress in reaching human clinical trials has been suggested in a mouse model of *MECP2* duplication using antisense oligonucleotides (ASO). These studies demonstrated improvements in hypoactivity, anxiety-like behaviors, motor abnormalities, and social behavior.

This raises the related issue of targeting specific symptoms versus correcting the duplication. An important issue regarding correcting the duplication is how to modulate the gene therapy so as to reduce MeCP2 expression correctly without overshooting and creating an RTT phenotype. Further, the reproducibility of preclinical models and improvements in QOL is critical to ensuring the development of drug development for humans.

This translation of preclinical results to human clinical trials is a universal problem when moving from animal models to humans. The animal models often fail to predict efficacy of potential therapies in humans. Thus, the third question regards the development of possible biomarkers for assessing disease progression. The absence of high-quality outcome measures including the ability to measure improvements across a broad functional range can lead to failure. In addition, absence of validated biomarkers and the inability to examine the target organ, that is, the brain make direct observation difficult. In order to obviate these problems, validated clinical measures and biomarkers need to be developed, and clinical trial readiness needs to be standardized across the clinical trial sites.

The key endpoints required are the establishment of typical developmental trajectories, development of measures sufficiently sensitive to detect improvement, and ability to detect minimally clinically significant differences.

## 2. Concluding remarks

Research in MDS has evolved steadily over the past nearly twenty years. Nonetheless, significant questions remain. How do the duplication size and the contiguous genes involved contribute to the clinical features and developmental trajectory? Can effective outcome measures and biomarkers (neurophysiologic or immunologic for example, be developed? Can we improve clinical assessments by capturing data from wearable devices?

Overall, these are the keys to development and assessment of effective therapies for conducting meaningful clinical trials.

### Competing interests

Dr. Peters has no competing interests.

### Author contributions

Dr. Peters is solely responsible for this review.

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# Recent Progress in Genotype-Phenotype Correlations in *FOXG1* Disorder

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Keywords: *FOXG1* disorder, hypogenesis of corpus callosum, pachygyria, phenotype-genotype, neuroimaging

*FOXG1* disorder (FD) was initially described as the congenital variant of Rett syndrome (RTT). The overlap between the two disorders was based on clinical features which included severe neurodevelopmental delay, postnatal microcephaly, absent language, epilepsy, a hyperkinetic-dyskinetic movement disorder, and features of autism. In addition, specific neuroimaging features emerged consisting of hypogenesis of the corpus callosum, simplification of the gyral pattern with mild frontal pachygyria, and hypomyelination with particularly reduced white matter volume in the frontal lobes. The clear distinction between the two disorders was the early developmental delay and the neuroimaging abnormalities in FD. The original description of the congenital variant of RTT by Rolando et al. [6] was followed up in 2008 by Ariani and colleagues in 2008 [48] and Kortüm et al. [49] in 2011 linking these clinical features with pathogenic variants in the autosomal dominant *FOXG1* gene at chromosome 14q12. In addition to the neuroimaging findings, FD differs from RTT by the striking presence of dyskinesias, a lack of regression, and lack of irregular breathing patterns, especially during wakefulness.

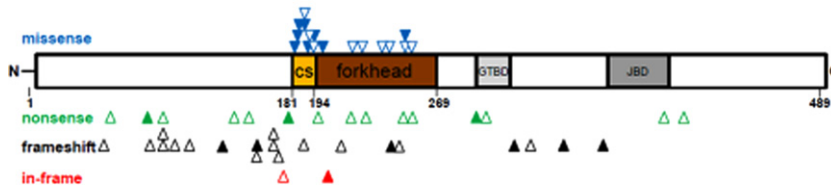
Subsequent efforts to delineate FD involved detailed analysis of the clinical and neuro-imaging phenotype and the development of clear genotype-phenotype correlation [50]. This arose from an analysis of 30 new individuals with pathogenic/likely pathogenic variants in *FOXG1* and absent microaberrations at 14q12 together with 53 previously reported individuals with FD aided by the application of a *FOXG1* severity score. From these two groups, 76 individuals were assigned to one of 5 genetic subgroups according to the location and type of their variant: 1) N-terminal domain frameshift or nonsense variants ( $n=37$ ); 2) forkhead domain conserved site 1 missense variants ( $n=12$ ); 3) forkhead domain excluding conserve site 1, frameshift and nonsense variants ( $n=9$ ); 4) forkhead domain except conserved site 1, missense variants ( $n=9$ ), and C-terminal domain frameshift and nonsense variants ( $n=9$ ). The phenotypic features included microcephaly in 24% at birth and 84% at last follow-up, sitting without support in 45% (5-108 months of life), walking without support in 15% (24-132 months of life), functional hand use in 40%, loss of motor skills in 18%, verbal expression in 21% with a maximum of 100 words, and epilepsy in 68% with onset ranging from 3-168 months (mean = 25 months of life). Among the entire cohort of 83 individuals, 54 different heterozygous variants were noted: 20 frameshift variants (37%); 17 missense variants (31%); 15 nonsense variants (28%); and 2 in-frame variations (4%). The frameshift and nonsense variants were distributed across the *FOXG1*

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### distribution of variants in the FOXG1 protein



54 different variants in 83 patients

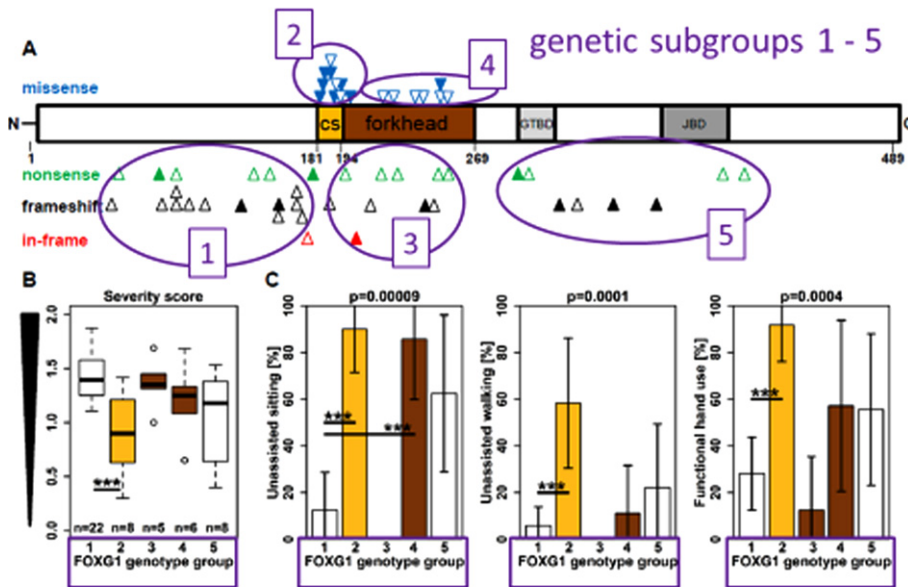
▲ = new variants (n=19)

△ = variants published previously (n=35)

Mitter et al., 2018 Genetics in Medicine

Fig. 1. FOXG1 variants across the protein domains.

### results III: genotype-phenotype associations

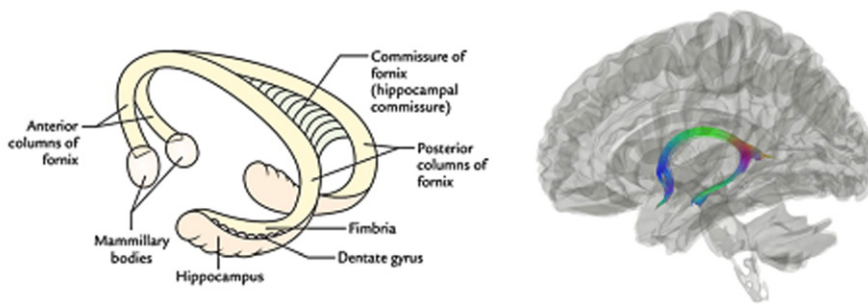


Mitter et al., 2018 Genetics in Medicine

Fig. 2. FOXG1 Genotype-Phenotype Correlations.

protein domains (Fig. 1). The resulting genotype-phenotype correlations were quite evident. (Fig. 2) More severe clinical phenotypes were associated with truncating variants in the N-terminal domain and forkhead domain except conserved site 1 (genetic subgroups 1 and 3). Milder phenotypes were seen in individuals with missense variants in the forkhead conserved site 1 (genetic subgroup 2).

## fornix cerebri



**C-shaped bundle of nerve fibers in the brain  
connecting hippocampus and corpus mammillare.  
Part of the limbic system.**

<https://www.earthslab.com/anatomy/limbic-system/>

© Yeh FC et al., 2018,  
NeuroImage, 178, 57-68.  
via Wikipedia

Fig. 3A. MRI Features of *FOXG1* Disorder: Fornix Cerebri.

Analysis of the neuroimaging findings were also quite informative [51, 52]. Cranial MRIs were available from 34 individuals with heterogeneous variants. Systematic evaluation of these neuroimages were made according to assignment to one of the five genetic subgroups. The MRI features of FD was described in six specific domains (Fig. 3A-3D: 1) corpus callosum anomalies (82%); 2) thickening of the fornices (74%); 3) simplified gyral pattern (56%); 4) enlargement of inner CSF spaces (44%); 5) hypoplasia of basal ganglia (38%); and 6) hypoplasia of frontal lobes (29%).

Utilizing a *FOXG1* severity score based on quantitation of the cerebral anomalies, statistical analysis of their genotype-MRI phenotypes was performed. The simplified gyral pattern was more frequent in those with early truncating variants. Higher FD MRI severity scores were associated with greater Clinical Severity scores.

Similar neuratomic features were found in a mouse model of *Foxg1* including a nearly 25% reduction in cross-sectional area of the telencephalon and a progressive increase (anterior to posterior) in the cross-sectional area of the fornix (Fig. 4A) and reductions in the corpus callosum diameter, cross-sectional area of the basal ganglia and striatum, and diameter of the cortex (Fig. 4B).

### 1. Concluding remarks

These results demonstrate more clearly that FD should be considered in infants and children with global neurocognitive delays, microcephaly, and hyper-/dyskinesias. Further, better understanding of the clinical consequences of different *FOXG1* variants is demonstrated along with the ability to provide more informed genetic counseling information to parents of a child with FD. The neuroimaging features reveal a combination of corpus callosal anomalies, simplified gyral patterns, and thickening of the fornix which seem to be pathognomonic. All three features were noted in 16/34 (47%) and two of the three features in an additional 9 (26%). Thus, 73% of the group demonstrated at least two. Further, these features provide a strong biologic biomarker for clinical trials in reversing the phenotype in FD.

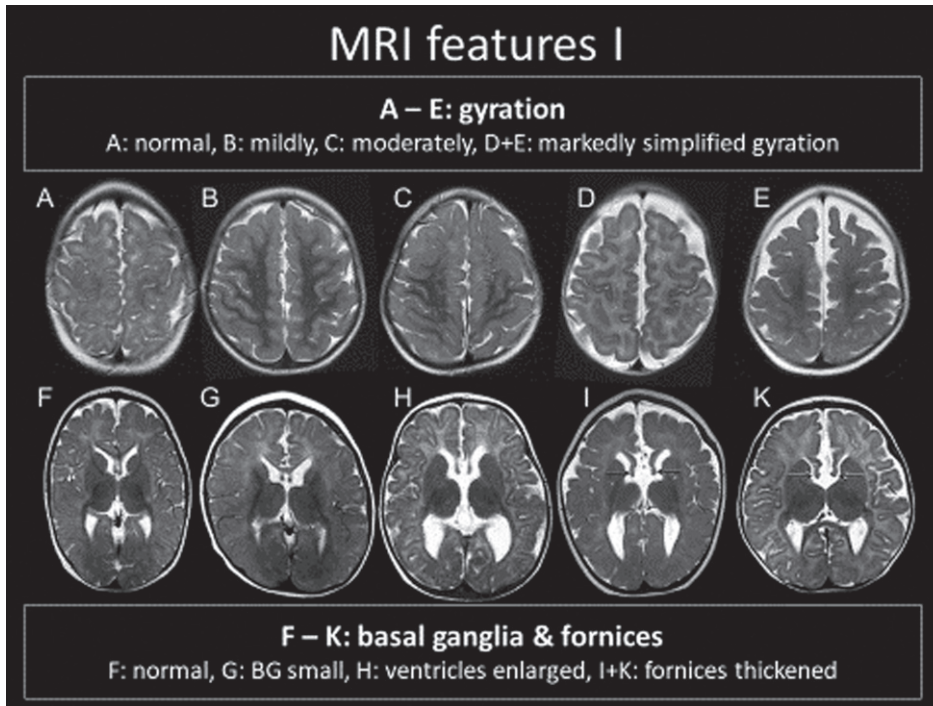


Fig. 3B. MRI Features of FOXG1 Disorder: Gyral Patterns.

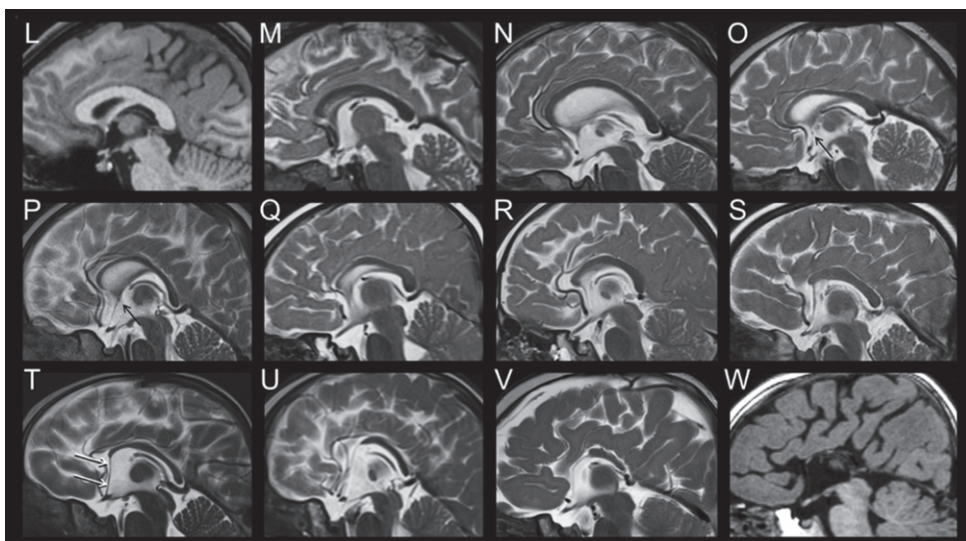


Fig. 3C. MRI Features of FOXG1 Disorder: Corpus Callosum Alterations.

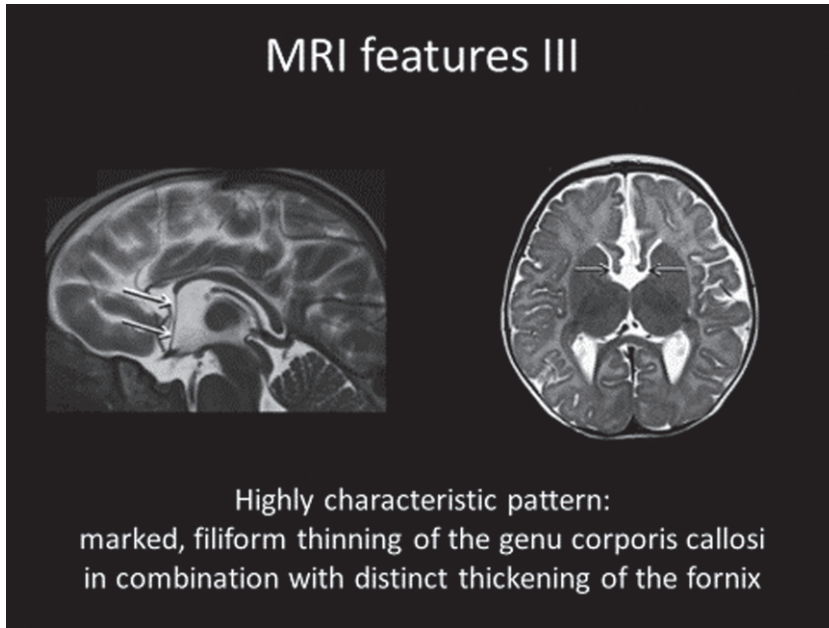


Fig. 3D. MRI Features of FOXG1 Disorder: Thickening of the Fornix.

results V: neuroanatomy of Foxg1+/- mice

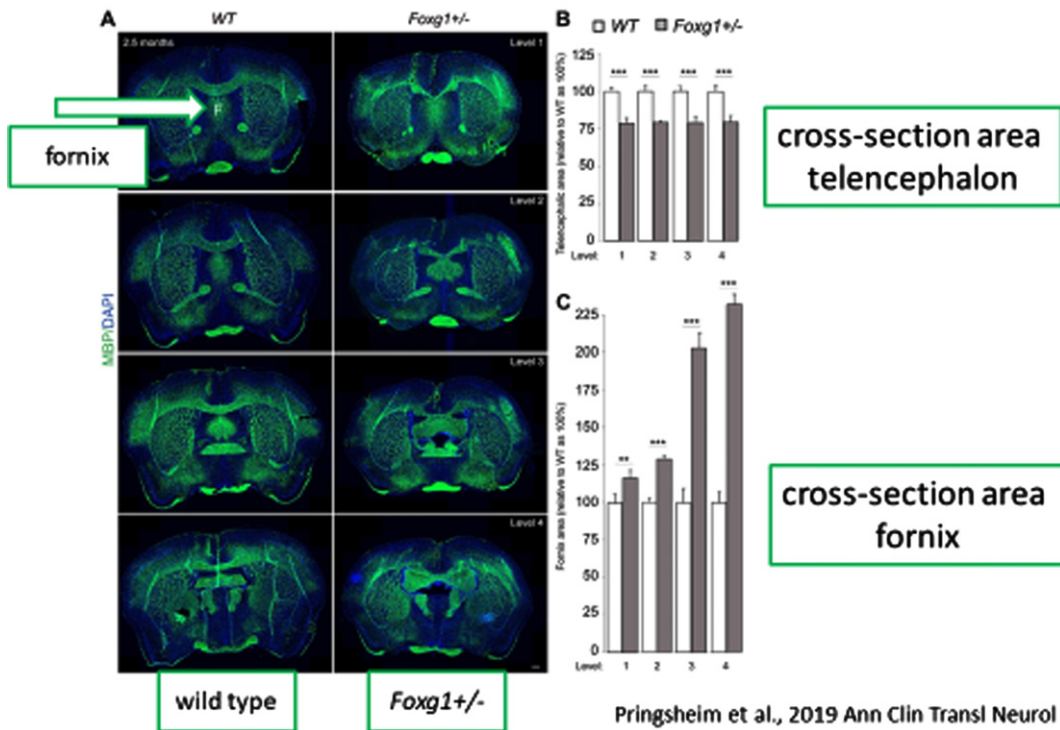


Fig. 4A. Neuroanatomy of FOXG1 Mouse Model: Cross-sectional area of Fornix.



## results V: neuroanatomy of Foxg1<sup>+/-</sup> mice

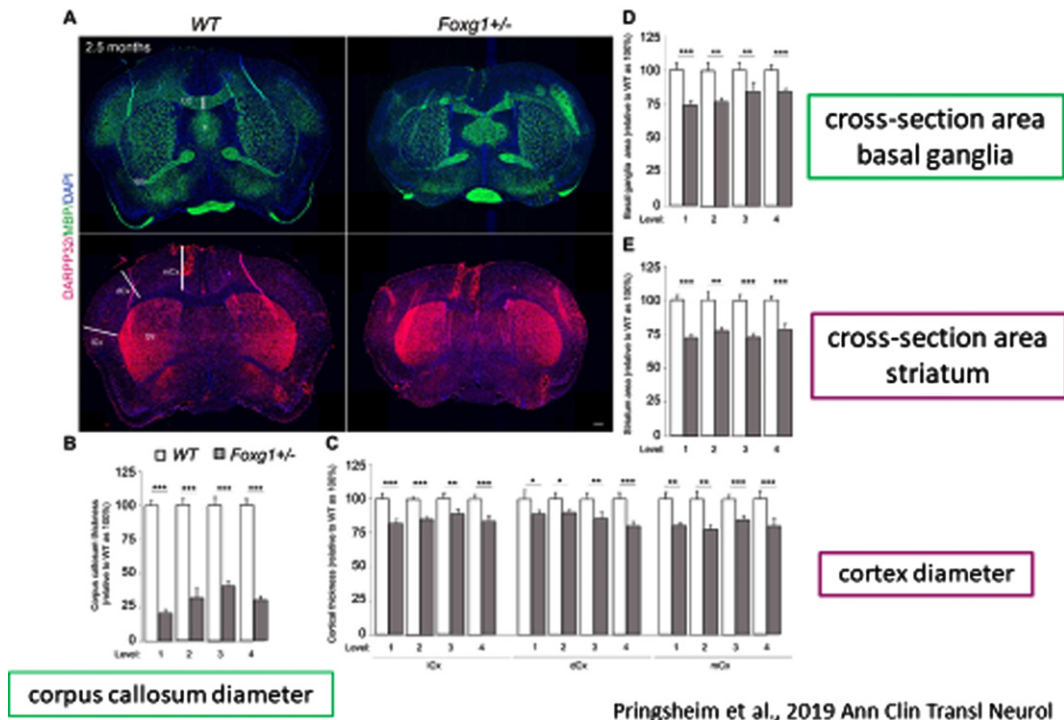


Fig. 4B. Neuroanatomy of FOXG1 Mouse Model: Cross-sectional area of Basal Ganglia and Striatum and Diameter of Cortex.

### Competing interests

Dr. Brockmann has no competing interests.

### Author contributions

Dr. Brockmann is solely responsible for this review.

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# Comparison of Core Features of the Developmental Encephalopathies From the Rett Natural History Study

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**Keywords:** *CDKL5* disorder, infantile epilepsy, hypsarrhythmia, phenotype-genotype, clinical trials, biomarkers, outcome measures

Comparison of the core clinical feature of Rett syndrome (RTT), *MECP2* Duplication Syndrome (MDS), *FOXG1* Disorder (FD, and *CDKL5* Deficiency Disorder (CDD) is critical to improve our understanding of the shared and distinct characteristics of these disorders. In this way, outcome measure development for assessing disease-modifying therapies can occur that both address unique but also shared features of these distinct clinical entities.

The three previous discussions dealt with RTT, MDS, and FD. CDD is described here in order to lay the groundwork prior to comparing and contrasting the characteristics of each.

CDD was first described as an infantile epilepsy variant of RTT with neurodevelopmental abnormalities [1]. While individuals with CDD have some features in common with RTT, the disorder was sufficiently distinct to warrant further molecular studies that resulted in identification of pathogenic variants in the *CDKL5* gene at Xp22.13 [2]. This finding allowed CDD to be regarded as a unique entity with an incidence of 1 : 41,000 births [3]. Unlike RTT, CDD occurs in both males and females with equal severity. Since 2014, 70 individuals with CDD have been followed in the US Natural History Study (NHS). The molecular genetic analyses of *CDKL5* (Fig. 1) indicate that the majority of mutations occur in the catalytic domain as missense mutations [4]. *CDKL5* is localized in the nucleus and the dendritic spines of excitatory glutamatergic synapses [5]. It is regarded as a putative nuclear kinase for MeCP2 and DNMT1. As a synaptic kinase, additional substrates are considered to be EB2, NGL-1, and amphiphysin. It stabilizes synapse formation and interacts with PSD-95, collybisin, Isyn1, NGL-1, and actin. Studies in animal models reveal altered behavior, excitatory synapses, and visual function.

Clinically, CDD is principally recognized as an infantile epileptic encephalopathy with medically refractory tonic seizures occurring as early as 2 weeks of life and infantile spasms without the EEG findings of hypsarrhythmia [6, 7]. Spasms and tonic-spasm sequences become prominent after 12 months and are persistent. A honeymoon period has been noted between ages 1-2 years that may last 12 months. In addition, prominent cortical visual impairment is evident with roving or dysconjugate eye movements and abnormal fixation [8, 9]. Global developmental delay is notable for markedly

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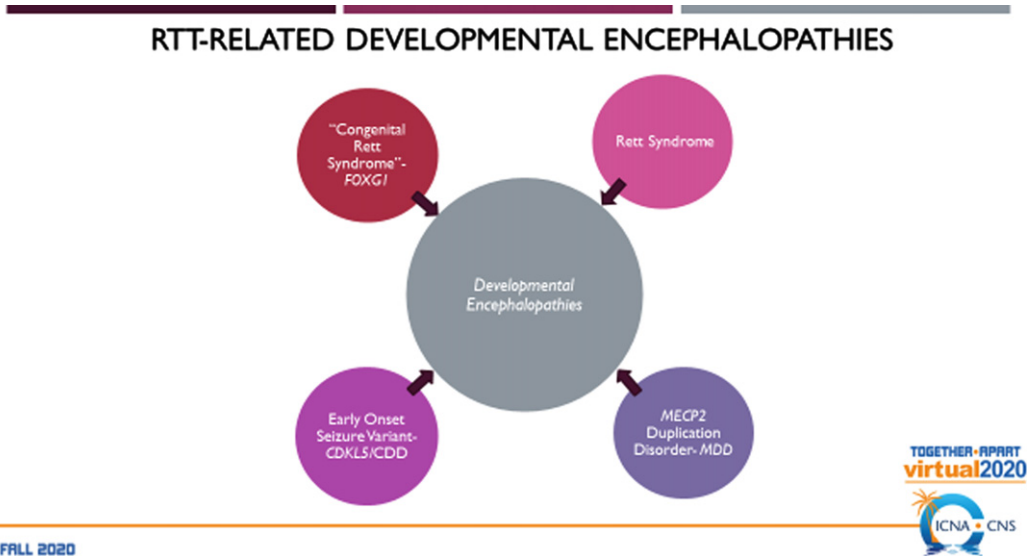


Fig. 2. The RTT-Related Developmental Encephalopathies: RTT, MDS, CDD, and FD.

## Comparison of Core Features in Four Developmental Encephalopathies in the Rett Natural History Study

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Table 1: Participant Demographics

	Total Number of Participants	Gender		Age at Baseline Visit (Years)		
		Female (n)	Male (n)	Median	IQR	P-value
T-RTT	535	529	3	13.5	7.2 - 20.9	–
A-RTT	39	38	1	9.8	4.3 – 21.5	0.037028
N-RTT	44	27	16	6	2.8-10.2	<0.000001
CDD	53	43	10	5.7	3.1 - 9.9	<0.000001
FOXG1	43	22	20	5.1	2.4 - 8.8	<0.000001
MDD	49	5	44	7.4	4.1 - 12.1	0.000002

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Fig. 3. Comparison of the RTT-related Developmental Encephalopathies: Participants.

Developmental encephalopathies was facilitated by the data collection from the US NHS involving 763 individuals (Fig. 3). By collecting the same data elements across these disorders, a direct and meaningful comparison could be performed [21]. The population of the NHS included 535 typical or classic RTT, 39 atypical RTT, 44 with *MECP2* mutations lacking features of RTT, 53 CDD, 43 FD, and 39 MDS. Overall severity assessed by the Clinical Severity Scale (CSS) (Fig. 4A) revealed a rank order (greater to lesser severity) of CDD, FD, RTT, atypical RTT and MDS, and N-RTT. Comparing the occurrence of regression in these disorders (Fig. 4B) revealed a quite different pattern with typical RTT (100%), atypical RTT (94.7%), FD (35.7%), CDD (34.0%), Non-RTT (30.2), and MDS (22.5%). Seizures represent yet another variable (Fig. 4C) occurring most prominently in CDD (98.1%) and FD



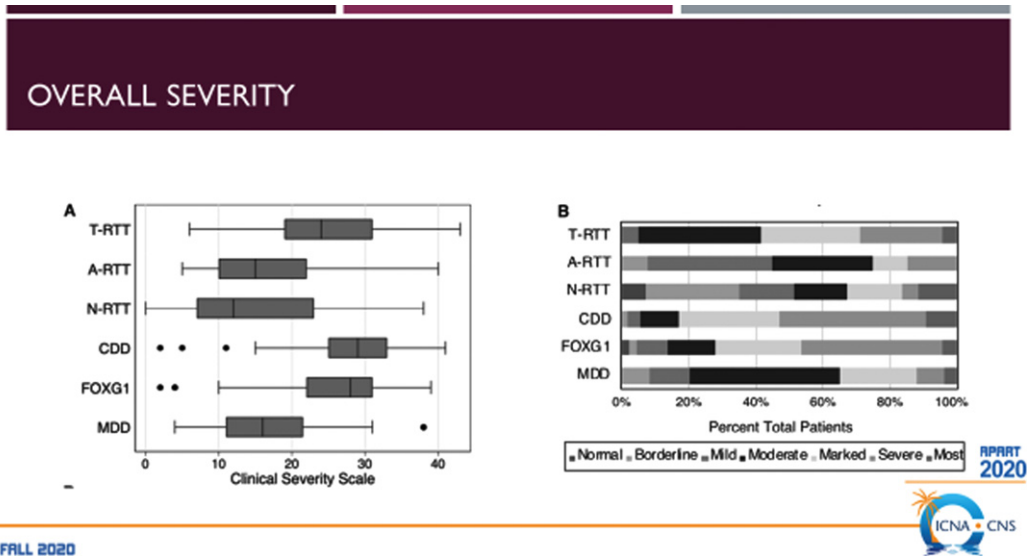


Fig. 4A. Comparison of the RTT-related Developmental Encephalopathies: Overall Severity.

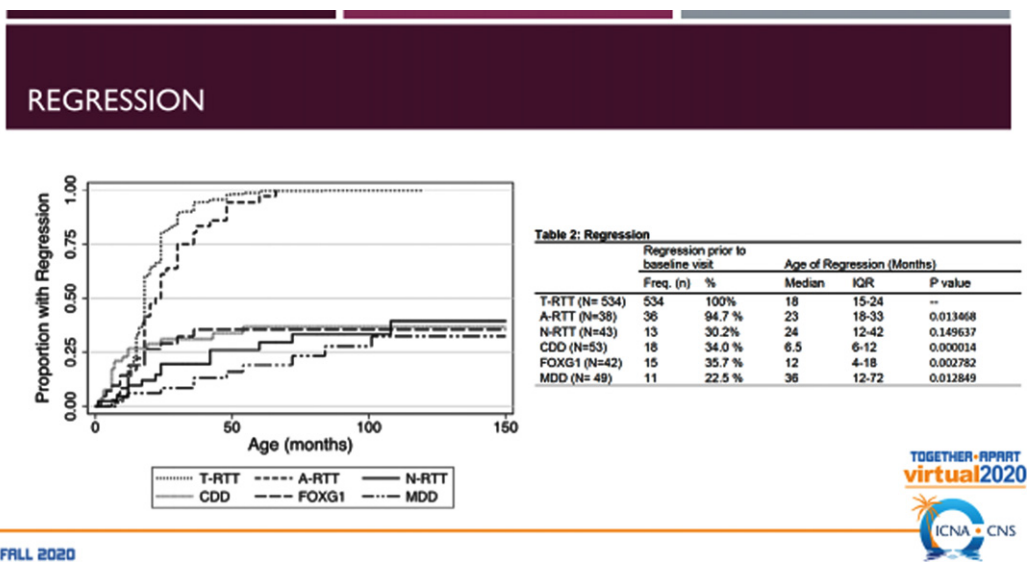
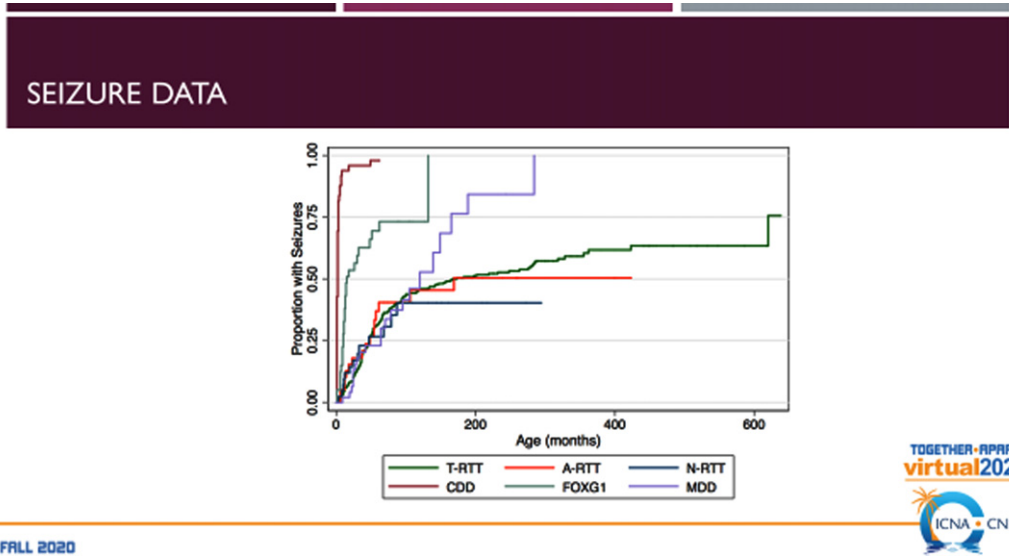


Fig. 4B. Comparison of the RTT-related Developmental Encephalopathies: Occurrence of Regression.

(69.8%) with smaller numbers in typical RTT (47.5%), MDS (44.9%), and atypical RTT (41.0%), and fewer in Non-RTT (29.5%). Early onset seizures were almost exclusively in CDD (88.5%), seen in only 10% of FD, and not at all in typical and atypical RTT and MDS. Average age at onset was also quite distinct with CDD and FD less than one year, two years for Non-RTT, nearly forty months for atypical RTT and nearly 4 years for typical RTT, but greater than 5 years in MDS. Overall occurrence is depicted in Fig. 4C.

The mean value of head circumference Z-scores was also distinctive being markedly reduced in FD, typical RTT, and CDD and essentially normal in MDS. Comparing the head circumference Z-scores with the CSS across all disorders revealed a significant inverse relationship.



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Fig. 4C. Comparison of the RTT-related Developmental Encephalopathies: Seizure Occurrence.

**SOME ACTIVE CLINICAL TRIALS IN DE**

▪ (See ClinicalTrials.GOV or sponsor website for full details)

DEE	Drug	Sponsor	Phase	Primary Outcome measures
RTT	Ketamine	RSRT	2	CGI, MBA, RSBQ, etc
RTT	Trofinetide	Acadia/Neuren	3	RSBQ, CGI, etc
RTT	Epidiolex	Greenwich	3	RSBQ, CGI
RTT	Anavex2-73	Anavex	2	RSBQ, CGI
CDD	Ganaxalone	Marinus	3	Seizure counts
CDD	TAK-935	Ovid/Takeda	2	Seizure counts

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Fig. 5. Current clinical trials in Neurodevelopmental Encephalopathies.

**2. Clinical trials**

Before clinical trials can be considered, it is critical to align the Natural History data for each disorder and generate outcome measures and biomarkers that can be effective within each disorder. Comparison of these measures across disorders is important as well to understand where convergence exists and to separate out those that are divergent. Examples of some on-going clinical trials in RTT and CDD demonstrate the distinct differences in outcome measures (Fig. 5). For RTT, the measures of Global Impression-Improvement and the Rett Syndrome Behavioral Quotient are accepted by the FDA in each trial. For CDD, similar measures are not used, instead utilizing seizure counts as a measure of efficacy as done recently for the first FDA approved drug for CDD, ganaxolone [22]. Clearly, much

work remains to establish effective outcome measures and biomarkers [23] for the developmental encephalopathies in order to demonstrate the effectiveness of potential disease-modifying therapies [15].

### Competing interests

Tim A. Benke: Consultancy for AveXis, Ovid, GW Pharmaceuticals, International Rett Syndrome Foundation, Takeda, Neurogene, Ultragenyx, Zogenix/UCB, GrinTherapeutics, Alcyone and Marinus; Clinical Trials with Acadia, Ovid, GW Pharmaceuticals, Marinus and RSRT; all remuneration has been made to his department.

Eric Marsh: Consultancy for Stoke therapeutics, Cipla pharmaceuticals. Clinical trials with Acadia, GW pharma, Marinus, RSRT, Biopharm, Stoke therapeutics, Zogenix Pharmaceuticals.

### Author contributions

Dr. Marsh and Dr. Benke are solely responsible for this review.

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