Gene discovery to clinical trials: How clinical and basic research have intersected to develop and test new therapies for Rett syndrome.

7th European Rett Syndrome Conference
Marseille, France

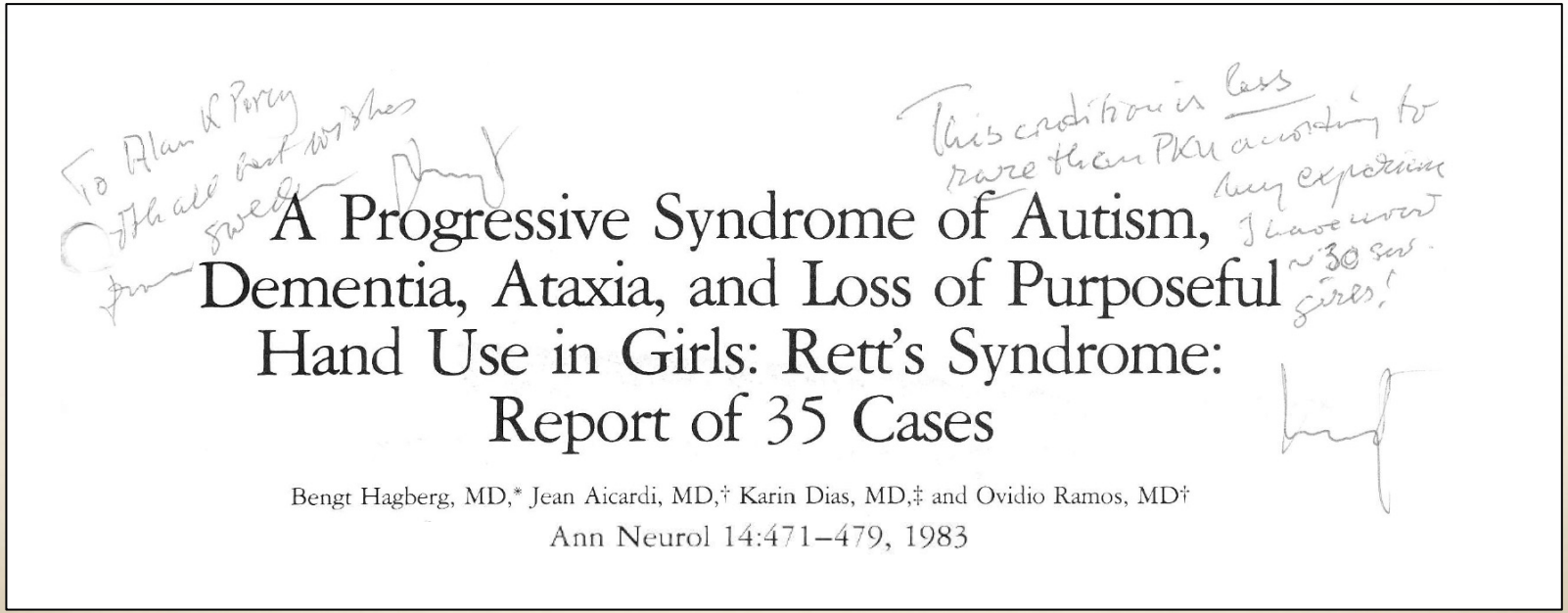
Jeffrey L. Neul M.D., Ph.D.

Director, Vanderbilt Kennedy Center
Annette Schaffer Eskind Professor
Departments of Pediatrics, Pharmacology,
and Special Education
Vanderbilt University Medical Center
Vanderbilt University

Rett Syndrome: History

Uber ein zerebral-atrophisches Syndrom bei Hyperammonamie in Kindesalter.

Andreas Rett, Vienna 1966

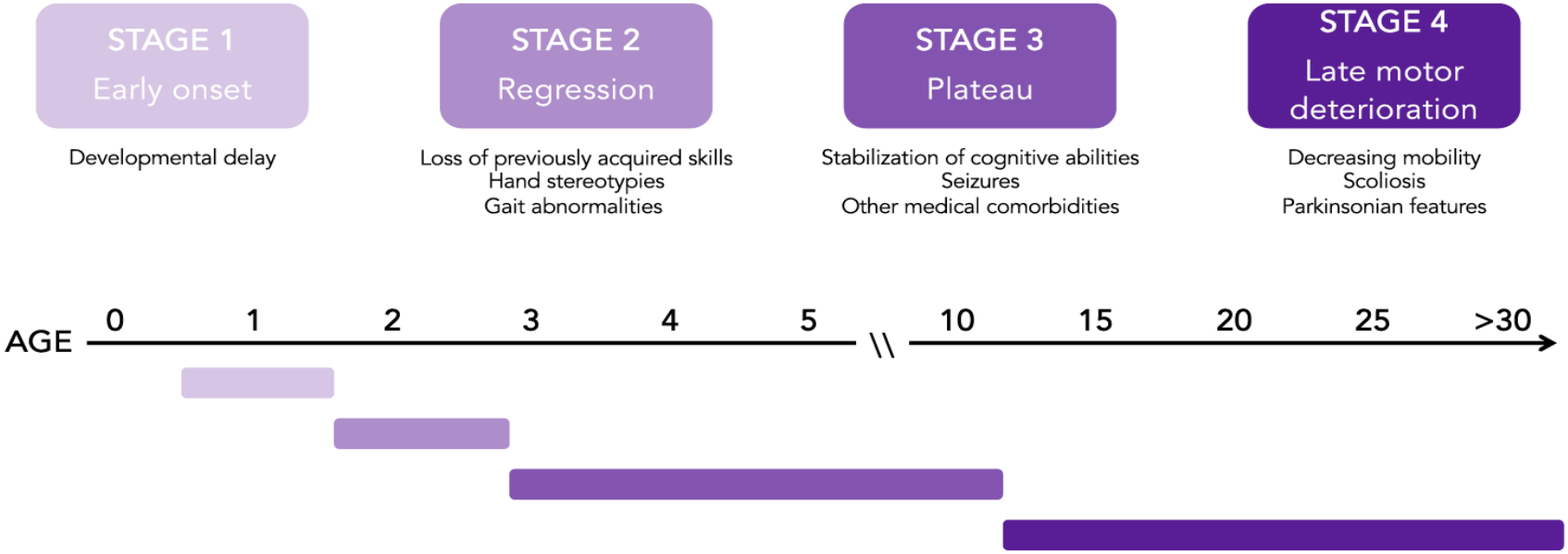


A Progressive Syndrome of Autism, Dementia, Ataxia, and Loss of Purposeful Hand Use in Girls: Rett's Syndrome: Report of 35 Cases

Bengt Hagberg, MD,* Jean Aicardi, MD,† Karin Dias, MD,‡ and Ovidio Ramos, MD†

Ann Neurol 14:471–479, 1983

What is Rett Syndrome?



- Regression followed by stabilization
- Gait abnormalities
- Stereotypic hand movements
- Primarily affects girls
- ~1:10,000 live female births

Neuropsychiatric Disease and Treatment 2022:18 2813–2835

Rett Syndrome: Revised Diagnostic Criteria and Nomenclature

Jeffrey L. Neul, MD, PhD,[1] Walter E. Kaufmann, MD,[2] Daniel G. Glaze, MD,[1] John Christodoulou, MB, BS, PhD, FRACP, FRCPA,[3] Angus J. Clarke, FRCP, FRCPCH,[4] Nadia Bahi-Buisson, MD, PhD,[5] Helen Leonard, MBChB,[6] Mark E. S. Bailey, PhD,[7] N. Carolyn Schanen, MD, PhD,[8] Michele Zappella, MD,[9] Alessandra Renieri, MD, PhD,[10] Peter Huppke, MD,[11] and Alan K. Percy, MD[12] for the RettSearch Consortium

ANN NEUROL 2010;68:944–950

Rett syndrome is caused by mutations in X-linked *MECP2*, encoding methyl-CpG-binding protein 2

Ruthie E. Amir[1], Ignatia B. Van den Veyver[2,3], Mimi Wan[5], Charles Q. Tran[3], Uta Francke[5,6] & Huda Y. Zoghbi[1,2,4]

nature genetics • volume 23 • october 1999

- 95-97% typical Rett syndrome patients have mutations in *MECP2*
  - 5% of Rett syndrome do not have mutations in *MECP2*
- Boys with *MECP2* mutations
  - Severe congenital encephalopathy
  - Expanded phenotype previously unrecognized
- Duplication of *MECP2* locus
  - Severe neurodevelopmental disorder
  - Mostly boys
  - Autism, seizures, absence speech, infections

Enrichment of mutations in chromatin regulators in people with Rett syndrome lacking mutations in *MECP2*

Samin A. Sajan, PhD[1,2,9], Shalini N. Jhangiani, MS[3], Donna M. Muzny, MS[3], Richard A. Gibbs, PhD[3,4], James R. Lupski, MD, PhD[3-5], Daniel G. Glaze, MD[1], Walter E. Kaufmann, MD[6], Steven A. Skinner, MD[7], Fran Annese, MSW[7], Michael J. Friez, PhD[7], Jane Lane, RN[8], Alan K. Percy, MD[8] and Jeffrey L. Neul, MD, PhD[1,2,4,9]

GENETICS in MEDICINE | Volume 19 | Number 1 | January 2017

The array of clinical phenotypes of males with mutations in *Methyl-CpG binding protein 2*

Jeffrey L Neul[1,2] | Timothy A. Benke[3] | Eric D. Marsh[4] | Steven A. Skinner[5] | Jonathan Merritt[1,2] | David N. Lieberman[6] | Shannon Standridge[7] | Timothy Feyma[8] | Peter Heydemann[9] | Sarika Peters[1] | Robin Ryther[10] | Mary Jones[11] | Bernhard Suter[12] | Walter E. Kaufmann[5] | Daniel G. Glaze[12] | Alan K. Percy[13]

Am J Med Genet. 2019;180B:55–67.

Autism and Other Neuropsychiatric Symptoms Are Prevalent in Individuals With *MECP2* Duplication Syndrome

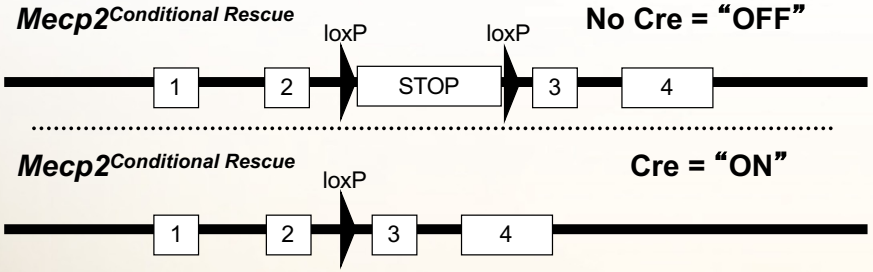
Melissa B. Ramocki, MD, PhD,*[1,2] Sarika U. Peters, PhD,*[1,2] Y. Jane Tavyev, MD,*[1] Feng Zhang, PhD,[3] Claudia M. B. Carvalho, PhD,[3] Christian P. Schaaf, MD,[3] Ronald Richman,[4] Ping Fang, PhD,[3] Daniel G. Glaze, MD,[1,2] James R. Lupski, MD, PhD,[2,3,5] and Huda Y. Zoghbi, MD[1,2,3,4,6]

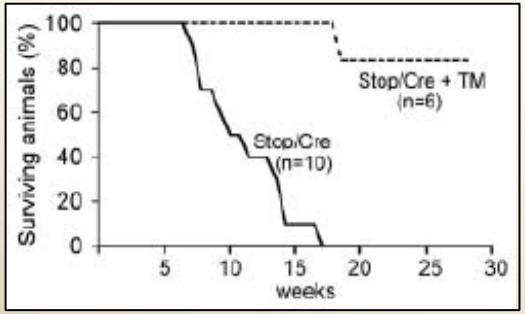
Ann Neurol 2009;66:771–782

Reversal of Neurological Defects in a Mouse Model of Rett Syndrome

Jacky Guy,[1] Jian Gan,[2] Jim Selfridge,[1] Stuart Cobb,[2] Adrian Bird[1]*

SCIENCE VOL 315 23 FEBRUARY 2007





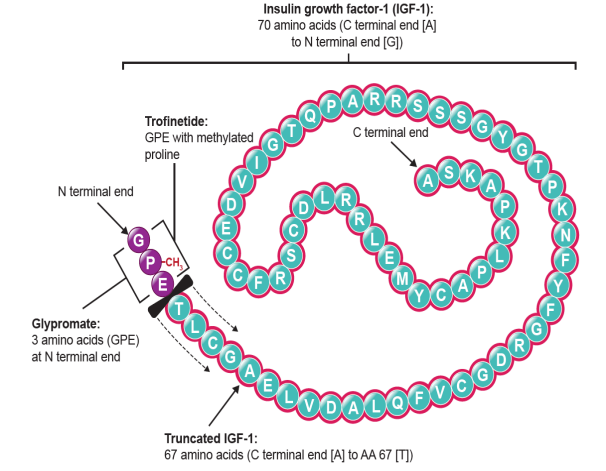
- Restoration of MeCP2 expression, even after symptom onset, reversed disease course
- Seen in both male and female animals
- Hope for meaningful therapy development

Disease targeted/modifying therapies

Partial reversal of Rett Syndrome-like symptoms in MeCP2 mutant mice

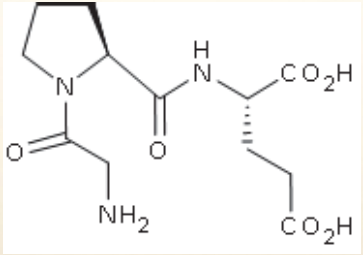
Daniela Tropea[a,1], Emanuela Giacometti[b,1], Nathan R. Wilson[a,1], Caroline Beard[b], Cortina McCurry[a], Dong Dong Fu[b], Ruth Flannery[b], Rudolf Jaenisch[b,c,2], and Mriganka Sur[a,2]

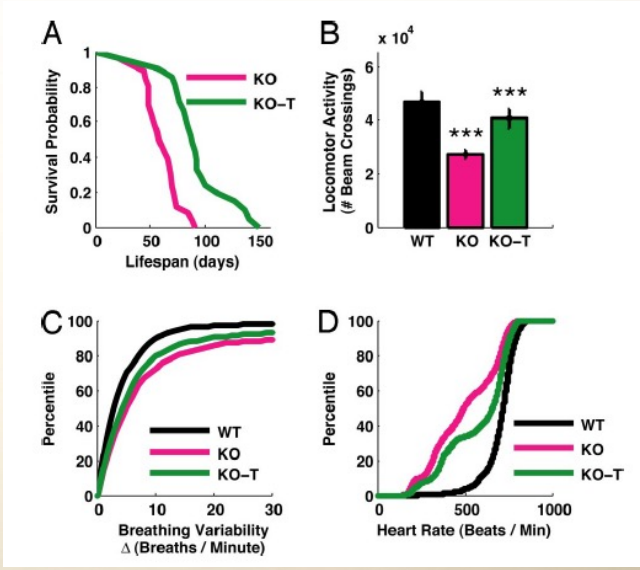
PNAS | February 10, 2009 | vol. 106 | no. 6 | 2033



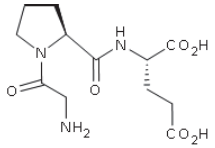
Male *Mecp2* mice treated with tripeptide from N-terminus of IGF-I

IGF-I [1-3] = Gly-Pro-Glu

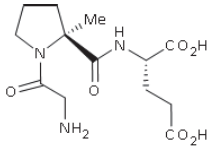




IGF1 [1-3] Glypromate



Trofinetide



A Double-Blind, Randomized, Placebo-Controlled Clinical Study of Trofinetide in the Treatment of Rett Syndrome

Daniel G. Glaze MD [a,*], Jeffrey. L. Neul MD, PhD [a,1], Alan Percy MD [b], Tim Feyma MD [c], Arthur Beisang MD [c], Alex Yaroshinsky PhD [d], George Stoms BS [d], David Zuchero MS, JD [e], Joseph Horrigan MD [f], Larry Glass BA [g], Nancy E. Jones PhD [g]

Double-blind, randomized, placebo-controlled study of trofinetide in pediatric Rett syndrome

Daniel G. Glaze, MD,* Jeffrey L. Neul, MD, PhD,* Walter E. Kaufmann, MD,* Elizabeth Berry-Kravis, MD, PhD, Sean Condon, DPH, George Stoms, BS, Sean Oosterholt, MSc, Oscar Della Pasqua, MD, PhD, Larry Glass, BA, Nancy E. Jones, PhD, and Alan K. Percy, MD,* on behalf of the Rett 002 Study Group

Design and outcome measures of LAVENDER, a phase 3 study of trofinetide for Rett syndrome

Jeffrey L. Neul [a,*], Alan K. Percy [b], Timothy A. Benke [c], Elizabeth M. Berry-Kravis [d], Daniel G. Glaze [e], Sarika U. Peters [a], Nancy E. Jones [f], James M. Youakim [g]

Contemporary Clinical Trials 114 (2022) 106704

Efficacy and safety of trofinetide for the treatment of Rett syndrome: results from the pivotal phase 3 LAVENDER study

Jeffrey L Neul[1], Alan K Percy[2], Timothy A Benke[3], Elizabeth M Berry-Kravis[4], Daniel G Glaze[5], Eric D Marsh[6], Kathie M Bishop[f], Serge Stankovic[f], James M Youakim[f]

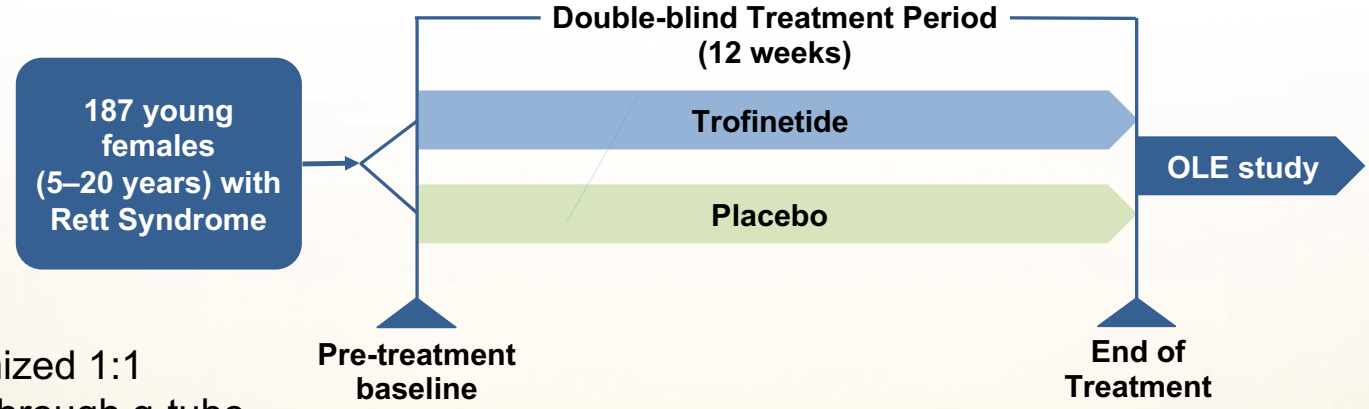
2022 American Academy of Neurology Emerging Sciences

Trofinetide Receives FDA Approval as First Drug for Rett Syndrome

JAMA. Published online March 22, 2023.

Phase 3 LAVENDER Study

Randomized, Double-blind, Placebo-controlled, Multi-center Study



- Randomized 1:1
- Oral or through g-tube
- Weight-based dosing

Co-primary efficacy endpoints

- Caregiver scale Rett syndrome Behavioral Questionnaire (RSBQ)
- Clinical Global Impression-Improvement (CGI-I)

Key secondary efficacy endpoint

- Caregiver communication scale: CSBS-DP-IT Social Composite

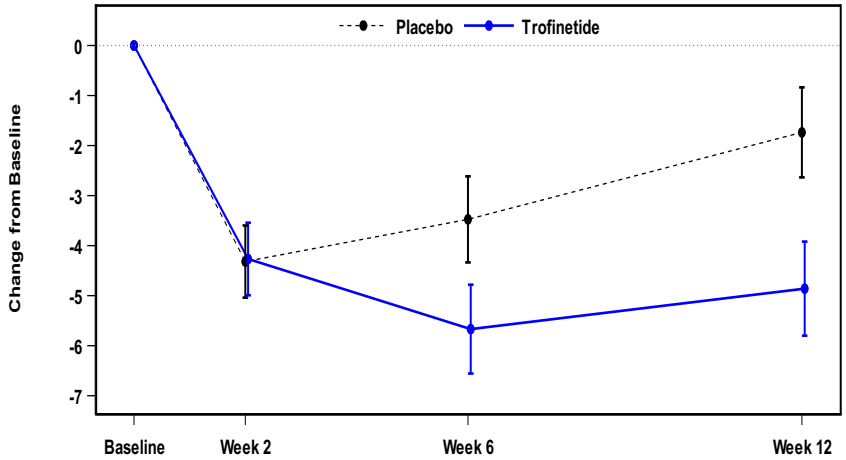
Trofinetide for the treatment of Rett syndrome: a randomized phase 3 study

Jeffrey L. Neul[1], Alan K. Percy[2], Timothy A. Benke[3], Elizabeth M. Berry-Kravis[4], Daniel G. Glaze[5], Eric D. Marsh[6], Tim Lin[7], Serge Stankovic[7], Kathie M. Bishop[7] & James M. Youakim[7]

Nature Medicine 29, 1468–1475 (2023)

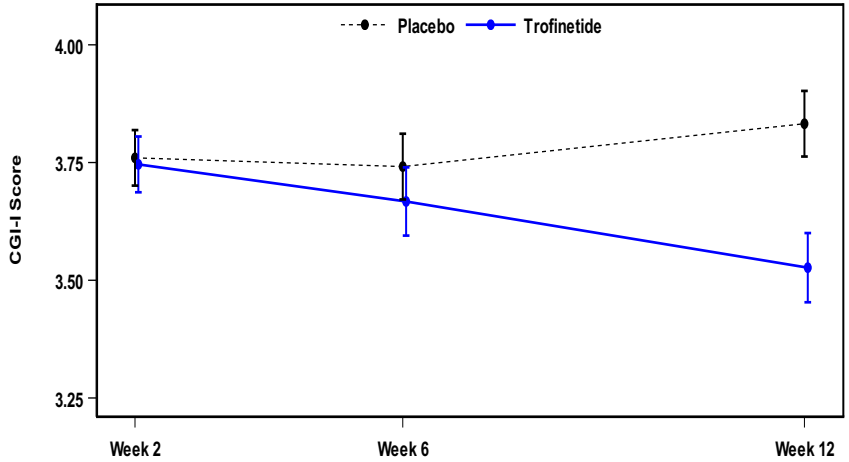
Phase 3 trial of Trofinetide – Top level results

Caregiver scale – Rett Syndrome Behavior Questionnare (RSBQ)



RSBQ change from baseline to week 12:
p-value = 0.0175*
Effect Size = 0.37

Clinician scale – Clinical Global Impression-Improvement (CGI-I)



CGI-I at Week 12:
p-value = 0.0030*
Effect Size = 0.47

Phase 3 trial of trofinetide – Treatment-Emergent Adverse Events

TEAE, n (%)	Placebo (n = 94)	Trofinetide (n = 93)
Any TEAE	51 (54.3)	86 (92.5)
Serious TEAE	3 (3.2)	3 (3.2)
TEAE leading to drug withdrawal Diarrhea	2 (2.1) 0	16 (17.2) 12 (12.9)
Fatal TEAE	0	0

Top TEAEs (Majority mild to moderate severity)
Diarrhea – 80.7% trofinetide, 19.2% placebo
Vomiting – 27% trofinetide, 9.6% placebo

Trofinetide Receives FDA Approval as First Drug for Rett Syndrome

JAMA. Published online March 22, 2023.

- For the treatment of Rett syndrome in patients over 2 years.
- Strawberry flavored liquid (200 mg/ml) for oral or g-tube use
- Recommended dosage is twice daily (weight-based)
  - with or without food.
- Most common side effects are diarrhea (82%) and vomiting (29%)

Patient weight	trofinetide volume
9kg to <12kg	25 ml twice daily
12kg to <20kg	30 ml twice daily
20kg to <35kg	40 ml twice daily
35kg to <50kg	50 ml twice daily
>50kg	60 ml twice daily

https://www.accessdata.fda.gov/drugsatfda_docs/label/2023/217026s000lbl.pdf

Management of side effects (diarrhea)

- Stop laxatives before starting trofinetide
- Decrease/switch sugar containing medications
- Start fiber (Metamucil) 1-3 times per day
  - children: 0.5-1 tsp in 4oz water
  - teenagers/adults: 1-3 tsp in 4 oz water
- Start at lower dose and titrate up over weeks
  - Split into 3 or 4 doses per day?
- At start of diarrhea:
  - Decrease trofinetide dose
  - Take loperamide (Imodium)

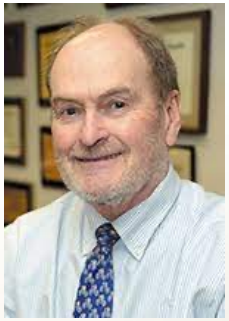
Availability of Trofinetide for people with RTT

Acadia Pharmaceuticals Acquires Ex-North American Rights to Trofinetide and Global Rights to Neuren's NNZ-2591 in Rett Syndrome and Fragile X Syndrome

- Announced July 13th, 2023
- Acadia says will file New Drug Submission in Canada within 18 months
- Europe and Asia plans to be announced later

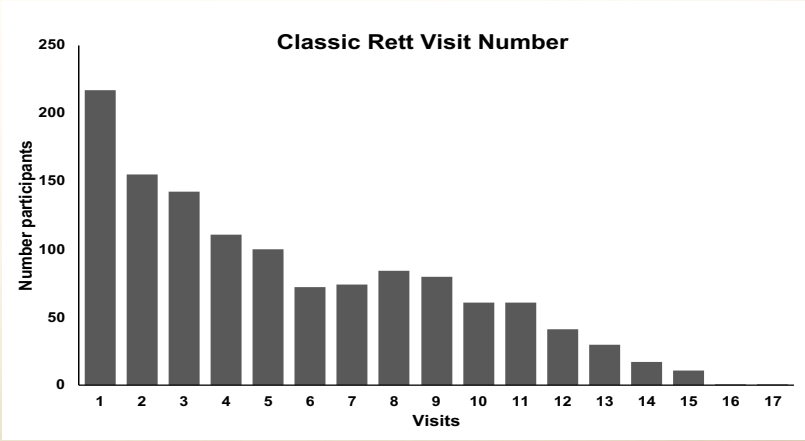
Rett Syndrome and related disorders Natural History Study

- Multi-center longitudinal study (2003-2021)
  - Alan Percy (University of Alabama, Birmingham) – PI
  - Jeffrey Neul (Vanderbilt) – Administrative Head
- RTT, CDKL5 deficiency disorder (CDD), FOXG1 Syndrome (FS), MECP2 Duplication Syndrome (MDS)
- Enrolled >1825 people, 8782 visits, 14 sites across US
  - Classic RTT – 1258, 6838 visits
  - 82.8% > 1 visit
  - 50% > 4 visits (average 5.4 visits)



Alan Percy, MD

Rett Consortium
Rett Syndrome,
MECP2 Duplication,
& Rett-Related Disorders



Timeline of critical events in Rett syndrome research

Rett syndrome is caused by mutations in X-linked *MECP2*, encoding methyl-CpG-binding protein 2

Ruthie E. Amir[1], Ignatia B. Van den Veyver[2,3], Mimi Wan[5], Charles Q. Tran[3], Uta Francke[5,6] & Huda Y. Zoghbi[1,2,4]

nature genetics • volume 23 • october 1999

Identification of genetic basis
Development of disease models

Initiation of Rett Syndrome Natural History Study

Reversal of Neurological Defects in a Mouse Model of Rett Syndrome

Jacky Guy,[1] Jian Gan,[2] Jim Selfridge,[1] Stuart Cobb,[2] Adrian Bird[1]*

SCIENCE VOL 315 23 FEBRUARY 2007

Potential of therapy even after disease onset

Partial reversal of Rett Syndrome-like symptoms in MeCP2 mutant mice

Daniela Tropea[a,1], Emanuela Giacometti[b,1], Nathan R. Wilson[a,1], Caroline Beard[b], Cortina McCurry[a], Dong Dong Fu[b], Ruth Flannery[b], Rudolf Jaenisch[b,c,2], and Mriganka Sur[a,2]

PNAS | February 10, 2009 | vol. 106 | no. 6 | 2033

Opportunity for treatment with a potential drug

Trofinetide Receives FDA Approval as First Drug for Rett Syndrome

JAMA. Published online March 22, 2023.

First successful Phase 3 trial in Rett syndrome

Goal of the Rett Syndrome Natural History Study

Develop ”Clinical Trial Readiness” for Rett syndrome

Goal of the Rett Syndrome Natural History Study

Develop ”Clinical Trial Readiness” for Rett syndrome

- Create a network of clinical sites able to do clinical research

14 sites across US

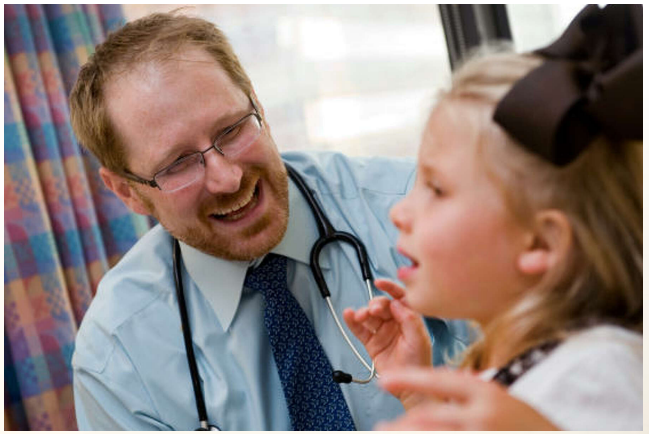
Enrolled people throughout US
>20 countries

Fundamental sites for industry sponsored trials

Goal of the Rett Syndrome Natural History Study

Develop ”Clinical Trial Readiness” for Rett syndrome

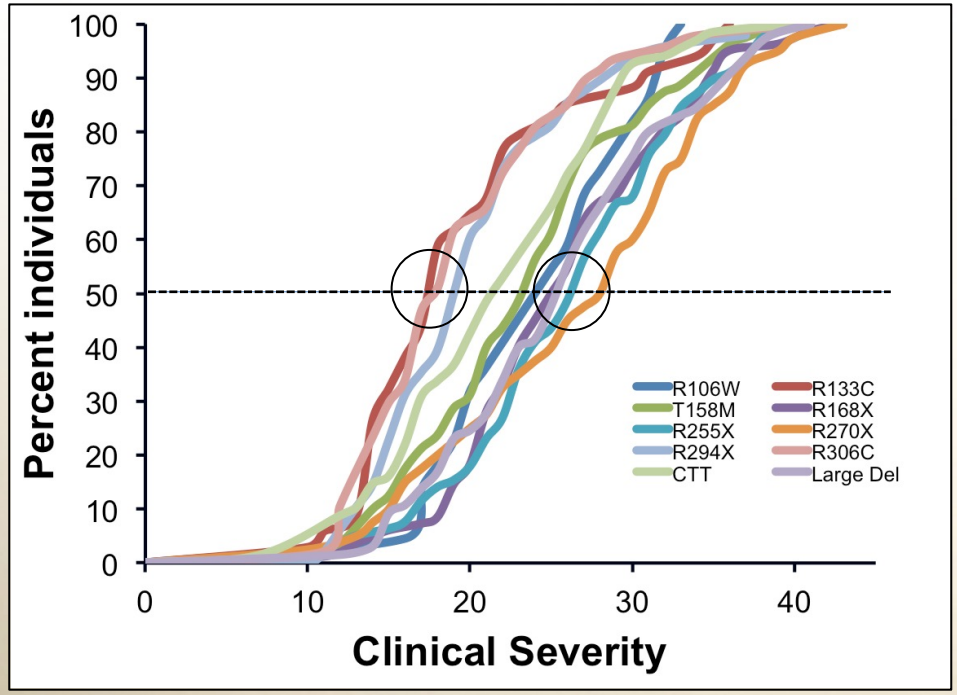
- Create a network of clinical sites able to do clinical research
- Characterize the clinical features and natural history



>50 manuscripts published
Multiple additional manuscripts submitted and in preparation

Clinical Features and Natural History of Rett syndrome

Genotype/phenotype relationships



Specific mutations in *Methyl-CpG-Binding Protein 2* confer different severity in Rett syndrome

Neurology® 2008;70:1313-1321

Methyl-CpG-binding protein 2 (*MECP2*) mutation type is associated with disease severity in Rett syndrome

J Med Genet 2014;**51**:152–158.

Clinical Features and Natural History of Rett syndrome

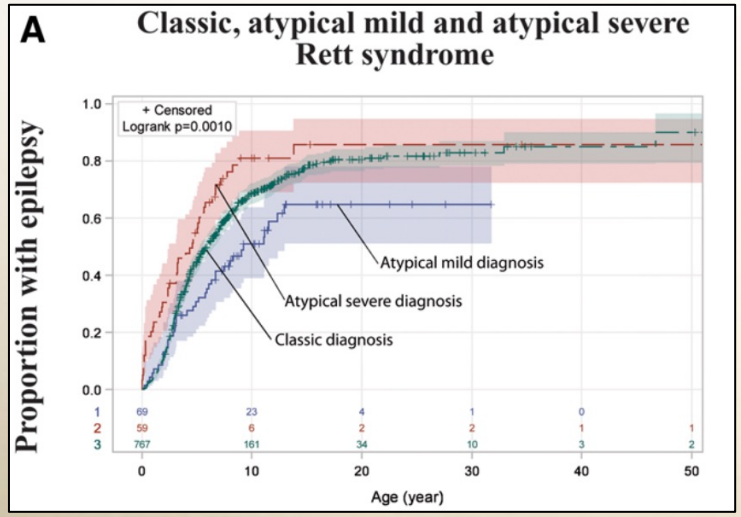
Clinical symptoms

doi:10.1093/brain/aww302 BRAIN 2017: 140; 306–318 | 306

BRAIN
A JOURNAL OF NEUROLOGY

Longitudinal course of epilepsy in Rett syndrome and related disorders

Daniel C. Tarquinio,[1] Wei Hou,[2] Anne Berg,[3] Walter E. Kaufmann,[4] Jane B. Lane,[5] Steven A. Skinner,[4] Kathleen J. Motil,[6] Jeffrey L. Neul,[7] Alan K. Percy[5] and Daniel G. Glaze[6]



The course of awake breathing disturbances across the lifespan in Rett syndrome

Brain & Development 40 (2018) 515–529

Hand stereotypies
Lessons from the Rett Syndrome Natural History Study

Neurology® 2019;92:e2594-e2603.

Evaluating Sleep Disturbances in Children With Rare Genetic Neurodevelopmental Syndromes

Pediatric Neurology 123 (2021) 30–37

Scoliosis in Rett Syndrome: Progression, Comorbidities, and Predictors

Pediatric Neurology 70 (2017) 20–25

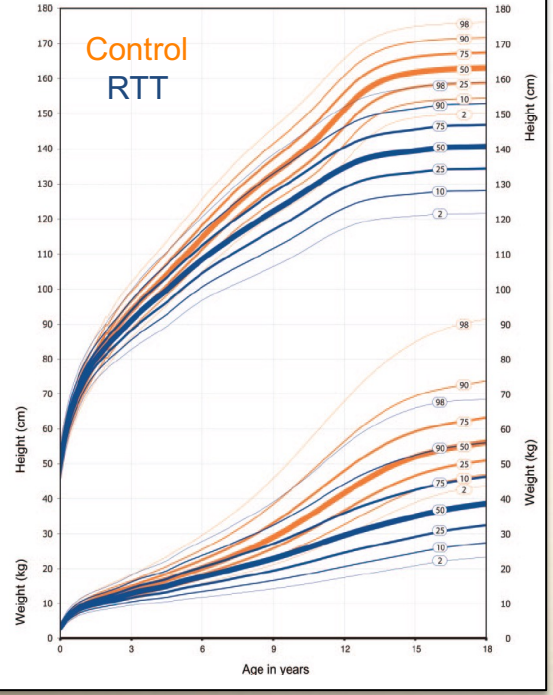
Developmental delay in Rett syndrome: data from the natural history study

Journal of Neurodevelopmental Disorders 2014, **6**:20

Clinical Features and Natural History of Rett syndrome

Growth Failure and Gastrointestinal-Nutritional issues

Height and weight



Growth failure and outcome in Rett syndrome

Specific growth references *Neurology* 2012;79;1653

Anthropometric Measures Correspond with Functional Motor Outcomes in Females with Rett Syndrome

Kathleen J. Motil, MD, PhD[1], Suzanne Geerts, BS[2], Fran Annese, BS, MSW[3], Jeffrey L. Neul, MD, PhD[4], Tim Benke, MD, PhD[5], Eric Marsh, MD, PhD[6], David Lieberman, MD[7], Steven A. Skinner, MD[3], Daniel G. Glaze, MD[8], Peter Heydemann, MD[9], Arthur Beisang, MD[10], Shannon Standridge, DO[11], Robin Ryther, MD[12], Jane B. Lane, RN, BSN[13], Lloyd Edwards, PhD[14], and Alan K. Percy, MD[13]

(J Pediatr 2022; ■:1-9)

Pubertal Development in Rett Syndrome Deviates From Typical Females

Pediatric Neurology 51 (2014) 769–775

Gastrointestinal and Nutritional Problems Occur Frequently Throughout Life in Girls and Women With Rett Syndrome

*Kathleen J. Motil, *Erwin Caeg, *Judy O. Barrish, †Suzanne Geerts, ‡Jane B. Lane, §Alan K. Percy, ||Fran Annese, ||Lauren McNair, ||Steven A. Skinner, ¶Hye-Seung Lee, *Jeffrey L. Neul, and *Daniel G. Glaze

JPGN • Volume 55, Number 3, September 2012

Biliary Tract Disease in Girls and Young Women With Rett Syndrome

*†‡Kathleen J. Motil, §||Jane B. Lane, †‡Judy O. Barrish, ¶Fran Annese, ||#Suzanne Geerts, ¶Lauren McNair, ¶Steven A. Skinner, **Jeffrey L. Neul, †‡Daniel G. Glaze, and §||Alan K. Percy

JPGN • Volume 68, Number 6, June 2019

Low Bone Mineral Mass Is Associated With Decreased Bone Formation and Diet in Girls With Rett Syndrome

Kathleen J. Motil, Judy O. Barrish, Jeffrey L. Neul, and Daniel G. Glaze

JPGN • Volume 59, Number 3, September 2014

Clinical Features and Natural History of Rett syndrome

Behavior and Quality of Life

Social impairments in Rett syndrome: characteristics and relationship with clinical severity
Journal of Intellectual Disability Research
VOLUME 56 PART 3 pp 233–247 MARCH 2012

Clinical severity and quality of life in children and adolescents with Rett syndrome
Neurology® 2011;77:1812-1818

Behavioral profiles in Rett syndrome: Data from the natural history study
Brain & Development 41 (2019) 123–134

Caretaker Quality of Life in Rett Syndrome: Disorder Features and Psychological Predictors
Pediatric Neurology 58 (2016) 67–74

Anxiety-like behavior and anxiolytic treatment in the Rett syndrome natural history study
Journal of Neurodevelopmental Disorders (2022) 14:31

Assessment of Caregiver Inventory for Rett Syndrome
J Autism Dev Disord (2017) 47:1102–1112

Goal of the Rett Syndrome Natural History Study

Develop ”Clinical Trial Readiness” for Rett syndrome

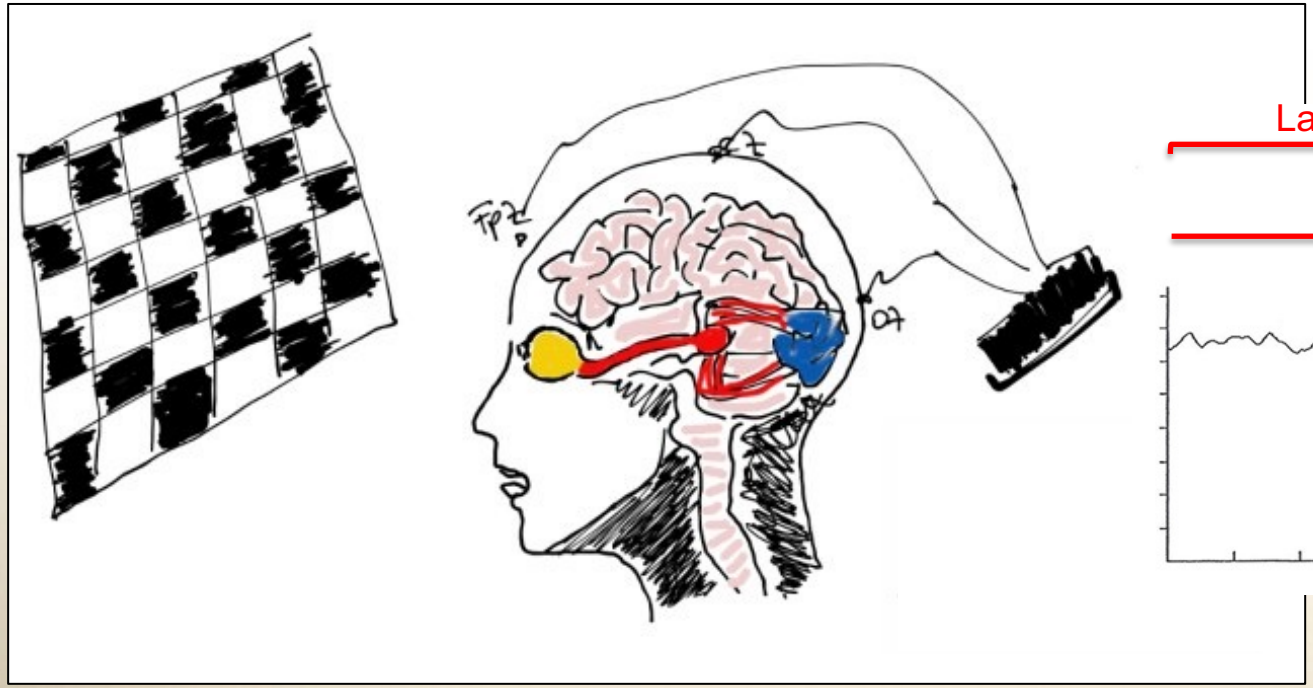
- Create a network of clinical sites able to do clinical research
- Characterize the clinical features and natural history
- Develop biomarkers and clinical outcome measures

“Biomarker”

Something measured that can:

- Show disease severity
- Identify people who will respond to a treatment
- Show changes before clinical improvement

Neurophysiological biomarkers – Evoked potentials

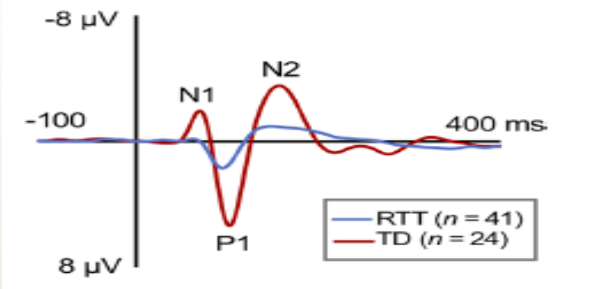


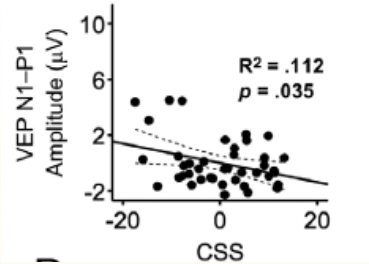
Multisite Study of Evoked Potentials in Rett Syndrome

Joni N. Saby, PhD,[1] Timothy A. Benke, MD, PhD,[2] Sarika U. Peters, PhD,[3]
Shannon M. Standridge, MD,[4] Junko Matsuzaki, PhD,[1] Clare Cutri-French, BA,[5]
Lindsay C. Swanson, MS CGC,[6] David N. Lieberman, MD PhD,[6] Alexandra P. Key, PhD,[7]
Alan K. Percy, MD,[8] Jeffrey L. Neul, MD PhD,[3] Charles A. Nelson, PhD,[9,10]
Timothy P.L. Roberts, PhD,[1] and Eric D. Marsh, MD, PhD[5]

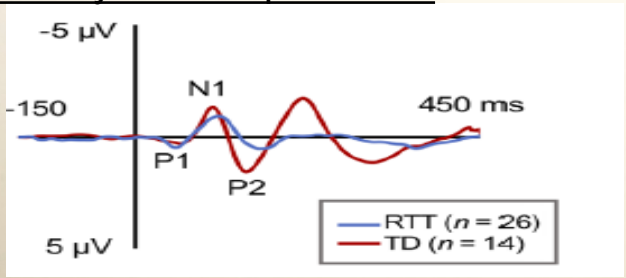
ANN NEUROL 2021;00:1–13

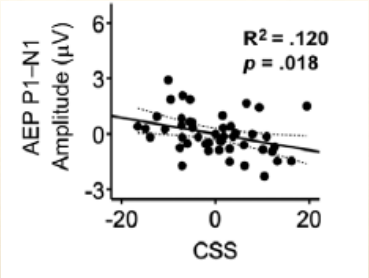
Visual evoked potentials





Auditory evoked potentials





Different from typically developing people.

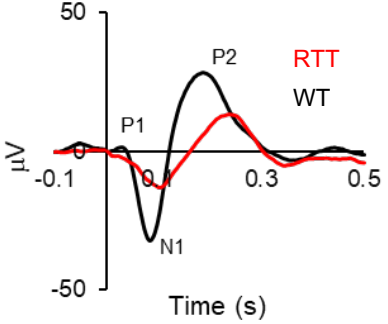
Correlated with severity in Rett syndrome.

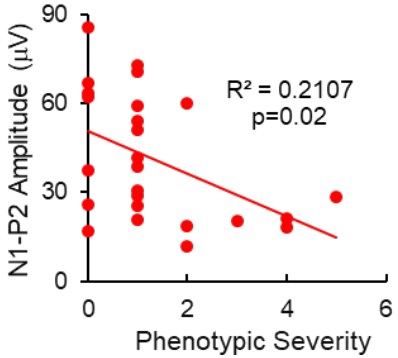
Detection of neurophysiological features in female R255X MeCP2 mutation mice

Hong-Wei Dong[a,b], Kirsty Erickson[a,b], Jessica R. Lee[a,b], Jonathan Merritt[a,b], Cary Fu[a,b], Jeffrey L. Neul[a,b,*]

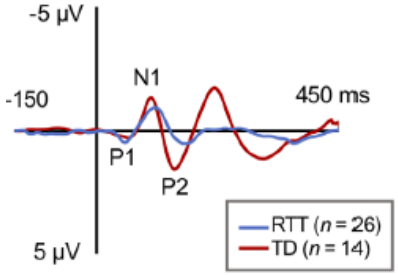
Neurobiology of Disease 145 (2020) 105083

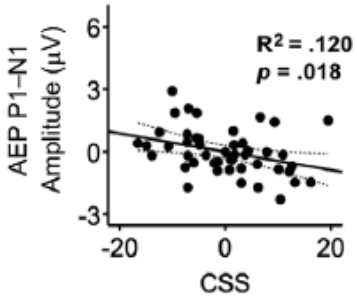
Mouse





Human





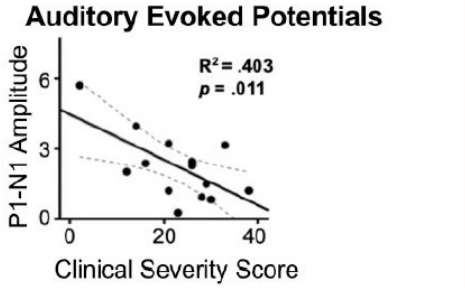
Similar in both people with Rett and mouse models

Opportunity to “translate” information

Electrophysiological biomarkers of brain function in CDKL5 deficiency disorder

BRAIN COMMUNICATIONS 2022

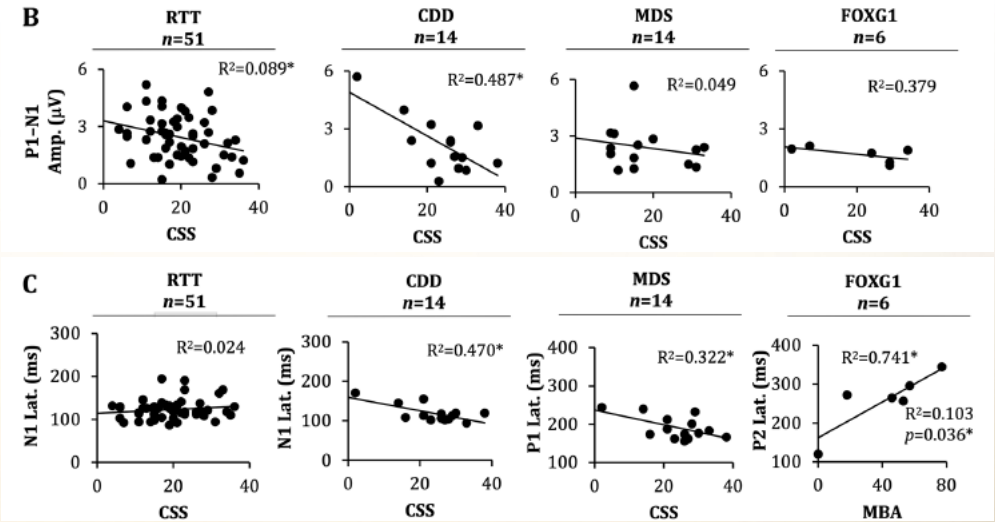
Associations with Clinical Severity



AEP Amplitude correlates with severity in CDKL5 disorder

Comparison of evoked potentials across four related developmental encephalopathies

Journal of Neurodevelopmental Disorders (2023) 15:10



AEP Latency correlates with severity in CDKL5, MECP2 Duplication Syndrome, and FOXG1 Disorder

Goal of the Rett Syndrome Natural History Study

Develop ”Clinical Trial Readiness” for Rett syndrome

- Create a network of clinical sites able to do clinical research
- Characterize the clinical features and natural history
- Develop biomarkers and clinical outcome measures
  - An outcome measure should measure what matters to people and their families

Improving Treatment Trial Outcomes for Rett Syndrome: The Development of Rett-specific Anchors for the Clinical Global Impression Scale

Jeffrey L. Neul, MD, PhD[1], Daniel G. Glaze, MD[2], Alan K. Percy, MD[3], Tim Feyma, MD[4], Arthur Beisang, MD[4], Thuy Dinh, MS, PA-C[1], Bernhard Suter, MD[1], Evdokia Anagnostou, MD[5], Mike Snape, PhD[6], Joseph Horrigan, MD[7], and Nancy E. Jones, PhD[7]

Journal of Child Neurology 2015 1-6

Caregiver top concerns

2. Below is a list of common features of Rett syndrome and related disorders. Using the list below, select the top 3 features that have had the greatest impact on your child's quality of life **in the past 6 months.**

1 (Biggest Problem):	If other not on the list, specify:
2 (Second Biggest Problem):	If other not on the list, specify:
3 (Third Biggest Problem):	If other not on the list, specify:

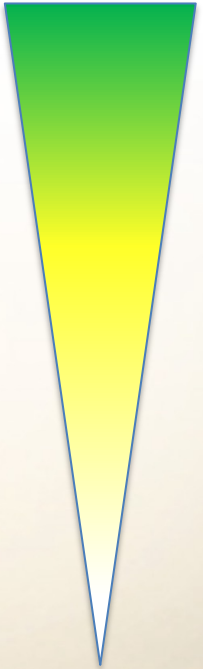
Common Features List

Lack of effective communication	Abnormal Walking/Balance Issues
Air swallowing/Bloating/Excessive Gas	Rapid breathing or breath holding while awake
Teeth Grinding (while awake	Problems with sleep
Lack of hand use	Repetitive hand movements (wringing, mouthing)
Scoliosis (curvature of the spine)	Poor weight gain
Lack of effective chewing or swallowing	Frequent infections
Seizures	Aggressiveness towards others
Constipation	Self abusive behaviors
Gastroesophageal reflux	Abnormal Movements (other than hand stereotypies)
Screaming episodes	Anxiety
Vision	Other (please specify above)

Top caregiver concerns: Classic RTT

Concern	Classic RTT
Lack of effective communication	25%
Seizures	11%
Lack of hand use	8%
Abnormal Walking/Balance Issues	8%
Constipation	8%
Repetitive hand movements	5%
Problems with sleep	4%
Rapid breathing or breath holding (awake)	4%
Air swallowing/Bloating/Excessive Gas	3%
Lack of effective chewing or swallowing	3%
Scoliosis/Kyphosis	3%
Screaming episodes	3%
Anxiety	2%
Teeth Grinding	2%
Gastroesophageal reflux	2%
Poor weight gain	2%
Abnormal Movements	1%
Other GI	1%
Dystonia/Rigidity/Contractures	1%
Other Behavior	0%

Green - more concern



White - less concern

Caregiver Impression of Function and Well-Being

Impression	Number visits	Percentage
Improved	349	27%
Unchanged	862	52%
Worse	271	21%

What are the top reasons for improvement or worsening?

What are the top caregiver concerns for different responses?

B. Overall Function and Well-Being Over the Past 6 Months

1. How would you describe your child's overall function?

- ○ Much improved
- ○ Improved
- ○ Unchanged (skip to question #3)
- ○ Worse
- ○ Much worse

If you answered any of the other options, please select ONE feature from the list below that MOST influenced your answer in the question above about your child's overall function in the past 6 months:

Example situation: "My child's function is much improved over the past 6 months because she began using her eyes to effectively communicate her needs and wants"

Example of answers: Question 1 – Answer: Much Improved
Question 2 – Answer: Effective Communication

○ Effective communication	○ Overall weight
○ Hand stereotypies	○ Hyperventilation and breath-holding
○ Air swallowing	○ Teeth grinding
○ Gastro-esophageal reflux	○ Hand use
○ Aggressiveness towards others	○ Seizures
○ Screaming episodes	○ Walking
○ Self-abusive behavior	○ Anxiety
○ Effective chewing and swallowing	○ Scoliosis
○ Constipation	○ Sleep
○ Vision	○ Frequent Infections
○ Involuntary movements (*other than hand stereotypies*)	○ Other:

Caregiver reasons for impression

Communication is main reason for improvement

Seizures is main reason for worsening

Improved Reason	
Effective communication	37%
Walking/Gross motor	12%

Worse Reason	
Seizures	20%
Walking/Gross motor	15%

Caregiver concerns vary by impression

Concern	Improved	Worse
Communication	30%	18%
Hand use	9%	2%
Seizures	8%	21%
Walking/Balance	7%	9%

Communication is top concern when caregivers note improvement

Seizures are top concern when caregivers note worsening

We need to make sure that outcome measures used in intervention trials matter to affected individuals

- Ask what is important and what things people would like to see change
- Important to know how much change matters to people who are affected
- Assess existing or develop outcome measures to align with top concerns

Top Caregiver Concerns in Rett syndrome and related disorders: data from the US Natural History Study

Jeffrey Lorenz Neul, Timothy A. Benke, Eric D. Marsh, Bernhard Suter, and 5 more

PREPRINT available at Research Square [https://doi.org/10.21203/rs.3.rs-2566253/v1]

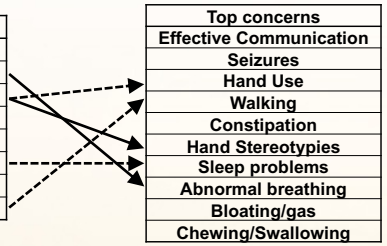
Current Outcome Measures in Rett Trials

Caregiver reported:
RSBQ

The Rett Syndrome Behaviour Questionnaire (RSBQ): refining the behavioural phenotype of Rett syndrome

Journal of Child Psychology and Psychiatry 43:8 (2002), pp 1099–1110

RSBQ Subscores	# items
General Mood	8
Breathing Problems	5
Hand Behaviors	6
Repetitive Face Movements	4
Body Rocking/Expressionless Face	6
Nightime behaviors	3
Fear/Anxiety	4
Walking/Standing	2



Top concerns
Effective Communication
Seizures
Hand Use
Walking
Constipation
Hand Stereotypies
Sleep problems
Abnormal breathing
Bloating/gas
Chewing/Swallowing

- Pros
  - Relatively simple to complete
  - Understood by regulators (FDA, EMA)
- Cons
  - Does not cover all top concerns

Clinician assessed:
Clinical Global Impression-Improvement

Improving Treatment Trial Outcomes for Rett Syndrome: The Development of Rett-specific Anchors for the Clinical Global Impression Scale

Journal of Child Neurology

Current Outcome Measures in Rett Trials

Caregiver reported:
RSBQ

The Rett Syndrome Behaviour Questionnaire (RSBQ): refining the behavioural phenotype of Rett syndrome

Journal of Child Psychology and Psychiatry 43:8 (2002), pp 1099–1110

Clinician assessed:
Clinical Global Impression-Improvement

Improving Treatment Trial Outcomes for Rett Syndrome: The Development of Rett-specific Anchors for the Clinical Global Impression Scale

Journal of Child Neurology

1	2	3	4	5	6	7
Very Much Improved	Much Improved	Mildly Improved	No Change	Mildly Worse	Much Worse	Very Much Worse

Can we use the Natural History Study data to develop additional outcome measures?

- Clinician's view of participant's global change
- Understood and accepted by regulators
- Need disease-specific anchors to guide raters
- Con: does not provide specifics of what improved

A Psychometric Evaluation of the Motor-Behavioral Assessment Scale for Use as an Outcome Measure in Rett Syndrome Clinical Trials

AMERICAN JOURNAL ON INTELLECTUAL AND DEVELOPMENTAL DISABILITIES
2020, Vol. 125, No. 6, 493–509

Motor Behavior Assessment (MBA)

- Clinician rated
  - 37 items
    - 5-point scale
    - higher score=worse
  - Collected throughout Natural History Study
  - Never evaluated as an outcome measure

→ **Psychometric evaluation**

Revised-Motor Behavior Assessment (R-MBA)

24 item clinician rated evaluation

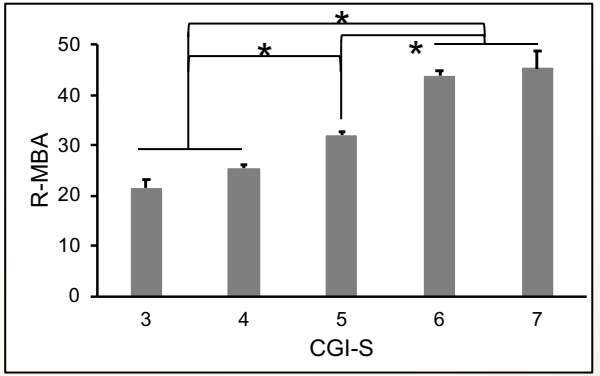
5 factors plus 3 important clinical features

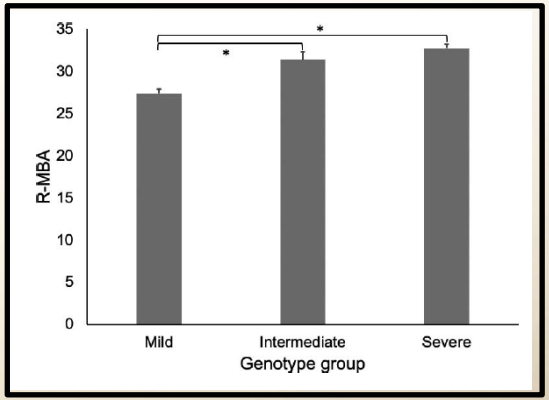
R-MBA Subscores
F1: Motor Dysfunction (stiff/scoliosis)
F2: Functional Skills (hand/speech/walking/feeding)
F3: Social Skills
F4: Behavior (aggression)
F5: Breathing problems
Seizures
Hand stereotypies
Body rocking

Using Natural History Study data to develop outcome measures

Revised-Motor Behavior Assessment (R-MBA)

- Higher score = more severe
- Correlated with clinical severity
- Correlated with caregiver assessment
- Correlation with MECP2 mutations





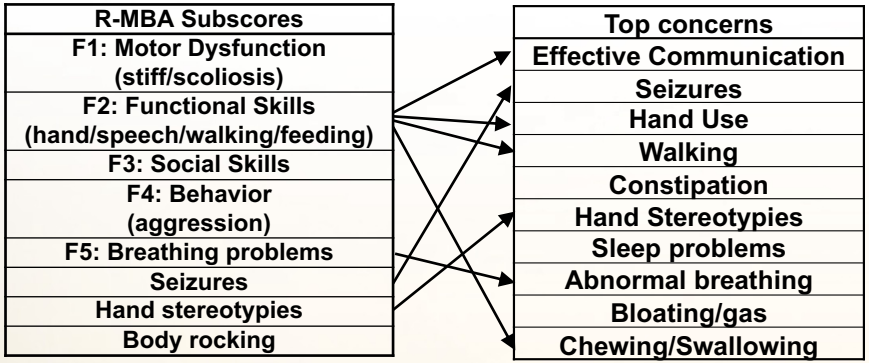
A Psychometric Evaluation of the Motor-Behavioral Assessment Scale for Use as an Outcome Measure in Rett Syndrome Clinical Trials

Melissa Raspa, Carla M. Bann, Angela Gwaltney, Timothy A. Benke, Cary Fu, Daniel G. Glaze, Richard Haas, Peter Heydemann, Mary Jones, Walter E. Kaufmann, David Lieberman, Eric Marsh, Sarika Peters, Robin Ryther, Shannon Standridge, Steven A. Skinner, Alan K. Percy, and Jeffrey L. Neul

AMERICAN JOURNAL ON INTELLECTUAL AND DEVELOPMENTAL DISABILITIES
2020, Vol. 125, No. 6, 493-509

Using Natural History Study data to develop outcome measures

How well does the R-MBA capture important clinical issues?



Can we use the Natural History Study data to develop a better clinician rating scale?

Can we use the Natural History Study data to develop a caregiver rating scale?

Issues still not completely
Full range of functional skills assessed limited

Using Natural History Study data to develop outcome measures

New Clinician-rated scale

Supported by Alcyone Therapeutics

Using Natural History Study data

31 items, 6 factors

"Catchy-name" Clinician Scale	# items
Motor dysfunction	8
Mobility	6
Hand skills and communication	7
Social Skills	3
Breathing	4
Behavior problems	3

New Caregiver-rated scale

Rett syndrome Caregiver Assessment of Severity And Symptoms

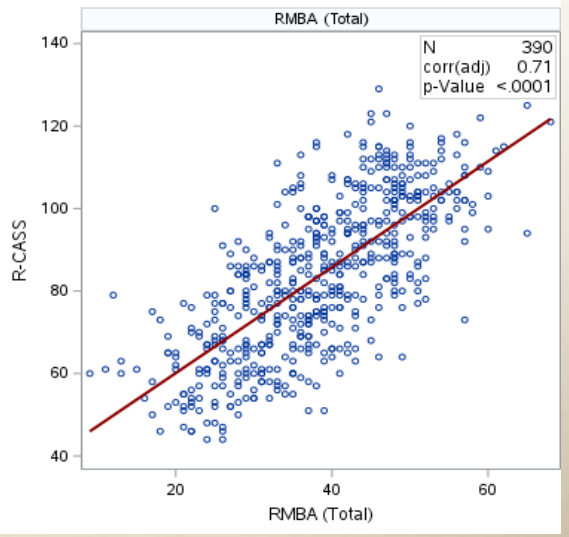
Supported by Rett Syndrome Research Trust

Using Natural History Study data

32 items, 4 factors

Correlates with clinician rated severity, age, genotype

R-CASS	# Items
Functional movement	10
Communication	8
Behavior problems	9
Rett-specific behaviors	5



Journal of Autism and Developmental Disorders

Psychometric Assessment of the Rett Syndrome Caregiver Assessment of Symptom Severity (RCASS)

--Manuscript Draft--

https://www.researchsquare.com/article/rs-2873717/v1

Using Natural History Study data to develop outcome measures

New Clinician-rated scale

Supported by Alcyone Therapeutics

Using Natural History Study data

31 items, 6 factors

"Catchy-name" Clinician Scale	# items
Motor dysfunction	8
Mobility	6
Hand skills and communication	7
Social Skills	3
Breathing	4
Behavior problems	3

Top concerns
Effective Communication
Seizures
Hand Use
Walking
Constipation
Hand Stereotypies
Sleep problems
Abnormal breathing
Bloating/gas
Chewing/Swallowing

Gastrointestinal Health Questionnaire for Rett Syndrome: Tool Development

JPGN • Volume 72, Number 3, March 2021

Evaluating Sleep Disturbances in Children With Rare Genetic Neurodevelopmental Syndromes

Pediatric Neurology 123 (2021) 30–37

New Caregiver-rated scale

Rett syndrome Caregiver Assessment of Severity And Symptoms

Supported by Rett Syndrome Research Trust

Using Natural History Study data

32 items, 4 factors

Correlates with clinician rated severity, age, genotype

R-CASS	# Items
Functional movement	10
Communication	8
Behavior problems	9
Rett-specific behaviors	5

Validating the Rett Syndrome Gross Motor Scale

PLOS ONE | DOI:10.1371/journal.pone.0147555 January 22, 2016

> Disabil Rehabil. 2020 Sep 15;1-8. doi: 10.1080/09638288.2020.1820084. Online ahead of print.

Characteristic behaviors associated with gait of individuals with Rett syndrome

ORCA

Observer-Reported Communication Ability Measure

Ongoing clinical trials in Rett syndrome

Anavex 2-73

ANAVEX®2-73 (blarcamesine), a Sigma-1 receptor agonist, ameliorates neurologic impairments in a mouse model of Rett syndrome

Pharmacology, Biochemistry and Behavior 187 (2019) 172796

ANAVEX®2-73 (Blarcamesine) AVATAR Phase 3 Trial met Primary and Secondary Efficacy Endpoints

Ketamine

- Multisite trial at NHS sites
- 5 days oral ketamine
- 4 escalating doses planned
  - Only 2 doses explored due to recruitment issues
- Safe and well tolerated
- Analyzing efficacy and biomarkers
  - EEG/breathing

2/2/2023

PharmaTher Holdings Announces FDA Grant of Orphan Drug Designation to KETARX™ (Ketamine) for the Treatment of Rett Syndrome

Reversal of Neurological Defects in a Mouse Model of Rett Syndrome

Jacky Guy,[1] Jian Gan,[2] Jim Selfridge,[1] Stuart Cobb,[2] Adrian Bird[1]*

SCIENCE VOL 315 23 FEBRUARY 2007

Gene therapy in RTT?

Systemic Delivery of MeCP2 Rescues Behavioral and Cellular Deficits in Female Mouse Models of Rett Syndrome

13612 • The Journal of Neuroscience, August 21, 2013 • 33(34):13612–13620

doi:10.1038/nature24058

Radically truncated MeCP2 rescues Rett syndrome–like neurological defects

Rebekah Tillotson[1], Jim Selfridge[1], Martha V. Koerner[1], Kamal K. E. Gadalla[2,3], Jacky Guy[1], Dina De Sousa[1], Ralph D. Hector[2], Stuart R. Cobb[2] & Adrian Bird[1]

Engineered microRNA-based regulatory element permits safe high-dose mini*MECP2* gene therapy in Rett mice

Sarah E. Sinnett,[1,2] Emily Boyle,[1] Christopher Lyons[1] and Steven J. Gray[1,2]

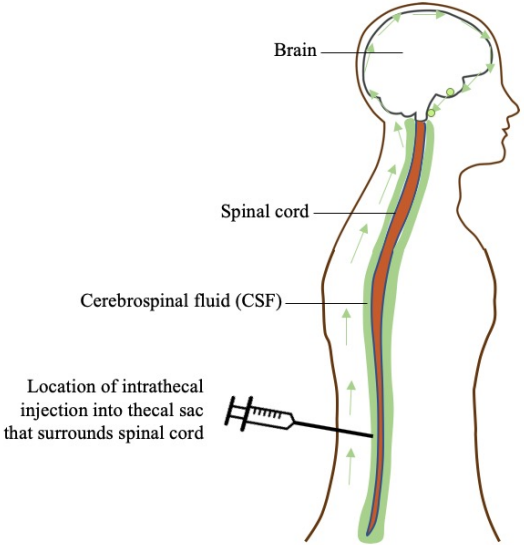
Gene therapy in RTT

TAYSHA GENE THERAPIES

TAYSHA GENE THERAPIES ANNOUNCES INITIATION OF CLINICAL DEVELOPMENT OF TSHA-102 IN RETT SYNDROME

Taysha Gene Therapies Announces First Patient Dosed with TSHA-102 in the REVEAL Phase 1/2 Trial Under Investigation for the Treatment of Rett Syndrome

Adults with Rett syndrome, intrathecal dosing
2 patients dosed so far
Taysha plans to submit Investigational New Drug application to FDA this year

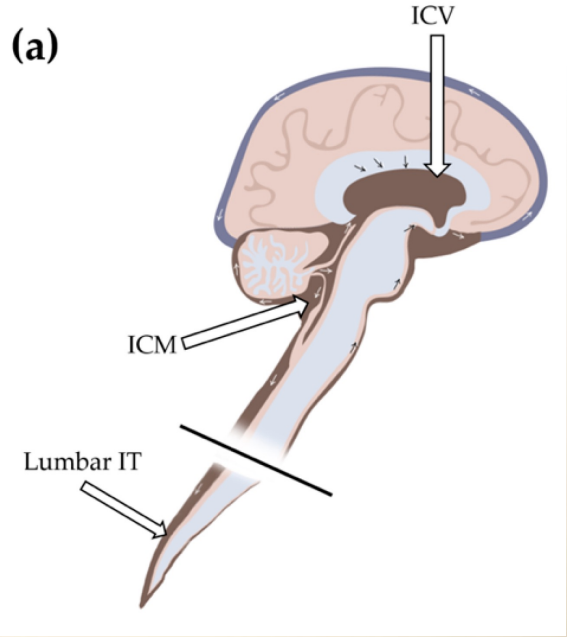


Gene therapy in RTT

NEUROGENE

Neurogene Announces FDA Clearance of IND for NGN-401 Gene Therapy for Children with Rett Syndrome

- US
- Age 4-10 years old
- 5 participants
- Intracerebral ventricular injection (ICV)
- Texas Children's Hospital (started)
- Children's Hospital Colorado
- Boston Children's Hospital



Alternative ways to restore MeCP2 activity

X-chromosome reactivation

A mixed modality approach towards Xi reactivation for Rett syndrome and other X-linked disorders

PNAS | Published online December 27, 2017 | E669

Tsix–Mecp2 female mouse model for Rett syndrome reveals that low-level MECP2 expression extends life and improves neuromotor function

PNAS | August 7, 2018 | vol. 115 | no. 32 | 8185–8190

Read-through therapy

GENERAL ARTICLE

Pharmacological read-through of R294X Mecp2 in a novel mouse model of Rett syndrome

Jonathan K. Merritt[1,2,3], Bridget E. Collins[4,5], Kirsty R. Erickson[2,3], Hongwei Dong[2,3] and Jeffrey L. Neul[1,2,3,*]

Human Molecular Genetics, 2020, Vol. 29, No. 15 2461–2470

DNA editing

High rate of HDR in gene editing of p.(Thr158Met) *MECP2* mutational hotspot

European Journal of Human Genetics (2020) 28:1231–1242

RNA editing

Site-directed RNA repair of endogenous Mecp2 RNA in neurons

PNAS | Published online October 16, 2017 | E9395–E9402

In Vivo Repair of a Protein Underlying a Neurological Disorder by Programmable RNA Editing

Cell Reports *32*, 107878, July 14, 2020

Department of Defense Clinical Trial Award

Umbrella Clinical Trial to Evaluate Repurposed Compounds in Rett Syndrome

- Recently awarded September 30th, 2023 (Neul PI)
- Studying FDA approved drugs that have preclinical evidence in Rett syndrome
  - Ketamine
  - Donepezil
  - Vorinostat
- Compared to common placebo
- 4 US sites
- Goal is to have a platform to accelerate clinical trials

Chronic Administration of the *N*-Methyl-D-Aspartate Receptor Antagonist Ketamine Improves Rett Syndrome Phenotype

Annarita Patrizi, Nathalie Picard, Alex Joseph Simon, Georgia Gunner, Eleonora Centofante, Nick Arthur Andrews, and Michela Fagiolini

Biological Psychiatry May 1, 2016; 79:755–764

Identification of a therapeutic candidate for Rett syndrome with a differentiated mechanism of action using a patient-derived cortical organoid screening platform

Cassiano Carromeu, PhD
Vyant Bio

Neuroscience 2022

Target-agnostic discovery of Rett Syndrome therapeutics by coupling computational network analysis and CRISPR-enabled *in vivo* disease modeling

bioRxiv preprint doi: https://doi.org/10.1101/2022.03.20.485056

The future…

- Success of Phase 3 Lavender trial of Trofinetide is promising
  - Proof that therapies can be developed for Rett and similar disorders
- New Rett syndrome targeted therapy
  - Exciting opportunities for additional treatments
- Natural History Study established foundation for clinical trials
  - Critical to understand what problems need to be addressed
- Analysis of Natural History data to enhance therapy development
  - Development of meaningful and robust outcome measures
- Continue collection of data to build upon these efforts

Rare Diseases Clinical Research Network

Office of Rare Diseases Research
National Center for Advancing Translational Sciences – NIH

NIH *Eunice Kennedy Shriver* National Institute of Child Health and Human Development

National Institute of Neurological Disorders and Stroke

IRSF International Rett Syndrome Foundation

Rett Consortium

Rett Syndrome,
MECP2 Duplication,
& Rett-Related Disorders

University of Alabama-Birmingham. Alan Percy
Vanderbilt University. Sarika Peters, Cary Fu, Jeffrey Neul
University of Colorado, Denver. Timothy Benke
Children's Hospital of Philadelphia. Eric Marsh
University of California, San Diego. Richard Haas
Baylor College of Medicine. Dan Glaze
Greenwood Genetics Clinic. Steve Skinner
Children's Hospital Boston. Mustafa Sahin, David Lieberman
Oakland Children's Hospital. Nancy Jones
Gillette Children's Hospital. Tim Feyma
Cincinnati Children's Hospital. Shannon Standridge
Cleveland Clinic. Sumit
Washington University. Robin Ryther, Judy Weisenberg
Rush University. Peter Heydeman

Thanks to my friends!