Bringing New Cures to Life

Rett Syndrome Investor Day

September 22, 2021 | 9:00 – 11:30 AM CT

Legal disclosure

FORWARD LOOKING STATEMENTS

This presentation contains forward-looking statements that involve substantial risks and uncertainties. All statements, other than statements of historical facts, contained in this presentation, including statements regarding our strategy, future operations, future financial position, future revenues, projected costs, prospects, plans and objectives of management, are forward-looking statements. The words “anticipate,” “believe,” “estimate,” “expect,” “intend,” “may,” “might,” “plan,” “predict,” “project,” “target,” “potential,” “will,” “would,” “could,” “should,” “continue,” and similar expressions are intended to identify forward-looking statements, although not all forward-looking statements contain these identifying words. These forward-looking statements are subject to a number of risks, uncertainties and assumptions. Risks regarding our business are described in detail in our Securities and Exchange Commission filings, including in our Annual Report on Form 10-K for the year ended December 31, 2020. We may not actually achieve the plans, intentions or expectations disclosed in our forward-looking statements, and you should not place undue reliance on our forward-looking statements. Actual results or events could differ materially from the plans, intentions and expectations disclosed in the forward-looking statements we make. The forward-looking statements contained in this presentation reflect our current views with respect to future events, and we assume no obligation to update any forward-looking statements except as required by applicable law.

This presentation includes statistical and other industry and market data that we obtained from industry publications and research, surveys and studies conducted by third parties as well as our own estimates of potential market opportunities. All of the market data used in this prospectus involves a number of assumptions and limitations, and you are cautioned not to give undue weight to such data. Industry publications and third-party research, surveys and studies generally indicate that their information has been obtained from sources believed to be reliable, although they do not guarantee the accuracy or completeness of such information. Our estimates of the potential market opportunities for our product candidates include several key assumptions based on our industry knowledge, industry publications, third-party research and other surveys, which may be based on a small sample size and may fail to accurately reflect market opportunities. While we believe that our internal assumptions are reasonable, no independent source has verified such assumptions.

Introduction

RA Session II

President, Founder & CEO

Unparalleled gene therapy pipeline focused exclusively on monogenic CNS disorders

PROGRAM		INDICATION	DISCOVERY	PRECLINICAL	PHASE 1/2	Pivotal	GLOBAL COMM. RIGHTS
NEURODEGENERATIVE DISEASES							
TSHA-120	*GRT*	Giant Axonal Neuropathy				Regulatory guidance YE 2021	TAYSHA GENE THERAPIES
TSHA-101	*GRT*	GM2 Gangliosidosis				Currently open CTA	
TSHA-118	*GRT*	CLN1 Disease				Currently open IND	
TSHA-119	*GRT*	GM2 AB Variant					
TSHA-104	*GRT*	SURF1-Associated Leigh Syndrome				IND/CTA submission 2H 2021	
TSHA-112	*miRNA*	APBD					
TSHA-111-LAFORIN	*miRNA*	Lafora Disease					
TSHA-111-MALIN	*miRNA*	Lafora Disease					
TSHA-113	*miRNA*	Tauopathies					
TSHA-115	*miRNA*	GSDs					
Undisclosed	GRT/*shRNA*	Undisclosed					
Undisclosed	GRT	Undisclosed					
NEURODEVELOPMENTAL DISORDERS							
TSHA-102	*Regulated GRT*	Rett Syndrome				IND/CTA submission 2H 2021	TAYSHA GENE THERAPIES
TSHA-106	*shRNA*	Angelman Syndrome					
TSHA-114	*GRT*	Fragile X Syndrome					
TSHA-116	*shRNA*	Prader-Willi Syndrome					
TSHA-117	*Regulated GRT*	FOXG1 Syndrome					
TSHA-107	*GRT*	Autism Spectrum Disorder					
TSHA-108	*GRT*	Inborn Error of Metabolism					
TSHA-109	*GRT*	Inherited Metabolism Disorder					
Undisclosed	GRT	Undisclosed					
Undisclosed	mini-gene	Undisclosed					
GENETIC EPILEPSY							
TSHA-103	*GRT*	SLC6A1 Haploinsufficiency Disorder					TAYSHA GENE THERAPIES
TSHA-105	*GRT*	SLC13A5 Deficiency					
TSHA-110	mini-gene	KCNQ2					
Undisclosed	mini-gene	Undisclosed					

GRT: Gene replacement therapy **miRNA**: microRNA **shRNA**: short hairpin RNA

Investor Mini-Series

TSHA-102 Rett Syndrome Investor Day	**September 22, 2021**
TSHA-106 Angelman Syndrome Investor Day	**October 26, 2021**

Agenda

Topic	Time	Presenter
Introduction	9:00 am CT	RA Session II
Clinical Features and Genetics of Rett Syndrome – Knowledge from the Natural History Study	9:15 am CT	Jeffrey Neul, MD, PhD
Disease Burden **Patient and Family Perspective**	10:10 am CT	Monica Coenraads
miRARE Platform and Preclinical Data	10:25 am CT	Steven Gray, PhD
Clinical Development Strategy	10:55 am CT	Suyash Prasad, MBBS, MSc, MRCP, MRCPCH, FFPM
Closing Remarks	11:15 pm CT	RA Session II

Speaker biographies

Jeffrey Neul, MD, PhD
Annette Schaffer Eskind Chair and Director, Vanderbilt Kennedy Center
Professor of Pediatrics, Pharmacology, and Special Education, Vanderbilt University Medical Center

- An internationally recognized expert in genetic neurodevelopmental disorders, who conducts clinical research and clinical trials on Rett syndrome, genetic research to identify other genetic causes of neurodevelopmental disorders, and translational research using disorder models to identify and test novel treatment modalities for these disorders

Monica Coenraads
CEO of Rett Syndrome Research Trust (RSRT)

- Co-founded the Rett Syndrome Research Foundation (RSRF) to stimulate scientific interest and research in Rett syndrome
- Launched the Rett Syndrome Research Trust (RSRT) and serves as a trustee for Reverse Rett
- On the Advisory Council for The Research Acceleration and Innovation Network (TRAIN) of FasterCures

Steven Gray, PhD
Associate Professor Department of Pediatrics at UTSW and Chief Scientific Advisor to Taysha

- Expertise in AAV gene therapy vector engineering, optimizing approaches to deliver a gene to the nervous system
- Research focus includes preclinical studies to apply AAV-based platform gene transfer technologies toward the development of treatments for neurological diseases such as Rett Syndrome, Giant Axonal Neuropathy (GAN), Tay-Sachs, Krabbe, AGU, and Batten Disease, and have expanded into human clinical studies to test a gene therapy approach for GAN and CLN7 Batten disease

Suyash Prasad, MBBS, MSc, MRCP, MRCPCH, FFPM
Chief Medical Officer and Head of Research and Development at Taysha

- Expertise in international drug development, including preclinical, Phase I-IV trials, regulatory filings, commercial application
- Former CMO of Audentes Therapeutics; led XLMTM AAV8 program from preclinical to initial positive clinical data
- Prior roles include Medical Affairs and Clinical Development at BioMarin, Genzyme Therapeutics, and Eli Lilly and Company
- UK board certified with postgraduate qualifications in Pediatrics, Internal Medicine, Pharmaceutical Development, and Translational Science

Clinical Features and Genetics of Rett Syndrome

Jeffrey Neul, MD, PhD

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

VANDERBILT KENNEDY CENTER

Clinical features and genetics of Rett syndrome: Knowledge from the natural history study

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

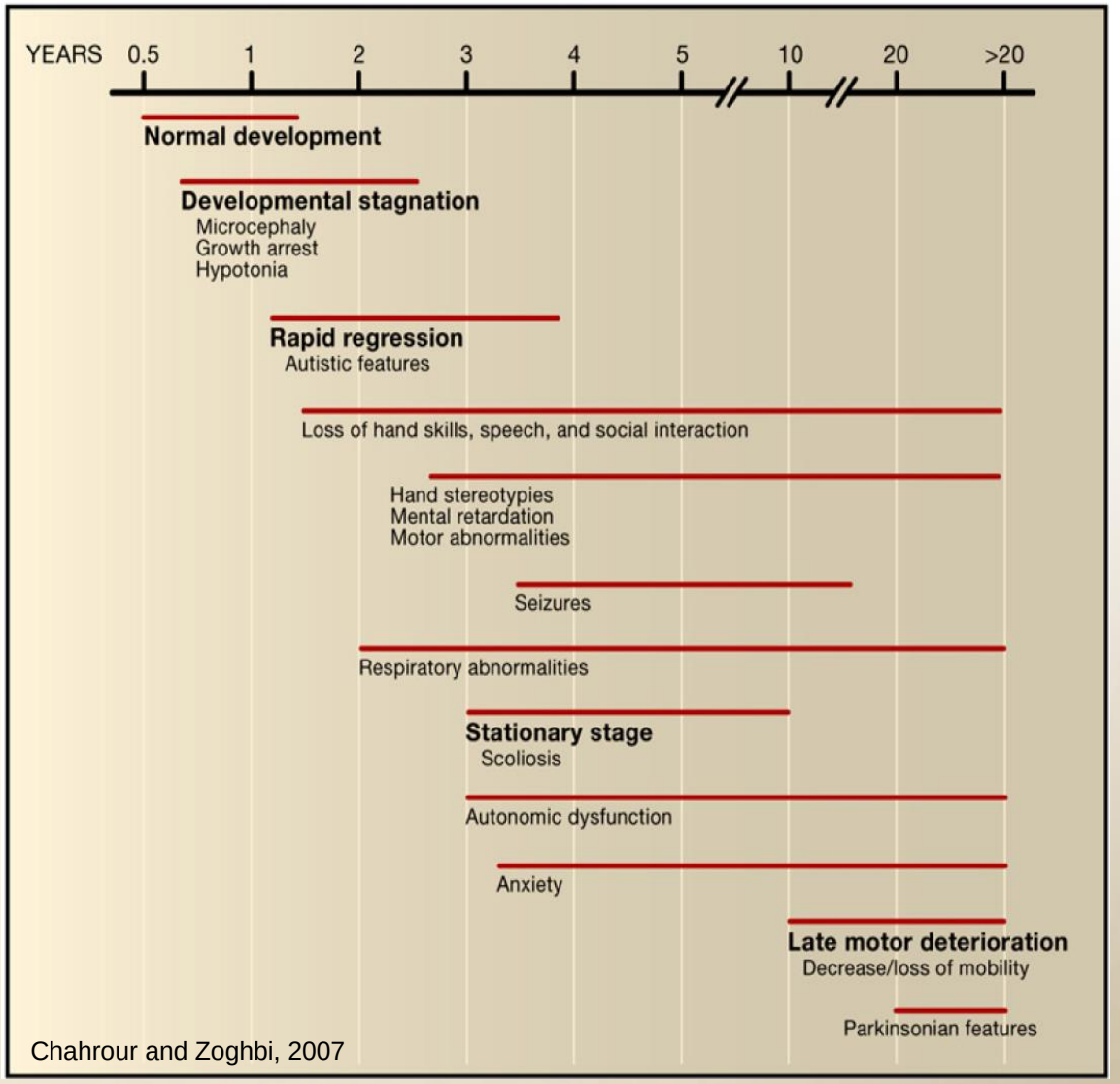
Director, Vanderbilt Kennedy Center
Annette Schaffer Eskind Professor
Department of Pediatrics, Pharmacology,
and Special Education
Vanderbilt University Medical Center
Vanderbilt University

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

- Regression followed by stabilization
  - Loss of purposeful hand skills
  - Loss of spoken language
- Gait abnormalities
- Stereotypic hand movements
- X-linked, primarily affects girls
- ~1:10,000 live female births



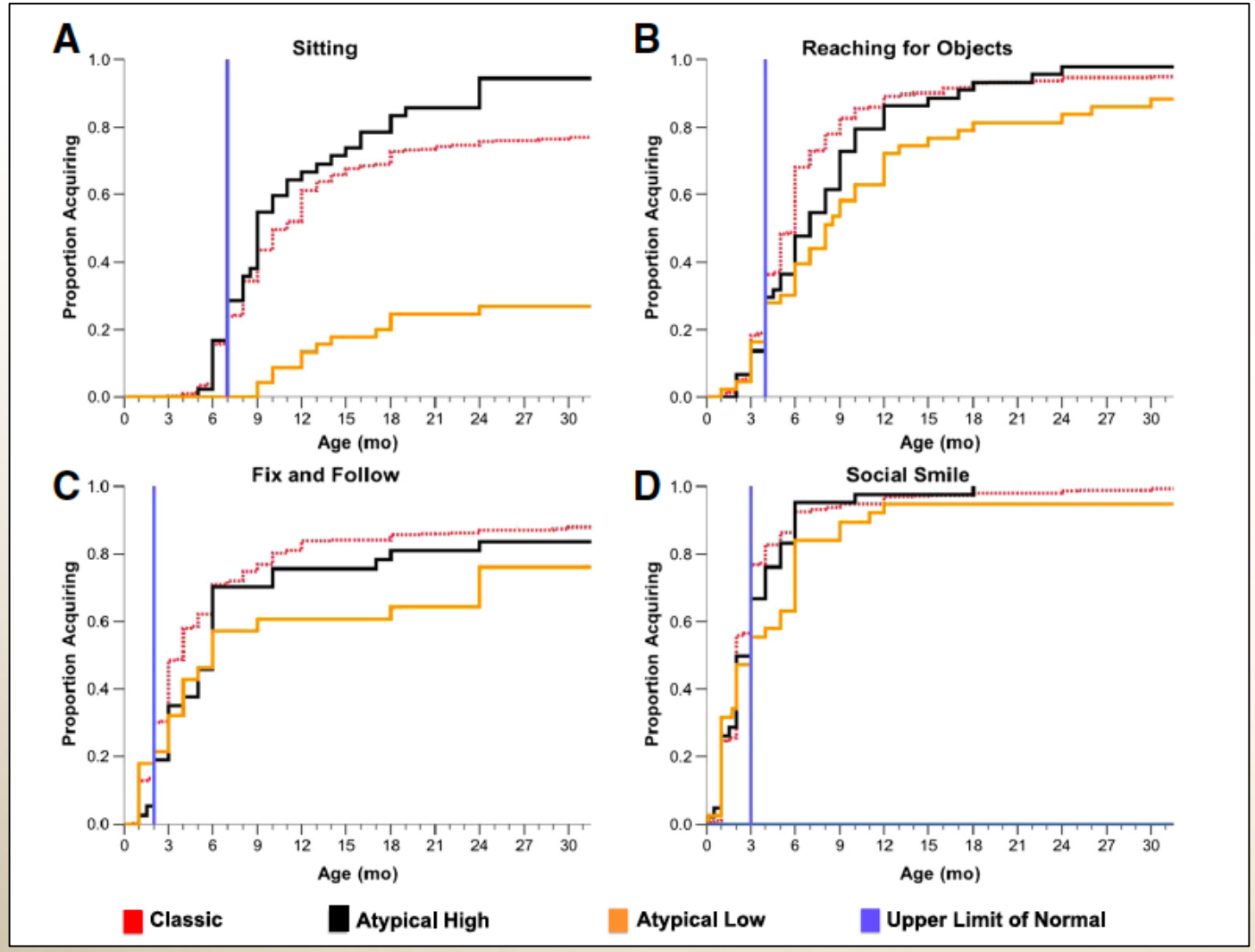
Chahrour and Zoghbi, 2007

Natural history study overview

- Multi-center longitudinal study
  - Alan Percy (University of Alabama, Birmingham) – PI
  - Jeffrey Neul (Vanderbilt) – Administrative Head
- U54 mechanism, funded by NIH, ORD, NICHD
- Enrolled >1800 people (>1250 with Classic Rett)
- Started in 2003 (officially enrolling in 2006)
  - 2003-2014: 5201 – Rett syndrome, Angelman syndrome, Prader-Willi syndrome
    - 4 primary enrolling sites, 4 travel sites
  - 2014-2021: 5211 – Rett syndrome, MeCP2 Duplication, CDKL5 deficiency disorder, FoxG1 syndrome
    - Revised many data collection forms
    - 14 sites across US

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

Acquired developmental milestones[1]



Neul *et al. Journal of Neurodevelopmental Disorders* 2014, **6**:20
http://www.jneurodevdisorders.com/content/6/1/20

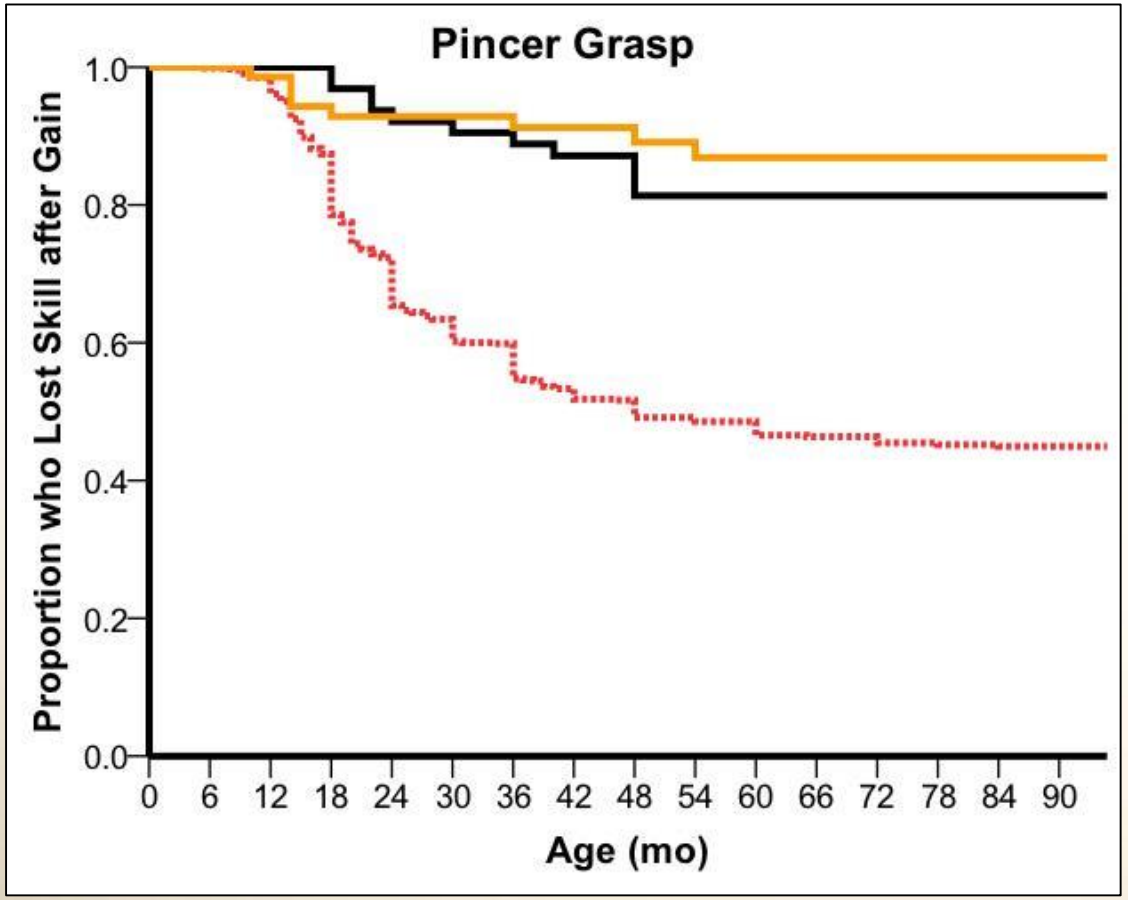
RESEARCH Open Access

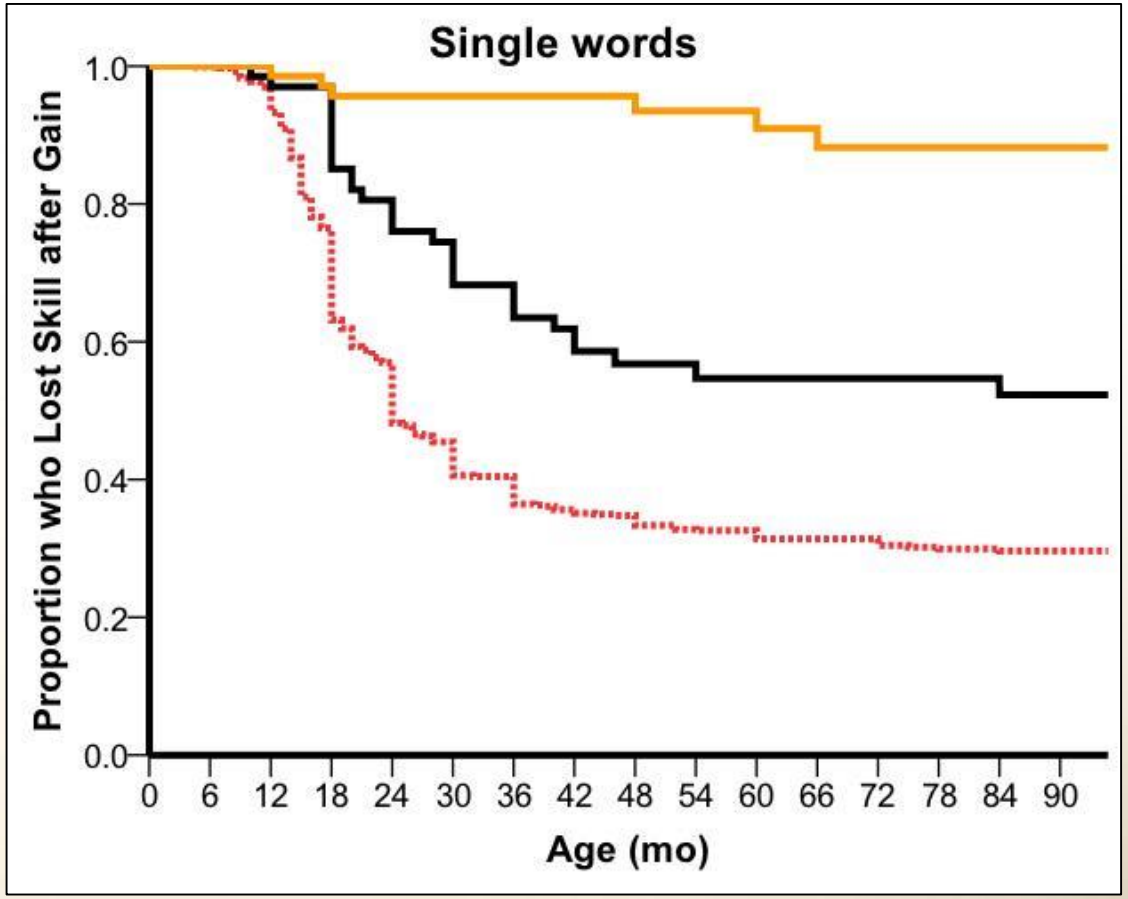
Developmental delay in Rett syndrome: data from the natural history study

Jeffrey L Neul[1,2], Jane B Lane[3], Hye-Seung Lee[4], Suzanne Geerts[3], Judy O Barrish[1], Fran Annese[5], Lauren McNair Baggett[5], Katherine Barnes[6], Steven A Skinner[5], Kathleen J Motil[1], Daniel G Glaze[1], Walter E Kaufmann[6] and Alan K Percy[3*]

1. Neul JL, Lane JB, Lee HS, et al. Developmental delay in Rett syndrome: data from the natural history study. *J Neurodev Disord*. 2014;6(1):20. doi:10.1186/1866-1955-6-20

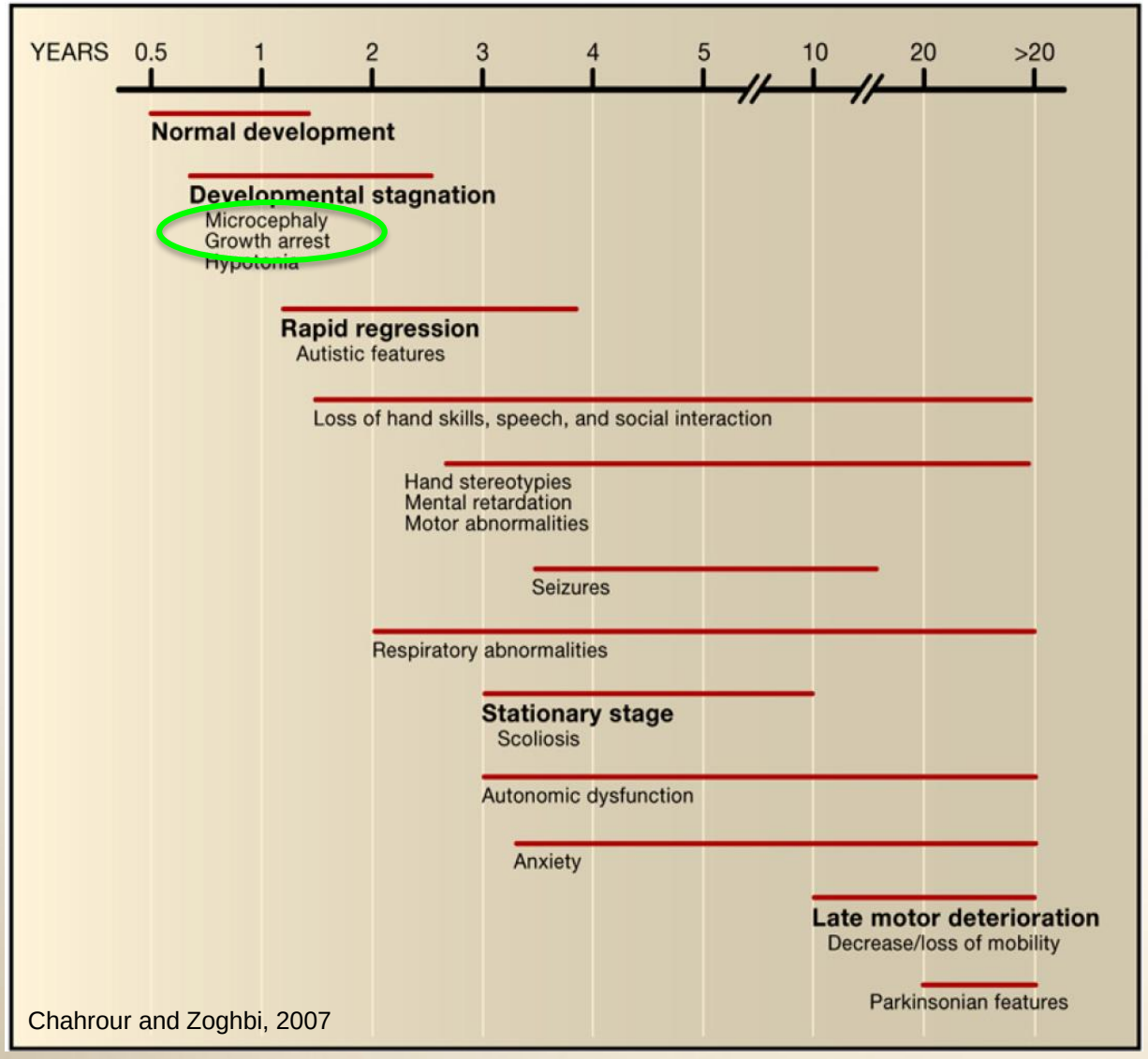
Acquired developmental milestones[1]





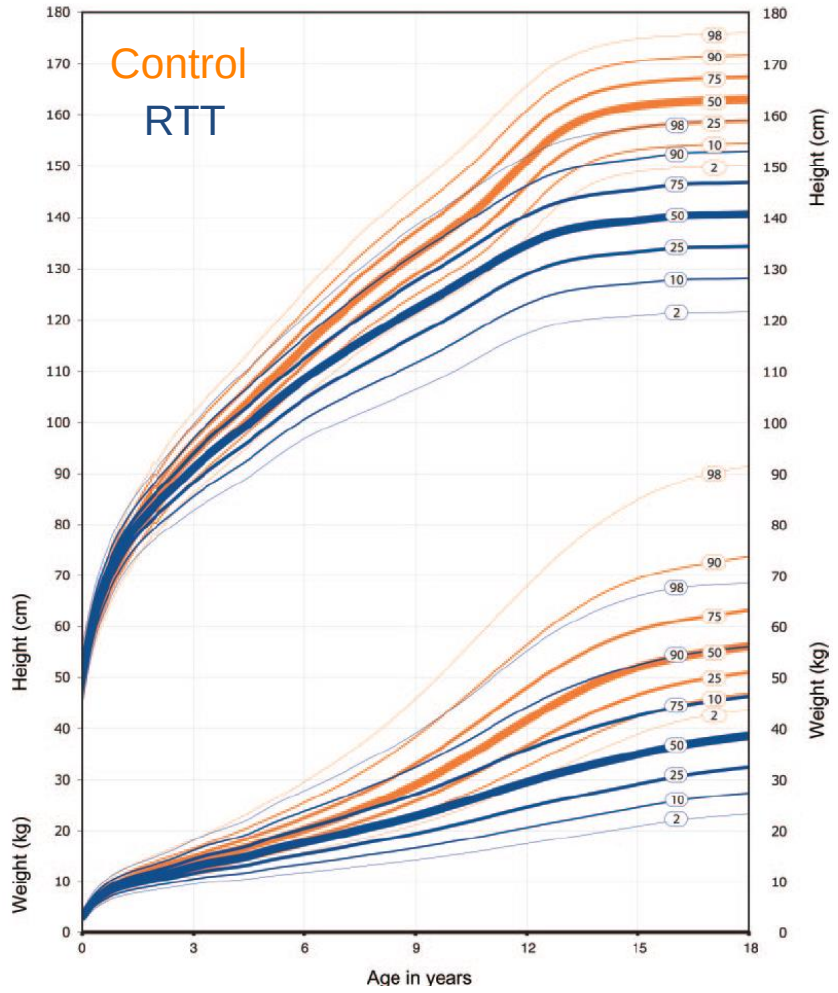
Classic Atypical High Atypical Low

1. Neul JL, Lane JB, Lee HS, et al. Developmental delay in Rett syndrome: data from the natural history study. *J Neurodev Disord*. 2014;6(1):20. doi:10.1186/1866-1955-6-20



Chahrour and Zoghbi, 2007

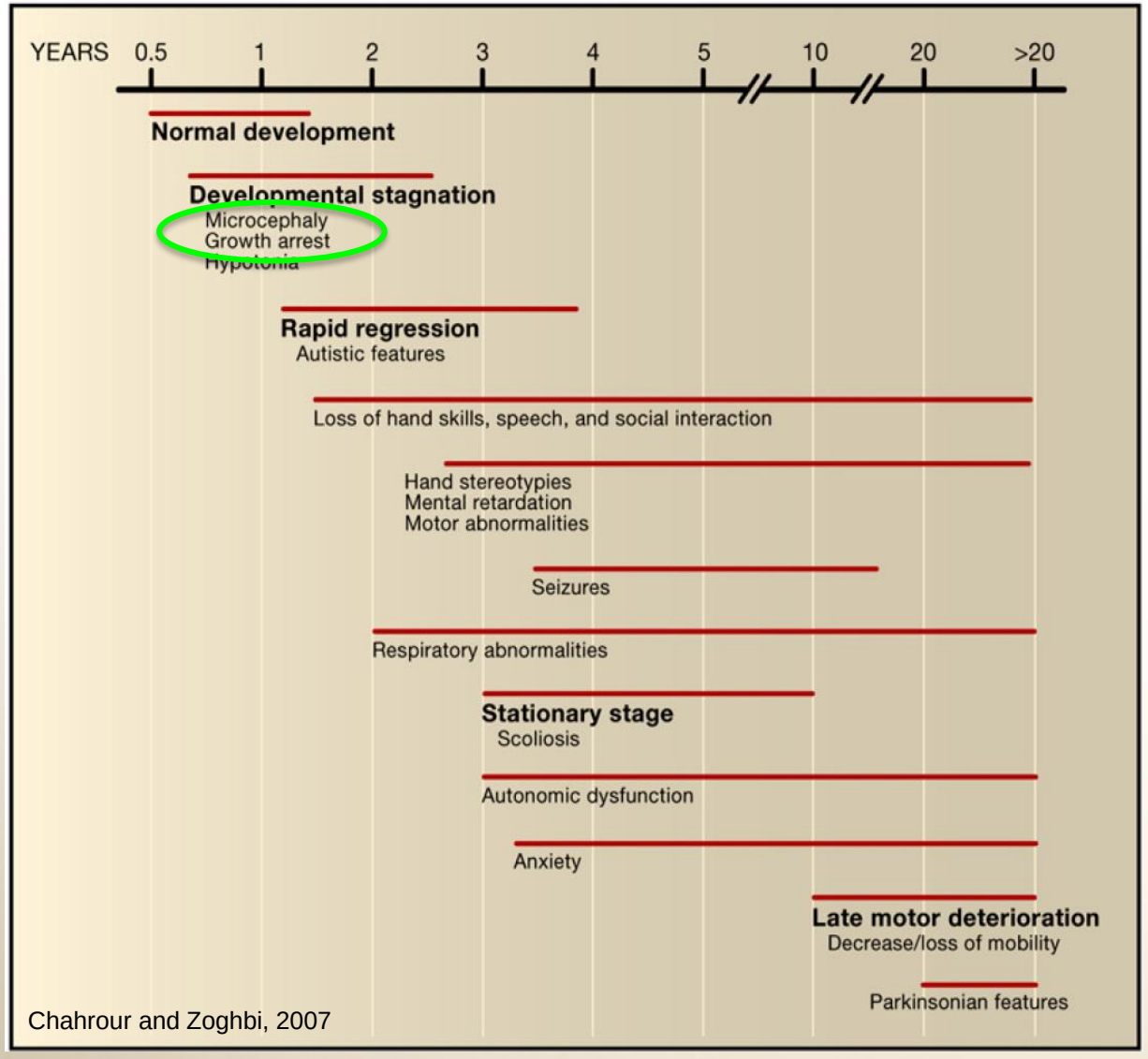
Height and weight[1]



Growth failure and outcome in Rett syndrome

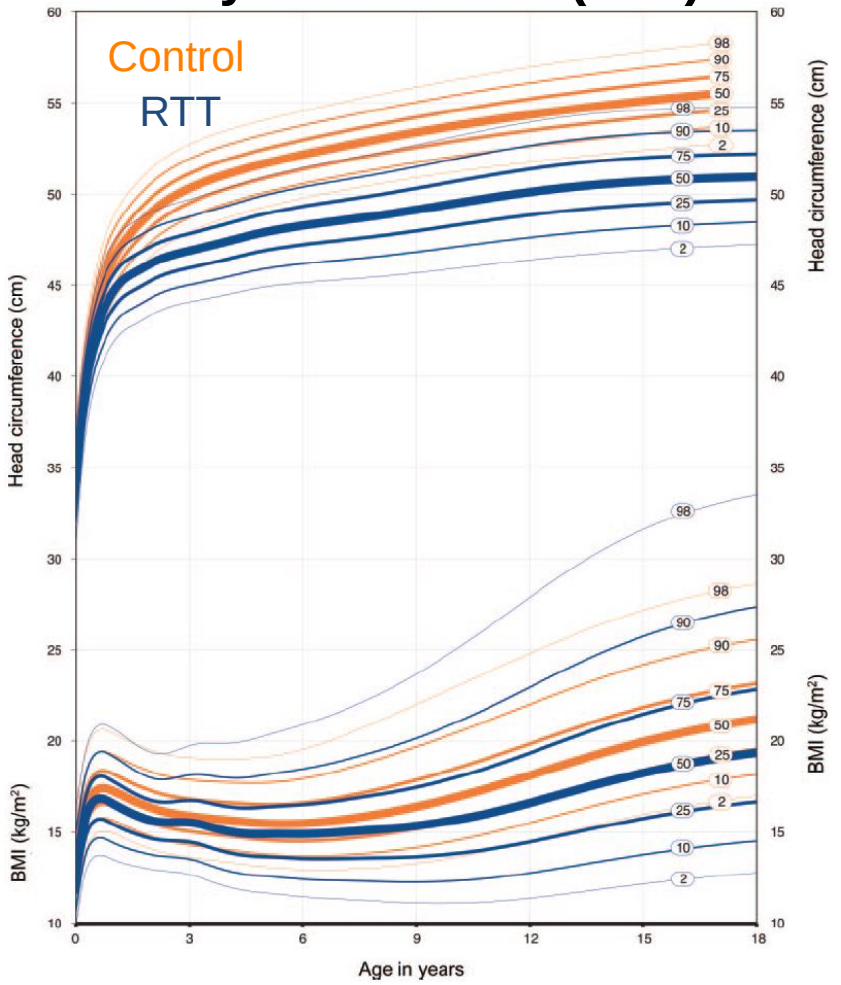
Specific growth references *Neurology* 2012;79;1653

1. Tarquinio DC, Motil KJ, Hou W, et al. Growth failure and outcome in Rett syndrome: specific growth references. Neurology. 2012;79(16):1653-1661. doi:10.1212/WNL.0b013e31826e9a70



Chahrour and Zoghbi, 2007

Head circumference and body mass index (BMI)[1]



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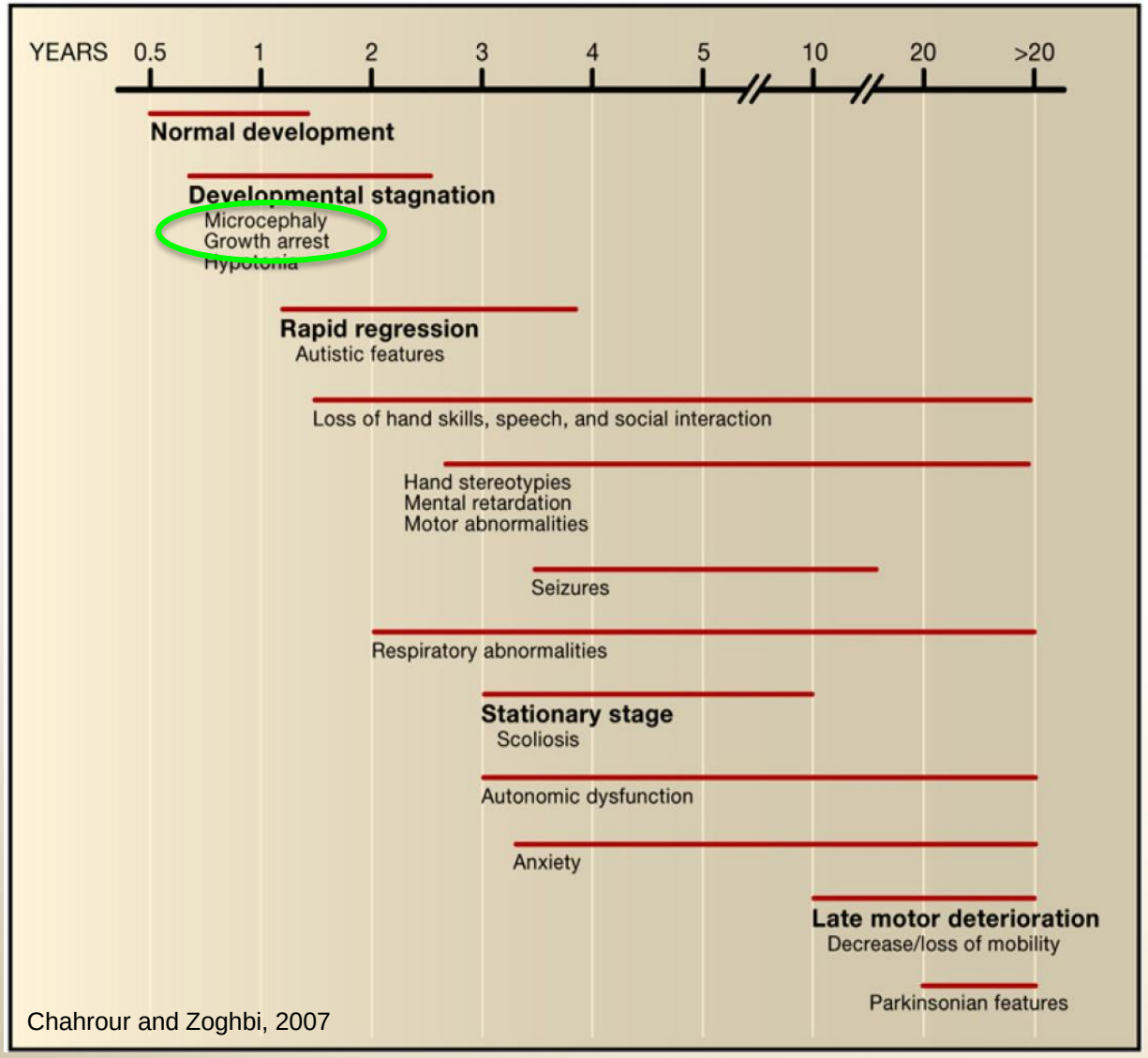
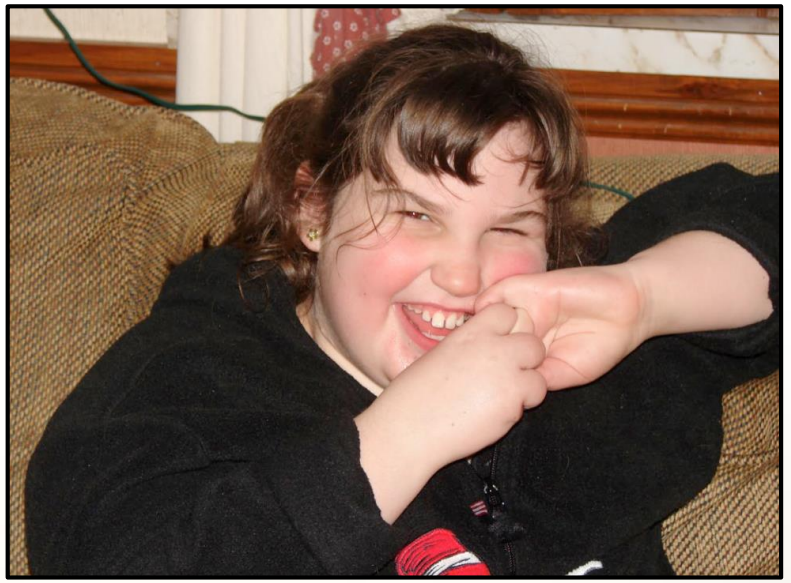
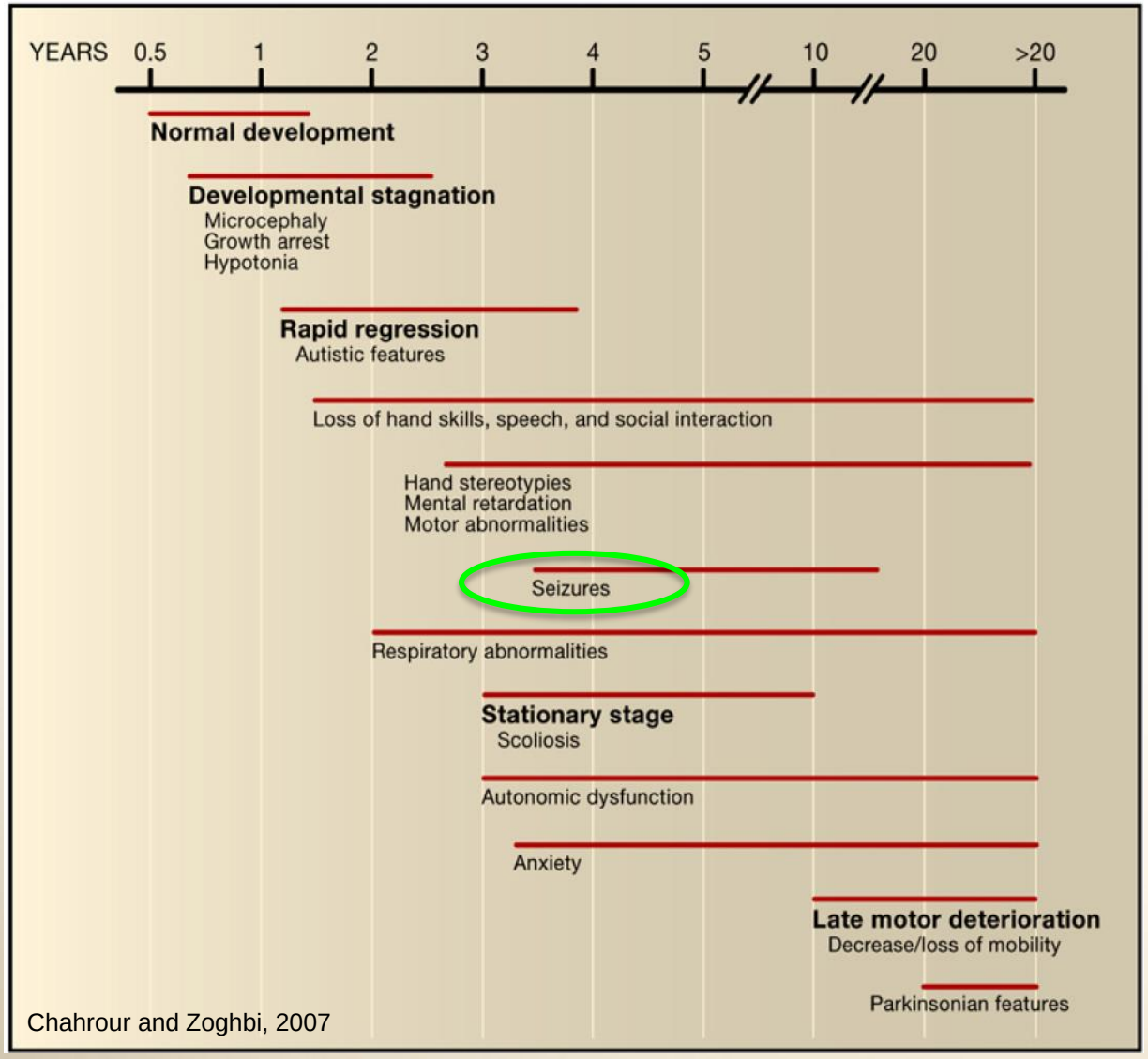


Chahrour and Zoghbi, 2007





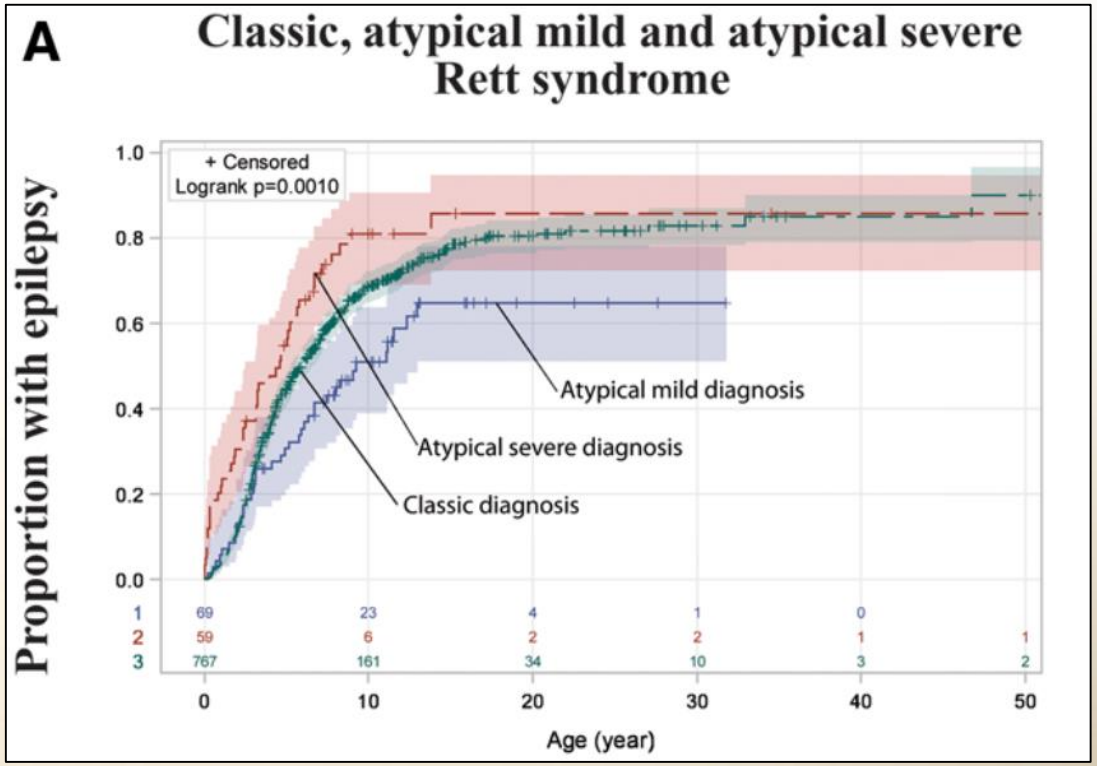
Chahrour and Zoghbi, 2007

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

BRAIN
A JOURNAL OF NEUROLOGY

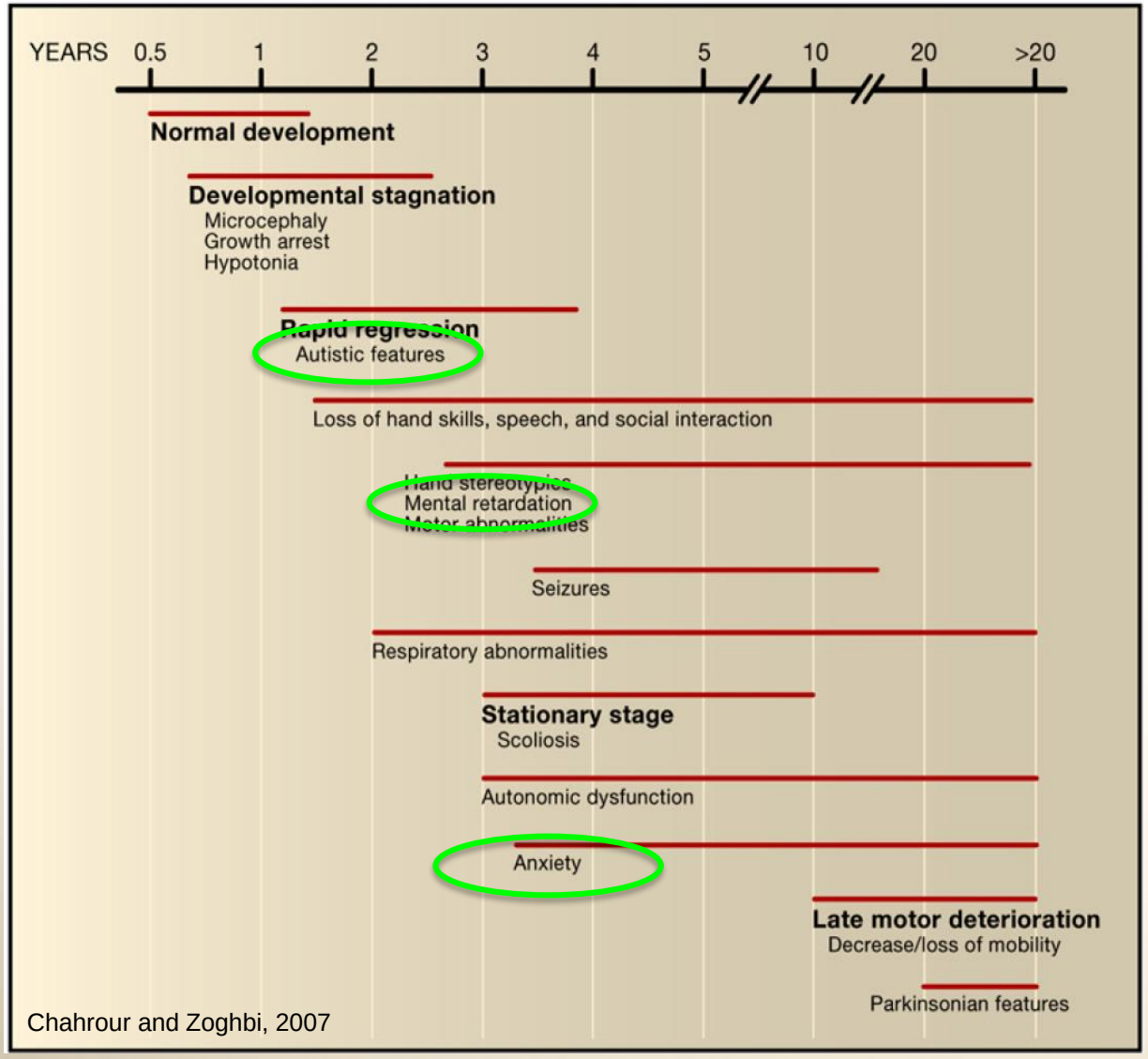
Longitudinal course of epilepsy in Rett syndrome and related disorders

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]



Tarquinio DC, Hou W, Berg A, et al. Longitudinal course of epilepsy in Rett syndrome and related disorders. Brain. 2017;140(2):306-318. doi:10.1093/brain/aww302

Characteristic clinical features: Movement abnormalities-dyskinesia



Chahrour and Zoghbi, 2007

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

- Autistic features may be present during regression but typically improve
  - Autistic features persist in higher functioning individuals
- Considered to have severe intellectual disability – IQ range unclear
  - Clearly have receptive language > expressive language

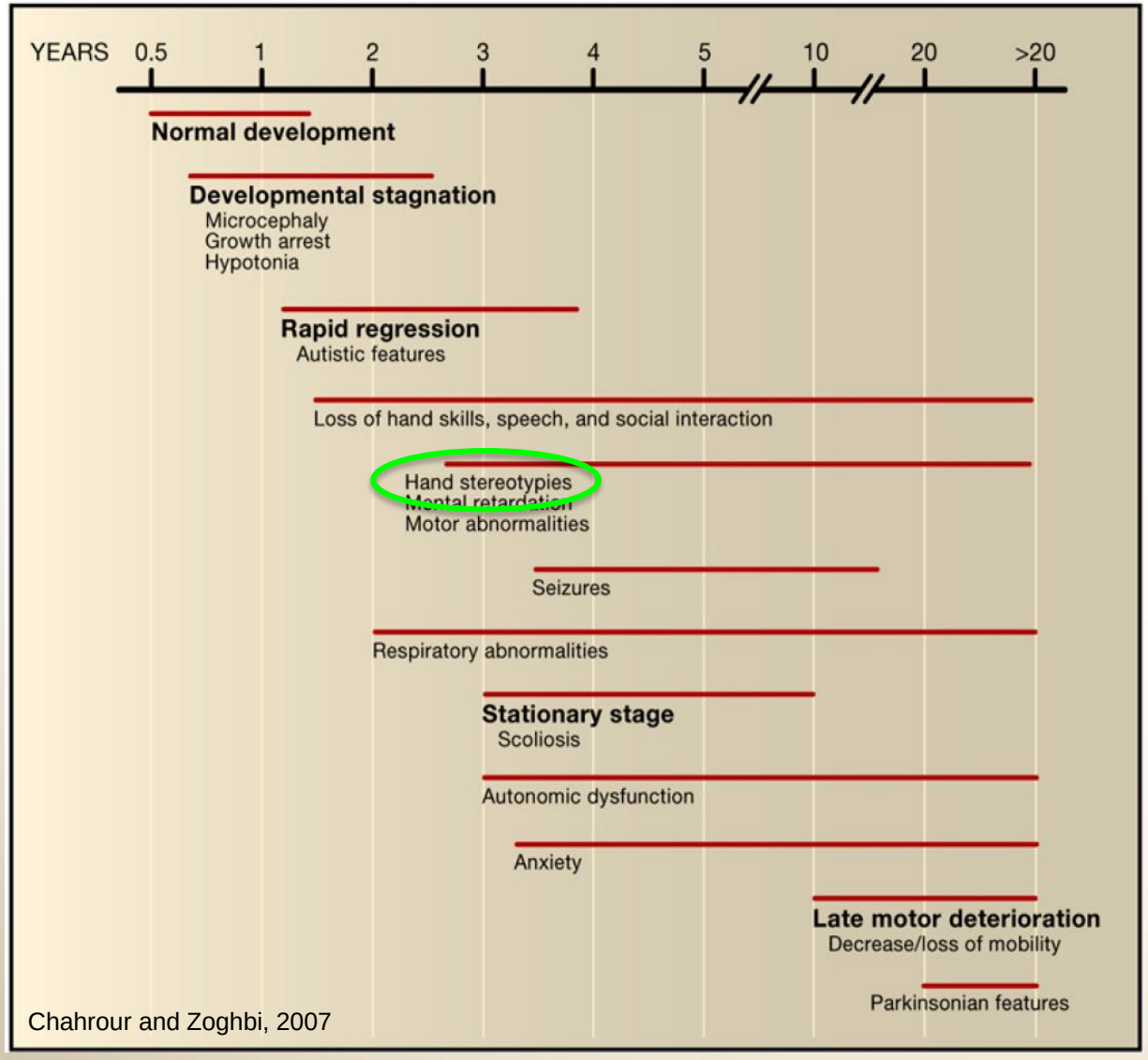
Behavioral profiles in Rett syndrome: Data from the natural history study

Brain & Development 41 (2019) 123–134

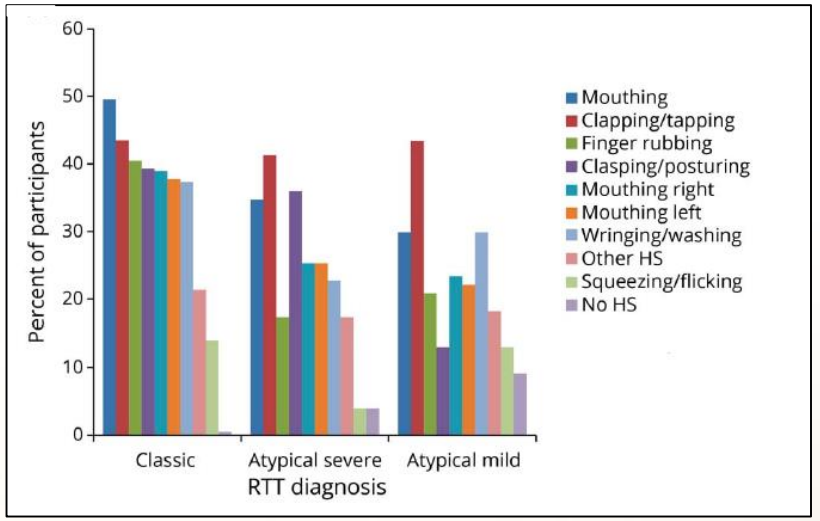
- Higher levels of mixed behavioral issues in younger and less affected individuals

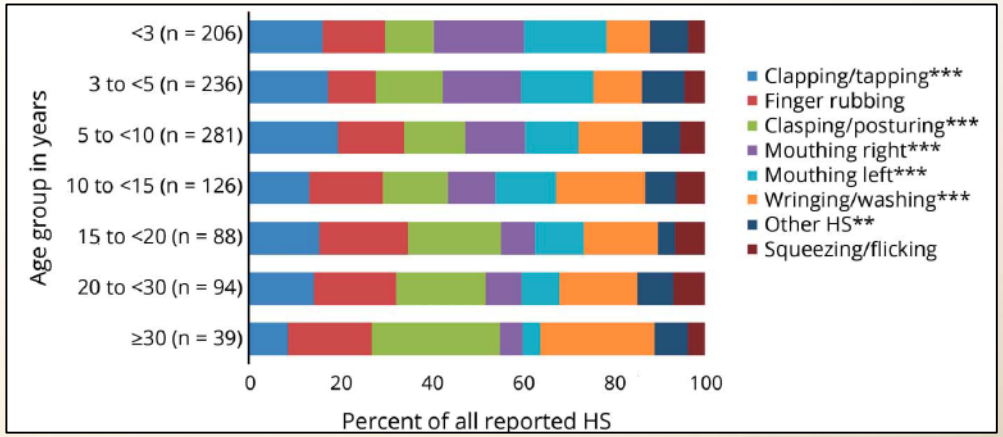
Kaufmann WE, Tierney E, Rohde CA, et al. Social impairments in Rett syndrome. J Intellect Disabil Res. 2012;56(3):233-247. doi:10.1111/j.1365-2788.2011.01404.x
Buchanan CB, Stallworth JL, Scott AE, et al. Behavioral profiles in Rett syndrome: Data from the natural history study. Brain Dev. 2019;41(2):123-134. doi:10.1016/j.braindev.2018.08.008

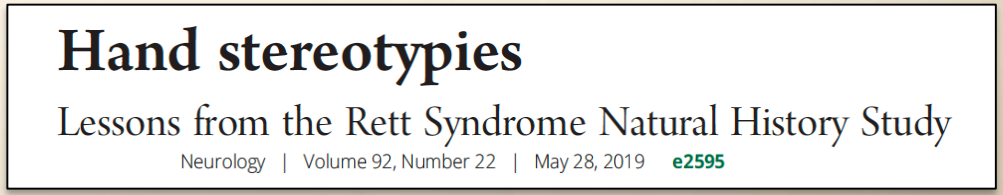
Abnormal stereotypical movements of the hands[1]



Chahrour and Zoghbi, 2007







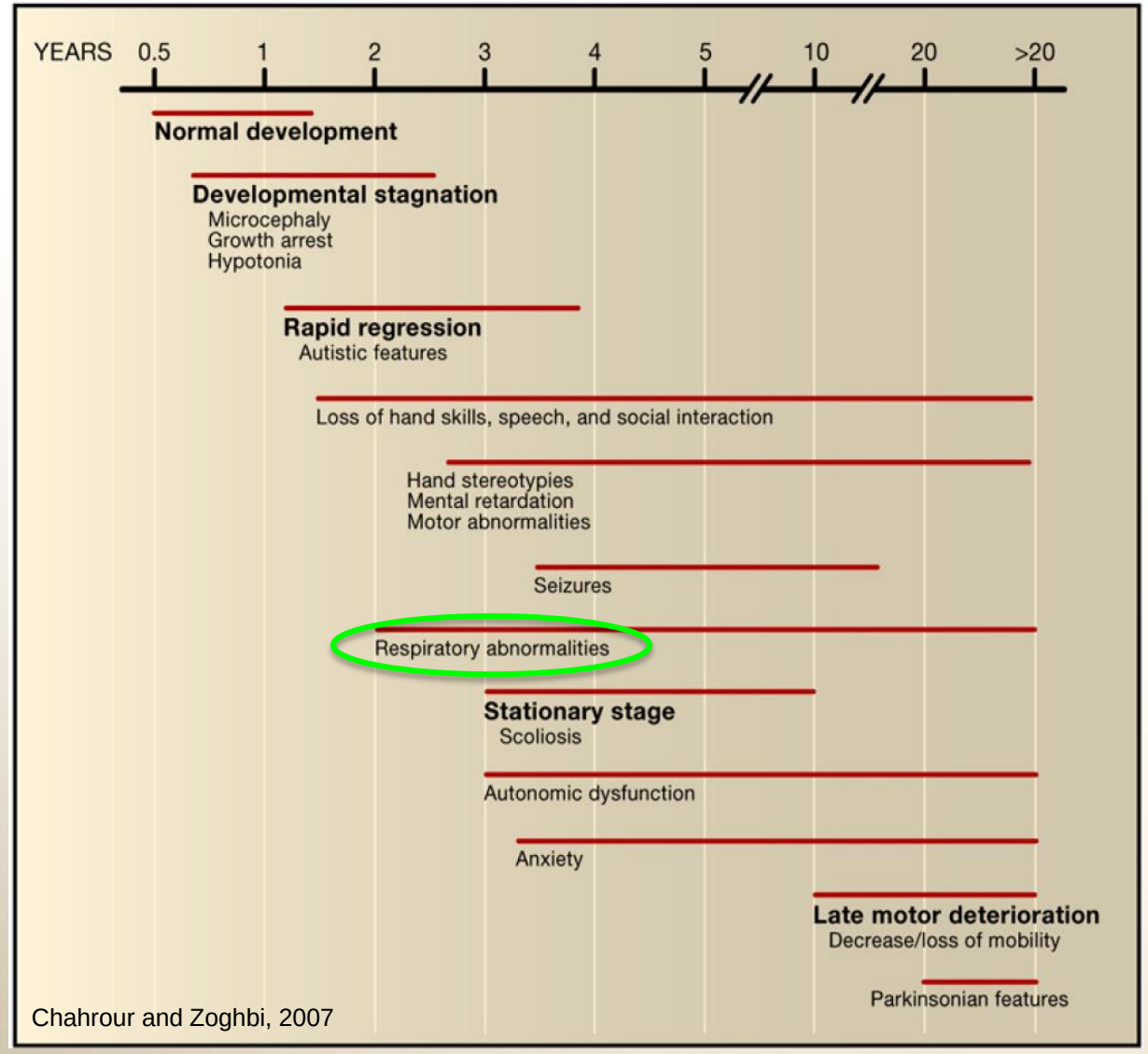
1. Stallworth JL, Dy ME, Buchanan CB, et al. Hand stereotypies: Lessons from the Rett Syndrome Natural History Study. Neurology. 2019;92(22):e2594-e2603. doi:10.1212/WNL.0000000000007560

Chahrour and Zoghbi, 2007

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

Brain & Development 40 (2018) 515–529

Tarquinio DC, Hou W, Neul JL, et al. The course of awake breathing disturbances across the lifespan in Rett syndrome. Brain Dev. 2018;40(7):515-529. doi:10.1016/j.braindev.2018.03.010



Chahrour and Zoghbi, 2007

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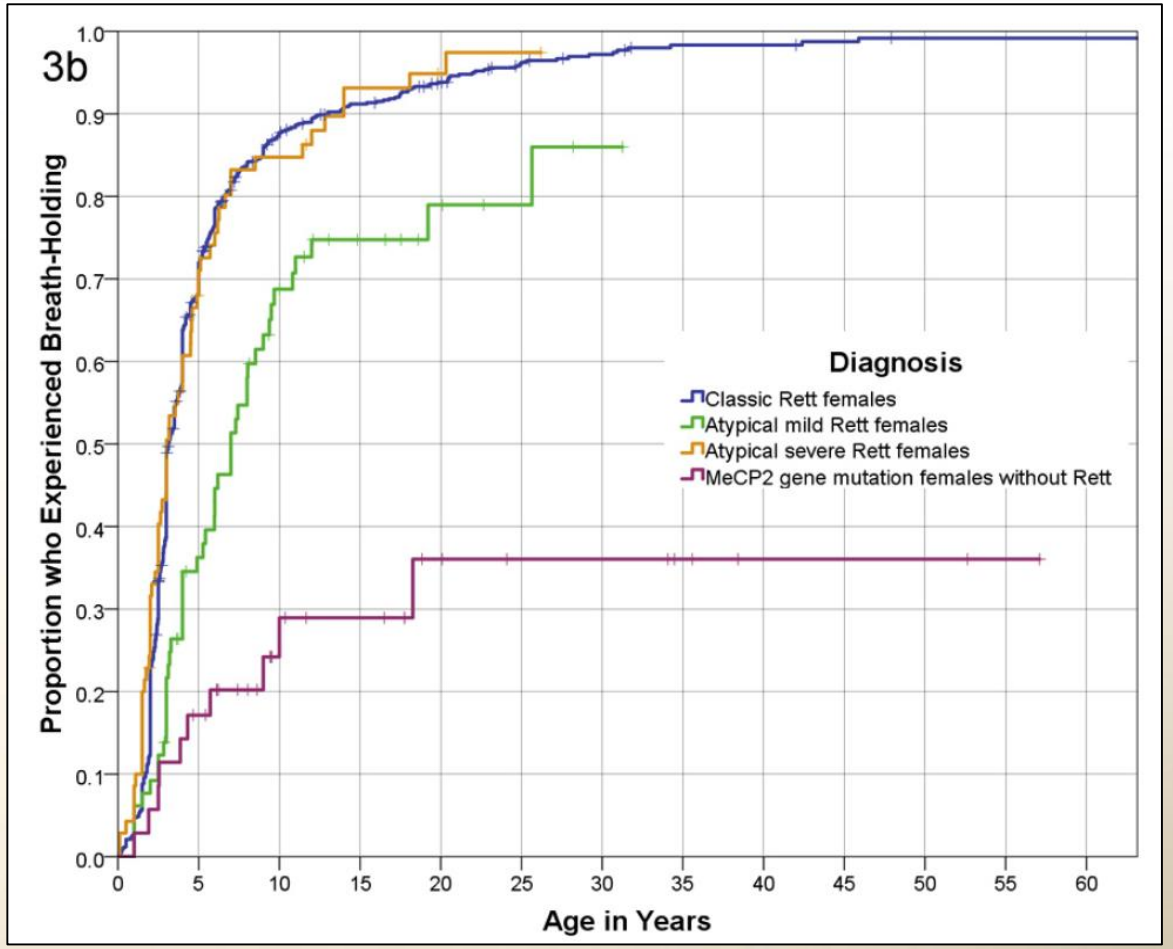

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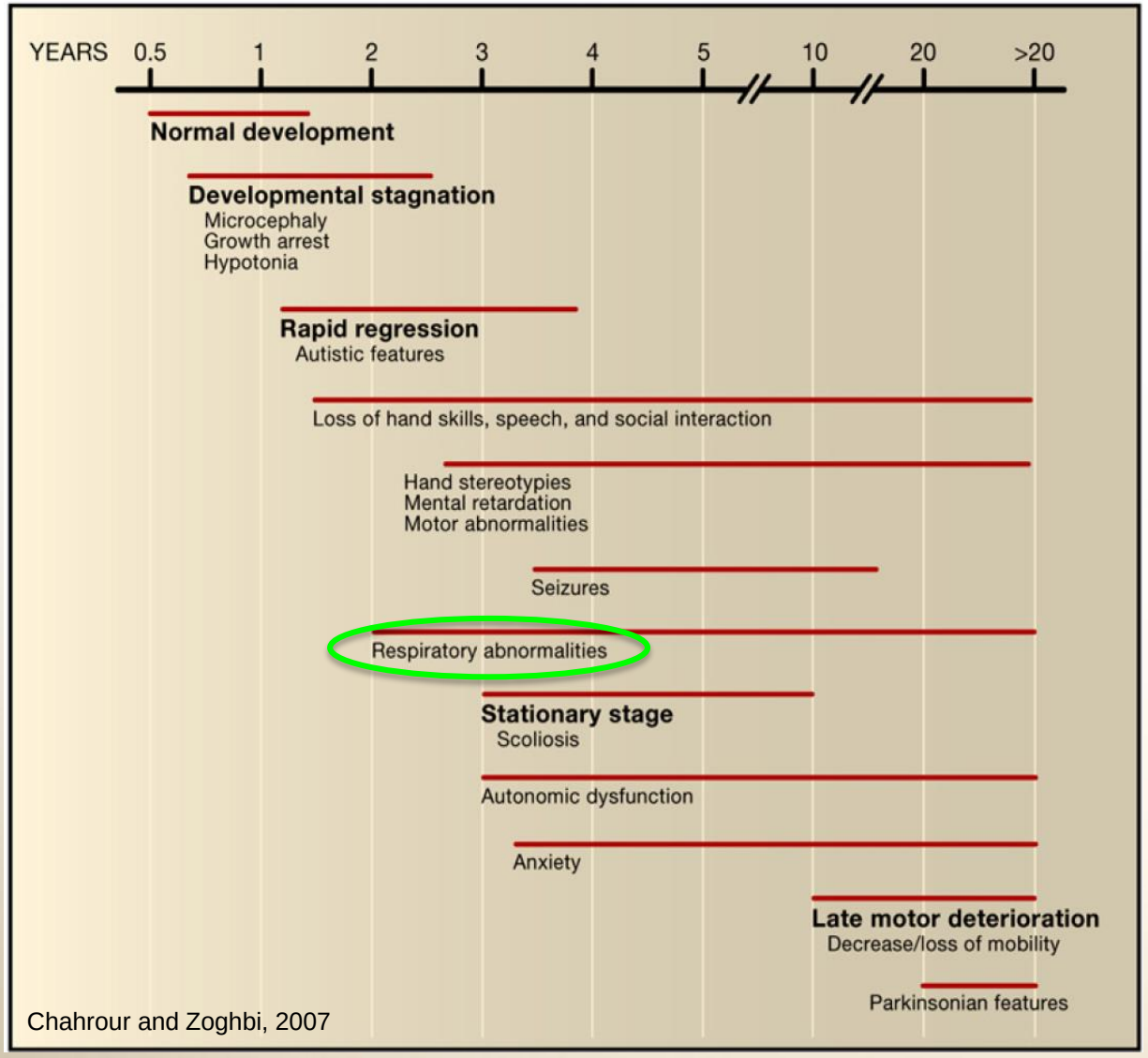

Chahrour and Zoghbi, 2007

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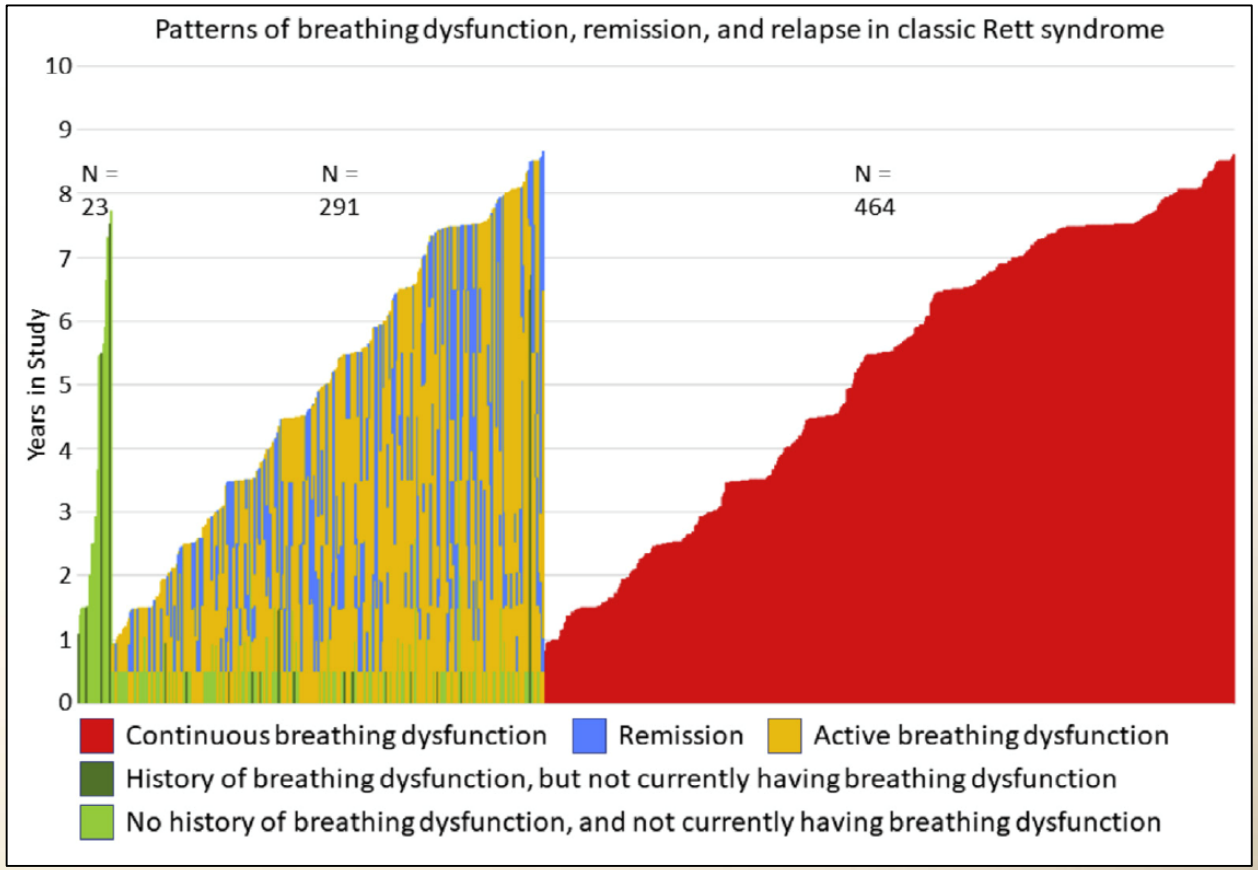

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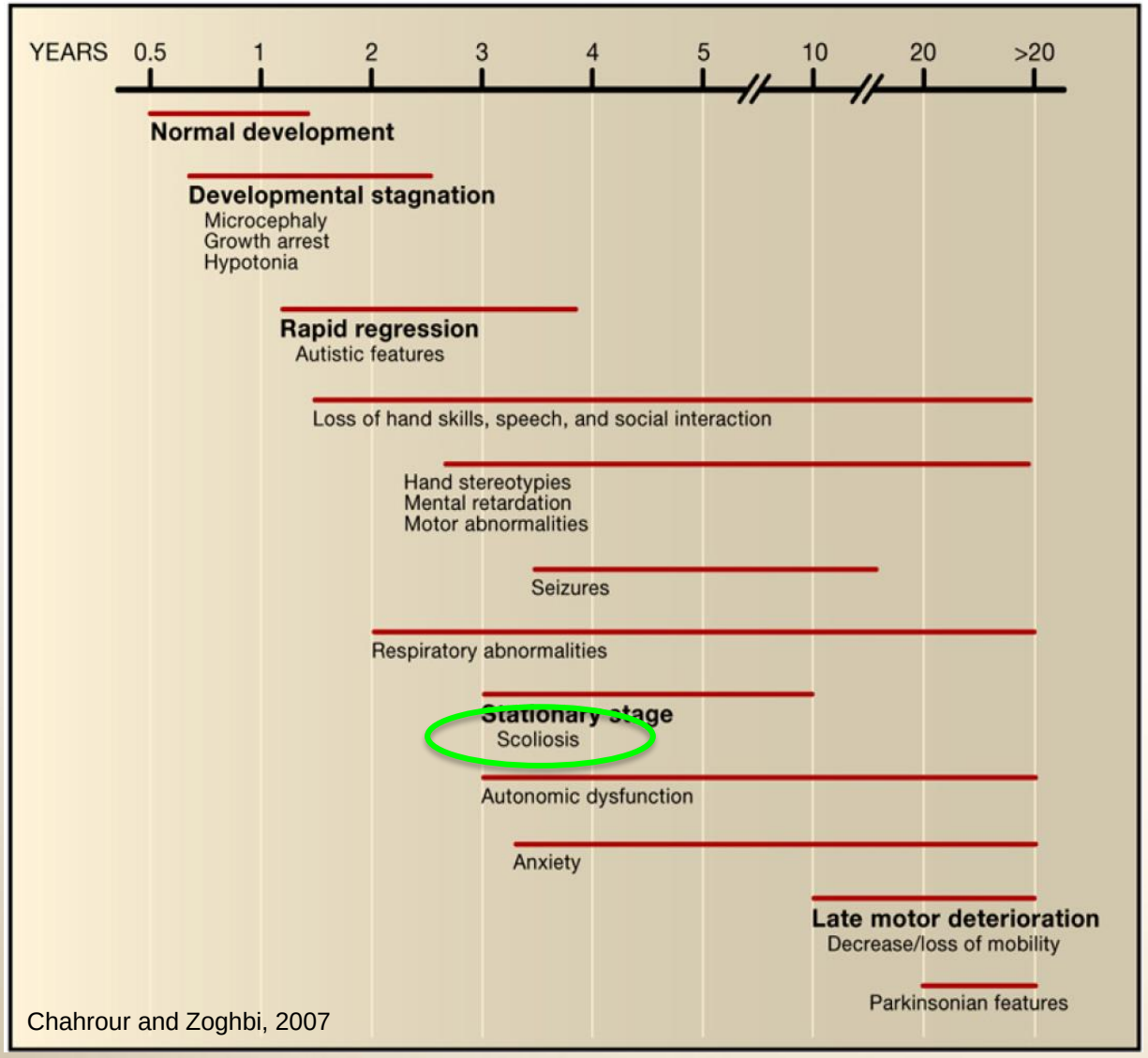


Chahrour and Zoghbi, 2007

Scoliosis in Rett Syndrome: Progression, Comorbidities, and Predictors

Pediatric Neurology 70 (2017) 20–25

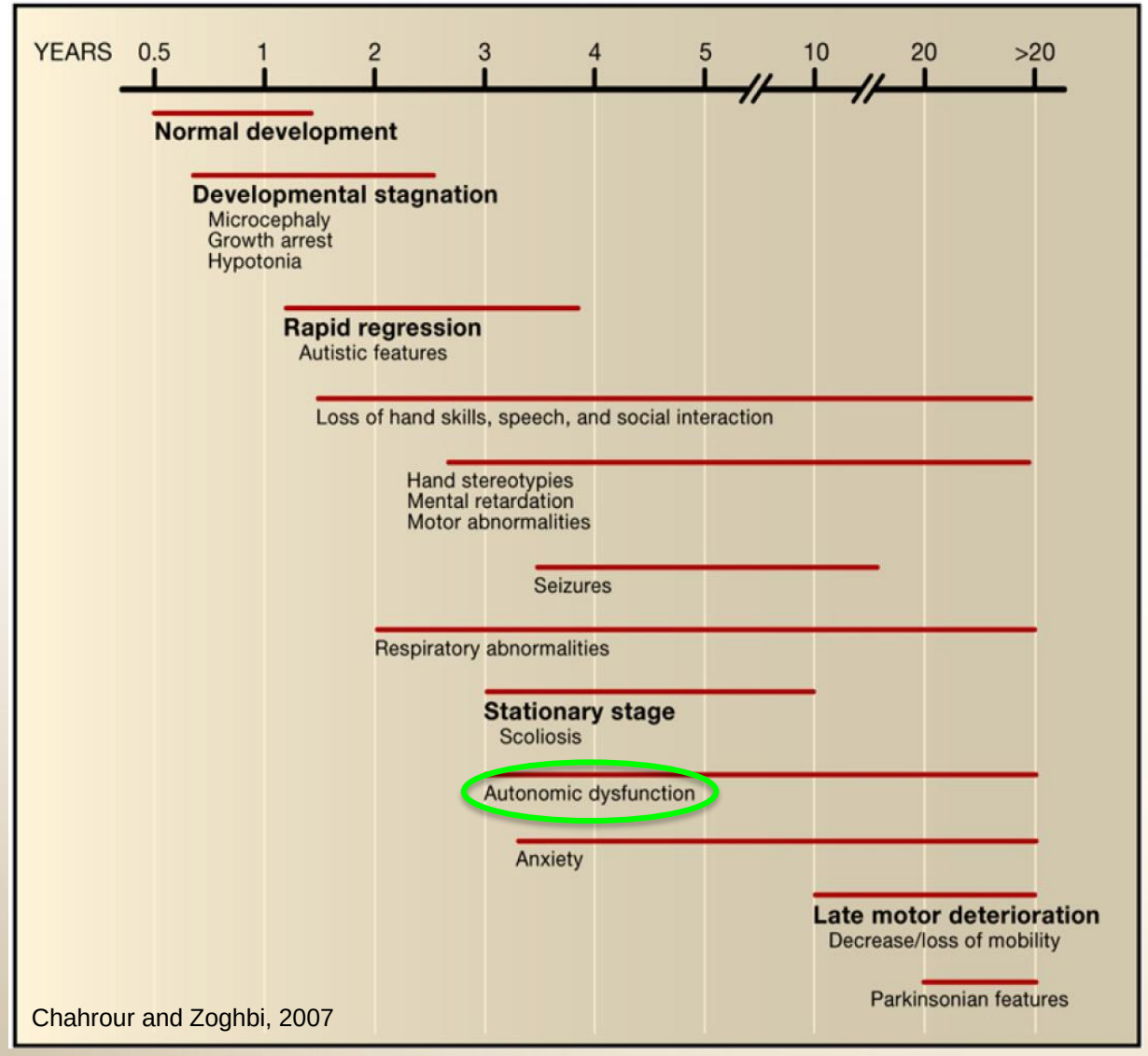
- Scoliosis severity increased with age
- Severe scoliosis in 27%
- Surgery in 18%

Killian JT, Lane JB, Lee HS, et al. Scoliosis in Rett Syndrome: Progression, Comorbidities, and Predictors. Pediatr Neurol. 2017;70:20-25. doi:10.1016/j.pediatrneurol.2017.01.032

Chahrour and Zoghbi, 2007

Autonomic features

- Vasomotor disturbances
  - Cold/blue feet/hands
- Gastrointestinal issues
- Decreased bone density



Chahrour and Zoghbi, 2007

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Chahrour and Zoghbi, 2007

Autonomic features

- Vasomotor disturbances
  - Cold/blue feet/hands
- Gastrointestinal issues
- Decreased bone density

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

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

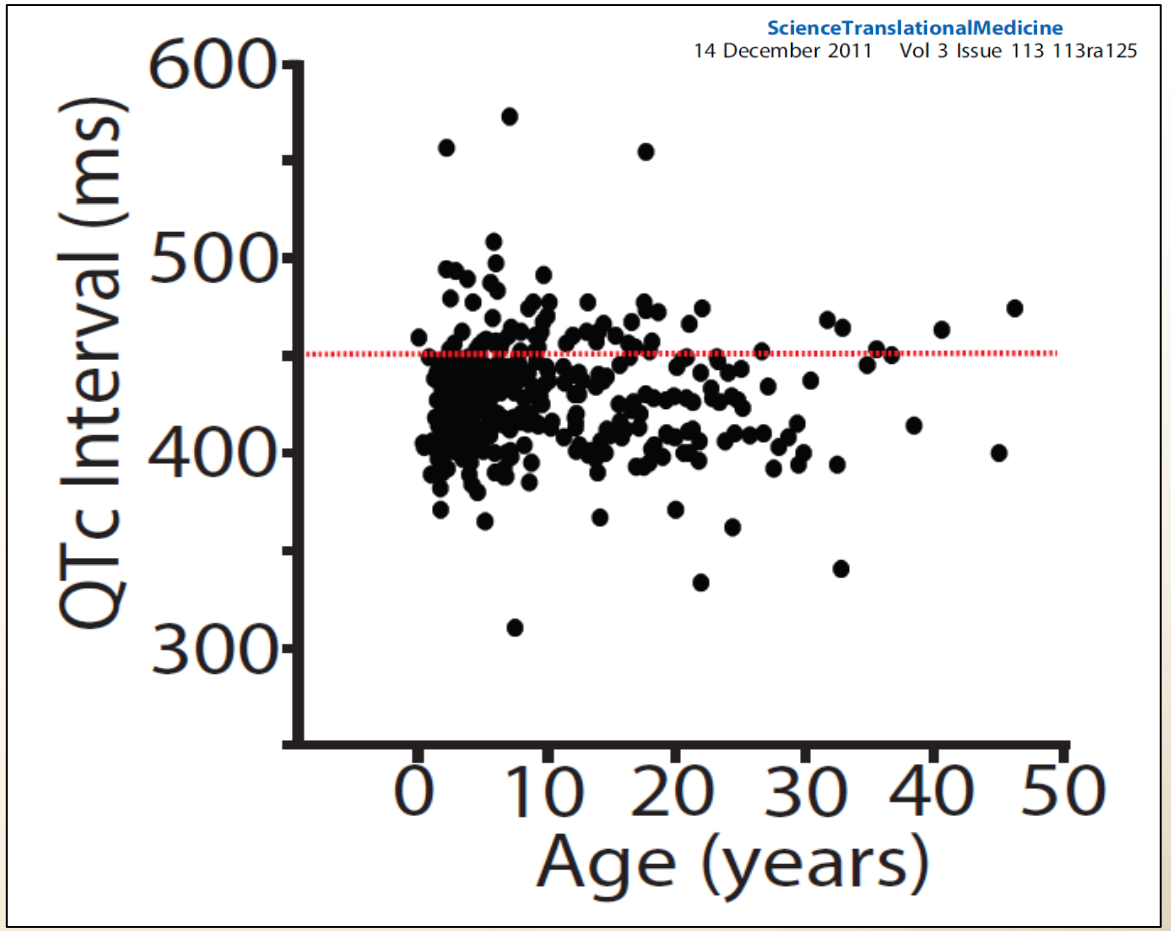
Kathleen J. Motil, Judy O. Barrish, Jeffrey L. Neul, and Daniel G. Glaze

JPGN • Volume 59, Number 3, September 2014

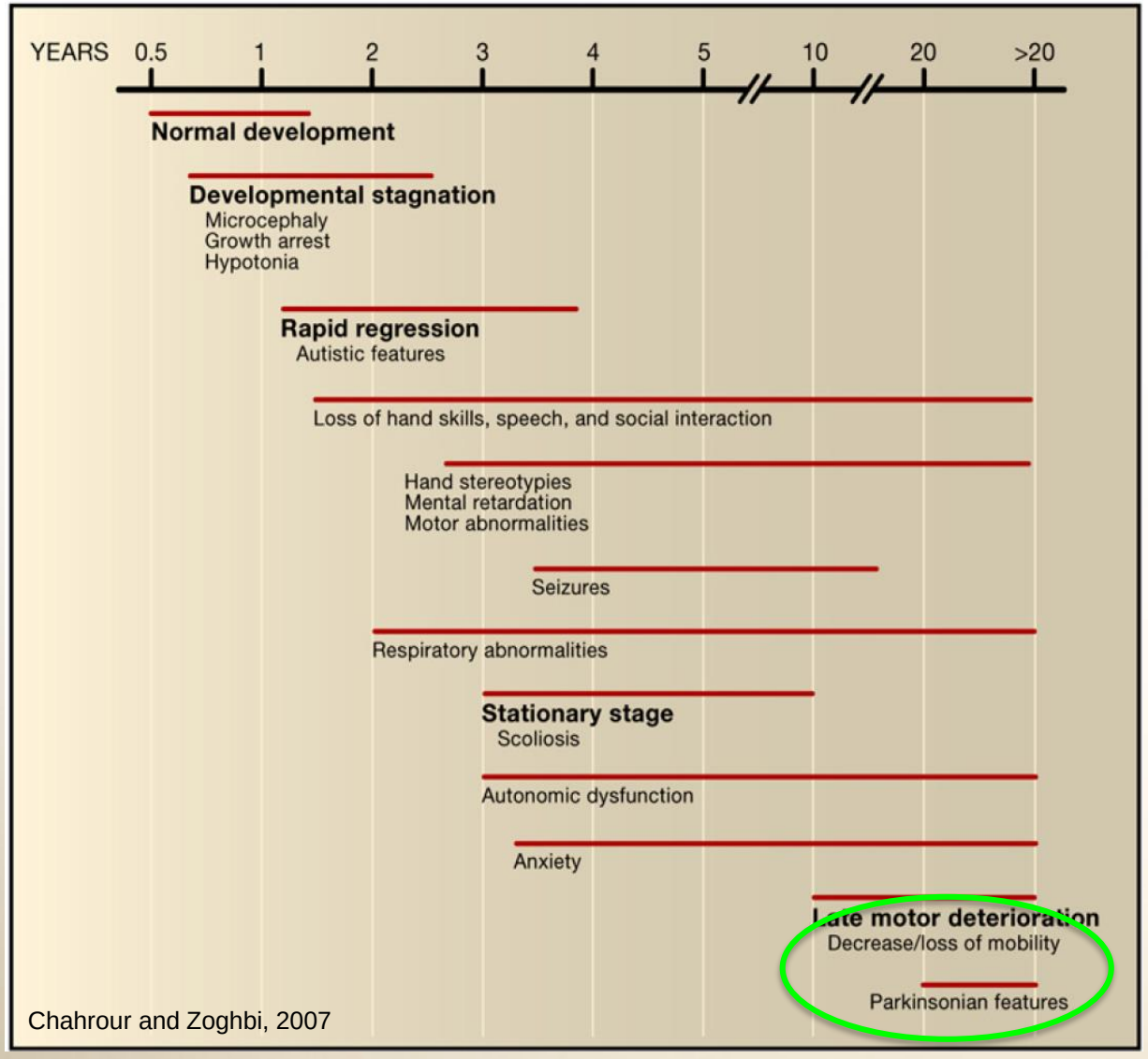
Other autonomic features

Cardiac abnormalities

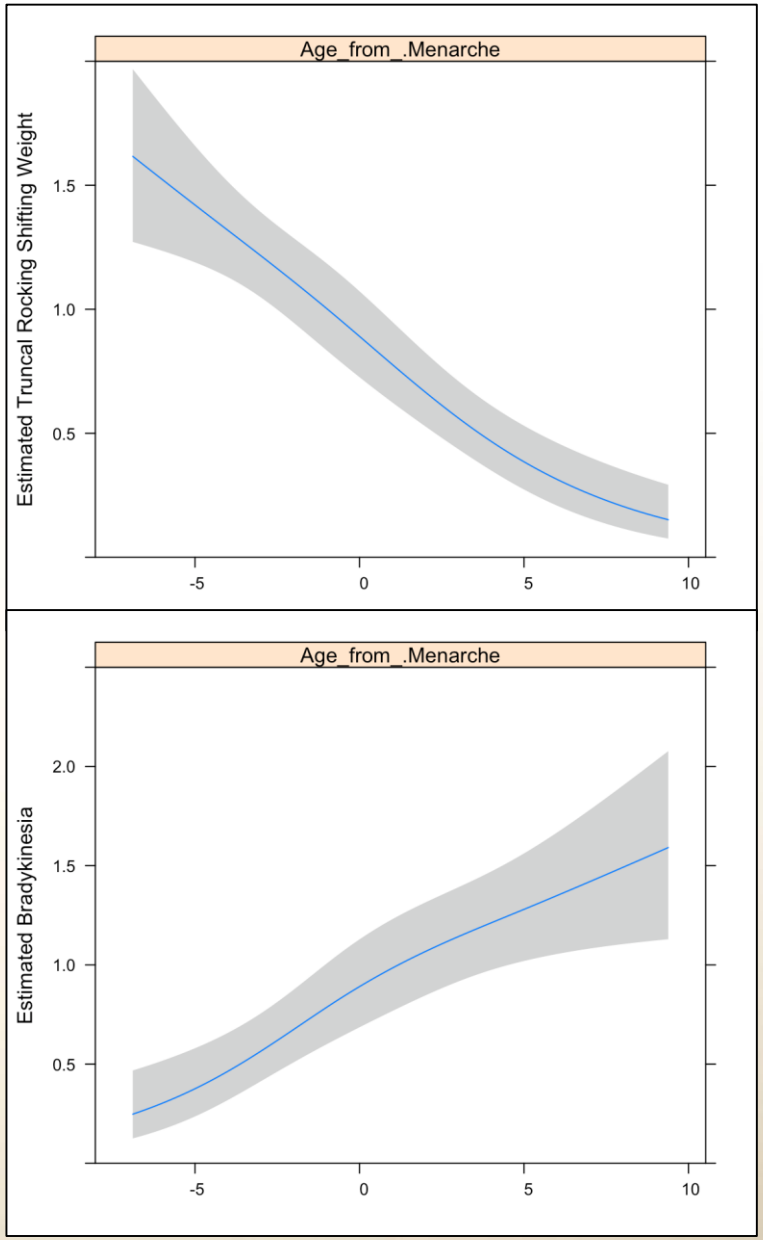
- Prolonged QTc
- Decreased beat-to-beat variability



McCauley MD, Wang T, Mike E, et al. Pathogenesis of lethal cardiac arrhythmias in Mecp2 mutant mice: implication for therapy in Rett syndrome. Sci Transl Med. 2011;3(113):113ra125. doi:10.1126/scitranslmed.3002982



Chahrour and Zoghbi, 2007



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*
  - Typically *de novo*
  - 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.

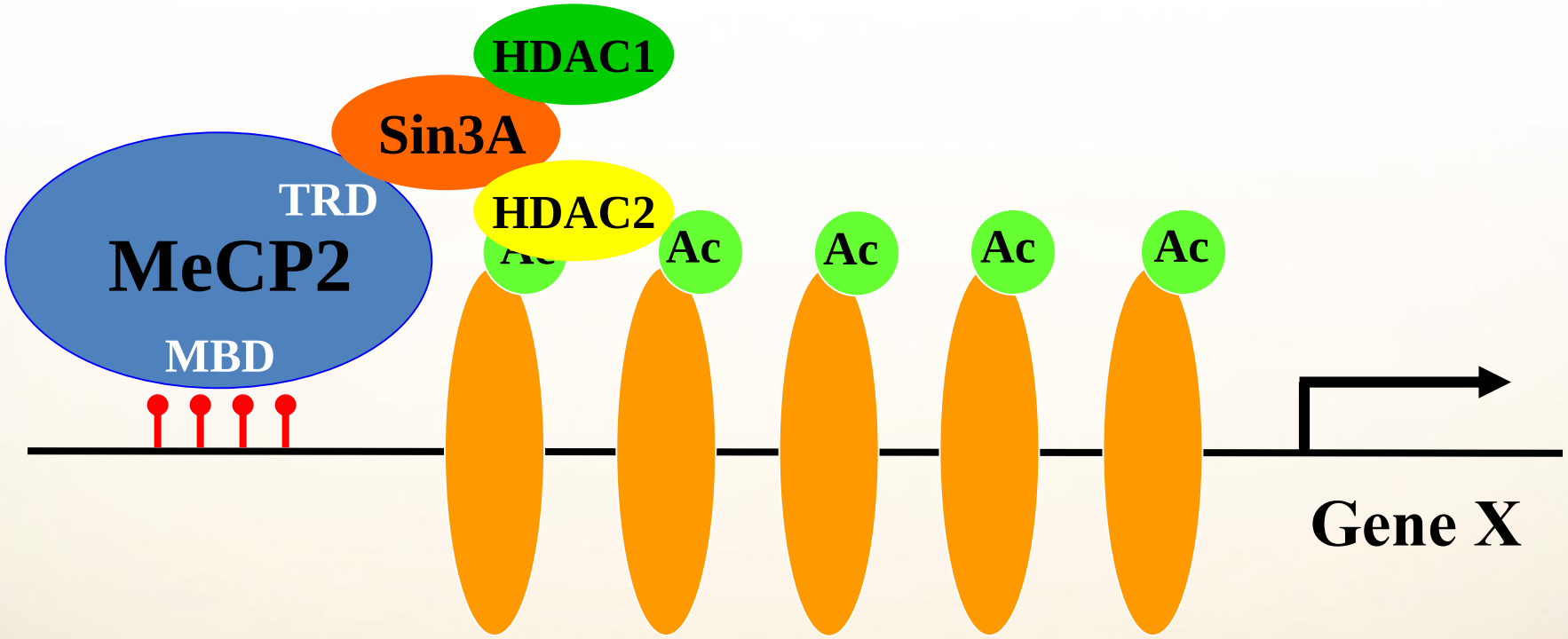
Autism and Other Neuropsychiatric Symptoms Are Prevalent in Individuals With *MECP2* Duplication Syndrome

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]

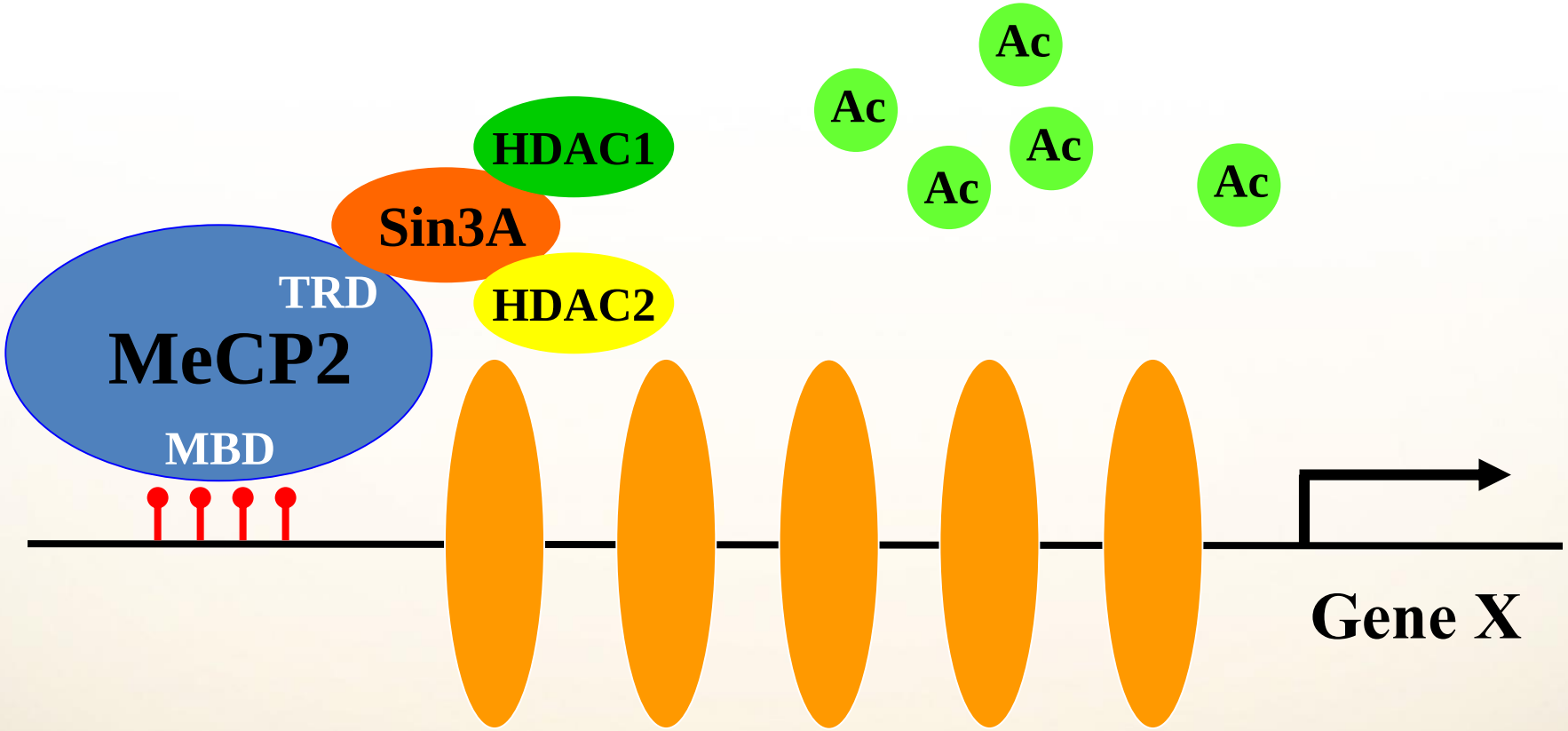
Ann Neurol 2009;66:771–782

Amir, R., Van den Veyver, I., Wan, M. et al. Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. Nat Genet 23, 185–188 (1999). https://doi.org/10.1038/13810

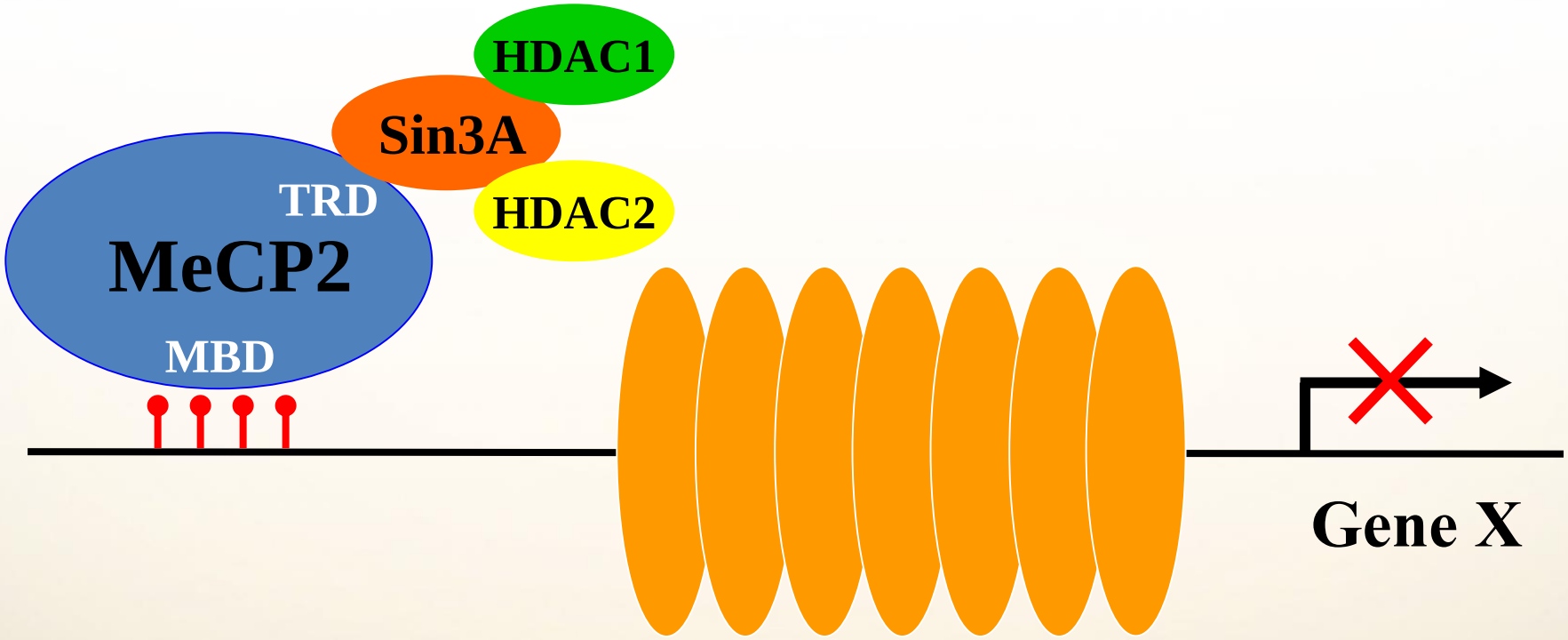
Methyl-CpG-binding protein 2 (MeCP2) function



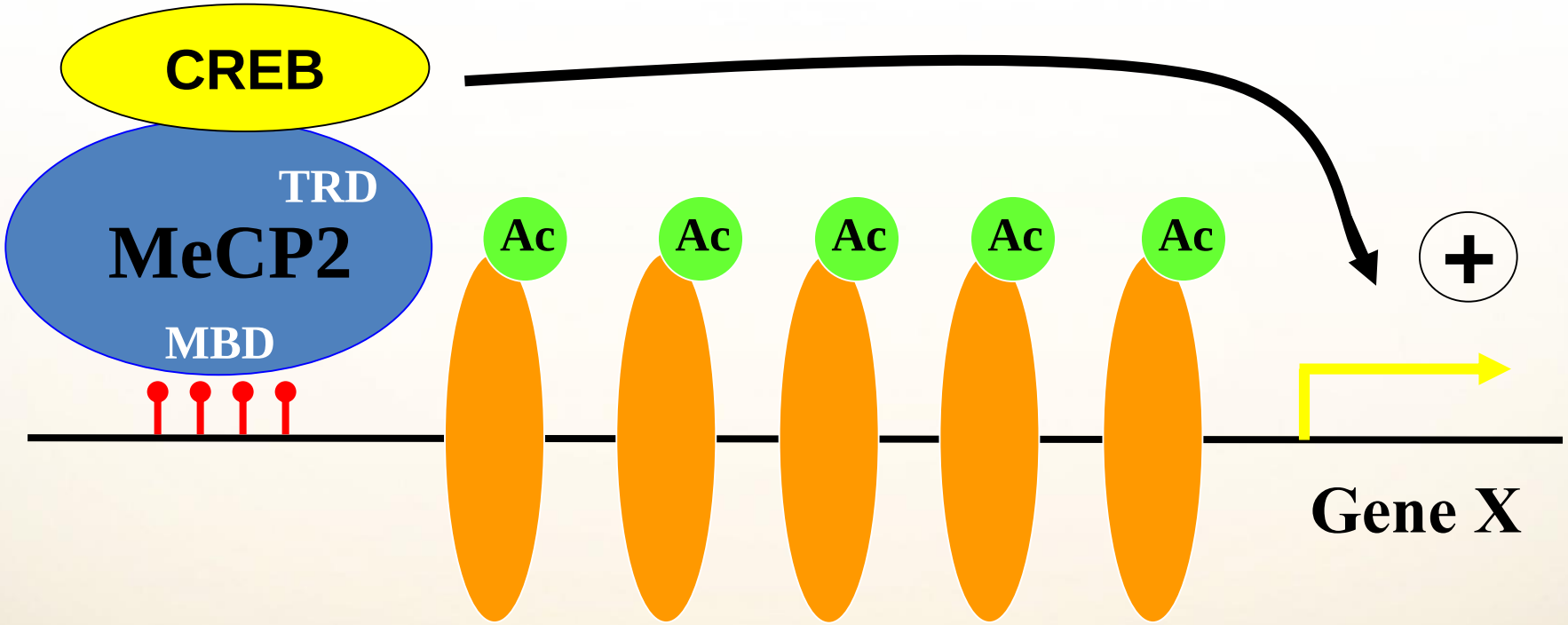
MeCP2 function



MeCP2 function



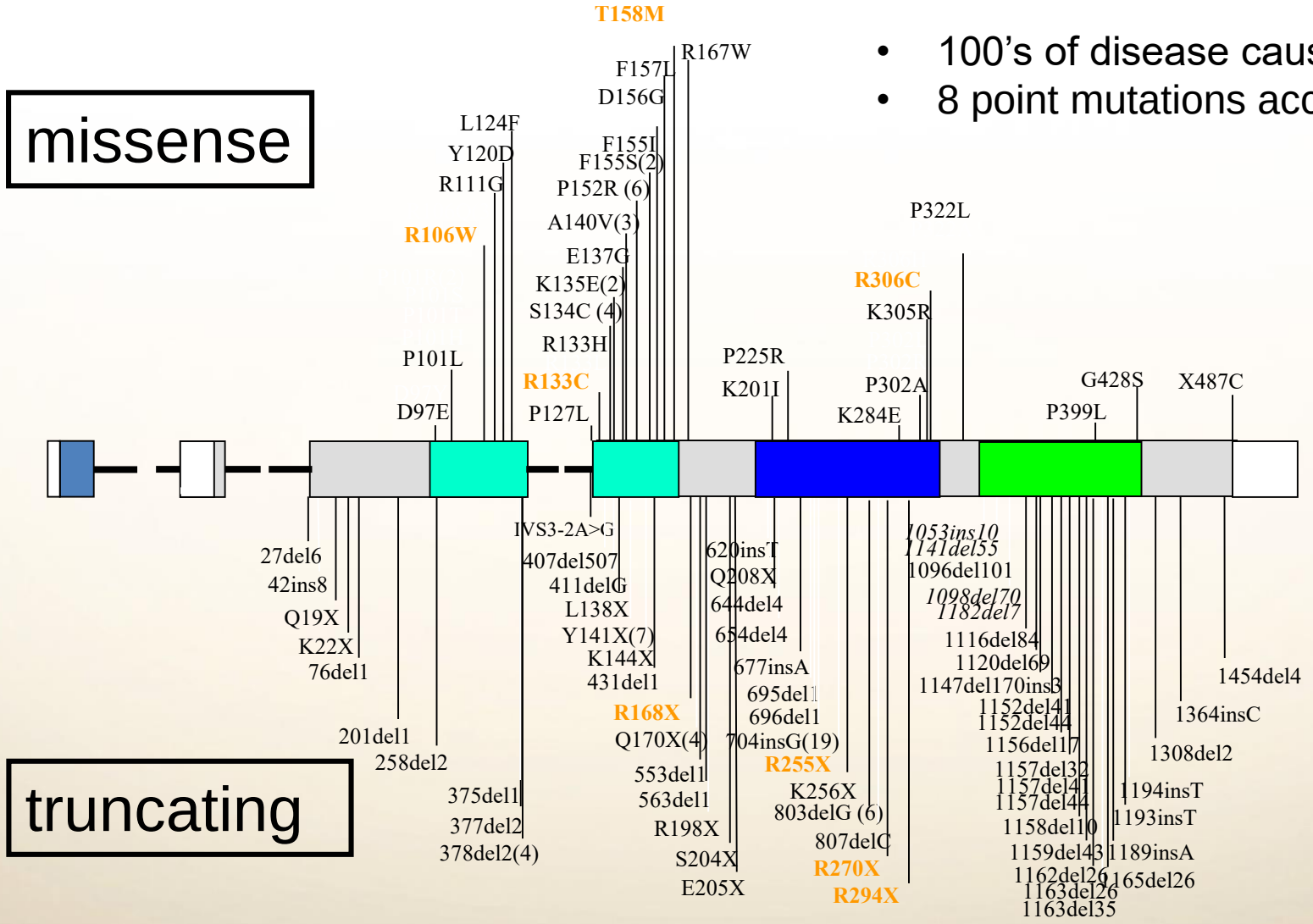
MeCP2 function: Activator



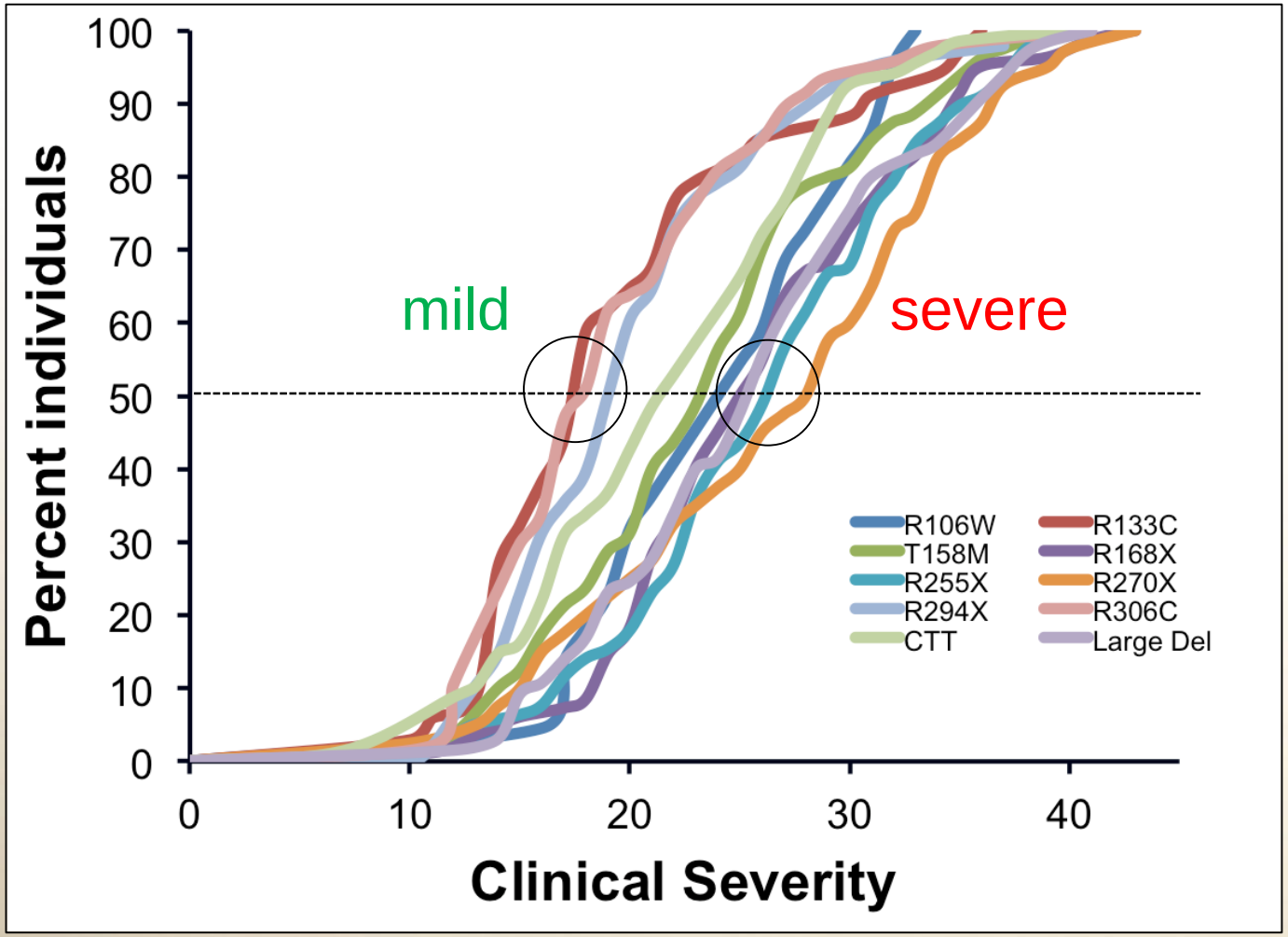
Yasui et al, PNAS 2007
Chahrour et al., Science 2008

MeCP2 mutations

- 100's of disease causing mutations
- 8 point mutations account for 65% cases



Genotype/phenotype relationship



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.

Rodent models of RTT reproduce clinical features

- **Weight/body size**
- **Lifespan**
- **Locomotor function**
- **Motor Coordination**
- **Gait**
- **Anxiety**
- **Social Interactions**
- **Repetitive behavior**
- **Breathing irregularities**
- **Cardiac rhythm abnormalities**
- **Electroencephalogram (EEG) changes and seizures**

- Multiple different alleles have been generated
  - Nearly all the common point mutations (7/8)
    - Show same genotype/phenotype relationship
  - Conditional knock out and rescue alleles
    - Cell-specific roles, temporal control
- Female animals have similar phenotypes
- Rat model shows phenotypic abnormalities

Female *Mecp2*$^{+/-}$ mice display robust behavioral deficits on two different genetic backgrounds providing a framework for pre-clinical studies

Rodney C. Samaco[1,2], Christopher M. McGraw[1,3], Christopher S. Ward[1,2], Yaling Sun[1,2], Jeffrey L. Neul[1,2,3,4] and Huda Y. Zoghbi[1,2,3,4,5,6,*]

Human Molecular Genetics, 2013, Vol. 22, No. 1

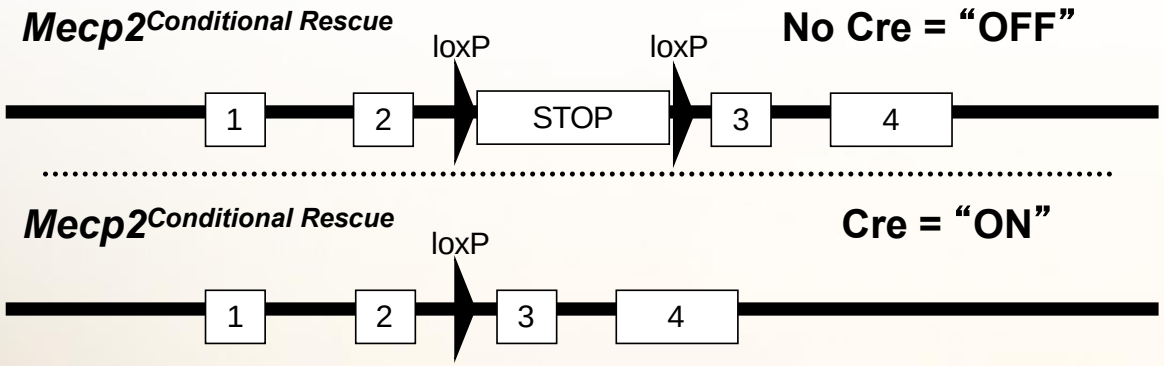
Loss of MeCP2 in the rat models regression, impaired sociability and transcriptional deficits of Rett syndrome

Human Molecular Genetics, 2016, Vol. 25, No. 15 3284–3302

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



Human Molecular Genetics, 2014, Vol. 23, No. 2 **303–318**
doi:10.1093/hmg/ddt421
Advance Access published on September 5, 2013

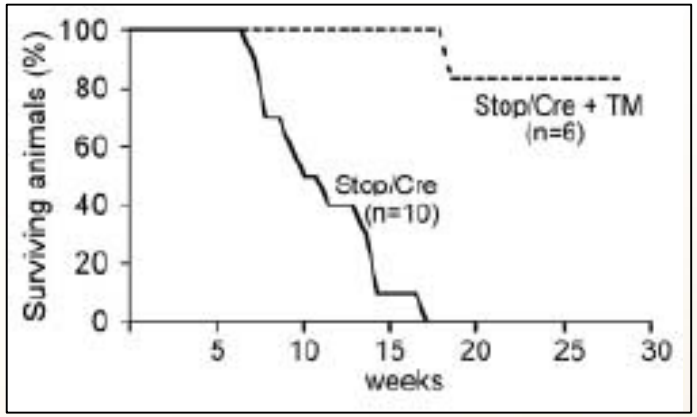
Rescue of behavioral and EEG deficits in male and female Mecp2-deficient mice by delayed *Mecp2* gene reactivation

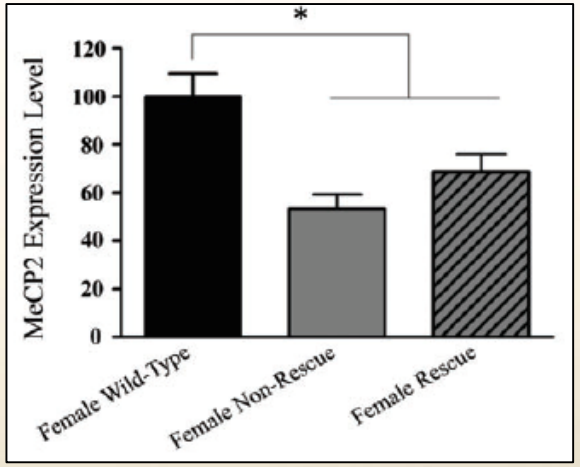
Min Lang[1,3], Robert G. Wither[1,3], Sinisa Colic[5], Chiping Wu[2], Philippe P. Monnier[1,3], Berj L. Bardakjian[5], Liang Zhang[2,4] and James H. Eubanks[1,3,6,*]

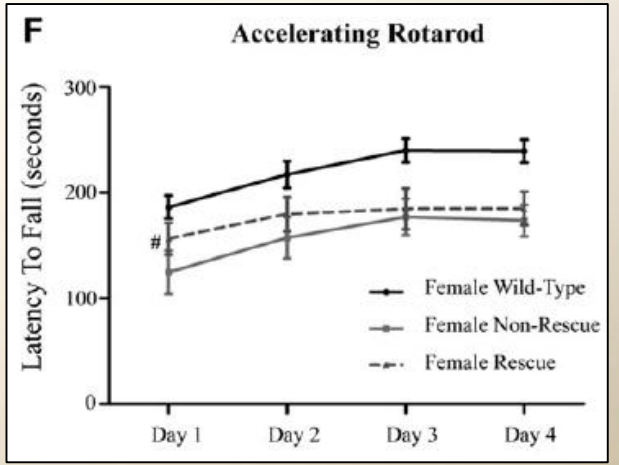
Morphological and functional reversal of phenotypes in a mouse model of Rett syndrome

Lianne Robinson,[1,*] Jacky Guy,[2,*] Leanne McKay,[3] Emma Brockett,[3] Rosemary C. Spike,[3] Jim Selfridge,[2] Dina De Sousa,[2] Cara Merusi,[2] Gernot Riedel,[1] Adrian Bird[2] and Stuart R. Cobb[3]

Brain 2012: 135; 2699–2710



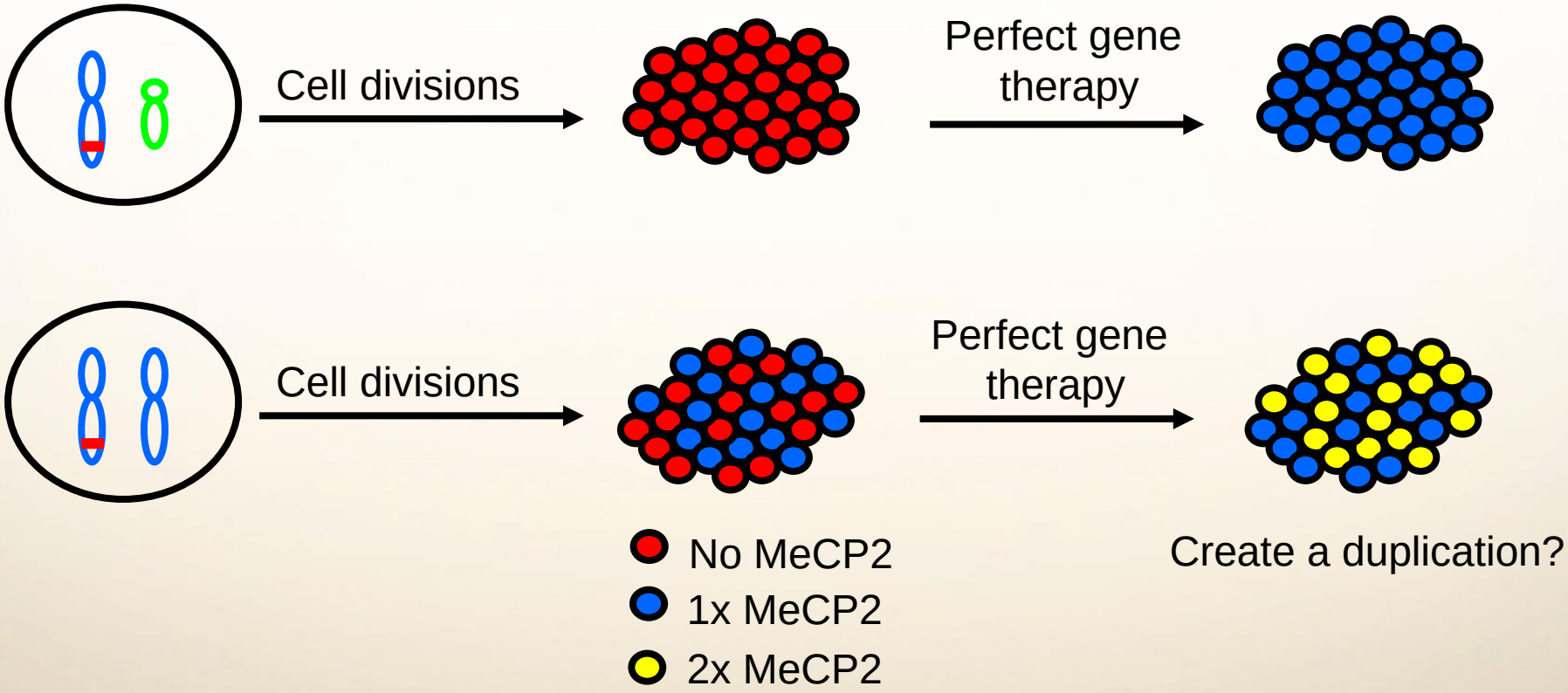




Gene therapy in RTT

Challenges?

- Delivery throughout the central nervous system (CNS)
- Overshoot



Gene therapy in RTT

Challenges?

- Delivery throughout the CNS
- Overshoot – Create MECP2 duplication

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

Improved *MECP2* Gene Therapy Extends the Survival of MeCP2-Null Mice without Apparent Toxicity after Intracisternal Delivery

Molecular Therapy: Methods & Clinical Development Vol. 5 June 2017

A codon-optimized *Mecp2* transgene corrects breathing deficits and improves survival in a mouse model of Rett syndrome

Neurobiology of Disease 99 (2017) 1–11

Development of a Novel AAV Gene Therapy Cassette with Improved Safety Features and Efficacy in a Mouse Model of Rett Syndrome

Molecular Therapy: Methods & Clinical Development Vol. 5 June 2017

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]

Need for clinical trials in RTT

- Outcome measures
  - Need psychometrically reliable and valid measures
  - **Should reflect relevant issues in RTT**
- Biomarkers
  - Disease severity
  - Identify treatment responders
  - Early predictive biomarkers of treatment response

Caregiver top concerns

Question added to caregiver-completed form (Interval History) in 2014 (5211)

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)

Symptoms	Classic RTT
Lack of effective communication	1
Seizures	2
Lack of hand use	3
Abnormal Walking/Balance Issues	4
Constipation	5
Repetitive hand movements	6
Problems with sleep	7
Rapid breathing or breath holding while awake	8
Air swallowing/Bloating/Excessive Gas	9
Lack of effective chewing or swallowing	10
Screaming episodes	11
Scoliosis (curvature of the spine)	12
Anxiety	13
Teeth Grinding (while awake)	14
Gastroesophageal reflux	15
Poor weight gain	16
Abnormal Movements	17
Frequent infections	18
Self-abusive behaviors	19
Other GI	20
Aggressiveness towards others	21
None indicated	22
Dystonia/Rigidity/Contractures	23
Other Behavior	24
Other Health Issue	25

Outcome measures

Clinician-reported Clinical Global Impression (CGI)

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

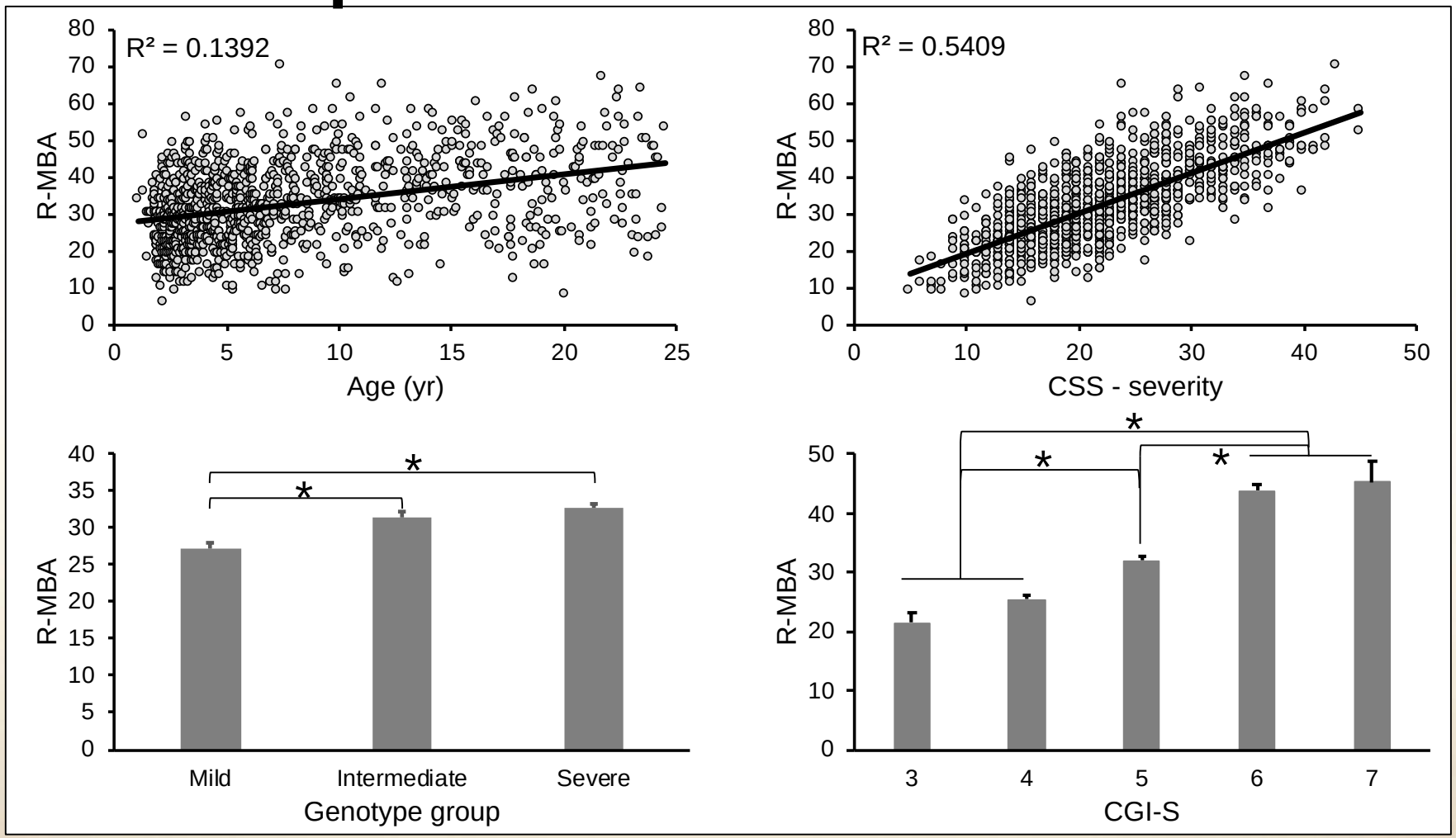
- Clinician's view of participants global functioning
- Split into 2 scales (both 7-point Likert scales)
  - Severity
  - Improvement
- Understood and accepted by FDA/EMA
- Establishing rater reliability is critical
  - Disease-specific anchors
  - Rater training
    - Written vignettes with gold-standard scores
  - Video-based vignettes would improve rater training and reliability assessment

Development of a new clinician reported outcome measure

- Formal psychometric analysis on Rett syndrome Motor Behavioral Assessment (R-MBA) using natural history data
- Factor Analysis reduced to 21 items in 5 factors
  - Motor dysfunction
  - Functional skills
  - Social skills
  - Aberrant behavior
  - Rett-specific behavior
- Total revised R-MBA score included 21 items plus 3 additional clinically important items
  - Stereotypical hand movements/hand-object mouthing
  - Truncal rocking
  - Seizures
- Reasonable internal consistency (Cronbach's α=0.55-0.8 for subscales, 0.78 for total)
- Factors correlated with parent reports of clinical domains (validity)

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

Development of a new clinician reported outcome measure

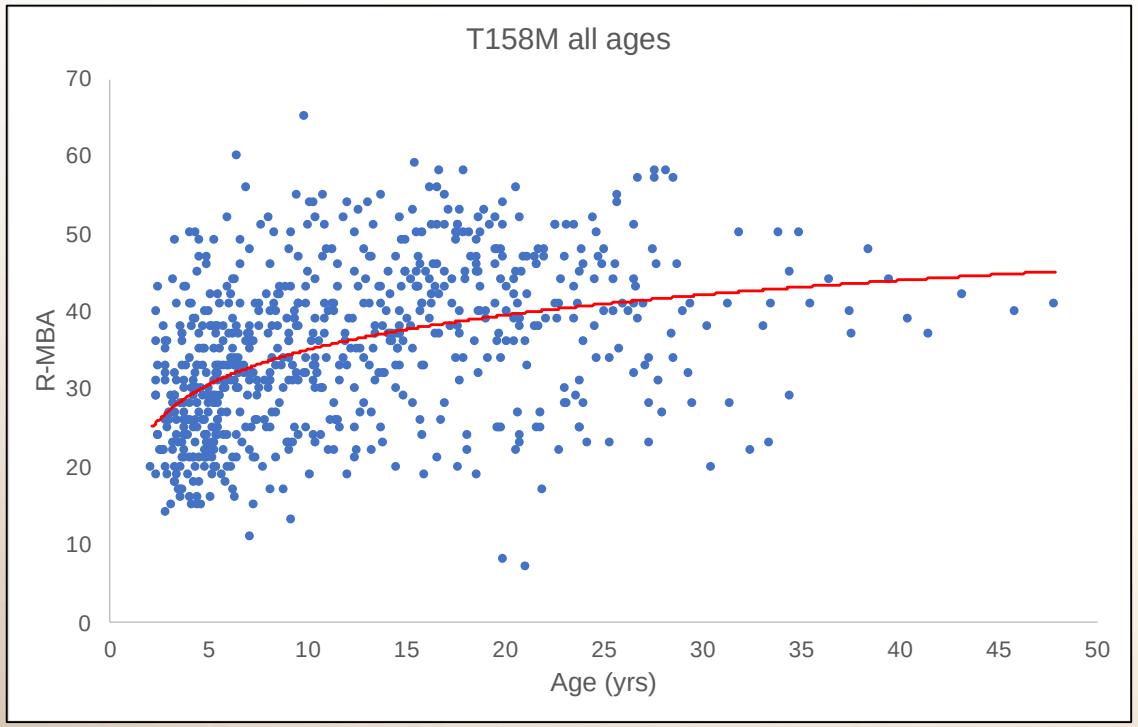


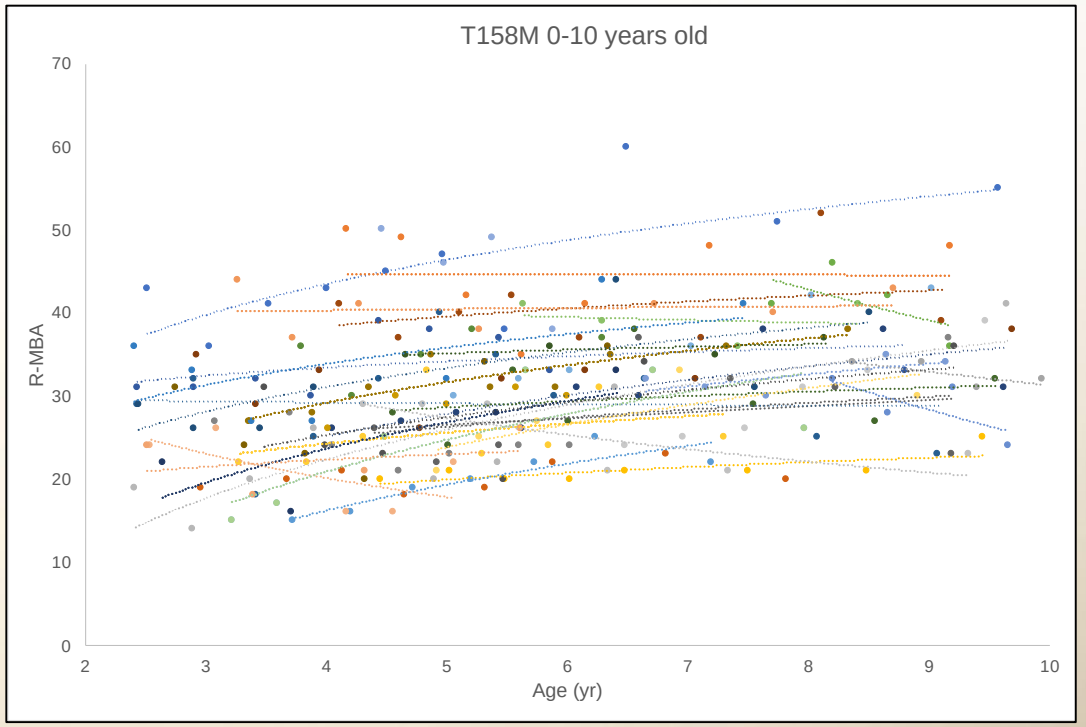
AMERICAN JOURNAL ON INTELLECTUAL AND DEVELOPMENTAL DISABILITIES
2020, Vol. 125, No. 6, 493–509

R-MBA next steps

Longitudinal assessment from natural history study data

- Evaluate trajectories over time
  - Compare across mutations
- Develop growth curves with confidence intervals





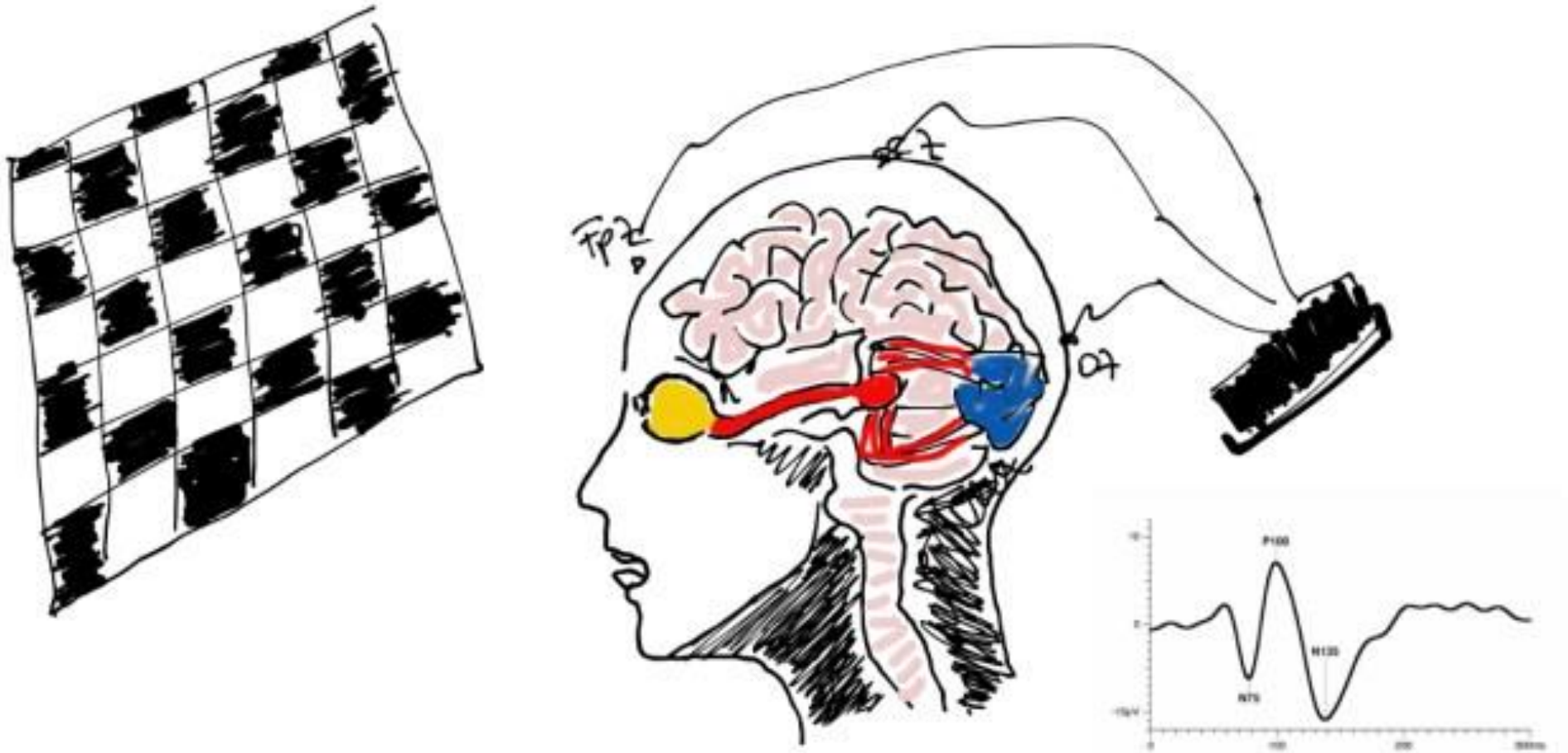
R-MBA next steps

- Establish reliability
  - Inter- and intra-rater reliability
  - Test-retest reliability
- External validity
- Development of video-based rater training and reliability platform
- Formal evaluation of sensitivity and responsivity
  - Establish the minimally clinically important difference
- Distribution based
  - Easy but based on assumptions on clinical relevance
- Anchor based
  - Tied to clinical meaningfulness
    - NHS Caregiver Impression of Improvement
    - Clinical Global Impression of Severity (CGI-S)
    - Clinical Global Impression of Improvement (CGI-I)

Current challenges for clinical trials in RTT

- Outcome measures
  - Need psychometrically reliable and valid measures
- **Biomarkers**
  - Disease severity
  - Identify treatment responders
  - Early predictive biomarkers of treatment response

Neurophysiological biomarkers – Evoked potentials



https://www.neuro-la-cote.info/uncategorized/do-not-forget-the-visual-evoked-potentials-pev/

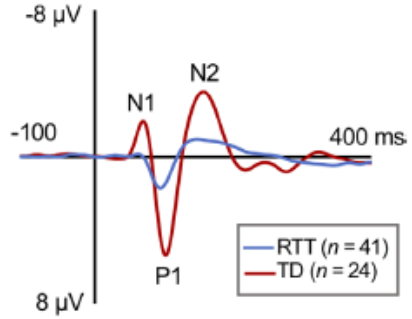
VANDERBILT KENNEDY CENTER

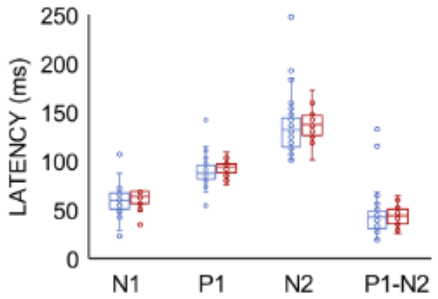
Multisite Study of Evoked Potentials in Rett Syndrome

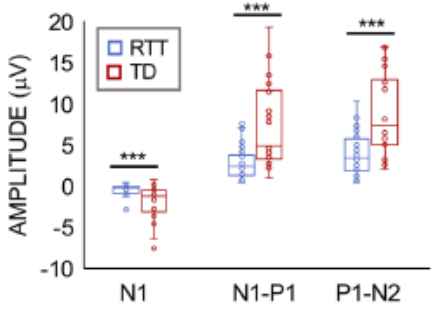
Joni N. Saby, PhD,[1] Timothy A. Benke, MD, PhD,[2] Sarika U. Peters, PhD,[3]
Shannon M. Standridge, MD,[4] Junko Matsuzaki, PhD,[1] Clare Cutri-French, BA,[5]
Lindsay C. Swanson, MS CGC,[6] David N. Lieberman, MD PhD,[6] Alexandra P. Key, PhD,[7]
Alan K. Percy, MD,[8] Jeffrey L. Neul, MD PhD,[3] Charles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

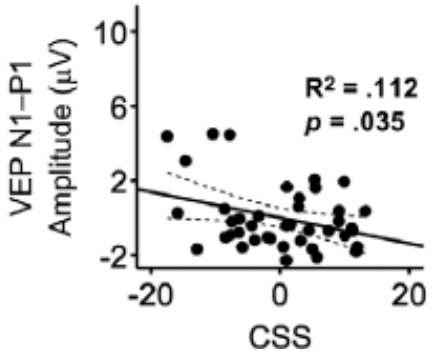
ANN NEUROL 2021;00:1–13

Visual evoked potentials

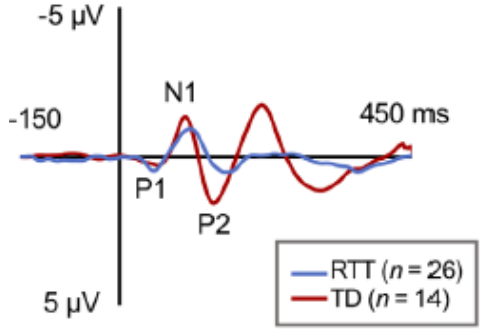


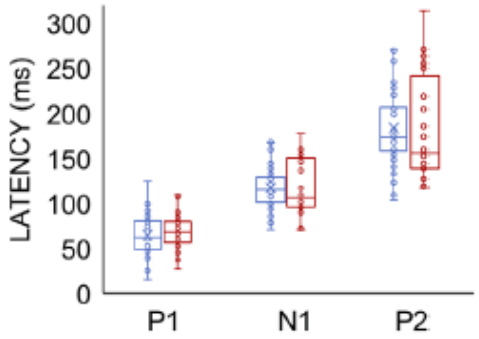


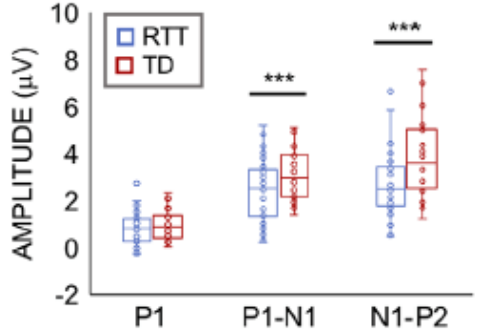


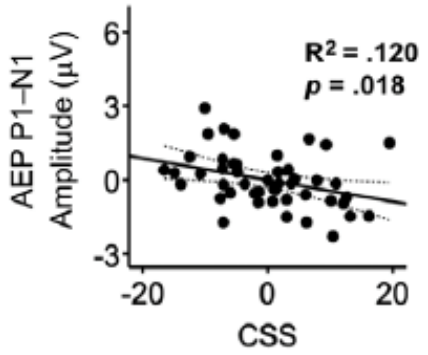


Auditory evoked potentials



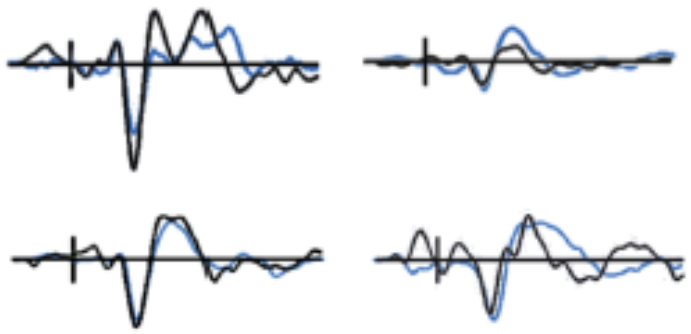


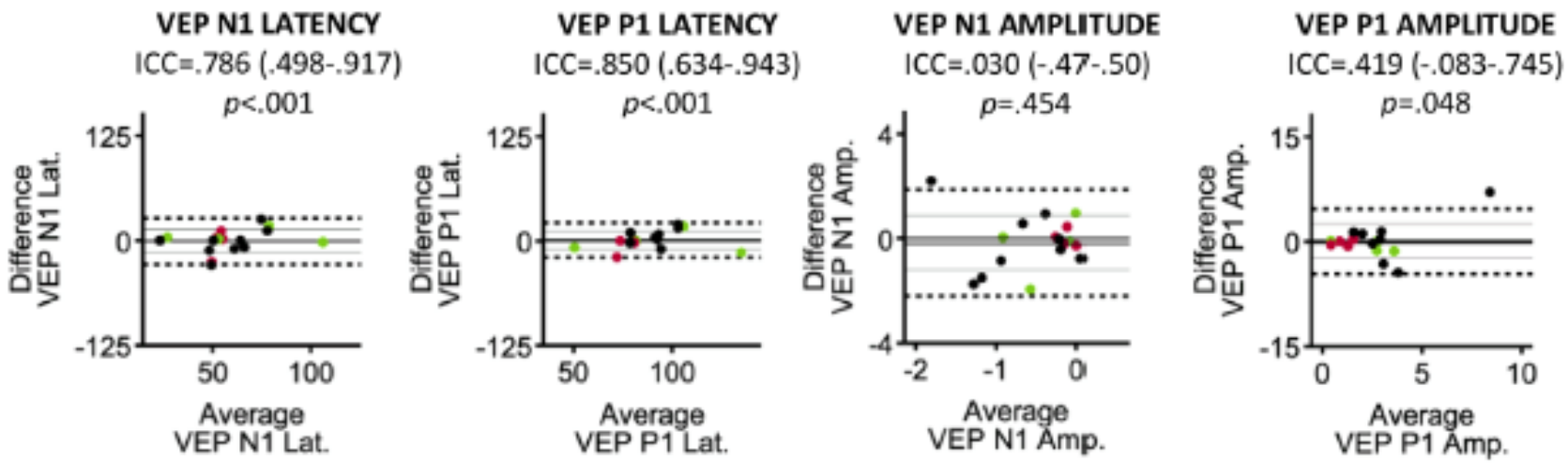




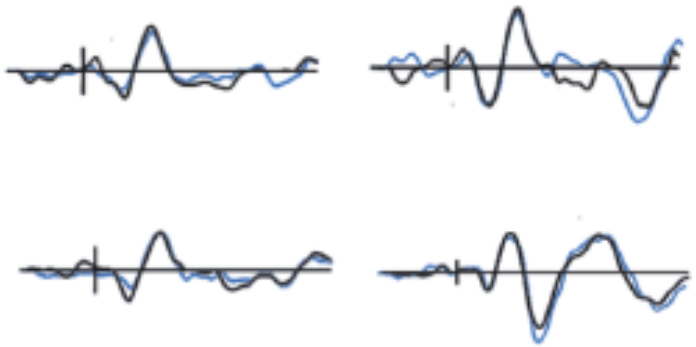
Repeated evoked potentials – 1 year interval

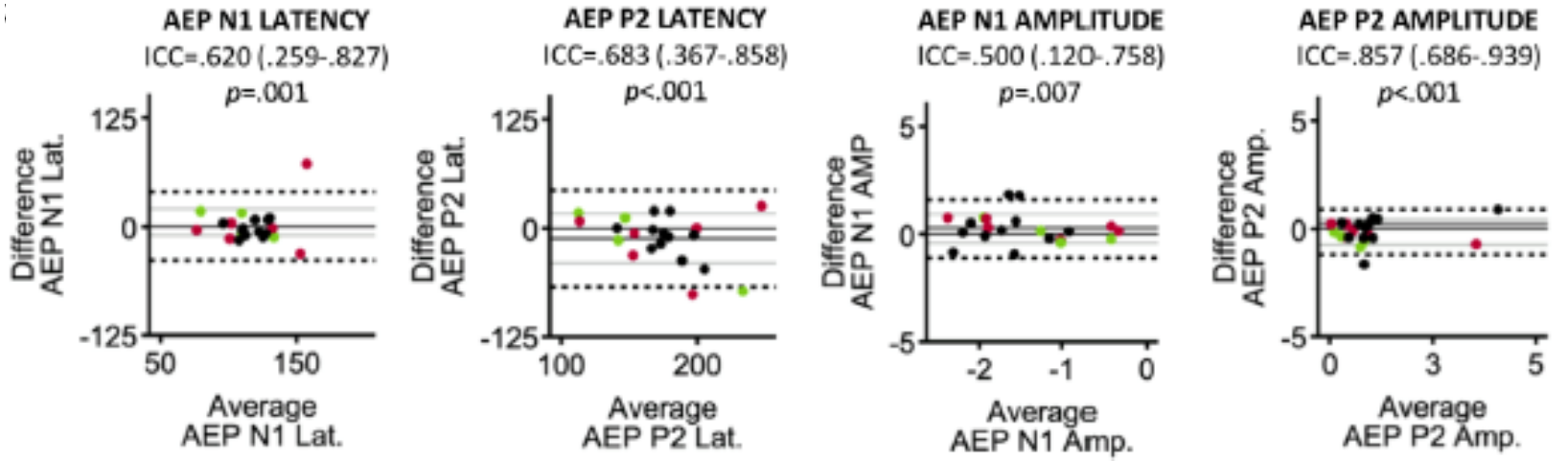
Visual evoked potentials





Auditory evoked potentials





ANN NEUROL 2021;00:1–13

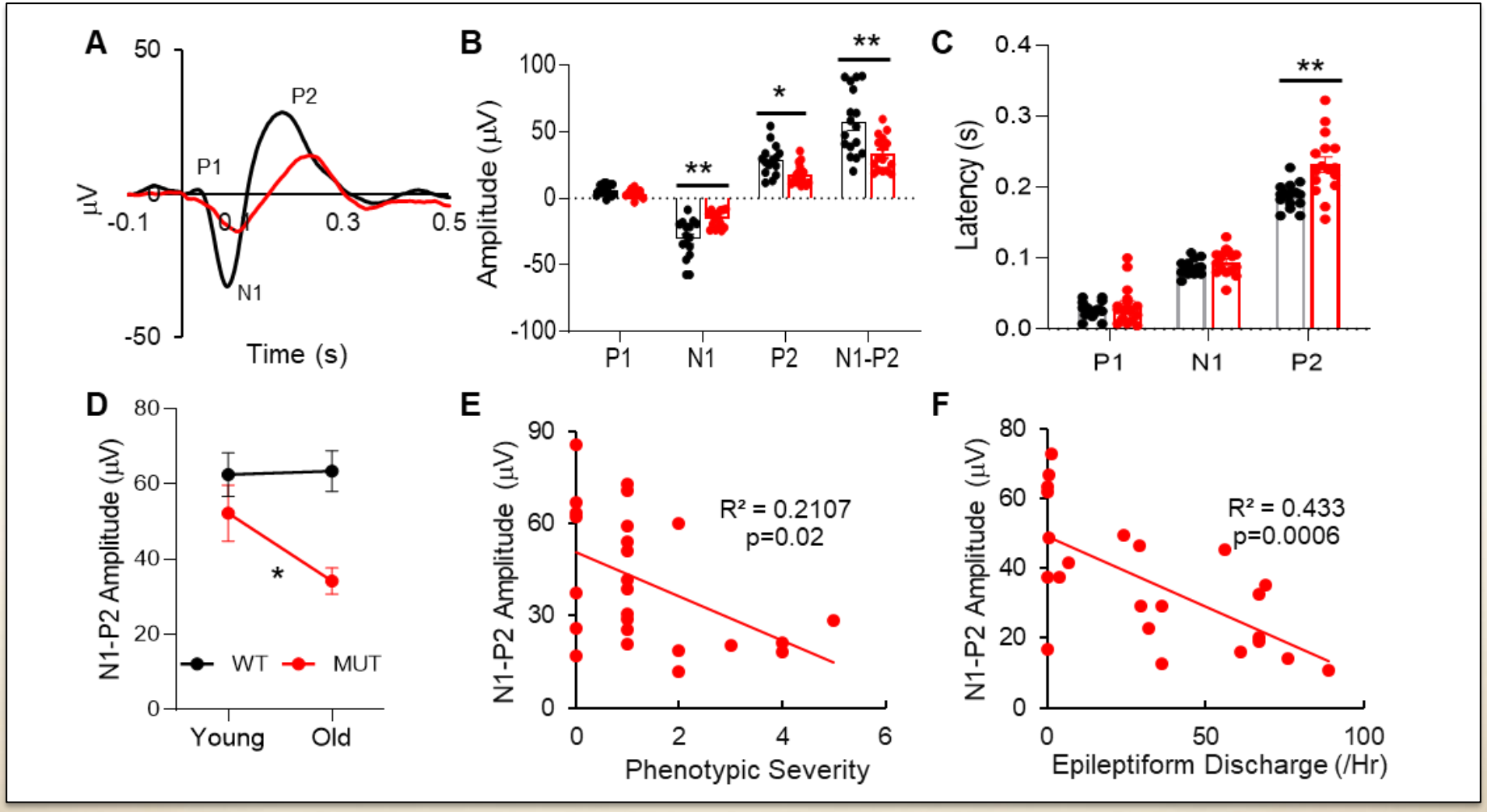
Detection of neurophysiological features in female R255X MeCP2 mutation mice

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

RTT

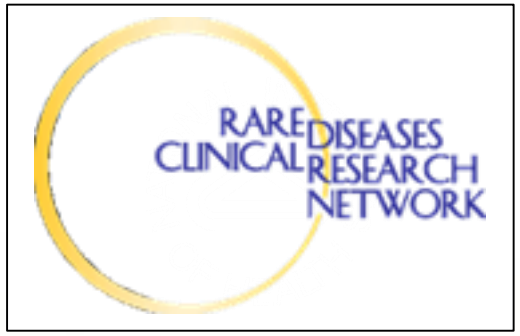
WT



Biomarkers – Neurophysiology

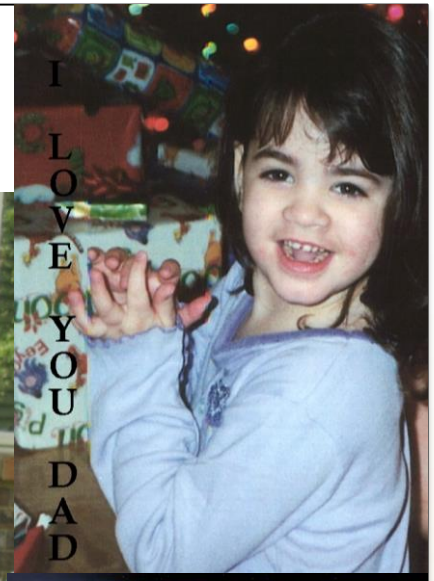
Next steps

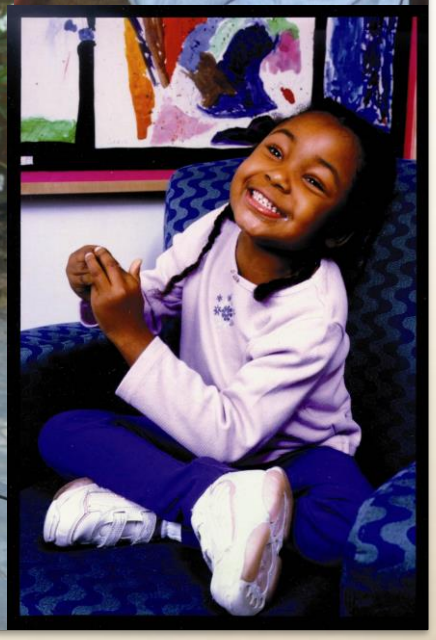
- Characterize neurophysiological features longitudinally in mouse models of Rett syndrome
- Restore MeCP2 expression post-symptomatically in mice and look at changes in neurophysiological features



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, Walter Kaufmann
Children's Hospital Boston
Mustafa Sahin, David Lieberman
Oakland Children's Hospital
Gillette Children's Hospital
Cincinnati Children's Hospital
Cleveland Clinic
Washington University
Rush University

Thanks to my friends!





Q & A

Disease Burden – Patient and Family Perspective

Monica Coenraads

CEO of Rett Syndrome Research Trust (RSRT)

Disease Burden – Patient and Family Perspective

Monica Coenraads

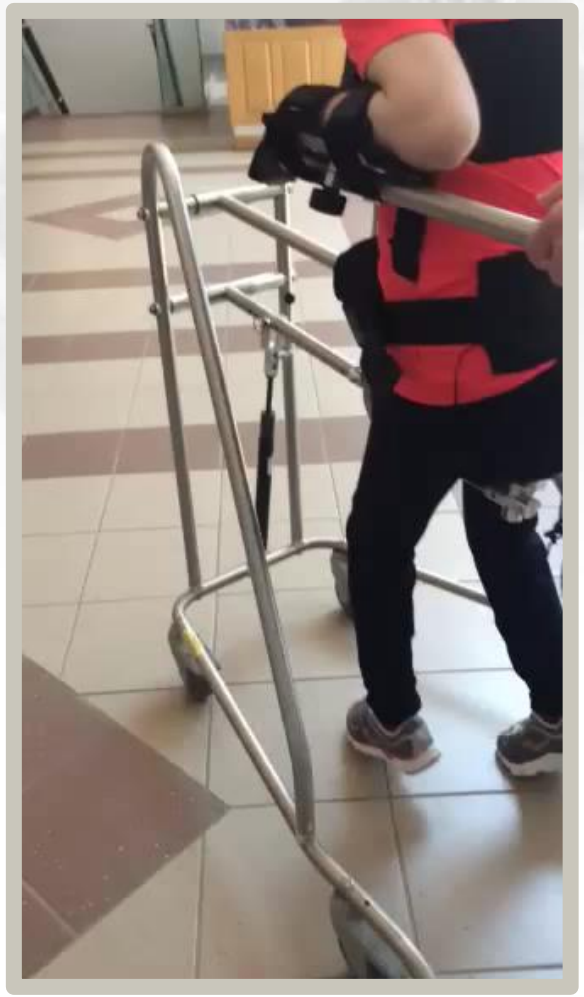
Chief Executive Officer,
Rett Syndrome Research Trust

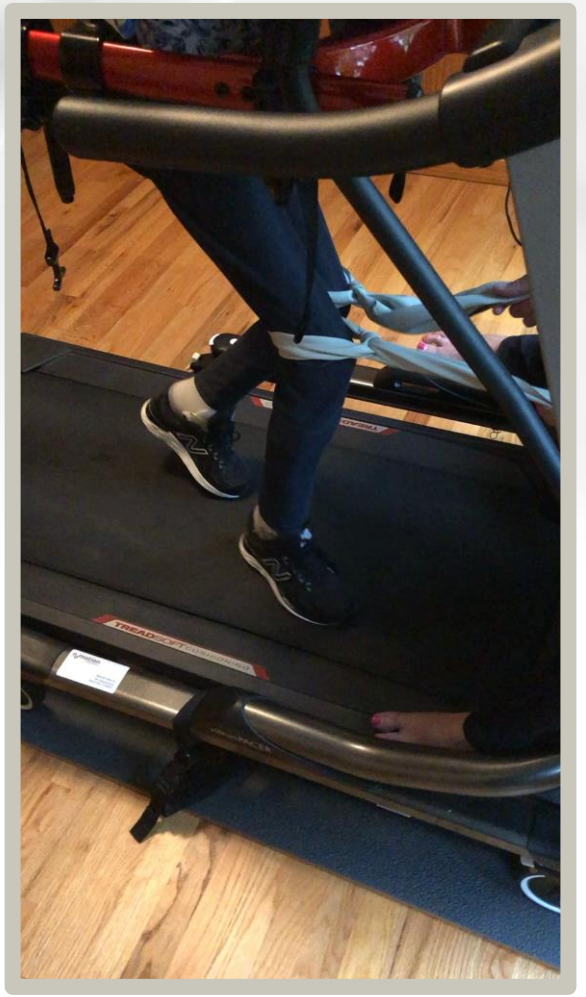
rett syndrome research trust

Early signs

Ambulation

Ambulation





Autonomic issues

- Disordered breathing
- Constipation / urine retention
- Oral motor difficulties
- Cold blue feet / hands
- Abnormal heart rate, long QT

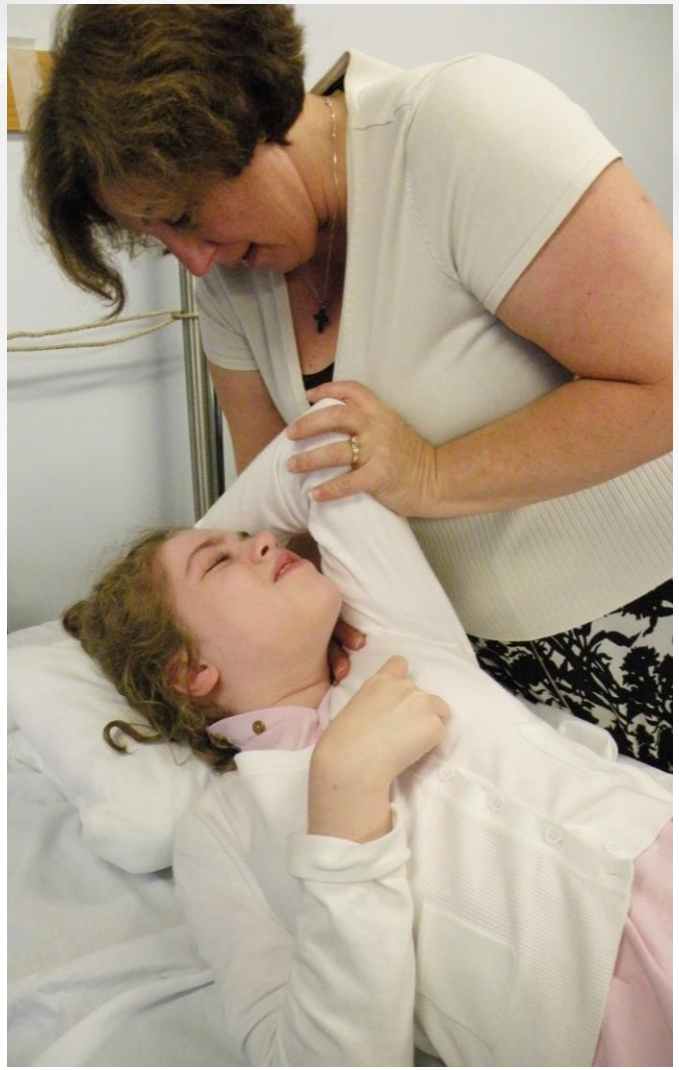
Movement issues

Other symptoms

- Seizures
- Scoliosis / kyphosis
- High tone
- Anxiety
- Sleep issues
- Teeth grinding

Patient care & medical management

- Patients require 24-hour care
- Frequent hospitalizations
- Surgeries for orthopedic and GI issues
- Multiple medications needed to manage CNS and other symptoms
- Need for special education, physical, speech and occupational therapy
- Extensive array of durable medical equipment needed
- Home health aides and nurses often required



Common medications

TYPES

- Anti-seizure
- Reflux
- Constipation
- Anxiety / sleep meds
- Depression
- Anti-psychotic
- Osteoporosis
- Movement disorder drugs
- Heart medications

CHELSEA'S MEDS

- Valproic acid / Lamictal
- Primidone
- Propranolol
- Baclofen
- Chloral hydrate / melatonin
- Clonazepam
- Botox
- Atrovent
- Inhaled steroids

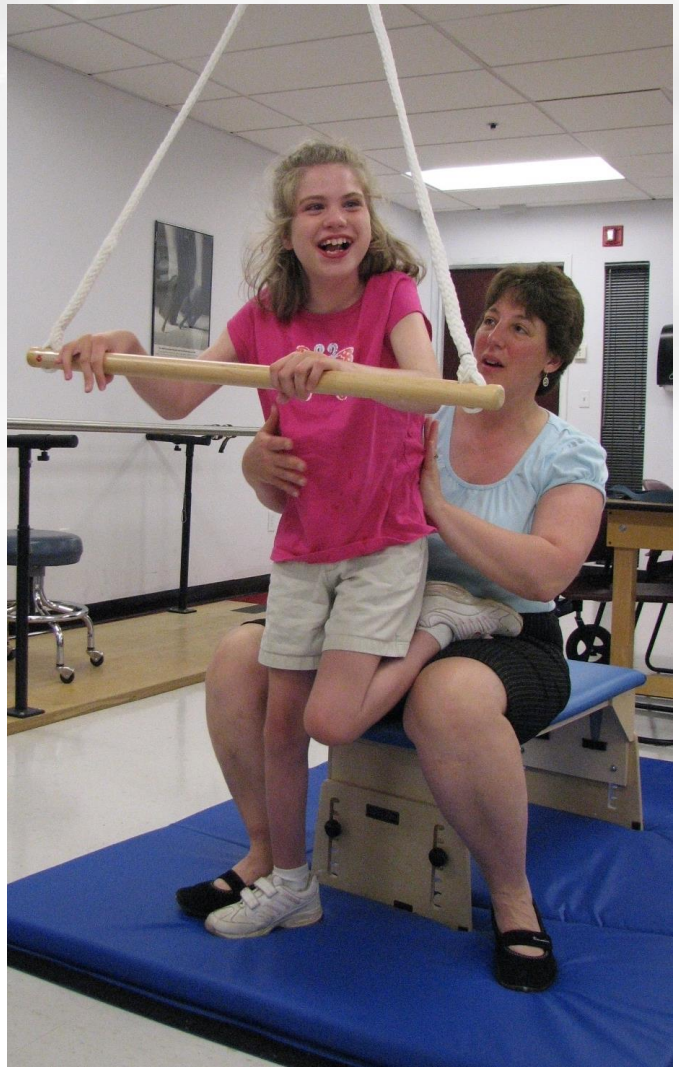
Therapies

MAINSTREAM

- Physical
- Occupational
- Speech

ALTERNATIVE

- Craniosacral
- Massage
- Hydrotherapy
- Hippotherapy
- Chiropractor
- Music therapy



Chelsea's stuff

Education

Leisure

Family life


MAYO
FAMILY HEALTH
GOLF IN AMERICA
STAMFORD HIGH SCHOOL 1980
DR. ATKINS
jack
STRAIGHT FROM THE GUT
JACK WELCH
STARZL
THE EINSTEIN FILE
BIGGEST AND BEST
GOOD TO GREAT
JACK WELCH WINNING
MICHAEL LEWIS MONEYBALL
LA PADRONA SI CASA
CANDLE

Patients are waiting

miRARE Platform and Preclinical Data

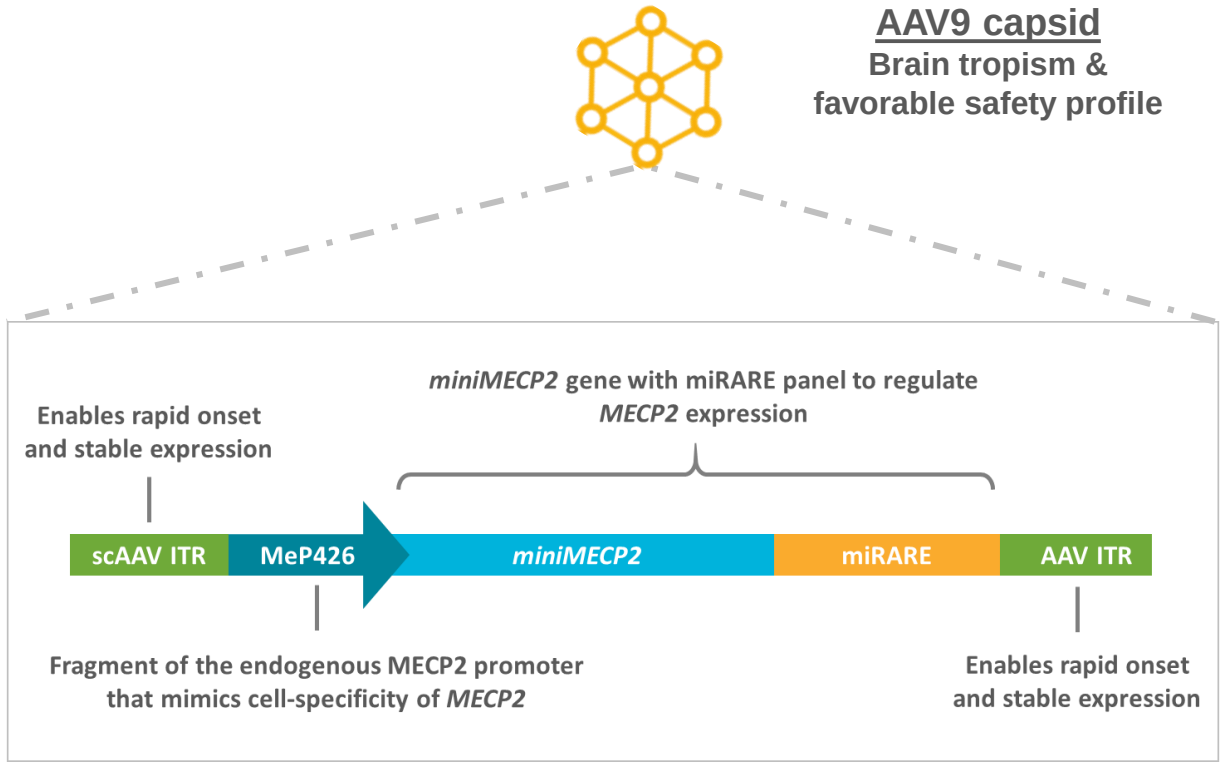
Steven Gray, PhD*

Associate Professor, Department of Pediatrics at UTSW
Chief Scientific Advisor, Taysha

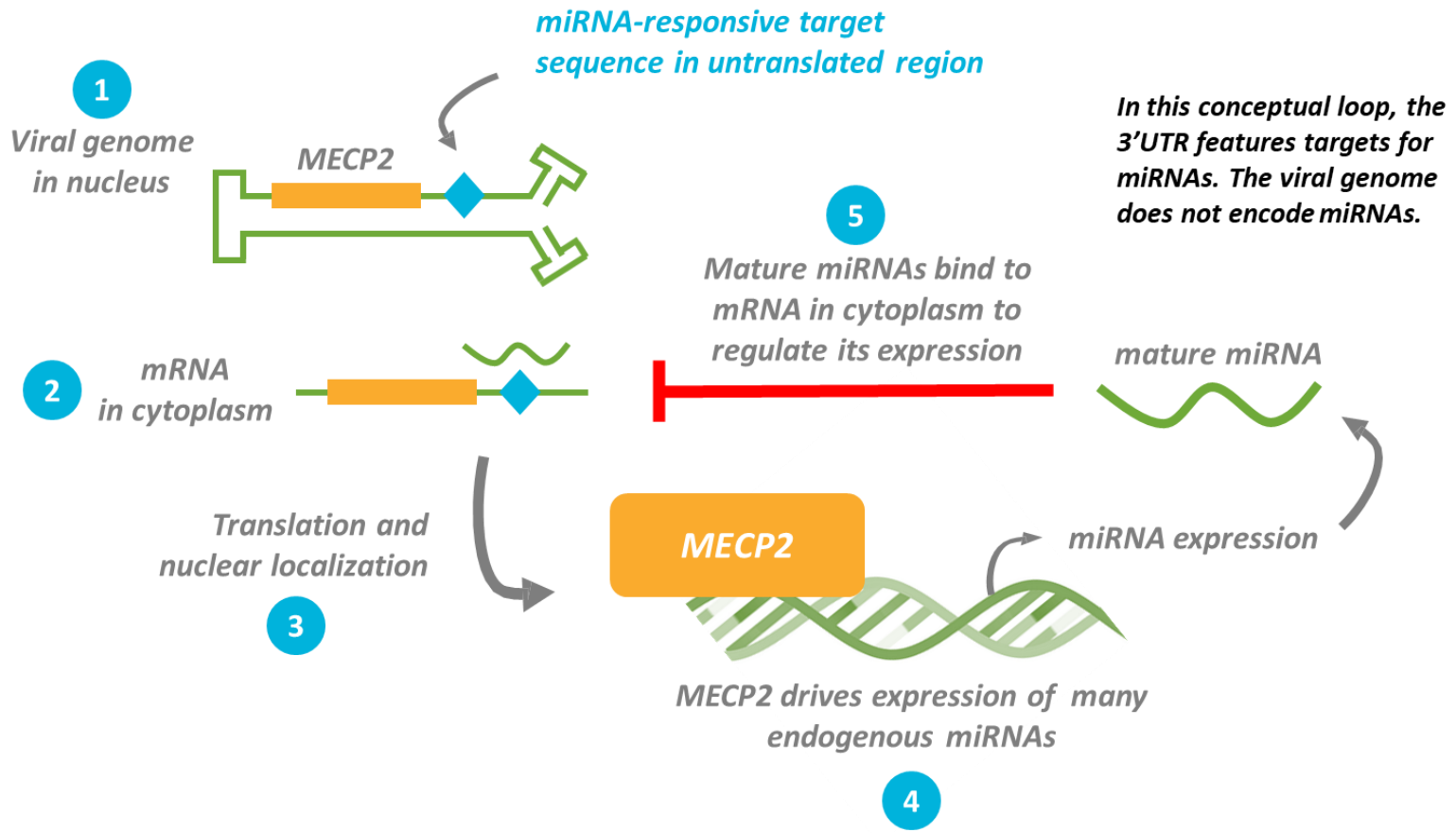
**miRARE developed with Sarah Sinnett, PhD, who leads ongoing preclinical studies for TSHA-102*

Development of a gene therapy for Rett syndrome requires regulated expression of MECP2

- AAV9/*MECP2* caused dose-dependent side effects after intraCSF administration in WT and KO mice
- We have developed a novel miRNA-responsive target sequence (miRARE) that regulates the expression of the *MECP2* transgene
- Our approach provides a superior therapeutic profile to that of unregulated *MECP2* gene replacement

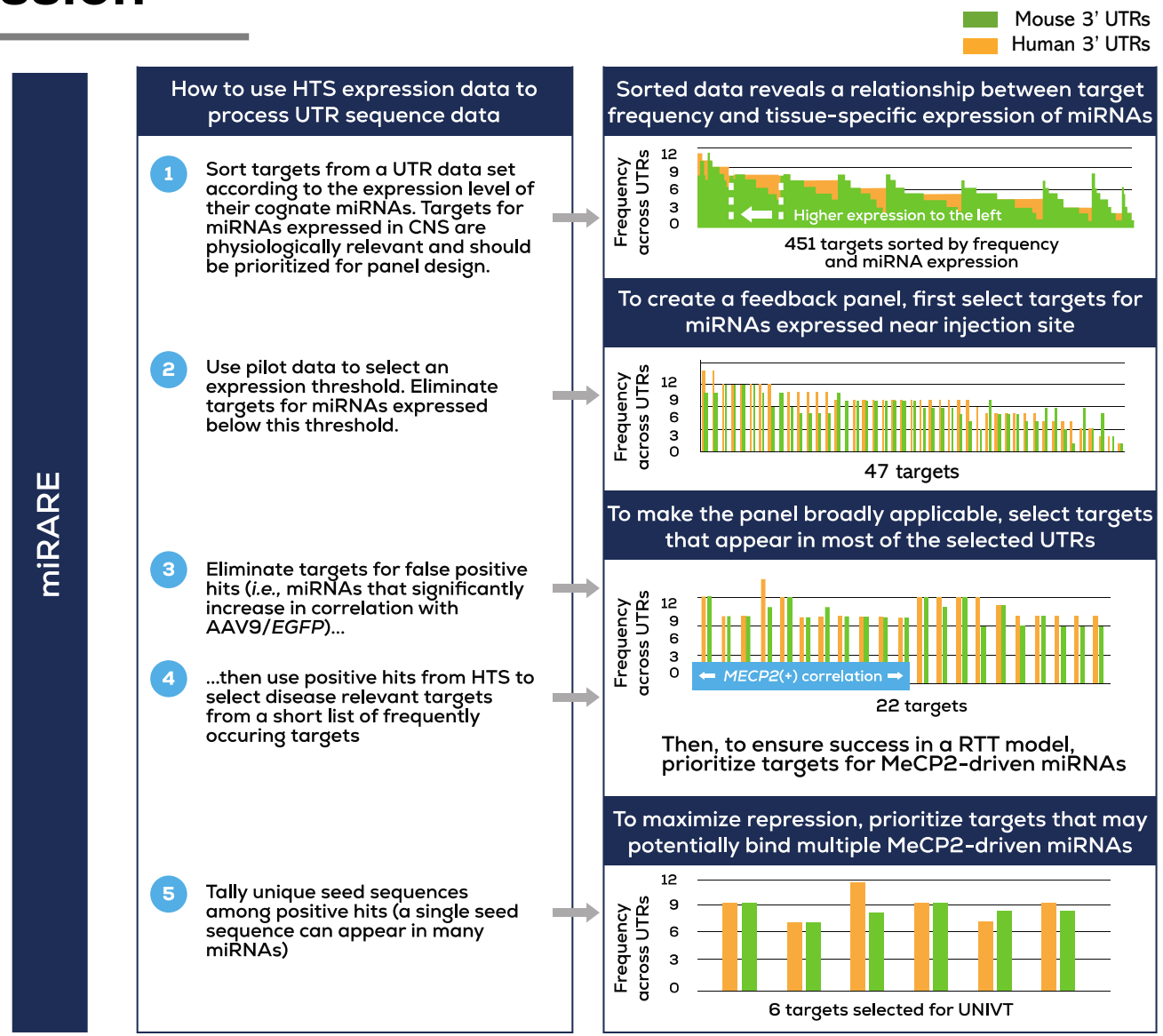


miRARE is a targeting panel for endogenous miRNAs which regulate MECP2 expression



Approaches to create a miRNA target panel for regulating MECP2 expression

- High-throughput screening of mouse CNS miRNAs upregulated by endogenous and/or exogenous MeCP2 after *MECP2* gene therapy overdose
- Identify endogenous miRNA targets that are conserved across species and appear frequently among the UTRs of dose-sensitive genes regulating intellectual ability
- Use positive results from high-throughput screening to filter and rank bioinformatics data
- Merge screening data and genomic sequence information
- Create a small synthetic (and potentially broadly applicable) regulatory panel



Preclinical data for myc-tagged version of TSHA-102 for Rett syndrome recently published in *Brain*

ACCEPTED MANUSCRIPT

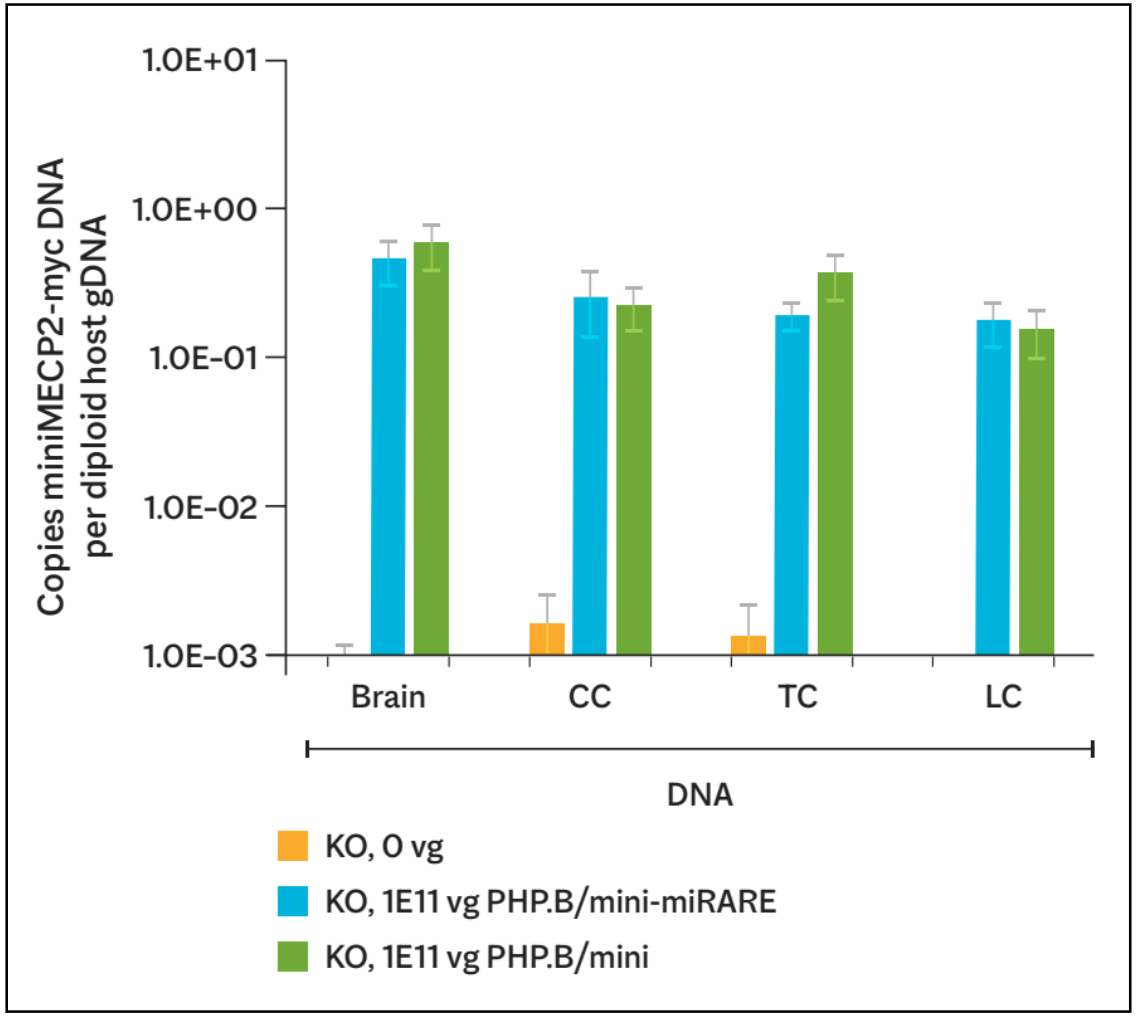
Engineered microRNA-based regulatory element permits safe high-dose mini*MECP2* gene therapy in Rett mice

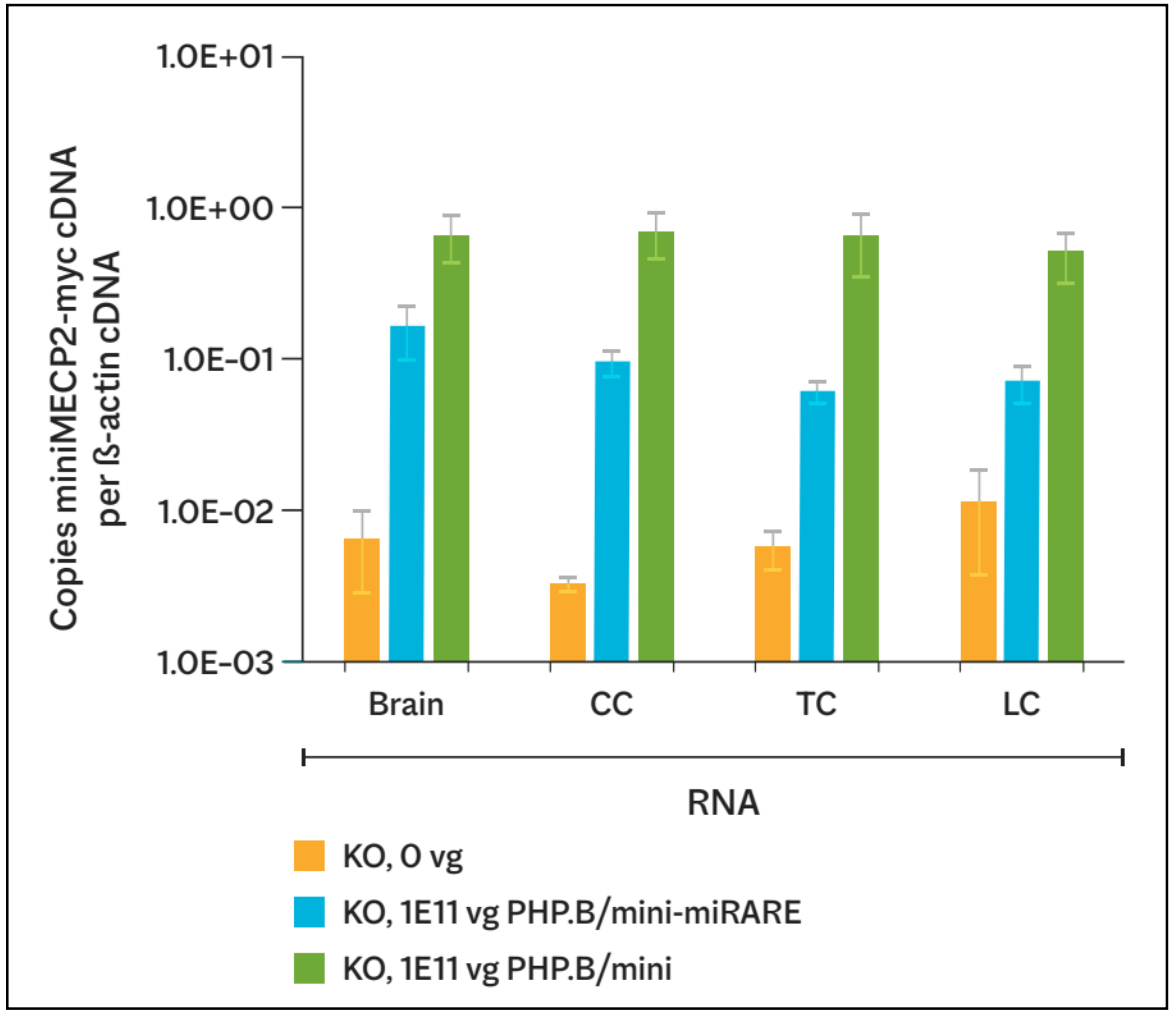
Sarah E Sinnett, Emily Boyle, Christopher Lyons, Steven J Gray

Abstract

MECP2 gene transfer has been shown to extend the survival of *Mecp2*$^{-/y}$ knockout (KO) mice modeling Rett syndrome (RTT), an X-linked neurodevelopmental disorder. However, controlling deleterious overexpression of MeCP2 remains the critical unmet obstacle towards a safe and effective gene therapy approach for RTT. A recently developed truncated mini*MECP2* gene has also been shown to be therapeutic after AAV9-mediated gene transfer in KO neonates. We show that AAV9/mini*MECP2* has a similar dose-dependent

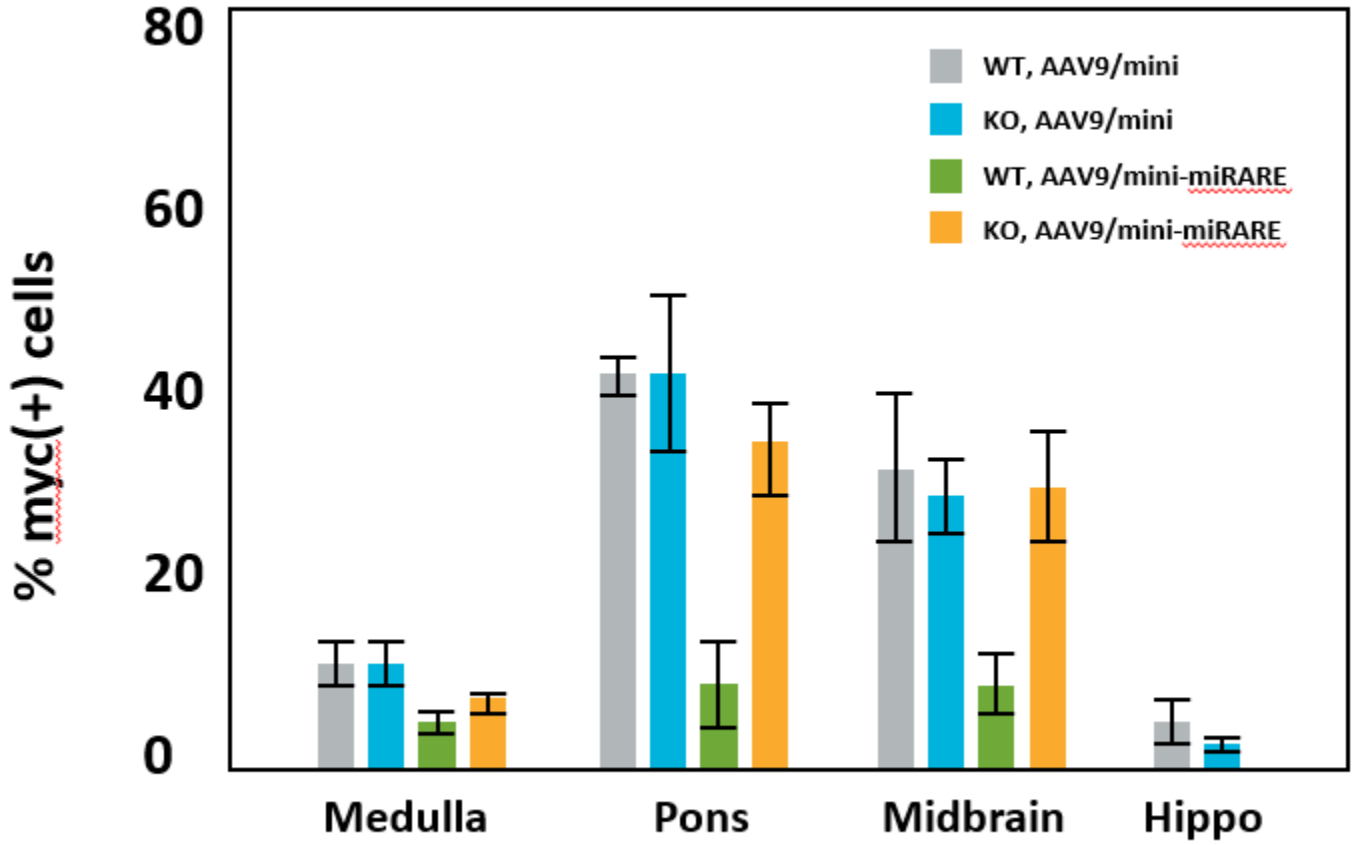
miRARE reduced overall expression of mini*MeCP2* transgene expression compared to unregulated mini*MeCP2* in WT mice



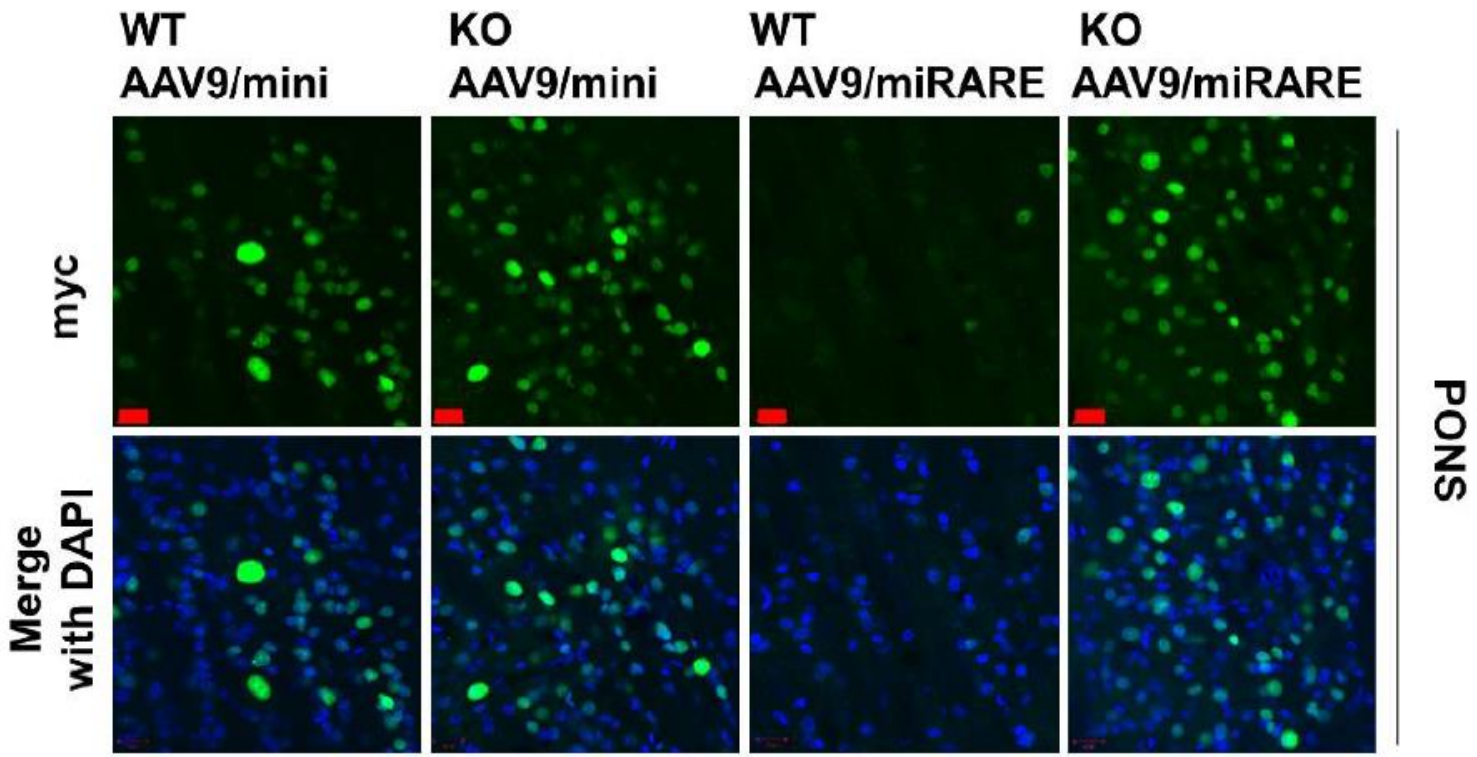


CC: Cervical Cord; TC: Thoracic Spinal Cord; LC: Lumbar Cord

miRARE regulated genotype-dependent *MECP2* expression across different brain regions in wild type and Rett KO mouse models



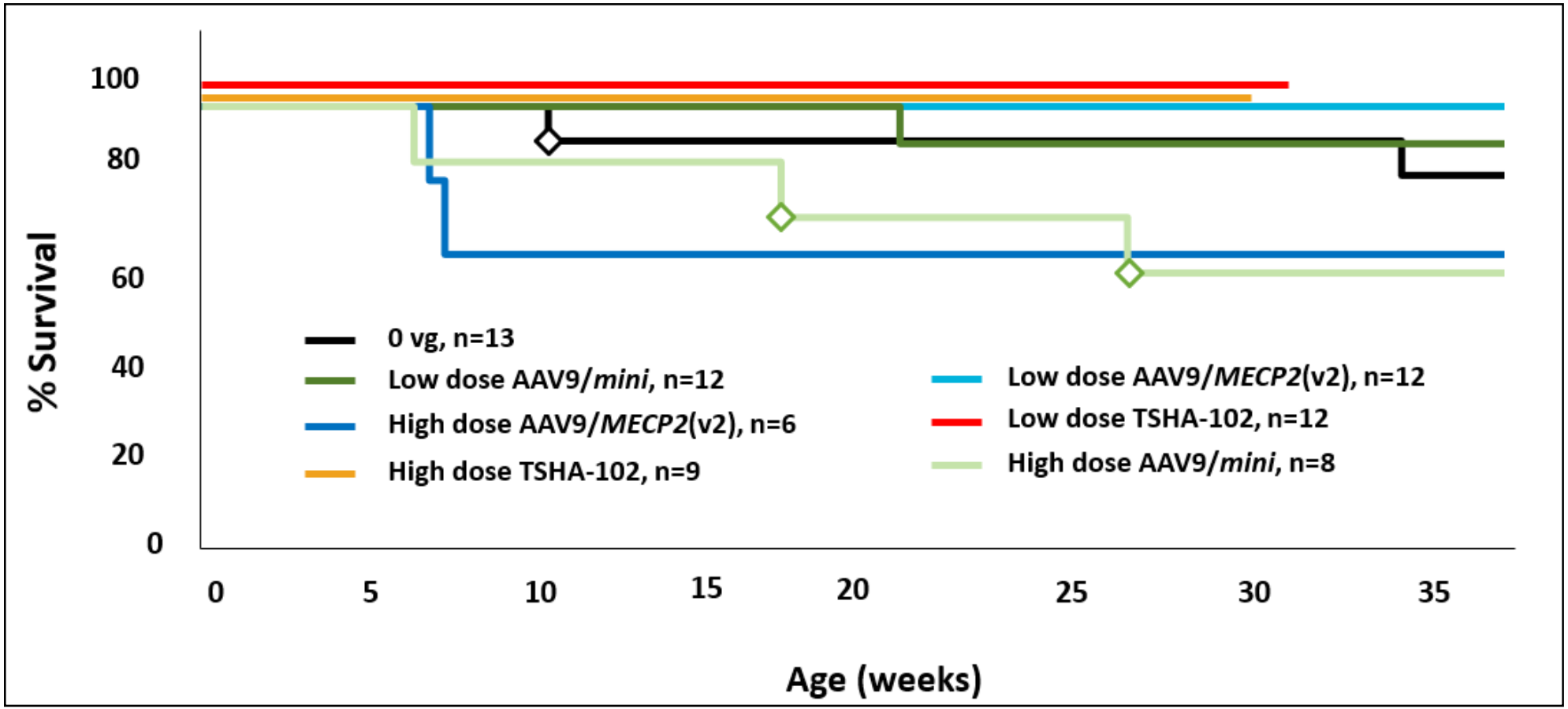
miRARE regulated expression in pons and midbrain based on a cell-by-cell basis



Overview of published MeCP2 gene therapy studies leading to the development of TSHA-102

#	Study Scope	Model	Age at dosing	RoA, Vector, & Dose (vg/animal)	Findings
1	Efficacy & Safety (Gadalla, 2013)	MECP2 KO male mice	Postnatal Day 0 to 3	Intracranial injection: ssAAV9-CBA-hMECP2-Myc 4.8 x 10^{10} vg/mouse ssAAV9-CBA-GFP	• Median survival was 16.6 weeks for vector treated versus 9.3 weeks for *GFP* treated mice. Vector treated mice also showed significant improvement in the phenotype severity score, in locomotor function, and in exploratory activity, as well as a normalization of neuronal nuclear volume in transduced cells • No improvement in respiratory phenotype (erratic breathing pattern, apnea)
		MECP2 KO male mice	4 to 5 weeks (juvenile)	scAAV9-MeP229-hMECP2-Myc (1st generation, v1): 1x10^{11} (low dose), 5x10^{11} (high dose) vg/mouse, IV scAAV9-MeP-GFP: 1x10^{10} vg/mouse (control), IV	• The intravenous delivery of scAAV9/MeP-MECP2 was less efficient in brain transduction • Still, median survival was significantly longer in the vector treated MECP2 KO mice in comparison to untreated MECP2 KO mice • There was no effect of scAAV9-MeP229-MECP2-Myc treatment on body weight
2	Efficacy & Safety with 1st and 2nd generation vectors (Gadalla, 2017)	WT, MECP2 KO male or $Mecp2^{T158M/y}$	4 to 5 weeks (juvenile)	scAAV9-MeP229-hMECP2-Myc (1st generation, v1): 1x10^{11} vg/mouse (low dose), IV 1x10^{12} vg/mouse (mid dose), IV 1x10^{13} vg/mouse (high dose), IV	• Increased survival at mid-dose; • Liver toxicity in WT mice at all doses • Increased survival and body weight in $Mecp2^{T158M/y}$ mice • No improvement of disease severity score
		MECP2 KO male mice	Neonatal	scAAV9-MeP426-hMECP2-Myc-RDH1pA (v2) (2nd generation vector): 1x10^{11} vg/mouse, ICV	• Median survival of the vector-treated mice extended to 38.5 weeks compared to 12.4 weeks in control mice; negligible impact on body weight • Clear improvement in disease severity score in the vector-treated mice
3	Efficacy & Safety with 2nd generation vector (Sinnett, 2017)	WT or MECP2 KO male mice	4 to 5 weeks	scAAV9-MeP229-hMECP2-Myc (v1), 1x10^{12}, IT or ICM scAAV9-MeP426-hMECP2-Myc-RDH1pA (v2), 1x10^{10}, 1x10^{11}, 1x10^{12} vg/mouse, ICM	• scAAV9-MeP229-MECP2-Myc vector was toxic in both routes • scAAV9-MeP426-MECP2-Myc-RDH1pA extended lifespan, increased body weight, increased growth rate. Increased adverse effects at 1x10^{12}
4	Efficacy & Safety (Sinnett, 2021)	MECP2 KO male mice	4 to 5 weeks	Percutaneous intrathecal or intracisternal injection 1x10^{11} to 1x10^{12} vg/mouse TSHA-102 (with myc tag) 1x10^{11} to 1x10^{12} vg/mouse hMECP2 (v2) 1x10^{11} to 1x10^{12} vg/mouse AAV9/miniMECP2	• TSHA-102 appeared to delay the approximate age of onset of severely abnormal gait by 4-5 weeks with a similar or lower frequency of occurrence in comparison to all other MECP2 KO groups.

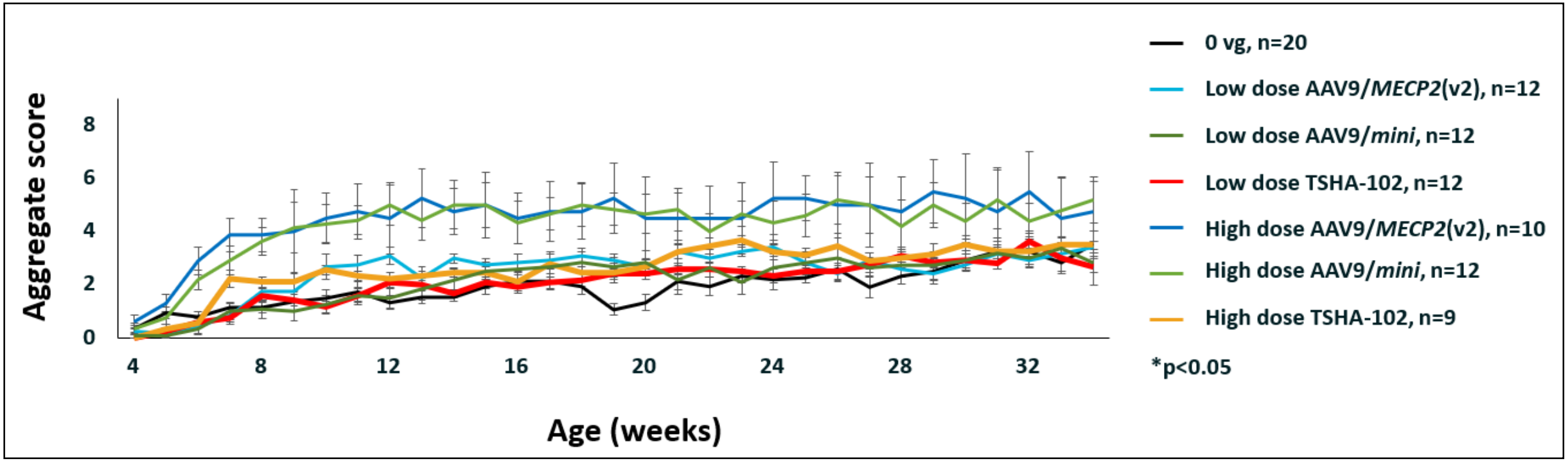
Safety – Intrathecal myc-tagged TSHA-102 was not associated with early death in WT mice



Mice were dosed P28-35

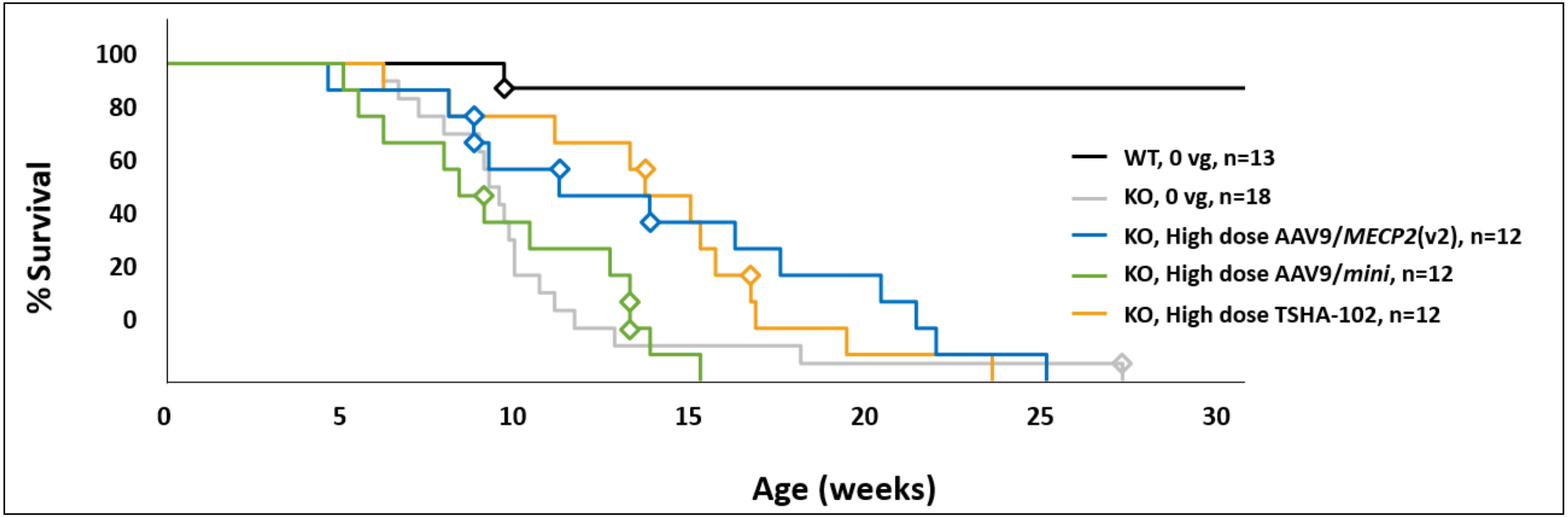
Diamond = vet-requested euthanasia for prolapse or bullying-related injury

Safety – myc-tagged TSHA-102 did not cause adverse behavioral side effects in WT mice



Mice were dosed P28-35

Efficacy – myc-tagged TSHA-102 outperformed unregulated AAV9/mini in *MECP2* KO mouse survival study



Mice were dosed P28-35

Diamond = vet-requested euthanasia, primarily for lesions. Lesions have been observed with varying frequencies among saline-treated KO mice, virus-treated WT and KO mice, as well as untreated RTT weanlings.

Q & A

Clinical Development Strategy

Suyash Prasad, MBBS, MSc, MRCP, MRCPCH, FFPM

Chief Medical Officer and Head of R&D

Rett syndrome TSHA-102 program designations

- Rare pediatric disease designation from the FDA
- Orphan drug designation from the FDA
- Orphan drug designation from the European Commission

TSHA-102 program completed milestones and updates

Conducted advisory boards in first half of 2021

Conducted patient focus groups in the early part of the year

Overall positive regulatory feedback with multiple key regulatory agencies

Completed GMP manufacturing using commercial process

Deployed clinical site feasibility assessments internationally in 2021

Advisory board overview

Scientific advisory board of preeminent international scientific and clinical thought leaders in Rett syndrome, gene therapy, CNS diseases, and developmental pediatrics

Feedback from global, rare disease expert KOLs during program advisory boards is used to inform clinical study design and plan regulatory interaction

Advisors provided insightful recommendations on the current clinical study design, preclinical results, and utility of available natural history data

Recommendations for patient selection, inclusion / exclusion criteria, and outcome assessments were obtained

Key thoughts from advisory board

MECP2

- Small amounts of MECP2 gene expression can lead to significant improvements in survival
- In contrast, excessive MECP2 gene expression can lead to toxicity (≥50% above null background leads to toxicity in preclinical models), but there is uncertainty as to what level precisely leads to adverse events both in animal models and humans
- Current preclinical data in male mice provides useful proof-of-concept data for the novel miRNA-responsive target sequence (miRARE), which regulates MECP2 transgene expression
- One of the primary challenges is the broad transduction across the central nervous system (CNS), which may require high doses; however, there is potential toxicity with over-expression of MECP2, getting the balance right will be critical

Nonclinical

- Treating neonatal mice would likely yield more robust/positive preclinical results
- A dose-range study in female mice would provide additional guidance for dosing in human trials; data needed in female mouse models including additional outcomes (e.g., levels of gene/protein expression) beyond mortality
- Data in nonhuman primates may translate more directly to humans (compared with mouse models)

Key thoughts from advisory board

Clinical Trial Design

- Dosing early in young patients will likely yield maximum efficacy; there is a potential benefit with initially testing in older children or adults to ensure safety and tolerability in humans
- High doses may be needed for broader distribution and transduction of more cells
- Select outcome measures specific to the patient and which produce clinically meaningful change for patients and families (important to FDA, EMA and all regulatory agencies in general)
- Potential safety concerns with TSHA-102 include dorsal root ganglion toxicity and immunologic reactions (specifically in patients with null mutations)

Outcome Measures

- Much work has been done in the assessment of outcome measures in Rett syndrome
- EEG recordings will be an important evaluation for RTT patients; up to 80% of girls with RTT have severe epilepsy and ongoing seizure abnormalities
- If the therapy is effective, changes in dendritic spines may be seen at the structural level
- Continuation of supportive rehabilitation therapies will be required in patients that show efficacy with TSHA-102

KOL advisors were enthusiastic and optimistic about Taysha's Rett syndrome gene therapy program

We work closely with patients and families to inform our clinical development plan

CURIOSITY

Understand the patient experience, including most challenging symptoms and QOL impacts

Identify patient-centric endpoints and meaningful outcomes

Uncover educational gaps for families about gene therapy and clinical trials

COLLABORATION

Develop clinical trial protocols based on patient and family insights

Partner with community to raise awareness and recruit clinical trials

Co-create the optimal clinical trial support to enhance experience and aid retention

Patient / caregiver input into TSHA-102 clinical study design

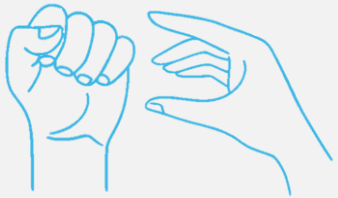
We routinely engage with caregivers of loved ones with rare diseases to learn about their experiences, needs, and therapeutic priorities as well as to inform clinical study design

Caregivers shared perspectives on Rett syndrome symptoms and priorities via an in-depth focus group. 20 caregivers of individuals with Rett Syndrome participated; 5 caregivers of children age 2-6, 6 caregivers of children age 7-12, 5 caregivers of children age 13-18, and 4 caregivers of adults age 19+, with the assistance of Rett Syndrome Research Trust and the International Foundation for Rett Syndrome

A confirmatory online survey was conducted with over 300 caregivers of loved ones with Rett syndrome ages 2-50+ years old

Most challenging symptoms over the years

While there are many impactful symptoms, caregivers (n=20): were asked to rank the 5 most challenging symptoms of Rett syndrome, showing both some key differences as well as similarities for Rett Syndrome over the years.



Loss of purposeful hand use and **loss of speech/communication** topped the list for nearly every single caregiver across age groups making them clearly the most challenging aspects of Rett syndrome regardless of disease progression or age.

Gastrointestinal issues was a significant challenge for caregivers of children aged 2-18 years; however, only one caregiver of a girl aged 19+ years found this to be one of the most challenging aspects. The 19+ age group was less severely impacted by Rett syndrome, perhaps because the girls who make it to adulthood have less severe forms of the disease.

Breathing issues are more significant for children younger than age 12 years. Typically, this symptom was breath-holding that was worrisome or swallowing that caused bloating and pain. For most families, breathing issues are symptoms that go away with time and caregivers find them less burdensome.

Mobility issues were cited by caregivers from all age groups but became more of a unanimous issue for caregivers of children aged 13 years and older. This is because mobility issues persist and worsen as girls grow older and at the same time girls become heavier and parents become older themselves, making the moving of girls for transfers, toileting, and daily needs significantly more challenging.

Anxiety was a significant issue for all age groups except age 13-18 years. Since it is suspected that the 19+ year age group was somewhat less severely impacted, anxiety may be a symptom that is replaced by symptoms that become more severe as girls age.

Involuntary hand movement was a significant challenge primarily for the youngest age group of children younger than age 6 years.

In their own words: Impact of symptoms

Speech and communication

"Not being able to hear her say what she wants or needs is truly **heartbreaking**." – Caregiver of child age 7-12

"Her lack of speech denies her the ability to let us know **what is wrong,** why she is happy, and what needs to be changed. It impacts the **entire family** and her **social abilities**." – Caregiver of child age 13-18

Loss of purposeful hand use

"It must be so **frustrating** for her to not be able to do the simplest of things; move a piece of hair out of her eyes, scratch an itch somewhere. Someone has to be **100% attentive to her 100% of the time**."
– Caregiver of child age 13-18

"It takes away so much **independence**, ability to **use her talker** more accurately, and **enjoyment of things like arts and crafts**."
– Caregiver of child age 2-6

Gastrointestinal issues

"Besides being uncomfortable, it has **caused us to stay home** at times and miss out on planned outings." – Caregiver of child age +19

"I wish her tummy didn't hurt so much all the time, and that she wasn't always so bloated and uncomfortable." – Caregiver of child age 7-12

Breathing issues

"She swallows SO MUCH air. I cannot imagine **how uncomfortable, how painful** her belly gets." – Caregiver of child age 7-12

Mobility issues

"I worry about how her lack of mobility affects her overall health. And this significantly impacts our daily lives because we have to **lift and transfer her to move her.**" – Caregiver of child age 13-18

Anxiety

"It is CRIPPLING and **destroys many days and experiences** for our family. Her screaming is unbearable sometimes."
– Caregiver of child age 7-12

"It's debilitating but it's last on this list**. She screams and yells when she can't handle her own anxiety** - just anything to get out of the situation. So hard when she **can't tell us what's wrong**."
– Caregiver of child age 7-12

Involuntary hand use

"**Therapies and doing day-to-day activities are difficult** because of her hand wringing and mouthing. It makes it difficult for her to play with toys, pick up her food or her bottle." – Caregiver of child age 2-6

Most desirable outcome

Hopes for gene therapy varies based on the child's age:

- **The youngest age finds treating the genetic root cause** most important, which is lower on the list of all other age groups. Perhaps this is because young children still have more abilities.
- **Symptom reduction and improvement of overall function** was most important to caregivers of children age 7 or older.
- **Stopping or slowing disease progression** was more important to the older age group in comparison to other ages.
- Unsurprisingly, **prolonging lifespan** was last on all lists. Most caregivers are concerned their daughters will outlive them and don't see value in more years without improvement.

Average rank (1-5)	Age 2-6	Age 7-12	Age 13-18	Age 19+
Improve overall function	2.6	1.67	1.4	1.25
Treat the genetic root cause	1.2	2.67	3.4	3.75
Treat and reduce symptoms	3.6	2	2	2.75
Stop or slow disease progression	3.8	4	3.6	3
Prolong lifespan	3.8	4.67	4.6	4.25

"I feel if we treat the root cause of the disease that will automatically slow down the progression. Slowing down the progression, I hope, would allow us to improve the overall function of things she was never able to do. Treating and reducing symptoms will prolong their life span."
– Caregiver of child age 2-6

"The biggest impact on her life and our lives would be if she can live more independently—if she could handle some basic functions on her own (getting up in the morning, eating, going to the bathroom, brushing teeth)." – Caregiver of child age 7-12

"At this point, improving her quality of life with improving her skills and decreasing her symptoms is most important. Treating the genetic root cause is very important for future generations. Slowing the disease and prolonging her life are important, but the quality of life would be better with improved overall function and decreased symptoms." – Caregiver of child age 19+

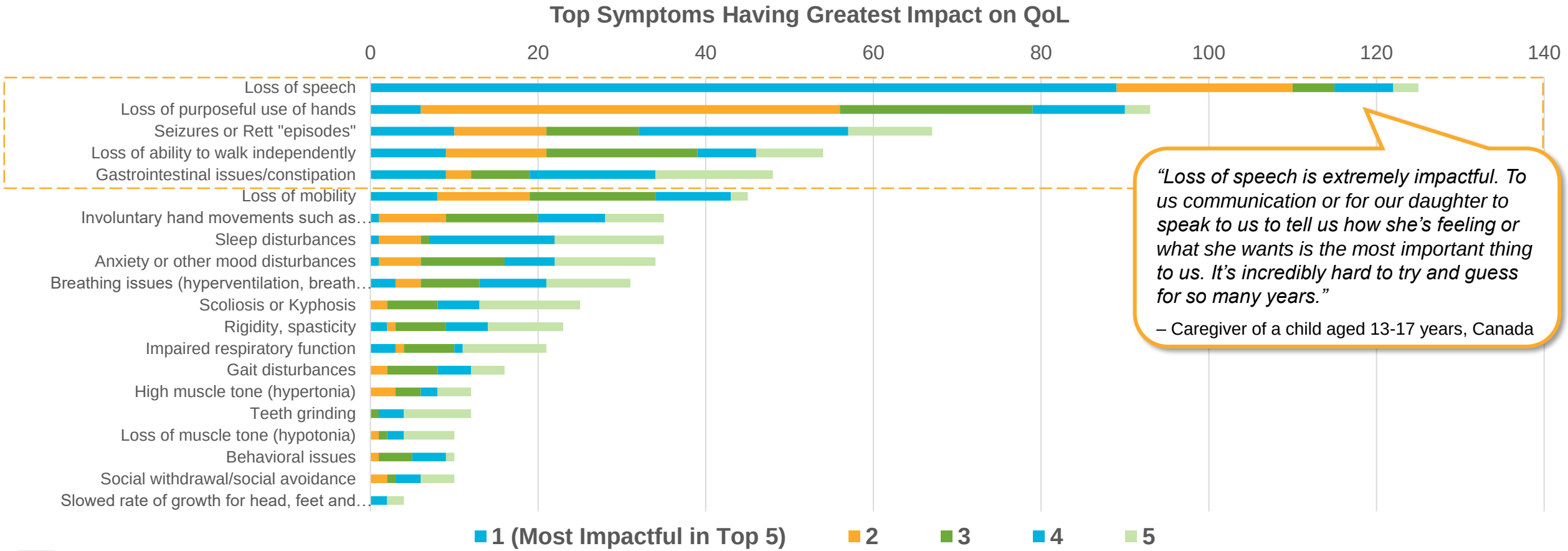
Meaningful improvements

An international survey of 323 caregivers of loved ones with Rett syndrome confirmed previous findings regarding most challenging symptoms and what would constitute a meaningful improvement

- **Communication**: The most important improvement evaluated by caregivers is the patient's ability to express their needs and opinions—it is invaluable
- **Hand Use**: Performing daily living activities, such as eating and grooming, are the most impactful to both patients and caregivers; becomes more challenging as patients age
- **Gastrointestinal Issues**: Dependence on medication and a reduction in overall pain and anxiety were the largest concerns; bowel issues were less concerning for respondents
- **Mobility**: Ability to stand and take steps become more concerning as patients age and become more difficult to carry and manage for caregivers
- **Overall Anxiety**: As patients age and patients become more agitated, the crying and screaming fits become more challenging for caregivers and families to manage
- **Breathing**: Breath-holding and hyperventilation are highly concerning for caregivers—a key source of anxiety

Symptoms of greatest impact on QoL: Patients 18+ years

Which symptoms of Rett have the greatest impact on your loved one's quality of life? Select top 5, with 1 being most impactful.



"Loss of speech is extremely impactful. To us communication or for our daughter to speak to us to tell us how she's feeling or what she wants is the most important thing to us. It's incredibly hard to try and guess for so many years."

– Caregiver of a child aged 13-17 years, Canada

N=142 patients 18+ years (US N=67; Canada N=21; Israel N=19; UK N=35)

TSHA-102 Phase 1/2 study design plan

Goals and Targets of Trial

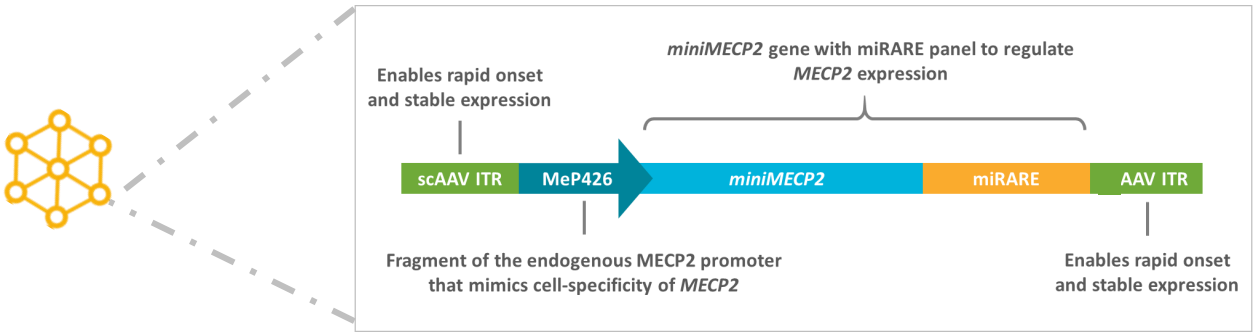
Goals

- **Primary** – Safety: clinical and laboratory assessments
- **Secondary** – Efficacy: pathologic, physiologic, functional and clinical markers

Target Recruitment

- Up to 18 subjects
- Adult females with pathogenic confirmation of mutation in *MECP2*

Product Details and Dose Cohorts



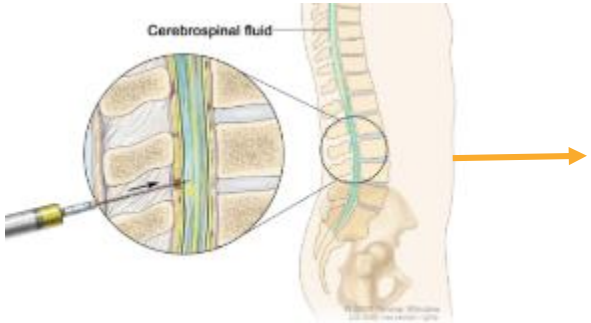
Dose Cohorts

- 3 + 3 study design, including 3 randomly selected delayed-treatment control (DTC) participants in each cohort, and each cohort may be expanded with up to 3 additional participants
- 5 x 10^{14} total vg (n=4)
- 1.0 x 10^{15} total vg (n=4)

Route and Method of Administration

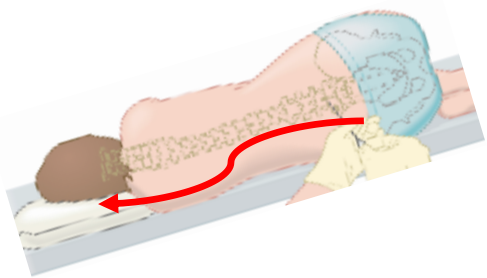
Administration

- Lumbar Intrathecal Infusion (IT)
- Amount and rate: 1 mL/min for total of 10-12 mL
- Immunosuppression regimen of prednisolone and sirolimus



Technique to Improve Transduction

- Trendelenburg position (15-30^{o})
- During infusion & 1 hour post infusion



Overview of primary efficacy endpoints

Revised Motor Behavior Assessment (R-MBA)	• A measure of onset or disease regression, growth, motor and communication skills, and disease behaviors adapted for Rett syndrome • This clinician-administered questionnaire is designed to evaluate current behavioral/social, orofacial/respiratory, and motor/physical symptoms • Higher scores indicate greater severity
Rett Syndrome Hand Function Scale (RSHFS)	• An eight-level scale designed to evaluate hand function in patients with Rett • Hand function is evaluated by an experienced physical therapist on the basis of videotaped sessions in which the participant's caregiver hands her both large and small objects so that the participant may demonstrate her ability to grasp, pick up, and hold the objects
Observer-Reported Communication Ability (ORCA), validated for Rett syndrome	• Captures overall communication ability observed by the caregiver over the previous 30 days • Items cover important communication behaviors in areas within expressive, receptive, and pragmatic communication • Plan to use a version of the ORCA specifically validated for use in Rett
Clinical Global Impression-Improvement (CGI-I) Scale, adapted to Rett syndrome	• A clinician-rated assessment tool used to establish global improvement or change in comparison to baseline following care, treatment, or intervention • A version of the CGI-I scale using anchors specific to Rett syndrome signs and symptoms will be used

Overview of secondary and exploratory endpoints

Rett-Specific
- Rett Syndrome Behavior Questionnaire (RSBQ)
- Functional Mobility Scale in Rett Syndrome (FMS-RS)
- Presence/absence and frequency of “Rett episodes” and seizures (if applicable) through review of seizure diaries
- Presence/absence, severity, and frequency of dystonic posturing present in each limb (if applicable), through review of diaries recorded by parents/caregivers

Global Assessments
- Clinical Global Impressions–Severity (CGI-S)
- Vineland Adaptive Behavior Scales (Vineland-3)

Biomarkers
- CSF and blood samples will be banked for potential future analysis

Neurophysiology and Imaging Assessments
- Quantitative EEG (qEEG) findings and auditory and visual evoked potentials (AEP and VEP)
- Brain volume per MRI of the brain
- Magnetic resonance spectroscopy (MRS) of the brain

Quality of Life/Other Assessment
- Parental Global Impressions–Improvement (PGI-I)
- Caregiver Top 3 Concerns via a Visual Analog Scale (VAS)
- Rett Syndrome Caregiver Burden Inventory (RTT-CBI)
- Caregiver quality of life via the 36-Item Health Survey (SF-36)
- Healthcare resource utilization outcomes

Key takeaways from regulatory interactions

- Positive feedback from multiple regulatory agencies supporting IND/CTA-enabling preclinical development plan
- Agreed with rationale around dose selection
- Noted comprehensiveness of preclinical data package
- Alignment around safety monitoring and efficacy endpoints
- Submission timing supports clinical trial initiation by YE 2021

Anticipated next steps for TSHA-102 by the end of 2021

Submit IND / CTA in 2H 2021

Initiate Phase 1/2 study by YE 2021

Clinical trial material release testing underway

Continue engagement with KOLs, patients, and UTSW at Rett center of excellence

Q & A

Closing Remarks

RA Session II

President, Founder & CEO

Focused on achieving anticipated near-term milestones in 2021 and building long-term value

GAN clinical program update, including 3.5 x 10^{14} total vg cohort
GM2 gangliosidosis preliminary biomarker data in 2H 2021
CLN1 program to dose first patient in 2021 under open IND

4 open IND/CTAs expected by the end of 2021, including Rett syndrome

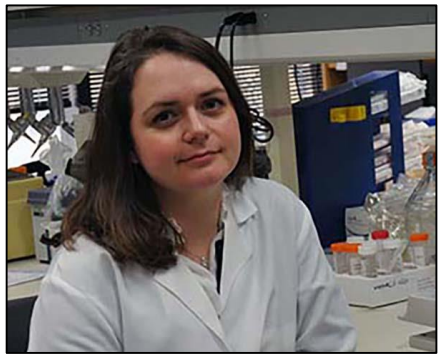
Initiated construction of internal cGMP facility in 1H 2021

5 additional programs currently in IND-enabling studies
TSHA-106 Angelman Investor Day October 26, 2021
Numerous value generating catalysts over the next 18 months

Thank you to our partners!

UT Southwestern Medical Center® | Research Labs

Sarah Sinnett Lab



Steven Gray Lab

International Rett Syndrome Foundation
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Thank you