The Developmental Plateau in Rett Syndrome: New Insights from the Natural History Study to Inform Novel Interventional Study Designs

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Aim

This study evaluated whether developmental milestone gain or regain in a developmental plateau population (age ≥6 years) from a trial-aligned RNHS analysis cohort could support a natural-history-informed null hypothesis and a plateau-anchored, baseline-adjusted aggregate responder analysis framework for use in future interventional studies

Background

- RTT is a rare neurodevelopmental disorder characterized by heterogeneous developmental trajectories, including variable gain, loss, retention and occasional regain of developmental milestones[1,2]
- Heterogeneity complicates interpretation of treatment-associated change in interventional studies, particularly when baseline function and developmental history differ across participants[3–6]
- PAOC approaches preserve individualized participant interpretation but do not inherently support prespecified group-level inference or powered aggregate analyses[7]
- Trial design challenges are compounded when evaluating an investigational gene therapy for RTT due to ethical and methodological concerns with sham controls (e.g., lumbar puncture without active agent), potential functional unblinding from required immunosuppression, and delayed-treatment control designs that may expose participants to unjust harm by postponing intervention during critical neurodevelopmental windows[8–14]
- The US Rett Syndrome Natural History Study (RNHS; >1800 participants with RTT and RTT-related disorders) provides longitudinal data on developmental milestone trajectories;[15,16] recent analyses indicated a clear developmental plateau beyond 6 years of age, with minimal spontaneous milestone gain/regain[15]
- The aims were to use the RNHS dataset to confirm a plateau in a trial-like analysis cohort; evaluate whether clinical, genotypic, and sociodemographic factors affect incidence of milestone gain in the plateau population; develop a plateau-anchored, baseline-adjusted aggregate responder analysis framework that uses an appropriate natural-history-informed single null hypothesis; and validate this null using 10,000 simulated control cohorts using the RNHS dataset.

Methods

Study design

- Cohort analysis of RNHS data using cumulative incidence of developmental milestone gain, loss, and regain over time
- Participants were female with typical RTT and confirmed *MECP2* mutation; inclusion/exclusion criteria were aligned to those typically used in interventional RTT trials

Developmental milestone selection

- Starting from 51 potential developmental milestones captured in the RNHS protocols (#5201, 2006–2014; #5211, 2015–2021),[4,16] RNHS study principal investigators, RTT specialists, and caregivers identified milestones that were linked to activities of daily living and that were independently meaningful based on caregiver concept elicitation work
- Finally, milestones with higher likelihood of spontaneous gain in an untreated population were also excluded, resulting in a selected set of 28 milestones for analysis (Figure 1)

Figure 1: 28 developmental milestones that reflect meaningful functional gains to caregivers, with low likelihood of being achieved without treatment, used in the study analysis

Communication
- Pointed for something they wanted
- Waved "Bye-bye"
- Followed a command with a gesture
- Identified body parts (pointed with eyes or fingers)
- Followed a command without a gesture
- Used word(s) with meaning
- Spoke in phrases (2 words or more) with meaning

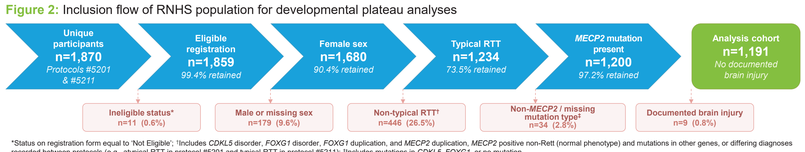
Fine motor
- Reached for toy
- Holds bottle unpropped
- Used raking grasp to retrieve an object
- Used pincer grasp (refined or modified)
- Transferred an object from one hand to another
- Finger feed
- Drank from a cup held without assistance
- Used a fork or spoon to eat with assistance
- Used a fork or spoon to eat without assistance

Gross motor
- Come to sitting
- Sat without support
- Stood while holding on
- Pulled to standing
- Stood independently
- Walked with support
- Walked independently
- Climbed up stairs with help
- Climbed down stairs with help
- Climbed up stairs without help
- Climbed down stairs without help
- Ran 10 feet without falling

Analysis cohort selection

- Parameters closely matching typical interventional RTT trial inclusion/exclusion criteria were applied to the RNHS dataset to select a trial-aligned analysis cohort representative of potential study participants (Figure 2)

Figure 2: Inclusion flow of RNHS population for developmental plateau analyses



*Status on registration form equal to 'Not Eligible'; †Includes *CDKL5* disorder, *FOXG1* disorder, *FOXG1* duplication, and *MECP2* duplication, *MECP2* positive non-Rett (normal phenotype) and mutations in other genes, or differing diagnoses recorded between protocols (e.g., atypical RTT in protocol #5201 and typical RTT in protocol #5211); ‡Includes mutations in *CDKL5*, *FOXG1*, or no mutation

Accounting for prognostic variables

- Prespecified prognostic covariates, identified a priori through literature review and endorsed by RNHS study PIs, were evaluated across sociodemographic, genetic, comorbidity, and birth characteristics to assess their impact on developmental milestone gain

Statistical analyses

- Cumulative incidence analyses were used to estimate the proportion of individuals who gained, lost, or regained developmental milestones as a factor of age and time, with gain defined from birth to first event, loss as the first loss after gain, and regain as the first regain after loss
- Multivariable Cox proportional hazards models were used to evaluate the effect of prespecified covariates on milestone gain, with results reported as hazard ratios (95% CIs) and p-values adjusted for multiple comparisons using Bonferroni correction

Designing aggregate responder analysis: Plateau-Anchored, Baseline-Adjusted (PABA) aggregate responder analysis

- RNHS cumulative incidence estimates were used to construct the PABA aggregate responder framework by defining milestone eligibility and deriving a conservative natural-history-informed null threshold. The highest observed spontaneous gain rate after age 6 years was 3% which, at N=15, corresponded to 0.45 expected spontaneous responders; however, as responder rate must be discrete, one spontaneous responder (6.7%) was selected as the null threshold and evaluated across feasible sample sizes and responder counts using power calculations

Trial control cohort simulations

- Using RNHS participants in the developmental plateau (age ≥6 years) cohort, bootstrap simulations (10,000 iterations) were used to generate trial-like untreated cohorts (N=15; 1-year follow-up) via repeated sampling of the RNHS analysis cohort under prespecified assumptions
- Simulations evaluated spontaneous milestone gain and regain in untreated cohorts, assuming a conservative null hypothesis of 1 spontaneous responder per cohort (6.7%) and assessed the probability of exceeding the null threshold for responder rate and event rate in untreated cohorts
- Each milestone was assessed to determine the minimum time-since-loss at which spontaneous regain falls below the defined null threshold

Results

Cohort profile and demographics

- The RNHS dataset comprises 1870 individuals. Parameters closely matching the inclusion/exclusion criteria typically used in interventional RTT trials were applied to select the analysis cohort (Figure 2)
  - 1191 (63.7%) participants were included, with a mean (SD) age at first study visit of 10.2 (9.3) years (Table 1)

Table 1: Cohort profile and demographics

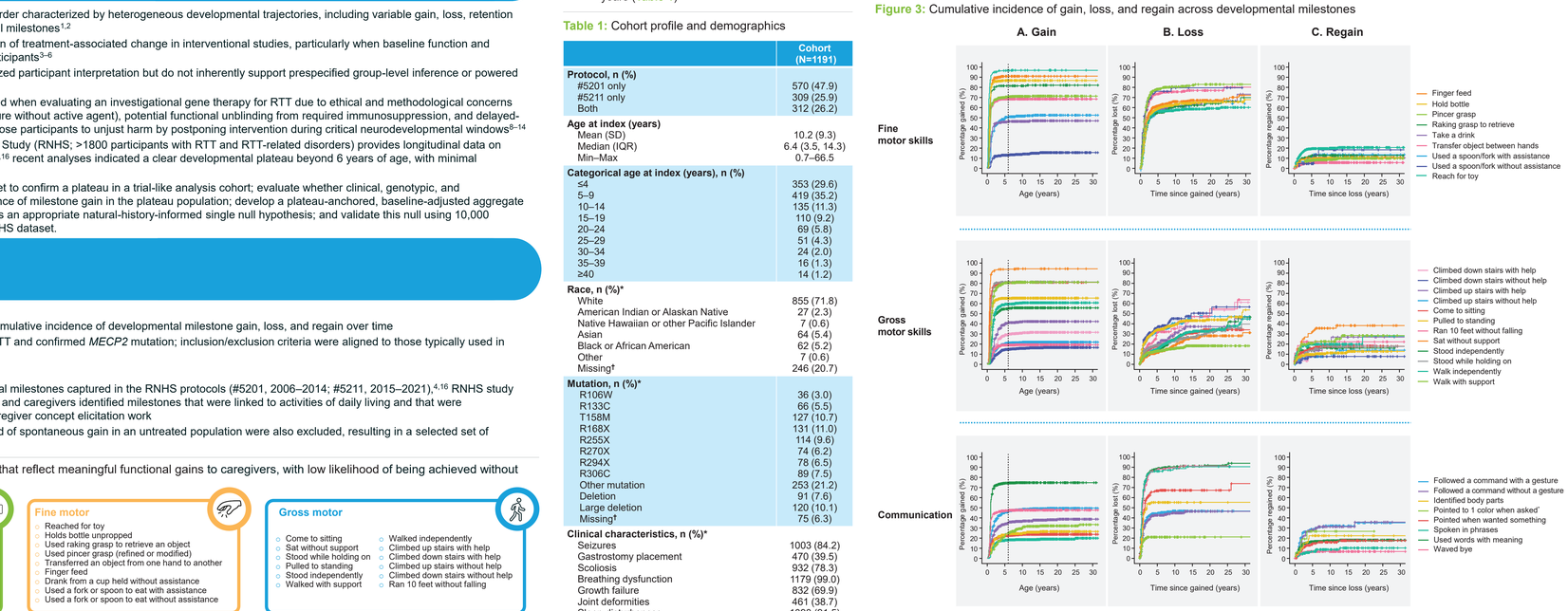
	Cohort (N=1191)
Protocol, n (%)	
#5201 only	570 (47.9)
#5211 only	309 (25.9)
Both	312 (26.2)
Age at index (years)	
Mean (SD)	10.2 (9.3)
Median (IQR)	6.4 (3.5, 14.3)
Min–Max	0.7–66.5
Categorical age at index (years), n (%)	
≤4	353 (29.6)
5–9	419 (35.2)
10–14	135 (11.3)
15–19	110 (9.2)
20–24	69 (5.8)
25–29	51 (4.3)
30–34	24 (2.0)
35–39	16 (1.3)
≥40	14 (1.2)
Race, n (%)*	
White	855 (71.8)
American Indian or Alaskan Native	27 (2.3)
Native Hawaiian or other Pacific Islander	7 (0.6)
Asian	64 (5.4)
Black or African American	62 (5.2)
Other	7 (0.6)
Missing†	246 (20.7)
Mutation, n (%)*	
R106W	36 (3.0)
R133C	66 (5.5)
T158M	127 (10.7)
R168X	131 (11.0)
R255X	114 (9.6)
R270X	74 (6.2)
R294X	78 (6.5)
R306C	89 (7.5)
Other mutation	253 (21.2)
Deletion	91 (7.6)
Large deletion	120 (10.1)
Missing†	75 (6.3)
Clinical characteristics, n (%)*	
Seizures	1003 (84.2)
Gastrostomy placement	470 (39.5)
Scoliosis	932 (78.3)
Breathing dysfunction	1179 (99.0)
Growth failure	832 (69.9)
Joint deformities	461 (38.7)
Sleep disturbances	1090 (91.5)

*Categories are not mutually exclusive; †Includes unknown or refused and those with no response for all listed categories

Developmental plateau

- Relatively high and variable rates of spontaneous first-time milestone gain were observed in children before 6 years of age, with rates as high as 97% in the youngest participants; most regain occurred within several years after loss, with milestone-specific variation (Figure 3A–C)
- A clear developmental plateau emerged in children aged ≥6 years, after which spontaneous gain, and regain after sustained loss, was rare (first time gain <3% across milestones; Figure 3A; regain shown in Figure 3C)
- Milestone loss continued to accumulate over time, with loss/regain often seen shortly (within years) after initial gain/loss, respectively (Figure 3A–C)

Figure 3: Cumulative incidence of gain, loss, and regain across developmental milestones



*Pointed to 1 color" milestone had higher incidence of gain and regain, so it was excluded from the final list of 28. Cumulative incidence curves representing milestones in the fine motor, gross motor, and communication domains by age of milestone for: A) Gain (initial gain); B) Loss (time to milestone loss from initial gain); and C) Regain (time to milestone regain from loss). Colors representing each milestone are shown in the legend, with censored data points shown as cross lines. Dotted black lines in A) denote the start of developmental plateau at 6 years of age

Prognostic factors

- No consistent significant predictors of gain/regain were identified across the 28 milestones, such as "finger feed" (Figure 4)
- HRs were generally close to 1, with no consistent direction or magnitude of effect observed

Figure 4: Association between covariates and milestone gain in multivariable Cox proportional hazards model for the milestone "finger feed"

Covariate	HR (95% CI)	p-value	Corrected p-value*
Age at index (years)	0.99 (0.97, 1.01)	0.430	1.000
Gestation age at birth (weeks)	1.01 (0.94, 1.07)	0.840	1.000
Birth weight (kg)	1.09 (0.84, 1.40)	0.520	1.000
Birth length (cm)	1.01 (0.98, 1.05)	0.550	1.000
Non-Caucasian [Ref: Caucasian]	1.03 (0.62, 1.72)	0.900	1.000
***MECP2* mutation [Ref: Other *MECP2*]**			
Deletion	0.48 (0.06, 3.62)	0.480	1.000
Large deletion	1.24 (0.65, 2.38)	0.510	1.000
R106W	0.85 (0.49, 1.49)	0.580	1.000
R133C	1.29 (0.77, 2.16)	0.330	1.000
R168X	0.90 (0.57, 1.42)	0.640	1.000
R255X	0.79 (0.47, 1.33)	0.370	1.000
R270X	0.94 (0.52, 1.72)	0.850	1.000
R294X	0.89 (0.48, 1.64)	0.700	1.000
R306C	0.96 (0.60, 1.51)	0.840	1.000
T158M	1.07 (0.71, 1.59)	0.760	1.000
Resource use			
Physical therapy	1.06 (0.71, 1.59)	0.760	1.000
Occupational therapy	0.99 (0.68, 1.44)	0.960	1.000
Speech therapy	1.01 (0.67, 1.52)	0.950	1.000
Clinical characteristics			
Seizures	0.93 (0.65, 1.32)	0.670	1.000
Gastrostomy tube	1.11 (0.84, 1.46)	0.480	1.000
Scoliosis	1.01 (0.72, 1.41)	0.970	1.000
Breathing dysfunction	0.94 (0.41, 2.14)	0.880	1.000
Growth failure	1.22 (0.92, 1.61)	0.170	1.000
Joint deformities	0.98 (0.73, 1.32)	0.900	1.000
Sleep disturbances	1.02 (0.56, 1.87)	0.940	1.000

Reference group for mutations was 'Other *MECP2*' mutations.
*p-values were adjusted for multiple comparisons using the Bonferroni correction method

Sample size feasibility

- The incidence of spontaneous gain during the developmental plateau (age ≥6 years) was <3% (Figure 3)
- For a sample size of 15, a 3% natural-history responder rate corresponds to less than one expected spontaneous responder
- To equate to whole participant numbers, a conservative null hypothesis of one spontaneous responder (1/15=6.7%) was selected and found to provide robust power to detect treatment effects exceeding natural history expectations (Table 2)

Table 2: Power calculation indicates that a minimum of five responders would be required to reject the natural-history-informed null hypothesis with a sample size of 15

Sample size* (*Spontaneous Responder (null)*)		15		
		1	*2*	*3*
		Statistical power		
Post-treatment responder count (treatment effect)†‡	1	0.01	0.00	0.00
	2	0.13	0.04	0.00
	3	0.35	0.16	0.02
	4	0.60	0.37	0.08
	5	0.79	0.60	0.20
	6	0.91	0.78	0.39
	7	0.97	0.90	0.60
	8	0.99	0.97	0.78
	9	1.00	0.99	0.90
	10	1.00	1.00	0.97
	11	1.00	1.00	0.99
	12	1.00	1.00	1.00
	13	1.00	1.00	1.00
	14	1.00	1.00	1.00
	15	1.00	1.00	1.00

Colors indicate thresholds for acceptable power to reliably detect a treatment effect, such that red values indicate poor power, amber values indicate moderate power, and green values indicate strong power
*Power calculations for the potential sample size of 15, considering a responder count of 1–15 individuals;
†Responder count reflects the number of participants with ≥1 gain/regain event; event counts represent total gain/regain events across participants. Given the rarity of spontaneous gain/regain, multiple events per cohort are uncommon in untreated populations;
‡Values in table indicate Power=(1-β) to detect an effect and avoid type I and II errors

Responder threshold

- Across 10,000 simulations (N=15; ≥6 years of age), only 1.8% of simulations had ≥2 responders (Table 3)
  - Across 150,000 simulated participants, this equated to a spontaneous responder rate of 1.39%
- The gain/regain event rate was extremely low (around 0.02 events per person), supporting ≥1 gain/regain as a responder threshold for a sample size of 15

Table 3: Distribution of milestone gain/regain responders and events across 10,000 simulated trials (N=15; ≥6 years of age)

Responder count	N	Number of responders	Percentage of simulations
0	8113	8113	81.13%
1	1699	1699	16.99%
2	179	358	1.79%
3	9	27	0.09%
Average spontaneous responder rate:		**2084***	**1.39%** *Likelihood of response per participant*
Event count	**N**	**Number of events**	**Percentage of simulations**
0	8113	0	81.13%
1	1332	1332	13.32%
2	385	770	3.85%
3	135	405	1.35%
3+	35	146	0.35%
Average spontaneous event rate:		**2653***	**0.02** *Average events per participant*

*This number is across 10,000 simulated trials that randomly sampled 150,000 participants in the developmental plateau (beginning at 6 years of age) from the RNHS analysis cohort

Conclusions

- RNHS data demonstrate a clear developmental plateau in the typical RTT population beginning at 6 years of age, after which spontaneous milestone gain or regain is highly unlikely and more predictable, supporting a stable population to serve as the plateau-anchor for interventional studies
- Before 6 years of age, milestone gain, loss, and regain were more variable and less predictable, with spontaneous first-time gain for a single milestone as high as 97%; inclusion of younger participants without explicit age accounting could increase background spontaneous gain and thereby increase type I error
- Time-since-loss parameters were important for interpretation of milestone regain because spontaneous regain likelihood varied as a function of time since milestone loss
- Power analyses and bootstrap simulations supported a conservative natural-history-informed null threshold of 6.7% at a feasible sample size of N=15 under modeled trial-like sampling assumptions for aggregate responder analyses using milestone gain or regain
- The proposed PABA framework preserves individualized participant interpretation while enabling prespecified group-level inference in future RTT interventional studies (Figure 5)
- This framework aligns with recent FDA guidance on baseline control and supports application of a PABA analysis framework to RTT trial design in populations that are robustly in the plateau stage (6+ years) where spontaneous milestone gain or regain rates are rare and can be statistically accounted for

Figure 5: Novel PABA aggregate analysis framework applied to RTT as a use-case

1 Robust Developmental Plateau Selection
Select participants **after ~6 years of age, when the developmental plateau becomes robustly evident *(plateau-anchor)*** and spontaneous milestone gain/regain is uncommon and predictable

2 Account for Participant Level Milestone History
Account for **each participant's prior milestone history *(baseline-adjustment 1)*** to address heterogeneity.
Require **milestones eligible for analysis are either never gained or lost long enough ago** to ensure a single null-hypothesis is applicable and trial results are interpretable

3 Account for Baseline Functional Ability
Ensure each milestone is properly assessed at baseline using the same methods that will be applied post-treatment *(baseline- adjustment 2)*, to verify current pre-treatment ability and account for this when determining milestones eligible for aggregate analysis.

4 Natural-History-Informed Aggregate Responder Thresholds
Define **responders as participants who have ≥1 *eligible* milestone gain or regain and apply a natural-history-informed, single null threshold** derived from CI curves and tested with power analyses and 10,000 trial-like, control cohort simulations

The PABA framework integrates **developmental plateau status**, **participant-level milestone history**, **baseline functional ability**, and **natural-history-informed aggregate responder thresholds** to support rigorous and interpretable **group-level inference** in future RTT studies

Well supported natural-history-informed null threshold: **6.7%**

Key takeaways

- **A robust RTT developmental plateau was observed after age 6 years, supporting use of natural-history-informed null thresholds in an aggregate responder analysis**
- **Novel PABA analysis framework preserves participant-level interpretation by evaluating each individual against their own baseline and developmental history while enabling prespecified group-level testing against a natural-history-derived null threshold**
- **Milestone eligibility is critical for interpreting milestone gain and regain in a trial setting, including accounting for time-since-loss because spontaneous regain rates vary by duration since loss**
- **Including participants younger than age 6 years may increase spontaneous milestone gain/regain rates and inflate type I error risk if not explicitly accounted for in the analysis framework**

The authors wish to thank all patients and caregivers, as well as the study staff who make this research possible

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Acknowledgments: Study was funded by Taysha Gene Therapies. Analytic support was provided by Mark Bennett, of Broadstreet HEOR, funded by Taysha Gene Therapies. Medical writing support for this poster was provided by Leigh O'Connor-Jones, of Avalere Health US Inc., funded by Taysha Gene Therapies.
Disclosures: MM is a paid consultant of Taysha Gene Therapies.

Abbreviations
CI, confidence interval; FDA, Food and Drug Administration; HR, hazard ratio; IQR, interquartile range; *MECP2*, *methyl-CpG-binding protein 2*; PABA, Plateau-Anchored, Baseline-Adjusted; PAOC, patient-as-own-control; RNHS, Rett Syndrome Natural History Study; RTT, Rett syndrome; SD, standard deviation

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