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RESEARCH ARTICLE

Distribution of hand function by age in individuals with Rett syndrome

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Abstract

Objective: We aimed to determine the longitudinal distribution of hand function skills in individuals with classic Rett syndrome (RTT), an X-linked dominant neurodevelopmental disorder, and correlate with *MECP2* variants.

Method: We conducted a longitudinal study of 946 girls and young women with typical RTT seen between 2006 and 2021 in the US Natural History Study (NHS) featuring a structured clinical evaluation to assess the level of hand function skills. The specific focus of this study was to assess longitudinal variation of hand skills from age 2 through age 18 years in relation to specific *MECP2* variant groups.

Results: Following the initial regression period, hand function continues to decline across the age spectrum in individuals with RTT. Specific differences are noted with steeper declines in hand function among those with milder variants (Group A: R133C, R294X, R306C, and C-terminal truncations) compared with groups composed of individuals with more severe variants.

Conclusions: These temporal variations in hand use represent specific considerations that could influence the design of clinical trials that test therapies aiming to ameliorate specific functional limitations in individuals with RTT. Furthermore, the distinct impact of specific *MECP2* variants on clinical severity, especially related to hand use, should be considered in such interventional trials.

Keywords: longitudinal assessment; *MECP2*; natural history

Introduction

Rett syndrome (RTT) is a rare neurodevelopmental disorder, occurring predominantly in females, >96% of whom have variations in the methyl-CpG-binding protein 2 (*MECP2*) gene at Xq28.[1] Initially described in 1966 by Andreas Rett[2] and called RTT after the landmark paper of Hagberg and colleagues in 1983,[3] RTT remains a clinical diagnosis with confirmation based on identifying a variant in *MECP2*. One of the core diagnostic features of typical or classic RTT is partial or complete loss of hand use during a discrete period of regression.[4] With the increasing promise and availability of pharmaceutical agents and vector-mediated gene replacement therapy, hand function is viewed as a top concern amongst caregivers of individuals with RTT (J. L. Neul, personal communication, manuscript in preparation) and will be a key endpoint in determining clinical improvement in interventional trials.

The US Natural History Study (NHS) of RTT and related disorders gathered data on various clinical components from historical accounts, physical examination, and global measures of clinical severity from 2006 through 2021. Thus, it is timely to assess the distribution of this specific skill across time to ascertain the range of abilities and the potential differences in this distribution over time. While the initial regression of hand skills, typically before 5 years of age, is well recognized,[3] subsequent progression has not been detailed. The demonstration of progressive decline of hand function across the age range from 2 to 18 years could have important implications in the development and stratification of the design of emerging clinical trials.

Methods

Patients and clinical evaluation

The NHS database was queried to document hand skills in girls and young women with classic RTT from ages 2 through 18 years. Individuals met the consensus diagnostic features of classic RTT as described in 2002[5] and modified in 2010.[4] Participants were evaluated longitudinally at intervals of six months to two years depending on their time of enrollment. As such, participants were analyzed as many as 15 times over the course of this NHS.

This report analyzed only female individuals with classic RTT as the numbers for individuals with atypical RTT, males with *MECP2* variants, and other disorders associated with *MECP2* variation were too small to allow accurate assessment. *MECP2* variants were found in far fewer (75%) individuals with atypical RTT who represent the extreme ends of the phenotypic spectrum, both milder and more severe than typical RTT. Thus, we excluded 40 female individuals who had complete *MECP2* variant analysis and completed severity scales but did not meet the criteria for typical RTT. Nevertheless, the overall results were unchanged when these individuals were included in the analysis (data not shown). In total, 946 females with classic RTT were included in this analysis (Table 1).

Table 1. Number of patients in each variant group for hand function levels and CSS/MBA.

	Variants				
Outcomes	Total	A	B	C	D
Six levels of hand function	946	298	166	296	186
CSS/MBA	945	298	165	296	186

Data were extracted from each visit from age 2 through 18 years assessing hand function utilizing four categories of data. These include the six options of hand function observed during the clinical assessment, the specific *MECP2* variant, and the two measures of clinical features common in RTT, the Clinical Severity Score (CSS) and the Motor Behavioral Assessment (MBA). In terms of clinical assessment, the six functions are pincer grasp, raking grasp, palmar grasp, reaching for objects, holding objects, or no attempt. Each of the six options are binary (dichotomous) outcomes with responses of yes/no (Table 1).

The specific *MECP2* variant and the CSS and MBA scores for each participant were included in the analyses. Estimation of disease severity at each visit using the two rating scales, both developed specifically for RTT, has been utilized throughout the NHS study. The CSS is a composite score based on 13 individual, ordinal categories measuring clinical features common in RTT.[6] All scores range from 0 to 4 or 0 to 5 with 0 representing the least severe and 4 or 5 representing the most severe findings. Data were extracted only for hand function. In the CSS, the best level of function is based on direct examination at the time of the assessment in five categories: acquired and conserved; holding object acquired on time and partially retained; holding objects acquired >10 months of age and partially retained; hand skills lost; and hand skills never acquired. A simplified scoring system was also used to compress the ordinal category measures into two bins: normal or partially conserved and lost or never acquired. The MBA, also an ordinal score based on a 0–4 range, 0 being best and 4 being worst, incorporates measures of behavior/social assessment (range = 0–64), orofacial/respiratory assessment (range = 0–28), and motor assessment/physical signs (range = 0–56).[6,7] In the MBA, hand function is evaluated in five categories: purposeful hand use; plays with toys or switches; uses a utensil or cup; finger feeds only; or no purposeful hand use. A simplified scoring system was also used to compress the ordinal category measures into two

bins: normal or partially conserved and lost or never acquired.

MECP2 variant analysis

Participants in this study had a complete *MECP2* variation analysis performed, including sequencing of all four exons and, if necessary, evaluation for large rearrangements involving one or more exons by Southern blotting or by multiple ligation-dependent probe amplification analysis. *MECP2* variation analysis was performed at Clinical Laboratory Improvement Amendments–approved laboratories. For the purposes of comparison by variant grouping, these were separated into four groups based on previous data showing significant group correlations[6,8]: Group A included R133C, R294X, R306C, and C-terminal truncations; Group B included T158M and other point variations; Group C included R106W, R168X, R255X, and R270X; Group D included large deletions, splice site variations, and early truncations (before nucleotide 850).

Statistical analysis

A generalized linear mixed model (GLMM) was used to assess the changes over age for each of the eight dichotomous outcomes in the study (MBA, CSS, pincer grasp, raking grasp, palmar grasp, reaching for objects, holding objects, or no attempt). The fixed effects in the GLMM were variant group, age, and variant group × age interaction. A random subject intercept was used. Since the outcomes are binary, a logistic link function was used assuming a binomial distribution for the outcome. Regression estimates are as log-odds. Higher quadratic and cubic age were explored, but linear age was selected as being most appropriate for the models. Two-sided p values are reported. Statistical significance was determined when the $p < 0.05$. Analyses were performed using SAS 9.4 (SAS Institute).

Human study approval

Human study approval was obtained from each participant prior to entry into the study. A Certificate of Confidentiality was provided by the National Institute of Human Development (NICHD).

ClinicalTrials.Gov

Two noninterventional clinical trial protocols (NCT00299312 and NCT02738281) were involved during this more than 16-year study.

Data sharing

As part of the Rare Disease Consortium under the National Center for Accelerating Translational Sciences, a data-sharing agreement was developed and signed by all grantees. These data are available through dbGap.

Results

Longitudinal comparison of six levels of hand function by *MECP2* variant group

The results of the GLMM-derived model for longitudinal progression of hand function from 2 through 18 years within the above-described specific variant groups are presented in Figure 1. Broadly, the analysis demonstrated an overall longitudinal reduction of hand skills beyond the initial period of regression, as indicated by reduced log-likelihood rates for the GLMM-derived model.

Hand function was assessed by six levels of ability: pincer grasp, palmar grasp, raking grasp, reaches for objects, holds objects, or no attempt. These clinical assessments were conducted longitudinally from age 2 through age 18 across each variant group. For each level of hand function (Figure 1A–F), Group A (R133C, R294X, R306C, and C-terminal truncations), revealed the best outcomes among the youngest ages, differing significantly from the other three groups. Group B (T158M and Other Point Variants) showed better outcomes than Group C (R106W, R168X, R255X, and R270X) and Group D (Large Deletions, Splice Site variations, and Early Truncations before nucleotide 850) for each hand function. However, for Group C versus Group D, the picture was mixed with similar levels of function at younger ages for pincer grasp and palmar grasp, but somewhat greater differences for the remaining levels of ability. In general, the respective abilities declined over the course of the study with the exception of pincer grasp for Groups C and D. However, the steepest rate of decline across the age range is evident for individuals in Group A. While individuals in Group A have superior function in the younger ages, they also represent those most likely to demonstrate the steepest decline in this function.

Examining the results across the six levels of hand function revealed important differences. For each level of hand ability assessed during the clinical evaluation (Figure 1A–F), the rank order by variant group was identical, with some qualifications, in terms of functional level, namely, Group A, Group B, Group D, and Group C. For pincer grasp, Groups C and D were identical at age 2 years and showed an improving trend over the subsequent 16 years, crossing Group B at ages 15 and 12 years, respectively. For palmar grasp, Groups A and B showed a difference in the loss of this skill, with Group A exceeding

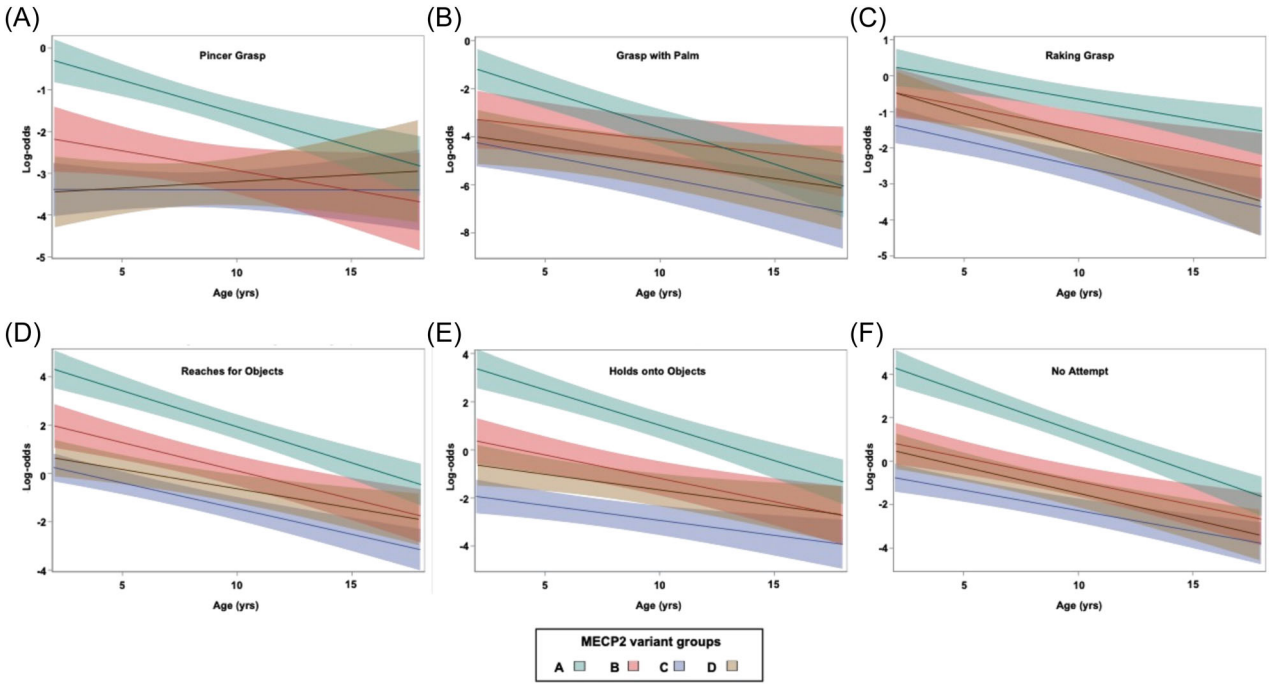

Figure 1. (A–F) Hand function from ages 2–18 by variant group. 1A is pincer grasp, 1B is palmar grasp, 1C is raking grasp, 1D is reaches for objects, 1E is holds objects, and 1F is no attempt. Mutation groups include specific variants: Group A includes R133C, R294X, R306C, and C-terminal truncations; Group B includes T158M and other point variations; Group C includes R106W, R168X, R255X, and R270X; Group D includes large deletions, splice site variations, and early truncations (before nucleotide 850). Hand function was assessed for 946 individuals from ages 2 through 18 years. A generalized linear mixed model (GLMM) was used to assess the changes over age for each of the six dichotomous outcomes. The fixed effects in the GLMM were mutation group, age, and mutation group × age interaction. Regression estimates are as log-odds. Two-sided *p* values are reported. Statistical significance was determined when the $p < 0.05$. Analyses were performed using SAS 9.4 (SAS Institute).

Group B after age 12 years. For raking grasp, Groups B and D were similar at age 2 years, but Group D showed a greater loss of skill through age 18. Reaching for objects and holding objects showed more rapid declines in these skills for Group B versus Group D, being essentially identical at age 18 years.

For no attempt, the same rank order, Group A, Group B, Group D, and Group C, is present initially. During the subsequent years, Group A declines more dramatically whereas the other groups have less steep but similar patterns of decline.

Comparison of six levels of hand function by *MECP2* variant group at age 2 and age 18

The decline in the six levels of hand function noted for individuals in each variation group, especially steep for those in Group A, next led us to examine these same parameters for participants who were age 2 and age 18 years. At age 2 years, each hand function level except raking grasp at age 2 (Table 2) showed that Group A was significantly different from Groups B, C, and D. For raking grasp (Table 2), Group A did not differ from Groups B and D but was significantly different from Group C. For pincer grasp (Table 2), Group B was significantly different from Group C and D. For palmar grasp (Table 2), Group B was not different from Groups C and D. For raking grasp (Table 2), Group B was significantly different from Group C, but not different from Group D. For reaches for objects (Table 2), Group B was significantly different from Group C and from Group D. For holding objects (Table 2) and no attempt (Table 2), Group B was significantly different from Group C, but not different form Group D. For pincer grasp (Table 2), palmar grasp (Table 2), and reaches for objects (Table 2), Groups C and D were not different. For raking grasp (Table 2), holding objects (Table 2), and no attempt (Table 2), Group C was significantly different from Group D.

However, at age 18 years the results differ dramatically. Pincer grasp revealed no differences; palmar grasp showed a significant difference for Group B versus Group C; raking grasp indicated the greatest differences with Group A being significantly different from Groups B, C, D; reaching for objects revealed a significant difference from Groups B, C,

Table 2. Hand function at age 2 and 18 by variant group.

		At 2 years old			At 18 years old		
	Comparison	Estimate (SE)	Exp. estimate	*p* (*t* value, DF)	Estimate (SE)	Exp. estimate	*p* (*t* value, DF)
Pincer grasp	A vs. B	−1.88 (0.48)	0.15	<0.0001 (−3.93, 3398)	−0.8599 (0.7004)	0.4232	0.2196 (−1.23, 3398)
	A vs. C	−3.09 (0.42)	0.05	<0.0001 (−7.36, 3398)	−0.5752 (0.615)	0.5626	0.3497 (−0.94, 3398)
	A vs. D	−3.15 (0.51)	0.04	<0.0001 (−6.19, 3398)	−0.1212 (0.7281)	0.8859	0.8678 (−0.17, 3398)
	B vs. C	1.21 (0.52)	3.35	0.0194 (2.34, 3398)	−0.2847 (0.7761)	0.7522	0.7137 (−0.37, 3398)
	B vs. D	1.27 (0.59)	3.56	0.0319 (2.15, 3398)	−0.7388 (0.8685)	0.4777	0.395 (−0.85, 3398)
	C vs. D	0.06 (0.55)	1.06	0.9103 (0.11, 3398)	−0.454 (0.8012)	0.6351	0.5709 (−0.57, 3398)
Palmar grasp	A vs. B	−2.09 (0.69)	0.12	0.0024 (−3.04, 3398)	1.0184 (0.8739)	2.7688	0.2439 (1.17, 3398)
	A vs. C	−3.06 (0.59)	0.05	<0.0001 (−5.19, 3398)	−1.0899 (0.8746)	0.3362	0.2128 (−1.25, 3398)
	A vs. D	−2.82 (0.66)	0.06	<0.0001 (−4.26, 3398)	−0.0817 (0.9709)	0.9216	0.9329 (−0.08, 3398)
	B vs. C	0.97 (0.65)	2.63	0.1364 (1.49, 3398)	2.1083 (0.9499)	8.2343	0.0265 (2.22, 3398)
	B vs. D	0.73 (0.72)	2.06	0.3166 (1, 3398)	1.1001 (1.0404)	3.0045	0.2904 (1.06, 3398)
	C vs. D	−0.24 (0.63)	0.78	0.6991 (−0.39, 3398)	−1.0082 (1.038)	0.3649	0.3315 (−0.97, 3398)
Raking grasp	A vs. B	−0.71 (0.44)	0.49	0.1071 (−1.61, 3398)	−0.966 (0.5721)	0.3806	0.0914 (−1.69, 3398)
	A vs. C	−1.61 (0.36)	0.19	<0.0001 (−4.43, 3398)	−2.1071(0.5267)	0.1216	<0.0001 (−4, 3398)
	A vs. D	−0.71 (0.41)	0.49	0.0816 (−1.74, 3398)	−1.9519 (0.6)	0.142	0.0012 (−3.25, 3398)
	B vs. C	0.91 (0.43)	2.47	0.035 (2.11, 3398)	1.1411 (0.6042)	3.1302	0.059 (1.89, 3398)
	B vs. D	0.01 (0.47)	1.01	0.9896 (0.01, 3398)	0.9859 (0.6693)	2.6803	0.1408 (1.47, 3398)
	C vs. D	−0.90 (0.40)	0.41	0.0247 (−2.25, 3398)	−0.1552 (0.6235)	0.8563	0.8035 (−0.25, 3398)
Reaches for objects	A vs. B	−2.3398 (0.5949)	0.09	<0.0001 (−3.93, 3398)	−1.2974 (0.7185)	0.2732	0.0711 (−1.81, 3398)
	A vs. C	−4.0671 (0.4961)	0.01713	<0.0001 (−8.2, 3398)	−2.6996 (0.6309)	0.06724	<0.0001 (−4.28, 3398)
	A vs. D	−3.6708 (0.5511)	0.02546	<0.0001 (−6.66, 3398)	−1.4514 (0.7084)	0.2342	0.0405 (−2.05, 3398)
	B vs. C	1.7273 (0.5492)	5.6253	0.0017 (3.15, 3398)	1.4022 (0.707)	4.064	0.0474 (1.98, 3398)
	B vs. D	1.331 (0.6008)	3.7847	0.0268 (2.22, 3398)	0.154 (0.7824)	1.1665	0.844 (0.2, 3398)
	C vs. D	−0.3963 (0.4851)	0.6728	0.414 (−0.82, 3398)	−1.2482 (0.6943)	0.287	0.0723 (−1.8, 3398)
Holds objects	A vs. B	−3.0097 (0.6393)	0.04931	<0.0001 (−4.71, 3398)	−1.4074 (0.7807)	0.2448	0.0715 (−1.8, 3398)
	A vs. C	−5.3196 (0.5692)	0.004894	<.0001 (−9.35, 3398)	−2.5995 (0.7059)	0.07431	0.0002 (−3.68, 3398)

(*Continued*)

Table 2. Continued.

		At 2 years old			At 18 years old		
	Comparison	Estimate (SE)	Exp. estimate	*p* (*t* value, DF)	Estimate (SE)	Exp. estimate	*p* (*t* value, DF)
	A vs. D	−4.01 (0.6049)	0.01813	<0.0001 (−6.63, 3398)	−1.374 (0.7745)	0.2531	0.0761 (−1.77, 3398)
	B vs. C	2.31 (0.6061)	10.0743	0.0001 (3.81, 3398)	1.192 (0.7888)	3.2938	0.1309 (1.51, 3398)
	B vs. D	1.0003 (0.652)	2.7191	0.1251 (1.53, 3398)	−0.033 42 (0.8599)	0.9671	0.969 (−0.04, 3398)
	C vs. D	−1.3097 (0.5558)	0.2699	0.0185 (−2.36, 3398)	−1.2254 (0.7818)	0.2936	0.1171 (−1.57, 3398)
No attempt	A vs. B	−3.4714 (0.6397)	0.03107	<0.0001 (−5.43, 3397)	−1.0486 (0.7736)	0.3504	0.1754 (−1.36, 3397)
	A vs. C	−5.0471 (0.5474)	0.006428	<0.0001 (−9.22, 3397)	−2.1564 (0.6777)	0.1157	0.0015 (−3.18, 3397)
	A vs. D	−3.8161 (0.5891)	0.02201	<0.0001 (−6.48, 3397)	−1.7929 (0.7546)	0.1665	0.0176 (−2.38, 3397)
	B vs. C	1.5757 (0.5895)	4.8341	0.0076 (2.67, 3397)	1.1078 (0.7736)	3.0278	0.1522 (1.43, 3397)
	B vs. D	0.3447 (0.6405)	1.4116	0.5905 (0.54, 3397)	0.7443 (0.8428)	2.1049	0.3773 (0.88, 3397)
	C vs. D	−1.231 (0.5325)	0.292	0.0208 (−2.31, 3397)	−0.3635 (0.7483)	0.6952	0.6271 (−0.49, 3397)

and D and Group B was significantly different from Group C; holding onto objects showed a significant difference from Groups B, C, and D; and no attempt revealed only a significant difference from Groups C and D. The tables describing the statistical assessments are shown in Supporting Information: Table 1A–1F.

Comparison of hand function using the CSS by *MECP2* variant group

Utilizing the GLMM modeling with linear age as the predictor, the four *MECP2* variant groups were compared for hand use based on the CSS binary scale (Figure 2). All groups showed declining hand skills from ages 2 to 18 years. Group A demonstrated the highest level of hand skills throughout the age range, although the pattern of greater decline for individual hand skills (Figure 1A–E) compared with the three other groups is again noted. Thus, from ages 2 to 18 years Group A, while remaining consistently better than Groups B, C, and D, did demonstrate the steepest slope in declining skill level.

When considering each group at age 2 years (Table 3), Group A was highly significantly different from Groups B, C, and D. Group B was highly significantly different from Group C and significantly different from Group D. Groups C and D were not different. When considering each group at age 18 years (Table 3), the results were different. Group A was still highly significantly different from Groups C and D, but only moderately significantly different from Group B. Group B was significantly different from Group C. Neither Group B versus Group C nor Group C versus Group D were significantly different.

To understand the intermediate age ranges between age 2 and age 18, age groups from 8–14 to 15–18 were examined in two ways (Table 4). CSS hand skill for each age group was evaluated for Group A versus Groups B, C, and D, both for each CSS level (0–4) and then for the simplified scoring of compressing the skills into two bins: normal or partially conserved and lost or never acquired. Group A is highly significantly different from Groups B, C, and D in both age groups, whether each level of hand function or presence or absence of hand function is considered. The table describing the statistical assessments for these groups are shown in Supporting Information: Table 2A.

Comparison of hand function using the motor behavioral assessment by *MECP2* variant group

Similar results were seen when using the MBA binary scale for the variant groups, although some differences from the CSS results were noted. All groups again showed declining hand skills from 2 to 18 years of age (Figure 2). Group A demonstrated the highest level of hand skills throughout the age range, although this

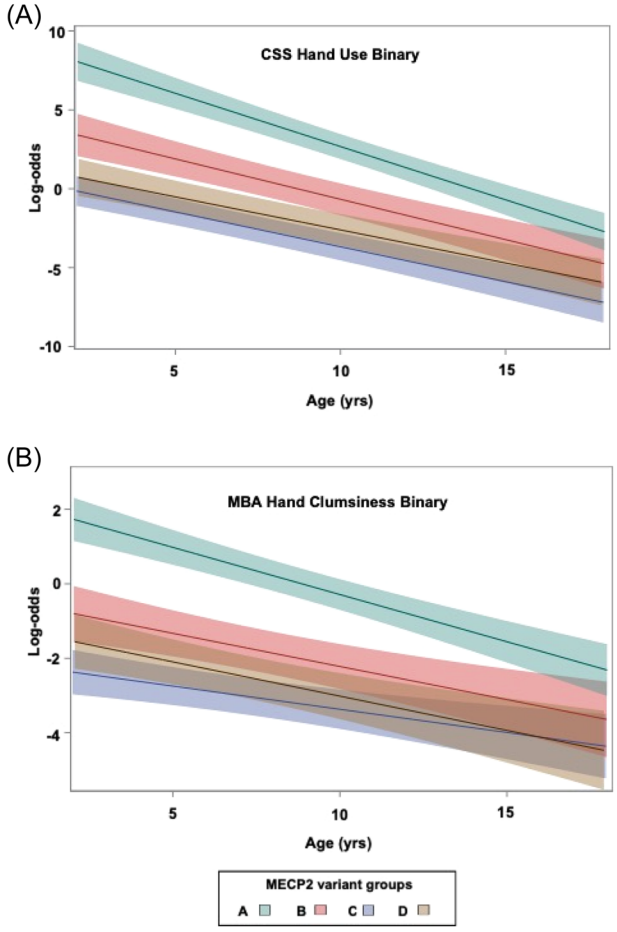

Figure 2. Hand function from age 2 to age 18 by variant group for CSS and MBA. (A) is CSS hand use and (B) is MBA hand clumsiness. Mutation groups include specific variant: Group A includes R133C, R294X, R306C, and C-terminal truncations; Group B includes T158M and other point variations; Group C includes R106W, R168X, R255X, and R270X; Group D includes large deletions, splice site variations, and early truncations (before nucleotide 850). Hand function was assessed for 946 individuals from age 2 through 18 years. A generalized linear mixed model (GLMM) was used to assess the changes over age for each of the two dichotomous outcomes. The fixed effects in the GLMM were mutation group, age, and mutation group × age interaction. Regression estimates are as log-odds. Two-sided p values are reported. Statistical significance was determined when the $p < 0.05$. Analyses were performed using SAS 9.4 (SAS Institute).

group again showed the steepest slope of decline, as shown for individual hand skills (Figure 1A–F), compared with the three other groups. While Group D was higher than Group C at age 2 years, the two curves intersect just after age 15 years and Group C is slightly greater than Group D at age 18 years. The description of the statistical assessments is shown in Supporting Information: Table 2B.

When considering each group at age 2 years (Table 4), Group A was highly significantly different from Groups B, C, and D. Group B was very significantly different from Group C but not different from Group D. Groups C and D were not different. At age 18 years (Table 4), Group A was significantly different from Group B and highly significantly different from Groups C and D. However, Groups B, C, and D were not different from each other.

To understand the intermediate age ranges between age 2 and age 18, as shown for the CSS, age groups from 8–14 to 15–18 were examined (Table 4). MBA hand skill for each age group was evaluated for Group A versus Groups B, C, and D, both for each CSS level (0–4) and then for the simplified scoring of compressing the skills into two bins: normal or partially conserved and lost or never acquired. Group A remained highly significantly different from Groups B, C, and D in both age ranges, whether compared across the MBA hand skill levels or collapsed into those with and without hand skills.

Discussion

This detailed examination of the array of hand function abilities in girls and young women with RTT confirmed our clinical observations that this critical skill is significantly limited following the initial regression and declines further throughout their first 18 years. These conclusions are based not only on findings from the structured clinical examination but also on two separate measures of clinical severity, the CSS and the MBA. The longitudinal examination of hand function skills based on specific variant groups demonstrated strong relationships between different classes of variants, confirming prior reports of critical variation group differences for overall functional skill assessments. As a group, individuals with point variations at R133C, R294X, R306C, and truncations distal to nucleotide 850 tended to have more preserved, albeit very abnormal, hand function ability. This statistically clear group difference may be influenced by variations in X-chromosome inactivation, overall genetic background, and environmental factors, making this information broadly applicable, although it is limited for individual considerations. These data appear to represent a seeming difference from those of Downs et al.[9] However, the results actually provide agreement that younger children do appear susceptible to loss of hand function more readily, and genotype does provide some predictive value in this process.

The gradual decline in hand skills over time, regardless of the position or type of *MECP2* variation albeit seemingly more rapid for Group A containing the milder variants, is evident and a potentially critical point regarding the impact of timing for pharmacologic interventions or gene

Table 3. CSS comparisons between variation groups at 2 and 18 years old.

	At 2 years old		At 18 years old	
Comparison	Estimate (SE)	*p* (*t* value, DF)	Estimate (SE)	*p* (*t* value, DF)
A vs. B	−4.6 (0.85)	<0.0001 (−5.4, 3937)	−2.1 (1.0059)	0.036 (−2.1, 3937)
A vs. C	−8.1 (0.83)	<0.0001 (−9.8, 3937)	−4.5 (0.89)	<.0001 (−5.1, 3937)
A vs. D	−7.3 (0.88)	<0.0001 (−8.2, 3937)	−3.3 (0.96)	0.0007 (−3.4, 3937)
B vs. C	3.5 (0.86)	<0.0001 (4.1, 3937)	2.4 (0.97)	0.013 (2.5, 3937)
B vs. D	2.6 (0.92)	0.0044 (2.9, 3937)	1.2 (1.0)	0.27 (1.1, 3937)
C vs. D	−0.88 (0.76)	0.25 (−1.2, 3937)	−1.3 (0.91)	0.17 (−1.4, 3937)
A vs. B	−2.5 (0.49)	<0.0001 (−5.2, 3938)	−1.3 (0.61)	0.029 (−2.2, 3938)
A vs. C	−4.1 (0.44)	<0.0001 (−9.3, 3938)	−2.1 (0.55)	0.0002 (−3.8, 3938)
A vs. D	−3.3 (0.49)	<0.0001 (−6.8, 3938)	−2.2 (0.63)	0.0006 (−3.4, 3938)
B vs. C	1.6 (0.48)	0.0011 (3.3, 3938)	0.71 (0.65)	0.27 (1.1, 3938)
B vs. D	0.73 (0.53)	0.16 (1.3, 3938)	0.83 (0.72)	0.25 (1.2, 3938)
C vs. D	−0.83 (0.47)	0.074 (−1.8, 3938)	0.13 (0.66)	0.85 (0.19, 3938)

replacement as well as the potential modification of X chromosome inactivation currently under evaluation in translational studies.

Of particular interest is that Group A, which contains the variants with overall mild involvement, had much better hand function at age 2 than the other three variant groups. However, it also had the steepest decline for all hand functions except raking grasp, indicating a more rapid decline in specific hand skills. As a result, by age 18 it was little different from the other variant groups and in the case of pincer and palmar grasp was equal to or worse than Group B. The precise basis for this is unresolved by our analysis and could be the basis for further study. What is clear is that the impact of treatment interventions, as noted above, may have a critical window for instituting.

Many factors may be responsible for this time-dependent deterioration in hand function, including the frequency and intensity of hand stereotypies that may dominate the girls' waking hours[10] and may lead to structural alterations in the hands as well as functional limitations in the upper extremities more generally. Alterations in muscle tone may have a significant impact as well. Muscle tone progresses from overall hypotonia to hypertonia and rigidity, along with concomitant contractures, further limiting the ability to reach and grasp. Periodic breathing disturbances while awake (breath-holding, hyperventilation, or both) increase during the school-age years and clearly interrupt volitional activities.[11] Other factors, including anxiety or agitation, dystonia, dyskinesias, or parkinsonian features, which are all known to be increased in RTT, are also potentially important considerations for worsening hand function with increasing age.[12,13]

These findings have important implications for ongoing or proposed clinical trials as alleviation of any of the above factors, such as the hand stereotypies, periodic breathing, and evolving dyspraxia, could represent a clinical benefit for these individuals and their families but do not mean improved hand use. Thus, close attention to the different factors that could improve as part of pharmacological or gene therapy trials is paramount, as modification of any aspect that interacts with hand function could provide some guidance on overall functional improvements. Assessment of hand function, ambulatory skills, periodic breathing frequency, behavior, and dyspraxia in general could represent key endpoints for assessing definitive clinical improvement.

A note of caution should be raised. Individuals with RTT who have partial improvement in hand skills or ambulation without corresponding improvement in other areas such as behavior or cognition might be more likely to engage in undesirable behaviors as previously noted.[14]

A potential limitation of this study is the specific assessment of hand function at each study visit. The child's abilities during this relatively limited observation period (30–60 minutes) could be modified by external factors and not reflect the skill level at home. Therefore, the six-point hand function scale may not represent the actual level of function. Similar evaluations of caregiver reports could be informative and warrant future assessment. For example, a child could have a modified pincer grasp at home that is not observed during the study visit. The above analyses were based on a linear model. Future work could evaluate a nonlinear methodology. In addition, the impact of occupational therapies, bracing, and parental encouragement are not accounted for in this study. While these concerns may have modest effects overall, the clear patterns observed in nearly 950 individuals suggest an overall decline in hand skills over time.

Table 4. CSS and MBA comparisons between variation groups at 2 and 18 years old.

		Ages 8–14					Age 15–18				
CSS	CSS hand use level	0	1	2	3	4	*p* value (χ^2)	0	1	2	3	4	*p* value (χ^2)
	A % (*n*)	1.4 (26)	45.4 (277)	9.8 (60)	39.7 (242)	0.82 (5)	<0.0001	7.6 (10)	35.6 (47)	9.1 (12)	46.2 (61)	1.5 (2)	0.0008
	BCD % (*n*)	1.1 (13)	22.4 (274)	10.0 (122)	63.1 (772)	3.51 (43)		2.5 (7)	22.4 (63)	6.4 (18)	45.8 (189)	1.4 (4)	
	CSS hand use level	0, 1, 2			3, 4		*p* value (χ^2)	0, 1, 2			3, 4		*p* value (χ^2)
	A % (*n*)	59.5 (363)			40.5 (247)		<0.0001	52.3 (69)			47.4 (63)		<0.0001
	BCD % (*n*)	33.4 (409)			66.6 (815)			31.3 (88)			68.7 (193)		
MBA	MBA hand clumsiness level	0	1	2	3	4	*p* value (χ^2)	0	1	2	3	4	*p* value (χ^2)
	A % (*n*)	1.5 (9)	16.9 (103)	25.1 (153)	19.3 (118)	37.2 (227)	<0.0001	2.3 (3)	13.6 (18)	21.2 (28)	24.2 (32)	38.6 (51)	<0.0001
	BCD % (*n*)	1.0 (12)	8.4 (103)	11.4 (140)	15.8 (194)	63.4 (778)		0.7 (2)	8.2 (23)	8.5 (24)	16.0 (45)	66.6 (187)	
	MBA hand clumsiness level	0, 1, 2			3, 4		*p* value (χ^2)	0, 1, 2			3, 4		*p* value (χ^2)
	A % (*n*)	43.4 (265)			56.6 (345)		<0.0001	37.1 (49)			61.9 (83)		<0.0001
	BCD % (*n*)	20.8 (255)			79.2 (972)			17.4 (49)			82.6 (232)		

Conclusions

The steady decline in hand skills over the first 18 years represents a key element in assessing the natural history of RTT. Specific *MECP2* variants have a significant impact on this functional level and need to be considered in the assessment of emerging therapies, including gene replacement. These factors alone, namely the steady decline with increasing age and the impact of the specific genetic variant, could require careful consideration of the timing of future gene replacement trials. The recent FDA approval of trofinetide (Daybue) deserves mention as these natural history data stand as an important benchmark against which to assess the future efficacy of this and future therapeutic agents.[15]

Author Contributions

Jeffrey L. Neul: Conceptualization; data curation; formal analysis; investigation; methodology; writing—review and editing. **Tim A. Benke**: Data curation; formal analysis; investigation; methodology; writing—review and editing. **Eric D. Marsh**: Conceptualization; data curation; formal analysis; investigation; methodology; writing—review and editing. **Jane B. Lane**: Investigation; methodology; supervision; writing—review and editing. **David N. Lieberman**: Investigation; methodology; writing—review and editing. **Steven A. Skinner**: Investigation; methodology; writing—review and editing. **Daniel G. Glaze**: Investigation; methodology; writing—review and editing. **Bernhard Suter**: Investigation; methodology; writing—review and editing. **Peter T. Heydemann**: Investigation; methodology; writing—review and editing. **Arthur A. Beisang**: Investigation; methodology; writing—review and editing. **Robin C. C. Ryther**: Investigation; methodology; writing—review and editing. **Richard H. Haas**: Investigation; methodology; writing—review and editing. **Lloyd J. Edwards**: Formal analysis; methodology; writing—review and editing. **Amitha Ananth**: Investigation; methodology; writing—review and editing. **Alan K. Percy**: Conceptualization; formal analysis; funding acquisition; investigation; methodology; project administration; writing—original draft.

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Conflicts of Interest

Dr. Neul has received research funding from the National Institutes of Health, the International Rett Syndrome Foundation (IRSF), and Rett Syndrome Research Trust (RSRT); personal consultancy for Acadia Pharmaceuticals Inc., Analysis Group, AveXis, GW Pharmaceuticals, Hoffmann-La Roche, Myrtelle, Neurogene, Newron Pharmaceuticals, Signant Health, Taysha Gene Therapies, and the preparation of CME activities for PeerView Institute and Medscape; serves on the scientific advisory board of Alcyone Lifesciences; is a scientific cofounder of LizarBio Therapeutics; and was a member of a data safety monitoring board for clinical trials conducted by Ovid Therapeutics. Dr. Benke has received research funding from GRIN2B Foundation, the International Foundation for CDKL5 Research, Loulou Foundation, the National Institutes of Health, and Simons Foundation; consultancy for Alcyone, AveXis, GRIN Therapeutics, GW Pharmaceuticals, the International Rett Syndrome Foundation, Marinus Pharmaceuticals, Neurogene, Ovid Therapeutics, and Takeda Pharmaceutical Company Limited; clinical trials with Acadia Pharmaceuticals Inc., GW Pharmaceuticals, Marinus Pharmaceuticals, Ovid Therapeutics, and RSRT; all remuneration has been made to his department. Dr. Marsh is site PI for Stoke Therapeutics, Zogenix Pharmaceuticals, Acadia Pharmaceuticals, Marinus Pharmaceuticals, Takeda Pharmaceuticals, and Epygenix Pharmaceuticals. He has received research support from NIH, Penn Orphan Disease Center, IRSF, International CDKL5 Research Foundation, and Curaleaf Inc.; is a consultant for Acadia Pharmaceuticals; and has prepared an educational program for Medscape. Ms. Lane has nothing to report. Dr. Lieberman was site PI for Acadia Pharmaceuticals, Anavex Life Sciences, and GW Pharmaceuticals. He received research support from the IRSF and RSRT. He has served as a consultant for Acadia Pharmaceuticals, Taysha Gene Therapies, and Neurogene. Dr. Skinner was a site PI for Acadia Pharmaceuticals and is a consultant with Acadia Pharmaceuticals. Dr. Glaze has nothing to report. Dr. Suter has consultancy for Neurogene and Taysha with all remuneration paid to his department. He has been an investigator for clinical trials with Acadia Pharmaceuticals, Marinus, and Newron Pharmaceuticals. Dr. Heydemann has no competing interests, but has been a consultant or PI for Newron Pharmaceuticals, GW Pharmaceuticals, Anavex Life Sciences, and Marinus Pharmaceuticals. Dr. Beisang is a consultant for Acadia Pharmaceuticals. Dr. Standridge has no competing interests. She has been a consultant for GW Pharma, Acadia, and Zogenix (bought by UCB). Dr. Ryther was site PI for Acadia Pharmaceuticals and a site sub-I for Marinus Pharmaceuticals. She has received research support from IRSF. Dr. Haas has nothing to report. Dr. Edwards has nothing to report in terms of competing interests. His support was in part by P30 BIGDATA core grant NIH P30AR072583. Dr. Ananth was a site PI for Acadia Pharmaceuticals. Dr. Percy has received research support from the National Institutes of Health and has been a site PI for Acadia Pharmaceuticals. He is a consultant for Acadia Pharmaceuticals, Taysha Gene Therapies, and Neurogene. He has prepared educational materials for WebMD, Medscape, Pharmacy Times Continuing Education, Prime Inc., and the CME Institute.

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Supporting Information

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