Hand stereotypies

Lessons from the Rett Syndrome Natural History Study

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Neurology® 2019;92:e2594–e2603. doi:10.1212/WNL.00000000000007560

Abstract

Objective

To characterize hand stereotypies (HS) in a large cohort of participants with Rett syndrome (RTT).

Methods

Data from 1,123 girls and women enrolled in the RTT Natural History Study were gathered. Standard tests for continuous and categorical variables were used at baseline. For longitudinal data, we used repeated-measures linear and logistic regression models and nonparametric tests.

Results

HS were reported in 922 participants with classic RTT (100%), 73 with atypical severe RTT (97.3%), 74 with atypical mild RTT (96.1%), and 17 females with *MECP2* mutations without RTT (34.7%). Individuals with RTT who had classic presentation or severe *MECP2* mutations had higher frequency and earlier onset of HS. Heterogeneity of HS types was confirmed, but variety decreased over time. At baseline, almost half of the participants with RTT had hand mouthing, which like clapping/tapping, decreased over time. These 2 HS types were more frequently reported than wringing/washing. Increased HS severity (prevalence and frequency) was associated with worsened measures of hand function. Number and type of HS were not related to hand function. Overall clinical severity was worse with decreased hand function but only weakly related to any HS characteristic. While hand function decreased over time, prevalence and frequency of HS remained relatively unchanged and high.

Conclusions

Nearly all individuals with RTT have severe and multiple types of HS, with mouthing and clapping/tapping decreasing over time. Interaction between HS frequency and hand function is complex. Understanding the natural history of HS in RTT could assist in clinical care and evaluation of new interventions.

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Go to Neurology.org/N for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

Glossary

CSS = Clinical Severity Scale; HS = hand stereotypies; LH = left-handed; MBA = Motor Behavioral Assessment; RDCRN = Rare Disease Clinical Research Network; RH = right-handed; RNHS = Rett Syndrome Natural History Study; RTT = Rett syndrome.

Rett syndrome (RTT) (OMIM #312750)^{1,2} is an X-linked neurodevelopmental disorder that typically affects females (approximately 1/9,000–1/10,000).^{3,4} Diagnosis of RTT is clinical and encompasses a phenotypic spectrum of severity.⁵ One of the 4 core diagnostic criteria is the presence of repetitive hand movements termed hand stereotypies (HS), which along with partial or complete loss of hand function, partial or complete loss of language, and impaired gait, are required for the diagnosis of classic RTT. Atypical RTT is diagnosed when at least 2 of these 4 main criteria are present plus 5 of 11 supportive criteria (breathing disturbances, bruxism when awake, impaired sleep, tone abnormalities, peripheral vasomotor disturbances, scoliosis/kyphosis, growth retardation, small cold hands and feet, inappropriate laughing/screaming spells, diminished pain response, and intense eye communication).⁶ These supportive criteria are highly prevalent in classic RTT. Though not a requirement for diagnosis, HS are very common in atypical RTT.⁵ RTT is usually associated with a pathogenic mutation in the methyl-CpG binding protein 2 (*MECP2*) gene⁷; genotype-phenotype correlations have led to groups of mutations with different levels of severity.^{8–11} Despite the prevalence and distinctive nature of HS in RTT, large-scale descriptive or longitudinal studies are lacking, and the underlying pathophysiology remains uncertain. Prior studies in smaller cohorts are consistent in reporting high prevalence and heterogeneity of HS in RTT.^{12–17} However, the relationship between HS and hand function is less clear, as is the progression of HS over time.^{5,12,14,16,18,19} Our study aims to provide an overview of the features of HS in RTT, including prevalence, frequency, number, and type of HS, and their longitudinal progression in the largest cohort of participants with RTT to date. The interaction between HS and hand function is also examined. Understanding the natural history of HS in RTT is critical to directing diagnosis, guiding prognosis, and testing the efficacy of new interventions in clinical trials.

Methods

Participants

From 2006 to 2015, the Rett Syndrome Natural History Study (RNHS) recruited and enrolled 1,123 females with a clinical diagnosis of RTT and/or a *MECP2* mutation; male participants (n = 50) and those missing key baseline data (n = 44) were excluded from this analysis. Assessments were conducted every 6 to 12 months as previously described.^{20,21}

Human studies approval

Each participating RNHS site obtained institutional review board approval for the study. Parental consent for study

conduct and publication of results was obtained prior to enrollment. The study has been registered with ClinicalTrials.gov NCT00299312 since March 3, 2006.

Diagnosis and genetic testing

Diagnoses of RTT and RTT-related disorders were performed by a study neurologist (A.K.P., J.L.N., W.E.K., D.G.G.) or geneticist (S.A.S.) with extensive clinical experience in RTT, based on published diagnostic criteria.^{5,22} Clinical Severity Scale (CSS) total scores were used to stratify individuals with atypical RTT into 2 phenotypic categories: atypical severe (score >20) and atypical mild (score ≤20).^{10,11} All participants had *MECP2* testing; mutations were categorized as severe (R106W, R168X, R255X, R270X, insertions, deletions, large deletions, and splice site), moderate (T158M), or mild (R133C, R294X, R306C, 3' truncations, and other point mutations) based on prior reports of genotype-phenotype correlations that have demonstrated distinctive severity profiles for the most common *MECP2* mutations.^{8–11} Participants with clinical RTT without a *MECP2* mutation were excluded from analyses involving *MECP2* mutation severity.

CSS and Motor Behavioral Assessment

The CSS and Motor Behavioral Assessment (MBA) are quantitative scales for determining severity of clinical manifestations in RTT as reported.²¹ Each individual item is scored from 0 to 4 (CSS, MBA) or 0 to 5 (CSS), with higher scores indicating greater severity. In addition to total scores, 5 specific MBA items were selected for additional analysis because of their obvious or potential relationship to HS: mouthing of hands and objects, hand clumsiness, HS frequency (percent of time engaged in HS, a measure of severity), bradykinesia, and hypertonia/rigidity. Associated comorbid conditions (seizures, dystonia, truncal rocking) were also selected from the MBA. Rater calibration on the CSS and MBA took place in the context of 2 clinical trials.^{23,24}

Assessment of HS, handedness, and hand function

At baseline, parents reported the age at onset of regression and the age at onset of each type of HS from 8 preselected categories. At each visit, the study clinician recorded the participant's hand preference, presence or absence of a pincer grasp, hand position, and how many of the 8 specific HS types were observed.

Statistical analysis

Descriptive comparisons and analyses were performed for baseline variables and for confirming consistency of data over

time (e.g., scores of same age participants with different ages at recruitment). Comparative analyses included Spearman correlations between continuous variables, nonparametric tests (Mann-Whitney, Kruskal-Wallis), Fisher exact test, Pearson χ^2 test, and the Somers *D* ordinal association test (i.e., proportion of individuals in a particular category). Longitudinal analyses of classic RTT consisted of mixed-effect regression models, linear for continuous variables and logistic for categorical variables. After exploring different models, all longitudinal analyses used visit as fixed categorical effect, age at assessment as covariate, and participant identification as random effect since these models were the best in terms of statistical parameters and hypotheses being addressed. HS categories were also analyzed by the McNemar test for paired nominal data, which compared annual visits and has been used for longitudinal analyses of diagnostic categories.^{25,26} Visits that occurred past 8 years (a total of 56 events) were excluded to avoid skewing caused by sparse data and limited cohort size. The *p* values less than 0.05 were considered significant without corrections for multiple comparisons and are reported as 2-sided. The analyses were performed using SPSS version 24 (IBM Corp., Armonk, NY).

Data availability

Data are housed at the NIH's Rare Disease Clinical Research Network (RDCRN) Data Management and Coordinating Center, and are available through RDCRN's data sharing policy. Data are also sent to the National Center for Biotechnology's Database of Genotypes and Phenotypes annually.

Results

Characteristics of the study population

Figure 1 summarizes the characteristics of participants in the RNHS, who ranged from age 1.3 to 66.4 years (mean age 9.54 years). Additional demographic information has been reported previously.²¹ The largest diagnosis group, classic RTT, was followed for an average of 4.22 years with a range of 0 to 9.5 years. Females with a *MECP2* mutation who did not meet criteria for RTT constituted a comparison group.

Characteristics of HS at baseline by diagnosis: Prevalence, frequency, number, and type

At study enrollment, 99.5% of participants with RTT had a history of HS, including all participants with classic RTT, 97.3% with atypical severe, and 96.1% with atypical mild RTT. Similar proportions of HS were observed on examination at baseline. The non-RTT *MECP2*-positive group confirmed the specificity of HS in RTT with only 35% having HS either in their history or observed at baseline ($p < 0.001$).

Mean HS frequency scores differed by diagnosis. Atypical severe and atypical mild groups had significantly lower scores than the classic RTT group (table 1). HS frequency scores did not demonstrate a normal distribution; more than half of the participants with classic RTT had the maximum score ("100% of

Figure 1 Flow diagram of the study

Study population and description of groups enrolled in the Rett Syndrome Natural History Study. *MECP2* = methyl-CpG binding protein 2; RTT = Rett syndrome.

the time"), while the majority of the non-RTT *MECP2*-positive group had the minimum score ("0% of the time") (figure 2).

Participants with RTT were reported to have a variety of the 8 preselected HS types (range 0–7; figure 3). The classic RTT group had the most HS types per participant (2.73 ± 1.3 SD), while both atypical RTT groups had fewer (mild: 2.00 ± 1.3 ; severe: 1.89 ± 1.0 ; $p < 0.001$). The non-RTT *MECP2*-positive group averaged less than 1 HS type per participant (0.61 ± 1.2 ; $p < 0.001$).

Clapping/tapping (41%–43%) was reported more often than wringing/washing (23%–37%) in each RTT diagnosis group at baseline (figure 3A). Regarding age groups, clapping/tapping was more common than wringing/washing until the 10- to 15-year age range in all participants with RTT at baseline (figure 3B). At baseline, 49.5% of all participants with classic RTT had mouthing; 54.5% of those involved both hands, while 23.9% mouthed only the right and 21.5% only the left. More than half of participants younger than 5 years had mouthing. Mouthing of hands and objects differed by diagnosis: scores for atypical severe RTT were higher than classic RTT, which were higher

Table 1 Hand stereotypy and hand use item scores based on diagnosis group

	HS frequency (range 0–4)	Mouthing of hands and objects (range 0–4)	Hand clumsiness (range 0–4)
Classic RTT (n = 922)	3.36 ± 1.0	1.28 ± 1.4	2.79 ± 1.2
Atypical severe RTT (n = 75)	2.85 ± 1.3 ^{↓b}	1.41 ± 1.6	3.19 ± 1.0
Atypical mild RTT (n = 77)	2.25 ± 1.4 ^{↓a}	0.84 ± 1.2 ^{↓c}	1.05 ± 1.1 ^{↓a}
Non-RTT <i>MECP2</i> -positive (n = 49)	0.50 ± 1.0 ^{↓a}	0.23 ± 0.7 ^{↓a}	0.50 ± 1.1 ^{↓a}

Abbreviations: HS = hand stereotypy; *MECP2* = methyl-CpG binding protein 2; RTT = Rett syndrome.

Values shown are mean scores ± SD. Higher scores indicate greater impairment. ↓ = values are significantly lower than the classic RTT group based on nonparametric Kruskal-Wallis test.

^a $p \leq 0.001$.

^b $p \leq 0.01$.

^c $p \leq 0.05$.

than atypical mild RTT. The lowest scores were seen in the non-RTT *MECP2*-positive group (table 1).

Characteristics of HS at baseline visit by *MECP2* mutation severity

When all participants with RTT were stratified by *MECP2* mutation severity, no difference was noted in HS prevalence (mild 98.1%, moderate 99.0%, severe 99.2%; $p > 0.05$), but other HS characteristics were affected. HS frequency and number were slightly higher in the severe *MECP2* mutation group relative to the mild group (table 2). Mouthing of hands

and objects was higher in the severe group (table 2); no other HS type was dependent on *MECP2* mutation severity.

Characteristics of HS at baseline visit by age groups

When participants younger than 21 years with RTT ($n = 949$) were compared as a group to participants 21 years and older ($n = 122$), HS prevalence and frequency did not differ ($p > 0.05$). The HS number and severity of mouthing of hands and objects were higher in the pediatric cohort ($p < 0.001$, table 2). Mouthing and clapping/tapping were more frequent in the pediatric group compared to clasping/posturing in the adult group (figure 3B).

Age at onset of HS

The age at onset of HS showed significant differences by diagnosis group: atypical severe (1.52 ± 1.1 years) had onset earlier than classic (1.87 ± 1.1 years), which in turn was earlier than atypical mild (3.06 ± 2.5 years; $p < 0.001$). Likewise, those with severe and moderate *MECP2* mutations exhibited earlier onset of HS relative to the mild mutation group. In addition, onset of HS and onset of regression occurred in varying order; the majority of participants with RTT demonstrated regression first (62.7%), but 24.1% demonstrated HS first with 13.3% having simultaneous onset of HS and regression reported.

Characteristics of HS by hand function

We assessed hand function using pincer grasp and hand clumsiness items, with the former as a measure of retained hand skill and the latter as a general purposeful hand use metric. These 2 items correlated well with each other (table 2). Presence of HS on baseline examination was associated with lack of pincer grasp ($p = 0.01$) and less purposeful hand use ($p < 0.05$). HS frequency was greater (table 2) and onset of HS earlier in participants without a pincer grasp

Figure 2 Hand stereotypy frequency scores (percent of time observed by clinician) based on diagnosis group

HS = hand stereotypy; *MECP2* = gene coding the methyl-CpG binding protein 2; RTT = Rett syndrome.

Figure 3 Hand stereotypies types in Rett syndrome

($p < 0.001$); however, correlations between these HS characteristics and purposeful hand use were negligible (Spearman $\rho < 0.3$; $p < 0.001$). Neither hand function measure correlated to number of HS (Spearman $\rho = -0.011$, NS; table 2) or presence of any particular HS type.

HS, overall clinical severity, and associated comorbid conditions

Correlations between HS characteristics and overall clinical severity scores were examined in all participants with RTT. HS prevalence was not related to CSS total or MBA total. HS frequency, number of HS types, mouthing of hands and objects, and onset of HS had only minor associations (Spearman $\rho < 0.3$) with these overall severity scores and with associated comorbidities such as seizures and other stereotyped and nonstereotyped movements. Individuals with clapping/tapping had less clinical severity (CSS and MBA) than those without clapping/tapping ($p \leq 0.001$); wringing/washing was associated with lower CSS total scores ($p < 0.01$).

Hand position and preference

For participants with RTT, 76.6% had midline hand position noted; 36.3% were right-handed (RH), 28.7% left-handed

(LH), 11.3% classified as variable, and 23.7% unable to determine hand preference. Hand position and preference differed by diagnosis. Midline hand position was noted in 78.4% with classic RTT, 73.3% with atypical severe RTT, and 58.4% atypical mild RTT ($p < 0.001$); LH preference did not differ by diagnosis group. Participants with midline hand position had higher prevalence, frequency, and number of HS than those without midline positioning ($p < 0.001$); no such differences were noted when participants with RTT and RH dominance ($n = 388$) were compared to those with LH dominance ($n = 307$). Both hand position and preference were related to some HS types. Wringing/washing, clapping/tapping, and clasping/posturing were more often associated with midline hand position than other HS ($p < 0.001$) and squeezing/flicking overrepresented in those with RH dominance ($p < 0.05$).

Longitudinal data

Characteristics of HS in the classic RTT group were evaluated over time; no changes in prevalence were found across each annual visit ($p > 0.05$). To further evaluate the effects of age, we performed repeated-measures logistic regression analyses with the HS characteristic's presence or absence as outcome.

Table 2 Hand stereotypy and hand use item scores in Rett syndrome based on *MECP2* mutation severity, presence or absence of pincer grasp, and age cohort

Cohort	HS frequency (range 0–4)	No. of HS types reported (range 0–7)	Mouthing of hands and objects (range 0–4)	Hand clumsiness (range 0–4)
Mild <i>MECP2</i> mutation (n = 419)	3.14 ± 1.1	2.53 ± 1.3	1.08 ± 1.4	2.16 ± 1.3
Moderate <i>MECP2</i> mutation (n = 105)	3.27 ± 1.0	2.45 ± 1.1	1.32 ± 1.5	2.93 ± 1.2 ^a
Severe <i>MECP2</i> mutation (n = 479)	3.39 ± 1.0 ^{†b}	2.74 ± 1.3 ^{†c}	1.40 ± 1.5 ^{†b}	3.16 ± 1.1 ^{†a}
Pincer grasp (n = 194)	2.95 ± 1.3	2.62 ± 1.4	1.11 ± 1.3	1.32 ± 1.0
No pincer grasp (n = 878)	3.31 ± 1.0 ^{†a}	2.61 ± 1.3	1.29 ± 1.5	3.00 ± 1.2 ^{†a}
Pediatric (n = 948)	3.25 ± 1.1	2.67 ± 1.3 ^{†a}	1.34 ± 1.5 ^{†a}	2.66 ± 1.3
Adult (n = 122)	3.20 ± 1.2	2.18 ± 1.2	0.61 ± 1.2	2.96 ± 1.2 ^{†c}

Abbreviation: HS = hand stereotypy.

Analyses on the entire Rett syndrome cohort, regardless of clinical presentation. Severe mutations = R106W, R168X, R255X, R270X, insertions, deletions, large deletions, and splice site; moderate mutation = T158M; mild mutations = R133C, R294X, R306C, 3' truncations, and other point mutations. Values expressed are mean ± SD. Higher scores indicate greater impairment for HS frequency, mouthing of hands and objects, and hand clumsiness. † = values are significantly higher than the mild group (for mutation severity groups) or than the comparison group (for pincer grasp and age groups) based on nonparametric independent-samples Kruskal-Wallis test.

^a $p \leq 0.001$.

^b $p \leq 0.01$.

^c $p \leq 0.05$.

These demonstrated that mouthing and, to a lesser extent, clapping/tapping decreased over time, while wringing/washing change throughout the study was not detectable (figure 4A). Similar analyses for other HS types were not feasible because of the relatively small number of observations of the events. Logistic regression models better predicted absence of HS (specificity), particularly during the first 2 years of follow-up. Figure 4A illustrates the dynamic of changes in mouthing and clapping/tapping as percentage of HS correctly predicted. Categorical analyses assessed using the McNemar test, divided at baseline into pediatric (<21 years) and adult (>21 years) groups, revealed that the decrease in mouthing was predominantly during the pediatric age group (figure 4B).

Linear mixed regression models were used to evaluate the effect of increasing age on several characteristics of HS and hand use through the 8-year visits in participants with classic RTT. Results indicate that, over time, HS frequency does not change but both number of HS and mouthing of hands and objects decreased. This contrasts with worsening of hand use, bradykinesia, and hypertonia/rigidity (table 3).

Discussion

HS are a defining feature in RTT.⁵ In this study, we aimed to characterize HS systematically in the largest cohort of females with the disorder. We confirmed that HS are present with high frequency (severity) in the majority of participants with classic and atypical RTT (>96%).^{12,14,17} Despite using different categorical HS descriptions from previous studies, we found a similar number of HS types (approximately 2 per participant).^{9,13,16,27} While we confirmed that mouthing is

common, we found that clapping/tapping is also prevalent and shows similar dynamics to mouthing, an interesting deviation from the traditional description of HS in RTT as midline hand wringing.^{13,27–29} This discrepancy may be explained by the recently reported low interrater reliability of HS categories.³⁰ Extending the findings of a previous study evaluating RTT diagnostic criteria,⁶ we found that in non-RTT *MECP2*-positive females not only is HS prevalence low but also HS frequency and mouthing, confirming the specificity of these features to RTT. Another observation with diagnostic implications is that, while HS presence is not required for the diagnosis of atypical RTT, HS were found to be both prevalent and severe in this group. One recent study found that individuals with atypical RTT had more diverse HS.¹⁷ In contrast, we observed greater HS heterogeneity in classic RTT. Our study, which evaluated HS among atypical mild and atypical severe RTT, demonstrated that differences in these diagnostic groups are reflected in HS characteristics, specifically higher prevalence, frequency, and mouthing in the severe group. One prior study¹⁴ reported that individuals with more severe *MECP2* mutations had less frequent HS and less mouthing; in contrast, we found that more severe mutations are associated with greater HS frequency and number, earlier onset, and increased mouthing. Of interest, overall clinical severity is not associated with HS frequency or other HS characteristics.

Previous studies have evaluated the timing of HS onset and the onset of developmental regression, with contradicting results.^{3,12,14–16,31,32} Here, we demonstrated that regression precedes HS in the majority of participants with RTT. However, at least one-third of individuals present with early

Figure 4 Longitudinal changes in prevalence of the most common types of hand stereotypies in Rett syndrome

HS onset. More detailed investigations of the timing of stereotypy appearance in affected individuals may reveal important clues about the pathophysiology of RTT. In this large, longitudinal study of HS in RTT, we demonstrated that HS prevalence and frequency remain high over time in classic RTT. We also expanded on previous reports of reduced HS heterogeneity with age,^{12,14,15,17} by identifying an age-dependent decrease in mouthing and clapping/tapping. Most important, however, are the longitudinal findings concerning the evolution of HS frequency and hand function. Earlier studies have reported that HS severity in individuals with RTT is correlated with hand function: decreased ability to manipulate objects exists when HS are more severe.^{12,14} We found instead that age at onset and frequency of HS seem to relate to level of achieved or retained hand function (pincer grasp), while the progressive decline in purposeful hand use appears to be independent of the relatively stable high severity of HS. Our analyses also suggest that increasing bradykinesia and hypertonia/rigidity may influence hand function and HS heterogeneity but not HS frequency. This complex

relationship between HS and hand function has prognostic and therapeutic implications. Targeting early developmental processes may lead to reduced HS severity and increased level of hand skills, while management of bradykinesia and increased muscle tone in childhood and adolescence could help to preserve existing hand function. Future studies evaluating the effects of medication and other associated comorbid conditions, such as sleep problems, could also help to provide additional clues for therapeutic interventions.

The RNHS represents the largest cohort of participants with RTT in the United States; thus, we believe our findings are generalizable to the broader RTT population. A limitation is that adults accounted for only 12% of the total study population. In addition, we did not include males in our analyses and excluded atypical RTT from longitudinal analyses; further studies could evaluate HS in these populations. For the longitudinal analyses, the use of the month of the visit and age at assessment could have led to slight overfitting in the models; however, since the goal of the analyses was descriptive rather

Table 3 Linear mixed-model analysis for hand stereotypy and hand function–related items over 8 years of study visits in classic Rett syndrome

A. Models with age as a covariate						
Item score (range)	Source	F	Numerator, df	Denominator, df	p Value	Coefficient
HS frequency (0–4)	Age at assessment	83.361	1	5,227	<0.001 ^a	–0.001
	Visit	2.453	16	5,227	0.001 ^b	All positive ^c
No. of types of HS (0–7)	Age at assessment	132.628	1	5,233	<0.001 ^a	–0.002
	Visit	4.515	16	5,233	<0.001 ^a	All negative ^c
Mouthing of hands and objects (0–4)	Age at assessment	174.871	1	5,228	<0.001 ^a	–0.002
	Visit	8.356	16	5,228	<0.001 ^a	All negative ^c
Hand clumsiness (0–4)	Age at assessment	0.032	1	5,228	0.857	0.00003
	Visit	8.812	16	5,228	<0.001 ^a	All positive ^c
Bradykinesia (0–4)	Age at assessment	754.502	1	5,228	<0.001 ^a	0.004
	Visit	3.436	16	5,228	<0.001 ^a	7 of 8 positive ^c
Hypertonia/rigidity (0–4)	Age at assessment	749.624	1	5,227	<0.001 ^a	0.005
	Visit	3.179	16	5,227	<0.001 ^a	6 of 8 positive ^c
B. Coefficients of fixed effects for visit by annual visit						
Visit, y	HS frequency	No. of types of HS	Mouthing of hands and objects	Hand clumsiness	Bradykinesia	Hypertonia/rigidity
1	0.116886	–0.17902	–0.06949	0.149584	0.097509	–0.04195
2	0.058842	–0.24398	–0.30571	0.270587	0.174919	0.094186
3	0.147807	–0.36796	–0.36553	0.402264	0.140124	0.099912
4	0.046016	–0.44126	–0.5622	0.43776	0.166666	0.18805
5	0.108625	–0.44461	–0.50421	0.534084	0.086852	0.173655
6	0.26638	–0.34615	–0.54334	0.520667	0.022559	0.320619
7	0.272646	–0.43053	–0.57568	0.467492	–0.00944	0.179859
8	0.476287	–0.10141	–0.36185	0.618161	0.176267	–0.16558

Abbreviations: df = degrees of freedom; HS = hand stereotypy.
^a $p < 0.001$.
^b $p < 0.01$.
^c See table 3B—coefficients of fixed effects for visit by annual visit (8 annual visits after baseline).

than predictive, there was little if any effect on the conclusions reached. Another limitation is the use of nomenclature and subjective reporting that may not be consistently used by clinicians and parents,³⁰ underscoring the need for standardization of terminology and methodologies. Our investigation may also be limited by the potential ceiling effect in the MBA measure of HS severity (HS frequency); the MBA has never been formally analyzed beyond face validity either. Technologies such as video analysis and wearable devices in the RNHS and other future studies could provide a more uniform categorization of HS type (including the 8 HS categories in the RNHS), longer sampling, and better quantification. In addition, only a cursory examination of *MECP2* mutation status and HS was conducted. Future investigations focusing on broadening the genotype-phenotype correlations

regarding HS and hand function would help improve mutation-specific prognosis. Although several hypotheses regarding the underlying pathophysiology of stereotypies in general have been postulated,^{33,34} the neurobiological bases of HS are still unknown. Mouse models of *Mecp2* deficiency show features similar to HS in RTT (hind-limb clasping).³⁵ Nevertheless, the presence of HS in some individuals with *CDKL5* or *FOXG1* mutations, or in those with RTT without *MECP2* mutations, suggest that *MeCP2* deficiency is not an essential requirement for the development of HS. The role of selective volumetric reductions in parietal cortex, the most distinctive neuroimaging feature of RTT, in HS pathophysiology is unknown.³⁶ Therefore, focused neuroimaging and neurophysiologic studies (e.g., diffusion tensor imaging) could provide valuable insight into this core RTT phenotype.

A greater understanding of HS may also contribute to the design of future clinical trials targeting this and other manifestations of RTT.

Author contributions

J.L. Stallworth: drafting or revising manuscript for intellectual content, analysis or interpretation of data. M.E. Dy: drafting or revising manuscript for intellectual content, analysis or interpretation of data. C.B. Buchanan: drafting or revising manuscript for intellectual content. C.-F. Chen: analysis or interpretation of data. A.E. Scott: analysis or interpretation of data. D.G. Glaze: drafting or revising the manuscript for intellectual content. J.B. Lane: drafting or revising the manuscript for intellectual content. D.N. Lieberman: drafting or revising manuscript for intellectual content. L.M. Oberman: drafting or revising manuscript for intellectual content, analysis or interpretation of data. S.A. Skinner: drafting or revising the manuscript for intellectual content. A.E. Tierney: drafting or revising manuscript for intellectual content, analysis or interpretation of data. G.R. Cutter: analysis or interpretation of data. A.K. Percy: drafting or revising the manuscript for intellectual content. J.L. Neul: drafting or revising the manuscript for intellectual content. W.E. Kaufmann: drafting or revising manuscript for intellectual content, analysis or interpretation of data, design or conceptualization of study.

Acknowledgment

The authors extend their sincerest appreciation to all of the participants and their parents whose contribution was vital to this research.

Study funding

Support is provided by grants from the International Rett Syndrome Foundation and from the NIH (RR019478), including the Angelman, Rett, Prader-Willi Syndrome Consortium and the Rett Syndrome, MECP2 Duplication Disorder, and Rett-like Syndrome Consortium (U54HD061222), a part of the NIH Rare Disease Clinical Research Network (RDCRN), supported through collaboration between the NIH Office of Rare Diseases Research (ORDR) at the National Center for Advancing Translational Science (NCATS), the Eunice Kennedy Shriver Child Health and Human Development Institute, and the National Institute of Neurological Diseases and Stroke. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

Disclosure

J. Stallworth reports no disclosures relevant to the manuscript. M.E. Dy received research support from Rettsyndrome.org (Mentored Clinical Fellowship under U54 HD 061222 grant). C. Buchanan received research support from Neuren and participates in clinical trials sponsored by Ovid. C. Chen and A. Scott report no disclosures relevant to the manuscript. D. Glaze is a consultant to Newron Pharmaceuticals and participates in clinical trials sponsored by Neuren and Newron Pharmaceuticals. J. Lane reports no disclosures relevant to the manuscript. D. Lieberman participates in clinical trials sponsored by

Neuren Pharmaceuticals. L. Oberman reports no disclosures relevant to the manuscript. S. Skinner is a consultant to AveXis and participates in clinical trials sponsored by Neuren and Ovid. A. Tierney reports no disclosures relevant to the manuscript. G. Cutter has received support from MO Pharmaceuticals, BioLineRx, Horizon Pharmaceuticals, Merck, Merck/Pfizer, OPKO Biologics, Neurim, Novartis, Ophazyme, Sanofi-Aventis, Reata Pharmaceuticals, Receptos/Celgene, Teva pharmaceuticals, NHLBI (Protocol Review Committee), NICHD (OPRU oversight committee), Atara Biotherapeutics, Axon, Biogen, Biotherapeutics, Argenx, Brainstorm Cell Therapeutics, Charleston Labs Inc., Click Therapeutics, Genzyme, Genentech, GW Pharma, Klein Buendel Inc., MedImmune, MedDay, Novartis, Roche, SciFluor, Somahlution, Teva Pharmaceuticals, TG Therapeutics, UT Houston. He is President of Pythagoras, Inc., a private consulting company located in Birmingham, AL. A. Percy is a consultant with Neuren Pharmaceuticals, Anavex, AveXis, and Teva and participates in clinical trials sponsored by Neuren and Newron Pharmaceuticals. J. Neul is a consultant to Ovid, AveXis, Biohaven, Teva, and Takeda Pharmaceuticals sponsored by Neuren and Newron Pharmaceuticals. W. Kaufmann is a consultant to Anavex, AveXis, Biohaven, Echo, Edison, EryDel, GW, Marinus, Neuren, Newron Pharmaceuticals, Ovid, Stalica, and Zynerba. Go to Neurology.org/N for full disclosures.

Publication history

Received by *Neurology* January 5, 2018. Accepted in final form January 25, 2019.

References

1. Hagberg B, Aicardi J, Dias K, Ramos O. A progressive syndrome of autism, dementia, ataxia, and loss of purposeful hand use in girls: Rett's syndrome: report of 35 cases. *Ann Neurol* 1983;14:471–479.
2. Rett A. On a unusual brain atrophy syndrome in hyperammonemia in childhood. *Wien Med Wochenschr* 1966;116:723–726.
3. Lee JYL, Leonard H, Piek JP, Downs J. Early development and regression in Rett syndrome. *Clin Genet* 2013;84:572–576.
4. Christodoulou J, Weaving LS. MECP2 and beyond: phenotype-genotype correlations in Rett syndrome. *J Child Neurol* 2003;18:669–674.
5. Neul JL, Kaufmann WE, Glaze DG, et al. Rett syndrome: revised diagnostic criteria and nomenclature. *Ann Neurol* 2010;68:944–950.
6. Percy AK, Neul JL, Glaze DG, et al. Rett syndrome diagnostic criteria: lessons from the Natural History Study. *Ann Neurol* 2010;68:951–959.
7. Amir RE, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY. Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nat Genet* 1999;23:185–188.
8. Bebbington A, Anderson A, Ravine D, et al. Investigating genotype-phenotype relationships in Rett syndrome using an international data set. *Neurology* 2008;70:868–875.
9. Cuddapah VA, Pillai RB, Shekar KV, et al. Methyl-CpG-binding protein 2 (MECP2) mutation type is associated with disease severity in Rett Syndrome. *J Med Genet* 2014;51:152–158.
10. Tarquinio DC, Hou W, Berg A, et al. Longitudinal course of epilepsy in Rett syndrome and related disorders. *Brain* 2017;140:306–318.
11. Neul JL, Lane JB, Lee HS, et al. Developmental delay in Rett syndrome: data from the Natural History Study. *J Neurodev Disord* 2014;6:20.
12. Temudo T, Oliveira P, Santos M, et al. Stereotypies in Rett syndrome: analysis of 83 patients with and without detected MECP2 mutations. *Neurology* 2007;68:1183–1187.
13. Temudo T, Ramos E, Dias K, et al. Movement disorders in Rett syndrome: an analysis of 60 patients with detected MECP2 mutation and correlation with mutation type. *Mov Disord* 2008;23:1384–1390.
14. Carter P, Downs J, Bebbington A, et al. Stereotypical hand movements in 144 subjects with Rett syndrome from the population-based Australian database. *Mov Disord* 2010;25:282–288.
15. Vignoli A, La Briola F, Canevini MP. Evolution of stereotypies in adolescents and women with Rett syndrome. *Mov Disord* 2009;24:1379–1383.

16. Temudo T, Freitas P, Sequeiros J, Maciel P, Oliveira G. Atypical stereotypies and vocal tics in Rett syndrome: an illustrative case. *Mov Disord* 2008;23:622–624.
17. Chin Wong L, Hung PL, Jan TY, Lee WT; Taiwan Rett Syndrome Association. Variations of stereotypies in individuals with Rett syndrome: a nationwide cross-sectional study in Taiwan. *Autism Res* 2017;10:1204–1214.
18. Nissenkorn A, Ben-Zeev B. Unilateral rhythmic hand tapping in Rett syndrome: is this stereotypy? *J Child Neurol* 2013;28:1210–1214.
19. Bumin G, Uyanik M, Kayihan H, Düger T, Topçu M. The effect of hand splints on stereotypic hand behavior in Rett's syndrome. *Turk J Pediatr* 2002;44:25–29.
20. Glaze DG, Percy AK, Skinner S, et al. Epilepsy and the natural history of Rett syndrome. *Neurology* 2010;74:909–912.
21. Tarquinio DC, Motil KJ, Hou W, et al. Growth failure and outcome in Rett syndrome: specific growth references. *Neurology* 2012;79:1653–1661.
22. The Rett Syndrome Diagnostic Criteria Group. Diagnostic criteria for Rett syndrome. *Ann Neurol* 1988;23:425–428.
23. Glaze DG, Neul JL, Percy A, et al. Double-blind, randomized, placebo-controlled clinical study of trofinetide in the treatment of Rett syndrome. *Pediatr Neurol* 2017;76:37–46.
24. Glaze DG, Neul JL, Kaufmann WE, et al. Double-blind, randomized, placebo-controlled study of trofinetide in pediatric Rett syndrome. *Neurol* 2019;92:e1912–e1925.
25. Hernandez RN, Feinberg RL, Vaurio R, Passanante NM, Thompson RE, Kaufmann WE. Autism spectrum disorder in fragile X syndrome: a longitudinal evaluation. *Am J Med Genet A* 2009;149:1125–1137.
26. Ozgen H, Helleman GS, Stellato RK, et al. Morphological features in children with autism spectrum disorders: a matched case-control study. *J Autism Dev Disord* 2011;41:23–31.
27. Hagberg B, Hanefeld F, Percy A, Skjeldal O. An update on clinically applicable diagnostic criteria in Rett syndrome: comments to Rett Syndrome Clinical Criteria Consensus Panel Satellite to European Paediatric Neurology Society Meeting, Baden Baden, Germany, September 11, 2001. *Eur J Paediatr Neurol* 2002;6:293–297.
28. Goldman S, Temudo T. Hand stereotypies distinguish Rett syndrome from autism disorder. *Mov Disord* 2012;27:1060–1062.
29. Cass H, Reilly S, Owen L, et al. Findings from a multidisciplinary clinical case series of females with Rett syndrome. *Dev Med Child Neurol* 2007;45:325–337.
30. Dy ME, Waugh JL, Sharma N, et al. Defining hand stereotypies in Rett syndrome: a movement disorders perspective. *Pediatr Neurol* 2017;75:91–95.
31. Kerr AM, Montague J, Stephenson JB. The hands, and the mind, pre- and post-regression, in Rett syndrome. *Brain Dev* 1987;9:487–490.
32. Temudo T, Maciel P, Sequeiros J. Abnormal movements in Rett syndrome are present before the regression period: a case study. *Mov Disord* 2007;22:2284–2287.
33. Harris AD, Singer HS, Horska A, et al. GABA and glutamate in children with primary complex motor stereotypies: an 1H-MRS study at 7T. *AJNR Am J Neuroradiol* 2016;37:552–557.
34. Houdayer E, Walthall J, Belluscio BA, Vorbach S, Singer HS, Hallet M. Absent movement-related cortical potentials in children with primary motor stereotypies. *Mov Disord* 2014;29:1134–1140.
35. Guy J, Hendrich B, Holmes M, Martin JE, Bird AA. A mouse *Mecp2*-null mutation causes neurologic symptoms that mimic Rett syndrome. *Nat Genet* 2001;27:322–326.
36. Carter JC, Lanham DC, Pham D, et al. Selective cerebral volume reduction in Rett syndrome: a multiple-approach MR imaging study. *Am J Neuroradiol* 2008;29:436–441.