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Rett Syndrome Behaviour Questionnaire: Psychometric characterization and revised factor structure

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Rett Syndrome Behaviour Questionnaire

in Children and Adults with Rett Syndrome:

Psychometric characterization and revised factor structure

Abstract

Rett syndrome (RTT) is a severe neurodevelopmental disorder associated with multiple neurobehavioral abnormalities. The Rett Syndrome Behaviour Questionnaire (RSBQ) was developed for pediatric RTT observational studies. Since its application has expanded to adult and interventional studies, we evaluated the RSBQ's psychometric properties in six pediatric (n=323) and five adult (n=309) datasets. Total and General Mood subscale scores had good reliability. Clinical severity had no influence on RSBQ scores. Exploratory and confirmatory factor analyses yielded 6 pediatric and 7 adult clinically relevant and psychometrically strong factors including the original Breathing Problems, Fear/Anxiety, and novel "Emotional and Disruptive Behavior" subscale composed of items from the original General Mood and Nighttime Behaviours subscales. The present findings support additional evaluations and improvements of an important RTT behavioral measure.

Key Words

Rett Syndrome (RTT), Rett Syndrome Behavioural Questionnaire (RSBQ), Adult, Pediatric, Psychometrics

Background

Rett Syndrome (RTT), a neurodevelopmental disorder affecting approximately 1 in 10,000 females, is the second most common cause of severe intellectual disability (ID) in females (Leonard et al., 1997). Over 95% of individuals with RTT have mutations in the X-linked methyl-CpG-binding protein (*MECP2)* gene (Amir et al., 1999), which encodes a protein (MeCP2) involved in synaptic development and maintenance (Gemelli et al., 2006; Kaufmann et al., 2005). The disorder is still diagnosed clinically as there are individuals who present with the RTT phenotype and do not carry pathogenic *MECP2* mutations as well as individuals with *MECP2* mutations who display non-RTT phenotypes. Genotype-phenotype studies have demonstrated clinical profiles, including overall severity, associated with the most common *MECP2* mutations (Cuddapah et al., 2014; Neul et al., 2008).

Diagnostic criteria for RTT have evolved over the past 40 years. Currently, core symptoms include a history of regression of purposeful hand use and spoken language, followed by a variable recovery or stabilization; gait abnormalities; and distinctive hand stereotypies. Presence of all 4 criteria is required for the diagnosis of “classic” or “typical” RTT and 2-3 criteria for the diagnosis of “variant” or “atypical” RTT. The latter also requires the presence of at least 5 out of 11 supportive criteria (e.g., breathing disturbances when awake, abnormal muscle tone, Inappropriate laughing/screaming spells), all of which are also common in individuals with classic RTT (Neul et al., 2010; Percy et al., 2010). Although abnormal behaviors are not among the core criteria for RTT diagnosis, they have been recognized as an important feature of the disorder (Hagberg et al., 1983) and have been included in the supportive diagnostic criteria (Neul et al., 2010). Early reports of behavioral abnormalities in RTT focused on autistic-like features, including social withdrawal during the regression period (Mount, Charman, et al., 2003; Olsson & Rett, 1990; Wulffaert et al., 2009; Young et al., 2008). During the subsequent decades, the range of abnormal behaviors associated with RTT has expanded to include

anxiety-like symptoms, mood instability, disruptive behavior, and repetitive and perseverative behaviors (Barnes et al., 2015; Buchanan et al., 2019; Robertson et al., 2006). Although some of these atypical behaviors may represent communicative attempts in individuals with minimal verbal communication, they have a negative impact on the quality of life of individuals with RTT and their families.

Several studies have pointed out the inconsistent and often confusing diagnostic terminology and the diverse methodology for evaluating behaviors as either traits or clinical problems in RTT. This is in part due to the early use of ad hoc instruments and unstructured data collection. Implementation of standardized surveys and questionnaires for abnormal behaviors, complemented by larger-scale natural history studies (Anderson et al., 2014; Buchanan et al., 2019; Cianfaglione et al., 2015), have allowed a more detailed and systematic characterization of RTT's behavioral phenotype (Mount et al., 2001; Mount, Hastings, et al., 2002, 2003). Standardized instruments have included the Anxiety, Depression, and Mood Scale (ADAMS) (Esbensen et al., 2003), and the Developmental Behaviour Checklist (DBC) (Einfeld & Tonge, 1995; Mount, Hastings, et al., 2003). Although such standardized instruments have been used in other neurologic disorders, their adequacy for RTT has been questioned due to its severe impairments in communication and motor function. The publication by Mount and colleagues of the Rett Syndrome Behaviour Questionnaire (RSBQ) (Mount, Charman, et al., 2002), the first disorder-specific instrument for evaluating behavior in RTT, represented a major advance in the field by providing a quantitative framework for assessment of a wide range of behavioral symptoms commonly seen in these individuals.

The original aim of the RSBQ was to determine whether there was a specific behavioral phenotype associated with RTT, which differentiated it from children with severe ID of other causes (Mount, Charman, et al., 2002). Their study included 143 girls with RTT and 85 girls with

severe ID of diverse or unknown etiology. The resulting 45-item, 8-domain, caregiver-completed assessment was derived through an iterative process. The researchers first narrowed down the items that were rated significantly higher in RTT than in severe ID. Next, item-total score correlations were conducted to ensure that the items in the scale were significantly associated with the total score. To define subscales, a principal component analysis (PCA) was conducted to define clusters of symptoms that tended to go together. Finally, test-retest reliability and internal consistencies were calculated for the derived subscales. Similar profiles were identified for individuals with classic RTT and for entire subject sample (Mount, Charman, et al., 2002).

The development of the RSBQ was undoubtedly a major contribution to the field. It has been applied to studies examining different aspects of behavior in RTT, including specific domains (e.g., anxiety, social impairment) (Barnes et al., 2015; Kaufmann et al., 2012), relationship with other impairments (e.g., sleep) (Leven et al., 2020), and genotype-phenotype correlations (Robertson et al., 2006). However, use of the RSBQ has been expanded far beyond its initial purpose to include observational studies in adults with RTT and assessment of efficacy in FDA-regulated clinical trials (Glaze et al., 2019; Khwaja et al., 2014; O'Leary et al., 2018). Several researchers have recently voiced concerns regarding the generalizability of the RSBQ to alternate contexts and its psychometric properties (Barnes et al., 2015; Hou et al., 2020). Specifically, as the scale was validated on children, items and/or subscales may not be appropriate for use in adults with RTT. Regarding psychometric properties, the RSBQ uses a limited range 3-point Likert scale (0=Not True, 1=Somewhat or Sometimes True, 2=Very True or Often True), which results in many items exhibiting floor or ceiling responses and non-normal distributions at the item and subscale levels. Additionally, the originally established subscales were defined based on a PCA. However, more recent exploratory factor analysis estimation methods (e.g., weighted least squares means and variance adjusted (WLSMV)) may be better suited for this type of non-normal, narrow-range, ordinal data. Availability of reference values on

the RSBQ for data interpretation of the wide range of applications of the instrument is also an unmet need in the field.

To address the aforementioned gaps in the RSBQ's psychometric characterization and provide data for potential refinements in its content and structure, we designed the current age-stratified, comprehensive cross-sectional psychometric analysis of RSBQ scores at the item, subscale and total score levels, using a large, international, multi-center RSBQ dataset representing in combination over 300 children and 300 adults with RTT. We aimed at answering the following research questions:

1. What is the range of item, subscale, and total scores in large pediatric and adult RSBQ datasets?
2. Which components of pediatric and adult RSBQ have strong psychometric features?
3. Are the original RSBQ factors replicated by exploratory and confirmatory factor analyses in large pediatric and adult samples?
4. If new RSBQ factors emerge, do they have better psychometric features than the original ones?

Methods

Sample

Data were obtained from six pediatric and five adult RSBQ datasets ("AussieRett", "Prescreening", "Danish", "InterRett", "RettBe" (pediatric only) and "UK") of individuals with RTT, representing in combination 323 children and 309 adults. Operationally, pediatric and adult components of each dataset were considered as separate units. Age was available for all subjects. Datasets came from previously approved observational studies of both clinic and community samples with both "classic" and "atypical" RTT. When available, genetic mutations were obtained from the original source data and were classified as either mild (p.Arg133Cys, p.Arg294X, p.Arg306Cys, and 3′ truncations), moderate (p.Thr158Met) or severe (p.Arg106Trp, p.Arg168X, p.Arg255X, p.Arg270X mutations, and large deletions) based on published genotype-phenotype severity profiles (Bebbington et al., 2008; Cuddapah et al., 2014; Neul et al., 2008). Details about sample characteristics, RSBQ administration, and other original study details can be found in Supplementary Tables 1 and 2.

No identifying data were available for any dataset. Local and reference Ethics committees reviewed and approved the sharing, combining, and analysis of these de-identified datasets.

Statistical Methodology

Descriptive Statistics

Descriptive statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS Subscription; IBM, Inc.). Pediatric and adult datasets were analyzed separately.

RSBQ scores were characterized at the item, subscale and total levels. Two overall scores were calculated. "Total" (i.e., the sum of all 45 items) and "Total subscale score" (TSS) (i.e., the sum of all subscale items included, based on the original subscales defined by Mount et al. (Mount,

Charman, et al., 2002) or on EFA-derived subscales). Standardized descriptive metrics included measures of central tendency, score variability, and dispersion of score distribution; tests of normality and equal variance; and measures of scale reliability (internal consistency) and relationships between individual items. Data was visualized using frequency histograms. For analytical purposes, a strong scale/subscale profile was defined as an entire range of possible scores, similar mean and median (up to 5% difference), absence of high (greater than +1 or less than -1) skewness or kurtosis, good to excellent Cronbach's alpha, non-significant Tukey's non-additivity test, and significant Hotelling's test (all last three, measures of reliability).

Following descriptive analyses of each individual dataset and the entire combined pediatric and adult datasets, subscale and total score comparisons were made between individual datasets and between clinical severity groups. The comparisons included data distribution and inferential analyses, specifically ANOVA with Tukey HSD post-hoc analyses, effect size and observed power, tests of equality of means and homogeneity of variances.

Factor Analyses

Exploratory (EFA) and Confirmatory (CFA) Factor Analyses were performed using SPSS and Mplus Version 8.4 (Muthén & Muthén, 1998-2017). To evaluate the validity of the original subscales in the current pediatric and adult combined datasets, we first replicated the original extraction method (PCA with orthogonal or Varimax rotation)(Mount, Charman, et al., 2002) limiting the model to 8 factors. Second, we used EFA extraction methods: Maximum-Likelihood (ML), Unweighted (Ordinary) Least-Squares (ULS), and WLSMV; first limiting the model to 8 factors, then allowing unlimited factors. For EFA, factor extraction employed Promax and Geomin (oblique) rotations (Norris & Lecavalier, 2010). We considered WLSMV the main analysis, as it is preferred for Likert scales with narrow score range and high correlation between items (Kidd et al., 2020; Norris & Lecavalier, 2010) as is the case with the RSBQ.

Across extraction methods, items included in the factors were based on significance factor loading of ≥ 0.4. Factors were selected based on models for which the root mean square error of approximation (RMSEA) was < 0.05 and the comparative fit index (CFI)/Tucker-Lewis index (TLI) was ≥ 0.95 (Aman et al., 2020; Hu & Bentler, 1999; Kidd et al., 2020).

PCA and EFAs were conducted initially in the entire combined pediatric and adult dataset respectively. For CFA, each pediatric and adult dataset was divided into 'development' and 'validation' subsets corresponding to a random selection of 60% and 40% of subjects, respectively, as reported (Raspa et al., 2020). The 'development' subset was used for factor extraction using the WLSWV method as described above. The 'validation' subset was employed for confirming the factor structure, also applying WLSWV but with slightly more relaxed fit parameters: RMSEA < 0.08 and CFI/TLI ≥ 0.90 as reported (Hu & Bentler, 1999; Raspa et al., 2020; Schreiber et al., 2006).

Following EFA and CFA, the best pediatric and adult RSBQ factor solutions were subjected to descriptive statistical characterizations comparable to those applied to the original factors/subscales. The proposed novel factor names are presented herein with quotation marks to differentiate them from the original factor names proposed by Mount and colleagues (Mount, Charman, et al., 2002).

Results

Descriptive Features

Pediatric Datasets

Descriptive analyses of pediatric RSBQ scores revealed substantial variability among datasets. A large proportion of items and subscales demonstrated positive skewing or negative kurtosis. Despite this variability, Total scores and TSS tended to be normally distributed and, in the case of the combined pediatric dataset, also showed good reliability parameters. Scores at the item level were less variable, in part because of the narrow possible range for each item (0-2). The only original subscale displaying a strong profile, reflecting a relatively normal distribution and good reliability, was the General Mood. The Breathing Problems subscale also displayed many of the 'strong features'; however, several datasets showed high negative kurtosis for this subscale.

Table 1 summarizes key descriptive parameters for the combined pediatric dataset: age, item-level scores, total scores (all 45 items), original subscale items (38 out of 45), and original subscale scores (8 subscales) while Supplementary Tables 1 and 2 contain descriptive statistics for the individual datasets and additional descriptive parameters for the combined dataset, respectively. Supplementary Figure 1 displays frequency histograms for the distributions of item-level, subscales, and total scores of the combined pediatric dataset.

Supplementary Table 3 summarizes two sets of comparisons involving Total, TSS and subscale scores. The first set of analyses corresponds to a comparison of the 6 individual pediatric datasets. The second to a comparison between groups of different clinical severity (mild, moderate, severe), based on their *MECP2* mutations. Reflecting the wide range of scores, Total scores and TSS were significantly different as determined by all applied tests. This variability

was reflected in most, but not all, subscales. Moreover, although mean and median variances were comparable for Total, TSS and most subscale scores, the Hand Behaviours and Walking/Standing subscales had significantly different variances. In contrast with these dataset comparisons, the clinical severity groups were not different in any parameter.

Adult Datasets

The adult RSBQ scores displayed a similar pattern to the pediatric data, with large proportion of items and subscales demonstrating positive skewing or negative kurtosis. Total scores and TSS were relatively normally distributed and had good reliability parameters, the latter also true for the General Mood subscale. Mutation-based clinical severity influenced only one adult subscale (Walking/Standing) with higher RSBQ scores associated with lower overall clinical severity. Similar to the pediatric datasets, scores at the item level were less variable, in part because of the narrow possible range for each item (0-2).

Table 1 summarizes key descriptive parameters for the combined adult dataset while Supplementary Tables 4 and 5 contain data for each of the 5 individual adult datasets and additional descriptive parameters for the combined dataset, respectively. Supplementary Figure 2 displays frequency histograms for the distributions of item-level, subscales, and total scores of the combined adult dataset.

Supplementary Table 6 summarizes the two sets of comparisons of the 5 individual adult datasets and the three clinical severity groups. As for the pediatric datasets and reflecting their score variability, adult Total scores and TSS were significantly different and differences between datasets were reflected in most but not all subscales. Moreover, half of the subscales had significant differences in their mean and/or median variance (including Hand Behaviours, Repetitive Face Movements, Nighttime Behaviours and Walking/Standing). In contrast with

these dataset comparisons, the *MECP2* mutation-based severity groups were only different in their Walking/Standing means with the mild group significantly higher than the severe group (2.04 vs. 1.07, $p < 0.001$).

Comparison of the combined pediatric and adult datasets revealed slightly higher scores at the item, subscale, and Total levels for the former, with exception of the Hand Behaviours and Walking/Standing subscales that showed higher means in the adult dataset. There was also greater variability in the adult dataset, as manifested by subscale mean and median variance and number of individual datasets with Total or subscale scores with high skewness or kurtosis: 38% for pediatric and 48% for adult datasets. Reliability was also lower in the adult combined dataset than in the pediatric one, affecting three subscales in the former (Hand Behaviours, Repetitive Face Movements, Walking/Standing) and only one in the latter (Body Rocking/Expressionless Face).

Factor Analysis

Pediatric factor analyses

We began by performing PCA on the combined pediatric dataset. The 8-factor PCA replicated three original factors: 2 (Breathing), 7 (Fear/Anxiety), and 8 (Walking/Standing) and approximated another two original factors (1, General Mood) and (3, Hand Behaviours). The unlimited analyses identified 11 factors, with two original factor replications (2 and 7) and several other approximated factors. Interestingly, Factor 1 in both 8- and 11-factor solutions constituted a combination of the original factors 1 and 6 (General Mood and Nighttime Behaviours, respectively), including mood abnormality-like and disruptive behaviors. Both novel PCA-based factor structures incorporated more items into factors than the original report (42 and 43 vs. 38 items in the original report). The novel 8-factor solution's contribution to score

variance was comparable to the original one (~53%) and, as expected, the 11-factor solution had a higher cumulative variance (~61%).

The most interpretable EFA solutions were the ones restricted to 8 factors, with the WLSMV-based showing the best fit parameters. Item loadings for the pediatric WLSMV 8-factor solution are presented in Table 2. Although unlimited EFA solutions made greater contributions to the cumulative variance or had slightly better fit parameters, they were more difficult to interpret or had lower clinical relevance. When compared to the original factor structure, the novel pediatric WLSMV 6-factor solution demonstrated mild reduction of variability and substantial increase of reliability (i.e., Cronbach's alpha and other parameters), as illustrated by the descriptive statistics in Supplementary Table 7. It was also characterized by a novel Factor 1 (12 items, "Emotional and Disruptive Behavior", average factor loading=0.68, Cronbach's alpha 0.90) composed of items from the original General Mood and Nighttime Behaviours factors (with originally published average factor loadings of 0.67 and Cronbach's alpha of 0.88 and 0.73 respectively). The WLSMV 8-factor solution also produced factors which replicated the original factor 2 in items, factor loadings, and Cronbach's alpha [Novel Factor 2 (5 items, "Breathing Problems", average factor loading=0.68, Cronbach's alpha 0.80)] and the original factor 7, but with improved factor loading and Cronbach's alpha [Novel Factor 4 (4 items, "Fear/Anxiety", average factor loading 0.64, Cronbach's alpha 0.77 versus the originally published with average factor loading of 0.57 and Cronbach's alpha of 0.66)]. Additional factors were named according to the symptoms represented by their items: Novel Factor 3 (5 items, "Rocking and Hyporeactivity", average factor loading=0.56, Cronbach's alpha=0.78), Novel Factor 5 (6 items, "Hand and other Stereotypies", average factor loading=0.61, Cronbach's alpha=0.68), and Novel Factor 6 (2 items, "Facial Movements", average factor loading=0.0.82, Cronbach's alpha=0.85). Novel Factor 7 (1 item) and Novel Factor 8 (4 items, two of them loading negatively) were not considered because only a single item loaded at the 0.4 threshold, items

loaded in different directions, or the relationship between items was limited. The number of items not loading onto EFA factors was larger than in PCA (for the 8- and 12-factor WLSMV solutions, 9 (or 11 for selected 6 factors) and 8, respectively, vs. 6 and 2 in the PCA 8- and 11-factor solutions).

Since WLSMV showed the best fit parameters, factors generated by this estimation method were used for the CFA. Supplementary Table 8 presents the factors generated with WLSMV EFA on the factor identification (Development) sample. For the CFA, only the 8-factor structure could be tested; the 12-factor solution led to no convergence, with lack of model confirmation despite a large number of iterations. The novel 8-factor solution was confirmed with moderate fit parameters: RMSEA 0.088, CFI 0.709, and TLI 0.687 (reference: RMSEA < 0.08, CFI/TLI ≥ 0.90) and a significant chi-square test of the model fit (chi-square = 1843.84, $p < 0.0001$).

Adult factor analyses

Overall, the 8-factor PCA replicated one original factor (2, Breathing) and approximated factor 7 (Fear/Anxiety). The unlimited analyses identified 12 factors, with two original factor replications (2 and 7). Similar to the pediatric PCA, Factor 1 in both 8- and 12-factor solutions constituted a combination of the original General Mood and Nighttime Behaviours subscales. Adult PCA-based incorporated 40 and 44 items into the 8-factor and 12-factor solutions, respectively. Similar to the pediatric PCA, the novel 8-factor solution's contribution to score variance was comparable to the original one (~52%) and, as expected, the 12-factor solution had a higher cumulative variance (~62%).

Adult EFAs, in particular the 8-factor solutions, were similar to the pediatric ones, with the WLSMV 8-factor solution consisting of the following subscales: Novel Factor 1 (11 items,

“Emotional and Disruptive Behavior”, average factor loading=0.70, Cronbach’s alpha=0.89), Novel Factor 2 (5 items, “Breathing Problems”, average factor loading=0.67, Cronbach’s alpha=0.78), Novel Factor 3 (4 items, “Fear/Anxiety”, average factor loading=0.60, Cronbach’s alpha=0.69), Novel Factor 4 (5 items, “Hand and other Stereotypies”, average factor loading=0.64, Cronbach’s alpha=0.69), Novel Factor 5 (4 items, “Social Interaction”, average factor loading=0.70, Cronbach’s alpha=0.77), Novel Factor 6 (4 items, “Walking/Standing & Rocking”, average factor loading=0.71, Cronbach’s alpha=0.72), and Novel Factor 7 (2 items, “Facial Movements”, average factor loading=0.83, Cronbach’s alpha=0.82). As for the pediatric WLSMV 8-factor solution, Novel Factor 8 (2 items) was not considered because the two items also loaded onto Novel Factor 6 and other items loading at the 0.2-0.4 level, loaded in different directions, or had weak relationships. Number of items not loading onto factors was larger in EFAs than in PCA solutions. Item loadings for the adult WLSMV 8-factor solution are presented in Table 3.

The CFA used exclusively WLSMV generated factors. The 8-factor solution for the EFA Development dataset was similar to the one generated with the entire sample, particularly in terms of replicating Factors 2 and 7, but the 12-factor solution was less comparable to its entire dataset counterpart. Nonetheless, both factor structures had adequate fit parameters (RMSEA < 0.05 and CFI > 0.95) and were confirmed by CFAs with moderate fit parameters (RMSEA 0.078 and 079, CFI/TLI 0.754/0.735 and 0.756/0.723, respectively; chi-square = 1605.27 and 1554.51, respectively, ps < 0.0001). Descriptive statistics for the novel adult WLSMV 7-factor solution are presented in Supplementary Table 9.

In sum, pediatric and adult factor analyses supported an 8-factor solution, which were confirmed by a CFA of WLSMV generated factors. Six and 7 factors were selected, respectively, as the most appropriate, including the clinical relevance of their items. Tables 2 and 3 present the item

loadings for the pediatric and adult WLSMV factor solutions, respectively. Supplementary Table 10 summarizes the factor analyses of RSBQ pediatric and adult data using EFA applying the WLSMV extraction method, including the proposed names for the novel subscales. Results of the PCA (both original and with the current datasets) and EFA using the alternative extraction methods (ML and ULS) are shown in Supplementary Table 11.

Discussion

Behavioral abnormalities are a feature of the RTT phenotype that has received greater attention in the last few years. Characterization of these atypical behaviors has been facilitated by the development and implementation of the disorder-specific RSBQ. However, its expanded use to adult RTT populations and as an outcome measure of treatment response in clinical trials has highlighted the need for an in-depth psychometric characterization of the RSBQ. In the present study, an international collaboration integrated 6 pediatric and 5 adult RSBQ cross-sectional datasets representing 323 children and 309 adults. We have delineated the range of scores of pediatric and adult RSBQ. Despite their relatively high variability, Total, TSS and General Mood (original and novel combined with Nighttime Behaviours) subscale scores demonstrated strong psychometric features in children and adults. Factor analyses replicated, in general, the original subscale structure, even in adults with RTT. The proposed EFA/CFA-based 6 pediatric and 7 adult factors showed clinical relevance, mild reduction of variability and substantial increase of reliability, particularly in pediatric RSBQ, with respect to the original ones.

Pediatric and adult samples showed considerable score variability within and between datasets. The most common feature was item and subscale positive skewing, a distinctive profile of instruments rating abnormal behaviors (e.g., ADAMS) (Rojahn et al., 2011), where a subset of individuals has substantially higher scores that lead to higher means than medians. This score

distribution suggests that these RSBQ components are clinically relevant, considering that positively skewed subscales (General Mood, Repetitive Face Movements, Nighttime Behaviours) represent more noticeable and disruptive externalizing behaviors. Negative kurtosis reflects a wide and homogeneous range of scores, without distinct subgroups of individuals with higher and lower scores. While flat distributions support the content validity of the item or subscale scores, their clinical utility, at least in isolation, is limited. The relatively normal distribution and good reliability (internal consistency) of Total, TSS and the General Mood subscale (original and in combination with Nighttime Behaviours) support their application to a wide variety of studies and, potentially, to clinical practice. While the overall profiles of pediatric and adult RSBQ profiles were similar, the latter was more variable and less reliable, potentially affecting its replication. The slightly lower RSBQ scores in adults with RTT are in line with clinical observations and a few studies demonstrating less prominent atypical behaviors, particularly externalizing ones, in this population (Buchanan et al., 2019). Although life expectancy of individuals with RTT has increased over the years (Tarquinio et al., 2015), the role of survivorship bias cannot be excluded. Thus, application of the RSBQ to adults with RSBQ requires a careful interpretation of scores. Clinical severity, as delineated by *MECP2* mutations, had minimal influence on RSBQ scores. This apparent lack of relationship between clinical severity and RSBQ scores, already reported in some initial studies (Barnes et al., 2015), may be explained by the fact that measures of clinical severity often rely on motor function, seizures and other clinical manifestations not assessed by the RSBQ. Dissociation between behavioral and motor severity in RTT is further supported by a recent study of the Motor-Behavioral Assessment (MBA) scale, an instrument that covers multiple aspects of the RTT phenotype. Though the “Motor Dysfunction” “Functional Skills” and “Social Skills” subscales correlated with each other, no correlation was found between MBA’s Aberrant Behavior subscale and any of the other MBA domains (Raspa et al., 2020).

Re-evaluation of RSBQ's subscales by factor analyses demonstrated that many elements of the original PCA-based structure remain after applying state-of-the-art EFA extraction methods such as WLSMV. Consistent with the originally reported subscales, an 8-factor solution was easier to interpret and led to the selection of subscales (6 pediatric, 7 adult) of higher clinical relevance than solutions with more factors and slightly better fit. The original Breathing Problems and Fear/Anxiety subscales and a combination of the original General Mood and Nighttime Behaviours subscales were either replicated or identified in pediatric and adult datasets using EFA, suggesting that they are the most consistent, cohesive, and clinically meaningful components of the RSBQ in both children and adults with RTT. Although the total number of items included into factors was smaller than in the original report and in the PCA analyses conducted in the present study, clinical relevance and psychometric validity of the WLSMV 6 pediatric and 7 adult factor solutions were greater. The new factor structures were not only confirmed by CFA but also demonstrated increased reliability with respect to the original report (Mount, Charman, et al., 2002). For instance, 5/6 novel pediatric and 5/7 novel adult factors had acceptable or higher levels of internal consistency in comparison with 4/8 and 3/8, respectively, in original pediatric and adult factors. Other statistical parameters were also slightly stronger in children than in adults (e.g., one factor at the Excellent level of internal consistency in the pediatric dataset and none at this level in the adult dataset). Of note is the "Emotional and Disruptive Behavior" subscale, representing a coherent group of previously reported externalizing behaviors in both children and adults with RTT (Buchanan et al., 2019), since it emphasizes the clinical relevance of the proposed factor structures.

In addition to its cross-sectional nature, which prevented examination of intra- and inter-rater reliability and score stability over time, the present study had multiple limitations. These included non-specified proportions of individuals with classic or atypical RTT, lack of use of instruments for assessing clinical severity (current study relied on *MECP2* mutation as a proxy of severity),

diverse enrollment criteria, unknown racial/ethnic background for many of the datapoints with a majority of the data coming from Caucasian individuals, and different methods of RSBQ administration (i.e., paper vs. online). The latter two limitations are important considerations as the predominantly Caucasian racial background may limit generalization to other racial or ethnic groups. Evaluation of the RSBQ in a broader range of racial and ethnic samples is an area for future research. Additionally, scoring may vary when questionnaires are applied as paper instruments versus electronically or as a phone or in-person interview. Level of guidance through written or verbal instructions may also have an impact on scores. However, recent studies of pain scales, which have a long track record with differential modes of administration, have shown that mode of administration does not have a high impact on score (Jibb et al., 2020). In the current study, this variation in the modes of administration of the RSBQ allowed for a more realistic assessment of the instrument and its variability across different forms of administration. Although our subject sample could be considered large for a rare genetic disorder like RTT, analyses performed on subsets of the pediatric or adult datasets (e.g., CFA including Development and Validation subsets) were relatively underpowered. Thus, validity of the novel factor structure should be confirmed in replication studies.

In summary, the analyses presented here support the use of the RSBQ in children and adults with RTT. Original and novel Total, TSS, and General Mood/Emotional and Disruptive Behavior, Breathing Problems, and Fear/Anxiety subscales have relatively strong psychometric properties, particularly in children. Because of this, and the overall greater reliability of the revised subscales, we consider the proposed novel pediatric and adult RSBQ factor structure suitable for use in research and clinical practice. Nonetheless, application of other original subscales and, in general, use of the RSBQ in adults with RTT should be conducted with caution considering their weaker psychometric properties. We expect the reported data will constitute

the basis for additional evaluations of the metric properties of the RSBQ and potential improvements to an instrument of increasing importance to the RTT community.

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Table 1 ***Pediatric and Adult Descriptive Statistics Combined Datasets***

	Total Pediatric Combined Dataset (N = 323)			Total Adult Combined Dataset (N = 309)		
	Mean (SD)	Median	Range (Min-Max)	Mean (SD)	Median	Range (Min-Max)
Age	10.51 (4.34)	10.95	1.60-18.24	26.48 (7.54)	24.53	18.00-64.02
Average item-level score	0.91 (0.75)	0.93	0-2	0.84 (0.75)	0.78	0-2
Total Score (45 items)	40.85 (15.72)	40.00	3-88	37.88 (14.23)	37.00	6-79
Subscale Items (38 items)	34.11 (13.32)	33.00	3-79	32.02 (12.20)	31.00	6-68
General Mood Subscale (8 items)	6.47 (4.23)	6.00	0-16	6.28 (4.17)	6.00	0-16
Breathing Problems Subscale (5 items)	4.47 (3.10)	4.00	0-10	4.20 (2.93)	4.00	0-10
Hand Behaviors Subscale (6 items)	7.97 (2.79)	8.00	0-12	8.08 (2.73)	9.00	0-12
Repetitive Face Movements Subscale (4 items)	3.40 (2.17)	3.00	0-8	2.61 (1.97	2.00	0-8
Body Rocking/Expressionless Face Subscale (6 items)	4.74 (2.28)	5.00	0-12	4.48 (2.18)	4.00	1-11
Nighttime Behaviors Subscale (3 items)	1.55 (1.66)	1.00	0-6	1.23 (1.57)	1.00	0-6
Fear/Anxiety Subscale (4 items)	3.97 (2.17)	4.00	0-8	3.43 (2.11)	3.00	0-8
Walking/Standing Subscale (2 items)	1.59 (1.42)	2.00	0-4	1.71 (1.54)	2.00	0-7

Table 2

Item Loadings for Proposed 6 Novel Pediatric Factor Solution derived from WLSMV 8-Factor EFA

RSBQ Item	Factor 1 (Emotional & Disruptive Behavior)	Factor 2 (Breathing Problems)	Factor 3 (Rocking & Hyporeactivity)	Factor 4 (Fear/Anxiety)	Factor 5 (Hand & other Stereotypies)	Factor 6 (Facial Movements)
1. Times when breathing is deep and fast	0.069	**0.600***	-0.201*	0.241*	0.009	0.025
2. Spells of screaming for no apparent reason during the day	**0.838***	0.115	0.092	-0.081	0.052	-0.050
3. Makes repetitive hand movements hands apart	0.234*	0.049	0.125	0.000	0.169	0.110
4. Makes repetitive hand movements involving fingers around the tongue	0.154	-0.136	0.124	-0.154	**0.404***	-0.086
5. Times when breath is held	-0.053	**0.709***	-0.035	0.227*	0.046	-0.036
6. Air or saliva expelled from mouth with force	-0.063	**0.486***	0.058	0.208*	0.242*	0.022
7. Spells of apparent anxiety/fear in unfamiliar situations	0.282*	0.040	-0.009	**0.712***	0.038	-0.051
8. Grinds teeth	0.140	0.168*	0.031	0.002	0.085	-0.038
9. Seems frightened when sudden changes in body position	0.075	0.110	0.027	**0.704***	0.044	-0.096

10. Times when parts of body held rigid	0.005	0.171*	0.338*	**0.535***	0.051	-0.024
11. Shift gaze with slow horizontal turn of head	-0.078	0.047	**0.473***	0.333*	-0.051	0.223*
12. Expressionless face	0.040	-0.026	**0.706***	0.051	-0.078	0.193*
13. Spells of screaming for no apparent reason during the night	**0.658***	0.088	0.331*	-0.016	-0.016	-0.046
14. Abrupt changes in mood	**0.793***	-0.100	-0.026	0.009	0.042	0.103
15. Certain periods when performs worse than others	**0.453***	-0.226*	0.099	0.303*	0.005	0.072
16. Times when miserable for no apparent reason	**0.819***	-0.165	0.034	0.259*	-0.150	0.072
17. Seems to look through people into the distance	0.214*	-0.028	**0.595***	0.178	0.031	0.079
18. Does not use hands for purposeful grasping	-0.131	0.129	0.344*	0.025	0.230	0.023
19. Swallows air	0.019	**0.826***	0.104	-0.007	-0.052	0.133
20. Hand movements uniform and monotonous	0.054	0.127	0.029	-0.122	*0.555*	-0.079
21. Has frequent naps during the day	-0.015	0.072	0.297*	0.105	-0.020	0.047
22. Screams hysterically for long periods of time and cannot be consoled	**0.770***	0.067	0.235*	-0.060	0.055	-0.083
23. Although can stand independently, tends to lean on objects or people	0.089	0.032	-0.106	0.021	**0.467***	-0.021

24. Restricted repertoire of hand movement	0.050	0.060	0.182	-0.065	0.342	0.015
25. Abdomen fills with air and sometimes feels hard	0.059	**0.795***	0.057	0.007	-0.010	0.148
26. Spells of laughter for no apparent reason during the day	**0.443***	0.137	-0.092	0.078	0.040	0.153
27. Has wounds on hands as a result of repetitive hand movements	0.266*	0.139	0.026	0.004	**0.460***	-0.078
28. Makes mouth grimaces	0.044	0.102	-0.003	-0.030	0.236	**0.796***
29. Times when irritable for no apparent reason	**0.830***	-0.128	-0.057	0.169	-0.008	0.102
30. Spells of inconsolable crying for no apparent reason during the day	**0.864***	0.010	-0.026	0.069	0.013	0.010
31. Uses eye gaze to convey feelings, needs and wishes (Reverse Coded)	0.093	0.002	-0.007	-0.199	-0.113	-0.071
32. Makes repetitive tongue movements	-0.045	-0.013	0.140	0.043	0.356*	0.164
33. Rocks self when hands are prevented from moving	-0.028	-0.183	-0.025	0.060	**0.921***	0.077
34. Makes grimacing expressions with face	0.061	-0.010	0.032	-0.007	0.248	**0.841***
35. Has difficulty in breaking/stopping hand stereotypies	0.140	0.135	-0.004	-0.034	**0.633***	0.034
36. Vocalises for no apparent reason	**0.414***	0.089	-0.089	0.198*	0.181	0.003
37. Spells of laughter for no apparent reason during the night	**0.526***	0.076	-0.101	0.083	-0.027	0.056

38. Spells of apparent panic	0.348*	-0.042	0.111	**0.615***	-0.029	0.031
39. Walks with stiff legs	0.003	0.167	0.029	0.200*	0.397*	0.079
40. Tendency to bring hands together in front of chin or chest	-0.085	0.003	0.071	0.174	0.307*	0.125
41. Rocks body repeatedly	0.012	-0.067	-0.047	0.029	**0.781***	0.025
42. Spells of inconsolable crying for no apparent reason during the night	**0.736***	-0.009	0.328*	-0.111	0.020	-0.011
43. Amount of time spent looking at an object is longer than time spent manipulating or holding	-0.027	-0.082	0.078	0.077	0.392	-0.061
44. Appears isolated	0.173	-0.116	**0.535***	0.257*	0.086	-0.064
45. Vacant 'staring' spells	0.124	0.115	**0.498***	0.116	0.037	0.133

Geomin rotation, CFI/TLI 0.973/0.960, RMSEA (0.033).
***Statistically significant (p<0.05)**

Table 3

Item Loadings for Proposed 7 Novel Adult Factor Solution derived from WLSMV 8-Factor EFA

RSBQ Item	Factor 1 (Emotional & Disruptive Behavior)	Factor 2 (Breathing Problems)	Factor 3 (Fear/Anxiety)	Factor 4 (Hand & other Stereotypies)	Factor 5 (Social Interaction)	Factor 6 (Walking/Standing & Rocking)	Factor 7 (Facial Movements)
1. Times when breathing is deep and fast	0.103	**0.563***	0.225*	-0.052	0.046	0.029	-0.022
2. Spells of screaming for no apparent reason during the day	**0.889***	0.083	0.006	0.003	-0.189*	0.025	0.003
3. Makes repetitive hand movements hands apart	0.048	0.018	-0.009	0.028	0.198*	0.149	0.061
4. Makes repetitive hand movements involving fingers around the tongue	-0.068	-0.087	0.073	0.236*	0.078	0.130	0.009
5. Times when breath is held	-0.053	**0.816***	0.070	-0.124	0.134	0.055	0.087
6. Air or saliva expelled from mouth with force	-0.047	**0.438***	0.073	0.013	0.159	0.176*	0.135
7. Spells of apparent anxiety/fear in unfamiliar situations	0.219*	0.052	**0.592***	-0.147	0.005	0.116	-0.020
8. Grinds teeth	0.101	0.047	0.084	0.052	0.219*	0.006	-0.108
9. Seems frightened when sudden changes in body position	0.012	0.057	**0.665***	0.170	0.067	-0.001	-0.149*
10. Times when parts of body held rigid	0.004	0.183*	**0.495***	0.230*	0.202*	-0.013	-0.007

11. Shift gaze with slow horizontal turn of head	-0.031	0.004	0.349*	0.198*	0.353*	-0.038	0.065
12. Expressionless face	0.020	-0.151	0.064	0.010	**0.782***	-0.052	0.056
13. Spells of screaming for no apparent reason during the night	**0.715***	-0.020	0.286*	-0.020	-0.053	0.027	0.028
14. Abrupt changes in mood	**0.723***	0.085	0.027	-0.032	0.055	0.119*	0.032
15. Certain periods when performs worse than others	**0.412***	0.048	0.220*	0.012	0.208*	-0.025	-0.057
16. Times when miserable for no apparent reason	**0.713***	-0.014	0.017	-0.001	0.248*	-0.053	-0.011
17. Seems to look through people into the distance	0.202*	0.025	0.001	0.002	**0.717***	-0.036	0.014
18. Does not use hands for purposeful grasping	-0.074	0.152	0.059	**0.508***	0.105	-0.350*	0.038
19. Swallows air	0.231*	**0.818***	-0.016	0.030	-0.053	-0.165	0.032
20. Hand movements uniform and monotonous	0.034	-0.024	-0.009	**0.823***	-0.022	-0.001	-0.016
21. Has frequent naps during the day	-0.018	0.164*	0.164*	-0.001	0.320*	-0.144	0.056
22. Screams hysterically for long periods of time and cannot be consoled	**0.960***	-0.021	0.050	-0.024	-0.197*	-0.088	0.013
23. Although can stand independently, tends to lean on objects or people	-0.009	0.055	0.041	-0.009	-0.020	**0.602***	-0.026
24. Restricted repertoire of hand movement	-0.056	0.249*	-0.056	**0.551***	0.109	-0.093	0.060

25. Abdomen fills with air and sometimes feels hard	0.352*	**0.710***	-0.032	0.086	-0.109	-0.219*	-0.015
26. Spells of laughter for no apparent reason during the day	0.393*	0.237*	-0.151*	0.033	0.314*	0.149	-0.038
27. Has wounds on hands as a result of repetitive hand movements	0.148	-0.021	-0.018	0.376*	-0.104	0.204*	0.099
28. Makes mouth grimaces	0.003	0.037	-0.035	0.119	0.045	-0.049	**0.861***
29. Times when irritable for no apparent reason	**0.789***	-0.004	-0.117	0.007	0.027	0.069	0.179*
30. Spells of inconsolable crying for no apparent reason during the day	**0.852***	-0.072	0.004	0.035	0.013	0.016	0.025
31. Uses eye gaze to convey feelings, needs and wishes (Reverse Coded)	-0.034	-0.241*	-0.141	-0.163*	0.166	-0.030	-0.056
32. Makes repetitive tongue movements	0.003	0.015	0.240*	0.323*	-0.020	-0.058	0.203*
33. Rocks self when hands are prevented from moving	-0.044	-0.006	0.015	0.139	-0.032	**0.898***	0.037
34. Makes grimacing expressions with face	0.135	0.051	0.124	-0.007	0.023	0.106	**0.801***
35. Has difficulty in breaking/stopping hand stereotypies	0.107	0.016	0.012	**0.874***	-0.017	0.108	-0.079
36. Vocalises for no apparent reason	**0.418***	0.011	0.038	0.209*	0.105	0.181*	0.034
37. Spells of laughter for no apparent reason during the night	**0.515***	0.174	0.022	0.086	0.123	0.035	-0.150*
38. Spells of apparent panic	0.194	-0.096	**0.639***	0.023	-0.104	0.175	0.139
39. Walks with stiff legs	-0.003	-0.055	0.097	0.186*	0.024	**0.481***	-0.012

40. Tendency to bring hands together in front of chin or chest	0.059	-0.005	0.024	**0.455***	-0.050	0.106	0.002
41. Rocks body repeatedly	0.085	0.083	-0.093	-0.041	0.043	**0.858***	0.033
42. Spells of inconsolable crying for no apparent reason during the night	**0.735***	-0.156*	0.292*	-0.026	0.085	-0.073	0.021
43. Amount of time spent looking at an object is longer than time spent manipulating or holding	0.009	0.016	-0.087	0.292*	0.082	0.041	0.004
44. Appears isolated	0.075	-0.101	-0.052	0.139*	**0.672***	0.062	0.037
45. Vacant 'staring' spells	0.063	0.148	0.036	-0.075	**0.634***	0.106	0.009

Geomin rotation, CFI/TLI 0.965/0.947, RMSEA 0.036.
***Statistically significant (p<0.05)**