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Anxiety-like behavior and anxiolytic treatment in the Rett syndrome natural history study

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Abstract
Background: Rett syndrome (RTT) is a neurodevelopmental disorder most often related to a pathogenic variant in the X-linked MECP2 gene. Internalizing behaviors appear to be common, but standard methods of diagnosing anxiety are not readily applied in this population which typically has cognitive impairment and limited expressive language. This study aims to describe the frequency of anxiety-like behavior and anxiolytic treatments along with associated clinical features in individuals with RTT.

Methods: Parental reports and medication logs provided data from 1380 females with RTT participating in two iterations of the multicenter U.S. RTT Natural History Study (RNHS) from 2006 to 2019.

Results: Most participants with RTT (77.5%) had at least occasional anxious or nervous behavior. Anxiety was reported to be the most troublesome concern for 2.6%, and within the top 3 concerns for 10.0%, of participants in the second iteration. Parents directly reported treatment for anxious or nervous behavior in 16.6% of participants in the second iteration with most reporting good control of the behavior (71.6%). In the medication logs of both RNHS iterations, the indication of anxiety was listed for a similar number of participants (15% and 14.5%, respectively). Increased use of anxiolytics and selective serotonin reuptake inhibitors (SSRIs) was related to more frequent anxiety-like behaviors (P < 0.001), older age (P < 0.001), and mild MECP2 variants (P = 0.002).

Conclusion: Anxiety-like behavior is frequent at all ages and is a significant parental concern in RTT. Older individuals and those with mild MECP2 variants are more likely to be treated with medications. Better diagnosis and treatment of anxiety in RTT should be a goal of both future studies and clinical care.

Trial registration: NCT00299312 and NCT02738281
Keywords: Rett syndrome, Natural history studies, Anxiety, Anti-anxiety agents, Methyl-CpG-binding protein 2

Background
Rett syndrome (RTT; OMIM 312750) is a genetic neurodevelopmental disorder that occurs in approximately 1 in 10,000 females [13]. Intellectual disability and communication and motor deficits occur after a period of regression with recovery or stabilization in early childhood [2, 11]. While individual presentations can vary greatly,
The prevalence of anxiety in RTT is unknown [1, 3, 8, 19], and C-terminal truncations) when compared to par-
pathogenic variants (R133C, R294X, R306C, MECP2
behaviors were more severe in participants with mild
epilepsy and in the general population. Internalizing
psychiatric diagnoses and worse than in patients with
were comparable to those in individuals with a range of
RNHS. Indeed, scores on the CHQ MH in classic RTT
clinically significant in RTT in the first iteration of the
ration, we characterize individuals with RTT reported
address the current gap in knowledge.

Anxiety is considered an internalizing behavior, along
with depressed mood, obsessive-compulsive behaviors,
and somatic complaints [6, 18, 30]. Although the exact
prevalence of anxiety in RTT is unknown [1, 3, 8, 19],
we previously reported that internalizing behaviors, as
defined by the Mental Health/Well-being (MH) scale
from the Child Health Questionnaire–Parent Form
50 (CHQ-PF50) as feeling lonely, feeling like crying,
acting upset or acting nervous [17], are common and
clinically significant in RTT in the first iteration of the
RNHS. Indeed, scores on the CHQ MH in classic RTT
were comparable to those in individuals with a range of
psychiatric diagnoses and worse than in patients with
epilepsy and in the general population. Internalizing
behaviors were more severe in participants with mild
MECP2 pathogenic variants (R133C, R294X, R306C,
and C-terminal truncations) when compared to par-
ticipants with either moderate (T158M and other point
mutations) or severe pathogenic variants (R106W,
R168X, R255X, R270X, early truncations, large dele-
tions, and splice site) [5]. Another study that exam-
ined the profiles of anxious behavior in girls with RTT
similarly reported that higher anxiety scores predomi-
nantly occurred in children with less severe neurologic
impairment and were associated with a lower quality of
life [3].

Existing diagnostic and assessment tools for anxiety
and other internalizing behaviors are often inappropriate
in RTT because of the cognitive impairment and limited
verbal communication of this population. Further com-
plifying the recognition of anxiety in individuals with
RTT is the common presence of anxiety-like behavior,
such as worsening of hyperventilation and breath hold-
ing, in situations that would not be expected to induce
anxiety. Other manifestations of anxiety in RTT may
include inability to relax, inconsolable crying, tenseness,
trembling, screaming episodes, avoidance of eye contact,
and social withdrawal [3, 8, 9, 12, 15, 19–21, 25, 27, 28].
Barnes et al. [3] demonstrated the feasibility of evaluat-
ing different subtypes of anxiety behaviors in children
with RTT using the Anxiety, Depression, and Mood Scale
(ADAMS) for individuals with intellectual disability. Nev-
ertheless, of the five ADAMS subscales, only the Social
Avoidance subscale showed the range of psychometric
properties suitable for clinical and research applications
in this population.

In spite of the general impression that anxiety-like
behavior is common in individuals with RTT, no system-
atic studies of anxiolytics use have been reported in this
population and guidelines for clinical management of
anxiety in RTT do not exist. Selective serotonin reuptake
inhibitors (SSRIs) have been suggested for the treatment
of anxiety and abnormal mood while benzodiazepines
have been recognized as a potential therapy for breathing
irregularities [7]. We conducted an exploratory analysis
of individuals being treated with anxiolytic medication
(e.g., SSRIs); in line with current perspectives on anxiety
in neurodevelopmental disorders, treated individuals had
more severe profiles of internalizing behaviors than their
non-treated counterparts [5].

Given the apparent high frequency of anxiety-like
behaviors in individuals with RTT, the significant
impact anxiety can have on quality of life, the shortage
of appropriate assessment tools for this mostly nonver-
bal population, and the lack of guidelines on the clinical
management of anxiety in RTT, the goal of this study was
to provide the first large-scale description of anxiety-like
behavior in RTT using data from the RNHS. In this inves-
tigation, we characterize individuals with RTT reported
to have anxious behavior or being treated for anxiety to
address the current gap in knowledge.
Methods
Participants
Participants were recruited through the multicenter RNHS to provide suitable longitudinal information that could serve as the foundation for conducting translational research including clinical trials. Assessments were conducted every 6 to 24 months by clinicians at one of eight sites in the USA from 2006 to 2015 (first iteration) and at 1 of 14 sites from 2015 to 2019 (second iteration). Data from 1380 females with RTT (718 in the first iteration only, 310 in the second iteration only, and 352 in both iterations) were included (Fig. 1). Medication log data from the first iteration were compared with parental report of anxiety-like behavior from the second iteration. Participants from both iterations allowed confirmation of parental report accuracy. Only baseline measures from each iteration were analyzed for this report. All female participants with a diagnosis of RTT (typical and atypical), regardless of MECP2 pathogenic variant status, were considered for inclusion in the analyses presented here. For this analysis, data were excluded from males, participants with a non-RTT diagnosis, and participants missing significant baseline data. The RNHS consortium is part of the Rare Diseases Clinical Research Network (RDCRN), an initiative of the Office of Rare Diseases Research, National Center for Advancing Translational Sciences, National Institutes of Health.

Human studies approval
Parental consent for study conduct and publication of results was obtained prior to entry into the study for all participants. Each participating institution retained institutional review board approval for the implementation of this study protocol and consent form (ClinicalTrials.gov identifiers: NCT00299312 and NCT02738281).

Diagnosis
Diagnoses of RTT were based on published diagnostic guidelines, whether classic (typical), variant (atypical), or other related phenotypes by a RNHS site Principal Investigator [22].

MECP2 pathogenic variants
MECP2 variants were reported from clinical laboratory testing. We categorized pathogenic variants as severe (R106W, R168X, R255X, R270X, early truncations, large deletions, and splice site), moderate (T158M and other point mutations), or mild (R133C, R294X, R306C, and C-terminal truncations) based on prior reports of genotype-phenotype correlations [4, 11, 23, 29]. Participants with clinical RTT without a MECP2 variant or with variants in exon 1 were excluded from analyses involving MECP2 severity.

Measures
Clinical severity scale (CSS)
The CSS is a clinician-completed questionnaire that uses a Likert-type scale to measure clinical features common in RTT, including age at onset of regression, somatic and head growth status, motor, communication, and RTT behaviors/other neurologic symptoms. Each item is

![Figure 1: Rett syndrome natural history study (RNHS) participants selected for analysis (n = 1380). Both = subjects participating in both the first and second iterations; cohort 1 = subjects only participating in the first iteration; cohort 2 = subjects only participating in the second iteration.](image-url)
ranked from either 0–4 or 0–5, with higher scores indicating greater clinical severity (range 1–58).

Motor behavioral assessment (MBA)
The MBA is a clinician-completed questionnaire for RTT that uses a Likert-type scale to score multiple items based on severity from 0 to 4, with a maximum total score of 148 (first iteration) or 136 (second iteration, with 3 previously low-scoring items removed). The scale includes measures of behavioral/social assessment, orofacial/respiratory assessment, and motor assessment/physical signs. A higher score indicates greater clinical severity.

Anxiolytic and/or SSRI treatment
The first iteration of the RNHS did not include direct parental assessment of anxiolytics use but the second iteration explicitly asked if the child was prescribed medication for being anxious or nervous. Both iterations of the RNHS collected comprehensive data regarding developmental and medical history, including medication use and indication. Medication logs from both iterations were reviewed for the indication of “anxiety” entered as either free text or as a SNOMED code, regardless of the type of medication associated with that entry. Medication logs were also reviewed for reported use of any SSRI, regardless of the indication associated with that entry.

Anxious behavior
The first iteration of the RNHS did not include specific questions about anxious behavior but the second iteration explicitly asked how frequently the child was anxious or nervous (with results on a scale of 0–4), whether the child was medicated for being anxious or nervous and, if yes, was the behavior well or poorly controlled. Parents were also asked to choose from a list of common features of RTT and select the top 3 features that have had the greatest impact on their child’s quality of life in the past 6 months.

Analytical strategy
Data from the second iteration of the RNHS constituted the primary source for the analyses, which included frequency of anxious/nervous and other abnormal behaviors, frequency and effectiveness of treatment for anxiety, drug type used to treat anxiety in RTT, and clinical characteristics of participants. Data from the first iteration of the RNHS were used as a potential source of convergent evidence for profiles of individuals with RTT treated pharmacologically for anxiety. Comparisons of direct report and medication logs for overlapping and non-overlapping subgroups of the second iteration were employed to assess comparability of first and second iteration datasets and accuracy of parental report of anxiety-like behavior.

Statistical analyses
Descriptive statistics were used for characterizing most parameters, with continuous variables described in terms of means, standard deviations (SD), and ranges and categorical variables mainly as frequencies. Groups’ age and clinical severity, based on CSS or MBA total scores, were compared using the Mann-Whitney $U$ or Kruskal-Wallis tests. Parent-reported frequency of anxious behavior in different groups was compared by Chi-square test or Fisher’s exact test. Frequency of other parameters and categorical variables, such as race, ethnicity, RTT diagnosis, MECP2 severity, parental report of anxiolytic use, medication log report of anxiolytic use, and medication log report of SSRI use, were also analyzed by these tests. Two-sided $P$ values less than 0.05 were considered significant; Bonferroni adjustments for multiple comparisons were used for analyses where appropriate. The analyses were performed using SPSS version 24.

Characteristics of the study population
Figure 1 displays the number of participants across the years of the first (2006–2015) and second (2015–2019) iterations of the RNHS, including the number of individuals unique to each study and those participating in both iterations. Table 1 displays the clinical features of the participants. Race and ethnicity differ between the study iterations, though most participants (> 85%) are White and non-Hispanic. Participants in the second iteration of the RNHS were older than the first iteration and displayed higher clinical severity scores, as expected from earlier studies. Older age was expected in the second iteration given the overlap of participants. Unexpectedly, when overlapping participants were removed from analysis, on average, participants in the second iteration were older than participants in the first iteration but had similar clinical severity. The type of RTT diagnosis (typical or atypical) and MECP2 variant severity did not differ between the first and second iteration.

Results
Frequency of anxiety-like behavior
During the second iteration of the RNHS, data were obtained specifically asking parents to report how often their child was anxious or nervous. A majority of participants (77.5%) were reported to be at least occasionally anxious/nervous, with 24.1% being reported as anxious/nervous frequently, very frequently, or constantly (Fig. 2). Frequency of being anxious or nervous
was not different based on type of RTT diagnosis (typical or atypical, \( P = 0.171 \)) or whether participants were enrolled in both iterations or only in the second (\( P = 0.406 \)). Frequency of being anxious showed no correlation with age or clinical severity based on MBA total score. Increased frequency of anxious behavior correlated with lower severity based on CSS total score, but the magnitude of the association was negligible (Spearman’s rho = −0.151, \( P < 0.01 \)). Participants with mild MECP2 variants had higher frequency of anxious

### Table 1  Baseline clinical characteristics of participants from the Rett syndrome natural history study (RNHS) analyzed in this study

| Characteristic | Category | First iteration (\( n = 1070 \)) | Second iteration (\( n = 662 \)) |
|---------------|----------|-----------------------------------|----------------------------------|
| Race**        | White    | 88%                               | 92%                              |
|               | Black or African American | 7%                           | 5%                              |
|               | American Indian or Alaska Native | 3%                           | 1%                              |
|               | Asian    | 5%                               | 5%                              |
|               | Native Hawaiian or Pacific Islander | (Not assessed) | 1%                              |
| Ethnicity*    | Not Hispanic, Latino, or Spanish Origin | 85%                           | 87%                              |
|               | Hispanic, Latino, or Spanish Origin | 15%                           | 11%                              |
| RTT diagnosis | Classic (typical) | 87%                           | 89%                              |
|               | Variant (atypical) | 13%                           | 11%                              |
| MECP2 severity| Mild     | 32.5%                            | 35.0%                            |
|               | Moderate | 16.7%                            | 17.0%                            |
|               | Severe   | 50.8%                            | 47.9%                            |
| Age***        |          | 9.1 years ± 8.8 (range 1–66)     | 15.2 years ± 10.7 (range 1–58)  |
| CSS total***  |          | 22.2 ± 7.8                       | 23.8 ± 8.3                       |
| MBA total**   |          | 46.9 ± 15.0                      | 48.8 ± 13.8                      |
| RTT diagnosis | Classic (typical) | 83.4%                        | 93.5%***                        |
|               | Variant (atypical) | 16.6%                        | 6.5%***                         |
| MECP2 severity| Mild     | 32.4%                            | 32.7%                            |
|               | Moderate | 16.4%                            | 17.1%                            |
|               | Severe   | 51.1%                            | 50.1%                            |
| Age           |          | 9.22 ± 8.6 (range 1–66)          | 8.84 ± 9.1 (range 1–48)         | 12.56 ± 10.7*** (range 1–52) |
| CSS total     |          | 22.92 ± 7.9                      | 20.79 ± 7.4***                  | 22.18 ± 8.4                   |
| MBA total     |          | 49.01 ± 15.2                     | 42.64 ± 13.6***                 | 47.84 ± 14.5                  |

Both Subjects participating in both the first and second iteration, Cohort 1 Subjects only participating in the first iteration, Cohort 2 Subjects only participating in the second iteration, CSS Clinical Severity Scale, First iteration Subjects enrolled 2006–2015, MBA Motor Behavioral Assessment, Mild MECP2 severity R133C, R294X, R306C, and C-terminal truncations, Moderate MECP2 severity T158M and other point mutations, RTT Rett syndrome, Severe MECP2 severity R106W, R168X, R255X, R270X, early truncations, large deletions, and splice site, Second iteration Subjects enrolled 2015–2019

1 More than one option could be selected so total > 100%; ***P < 0.001, **P < 0.01, *P < 0.05 compared to first iteration or cohort 1

* Baseline data from first iteration

![Fig. 2](image-url) Responses to: How often is your child anxious or nervous? From 662 participants in the second iteration of the RNHS. First iteration = subjects enrolled 2006–2015; second iteration = subjects enrolled 2015–2019
behavior than participants with more severe variants ($P < 0.001$).

**Severity of anxiety-like behavior**

Severity was assessed in the second iteration through parental report of anxious/nervous behavior as a major concern and through direct parental report of treatment. Specifically, the second iteration of the RNHS asked parents to select “the three biggest problems” affecting their child’s quality of life in the past six months from a list including both behavioral and medical complications of RTT: anxiety was the greatest concern for 2.6% and was within the top 3 concerns for 10% of participants (data not shown). Parents also reported whether their child displayed certain problem moods or behaviors, and whether those required treatment. Being anxious/nervous was the most common behavioral problem reported and treated, with a majority of those reporting good control of symptoms with treatment (Table 2). The frequency of anxious/nervous behavior was greater in those participants reporting treatment and in those with reported poor control (Fig. 3). Participants treated for anxiety by direct parental report were older than those without treatment ($P < 0.001$). In addition, participants with good control of anxious/nervous behavior were also older than those with poor control or no treatment ($P < 0.001$). Parental report of treatment frequency differed by MECP2 variant severity ($P < 0.001$); participants with mild variants were more than twice as likely as participants with severe variants to report treatment for being anxious or nervous (23.5% vs 10.1%). The percent of participants reporting treatment for being anxious did not differ based on RTT diagnosis type (typical or atypical) ($P = 0.795$), study enrollment (both or second iteration only, Table 2).

**Table 2** Rett syndrome natural history study (RNHS) participants’ direct report of treatment for behavioral problems ($n = 655$)

| Is your child treated for:                          | No     | Yes, my child is treated for this and it is well controlled | Yes, my child is treated for this but it is poorly controlled |
|---------------------------------------------------|--------|-------------------------------------------------------------|------------------------------------------------------------|
| Being anxious or nervous                          | 83.4%  | 11.9%                                                       | 4.7%                                                       |
| Being sad, miserable, or uncomfortable             | 87.8%  | 8.1%                                                        | 4.1%                                                       |
| Rapid changes in mood                             | 91.0%  | 6.1%                                                        | 2.9%                                                       |
| Being irritable                                   | 91.8%  | 5.5%                                                        | 2.7%                                                       |
| Screaming episodes                                | 93.4%  | 4.1%                                                        | 2.4%                                                       |
| Self-abusive behaviors                             | 95.9%  | 2.9%                                                        | 1.2%                                                       |
| Being aggressive and abusive to others            | 96.6%  | 2.1%                                                        | 1.2%                                                       |
| Being excessively active                          | 98.5%  | 1.1%                                                        | 0.5%                                                       |
| Having a low level of activity                    | 99.2%  | 0.3%                                                        | 0.5%                                                       |

**Fig. 3** Frequency of anxious/nervous behavior based on direct report of anxiolytic treatment. Frequency values: 0 = “never”, 1 = “occasionally”, 2 = “frequently”, 3 = “very frequently”, 4 = “constantly”
$P = 0.356$), or clinical severity based on CSS or MBA total scores ($P = 0.186, P = 0.993$).

**Frequency of anxiolytic use by medication logs**
Review of medication logs found “anxiety” noted as an indication for a similar number of participants in both iterations of the RNHS: 15.0% of participants in the first and 14.5% in the second ($P = 0.835$). The most commonly used drugs were escitalopram, sertraline, fluoxetine, and buspirone. Anxiolytic treatment according to medication log entries did not vary by type of RTT diagnosis (typical or atypical) ($P = 0.759$). Participants on a medication for the indication of anxiety were older than their untreated counterparts (Table 3, Fig. 4). The proportion of participants on anxiolytic treatment was higher for participants over 18 years of age (21.3%) than for pediatric participants (12.9%; $P < 0.001$). Anxiolytic treatment was higher at younger ages in the first iteration than in the second iteration (Fig. 4). In the first iteration, anxiolytic use by medication log was also associated with lower clinical severity, but this was not replicated in the second iteration (Table 3). Similar to direct parental report, medication logs reported more anxiolytic use in participants with mild MECP2 variants than severe variants in both first (20.1% vs. 10.9%, $P = 0.001$) and second (18.6% vs. 10.6%, $P = 0.034$; Fig. 5) iterations.

**Table 3 Clinical features of participants based on treatment recorded in medication logs**

| Study Feature | Any anxiolytic | SSRI | Significance | Significance |
|--------------|----------------|------|--------------|--------------|
| Age (years)  | 11.3 ± 9.7 8.7 ± 8.6 $P < 0.000^*$ | 11.3 ± 9.8 8.76 ± 8.6 $P < 0.000^*$ | | |
| CSS total score | 19.9 ± 7.1 22.6 ± 7.8 $P < 0.000^*$ | 18.9 ± 6.9 22.7 ± 7.8 $P < 0.000^*$ | | |
| MBA total score | 44.0 ± 14.1 47.4 ± 15.1 $P = 0.006^*$ | 43.8 ± 14.0 47.4 ± 15.1 $P = 0.006^*$ | | |
| Age (years)  | 18.7 ± 10.6 14.6 ± 10.6 $P < 0.000^*$ | 20.2 ± 10.6 14.5 ± 10.5 $P < 0.000^*$ | | |
| CSS total score | 24.0 ± 7.1 23.8 ± 8.5 $P = 0.790$ | 23.0 ± 7.2 23.9 ± 8.4 $P = 0.324$ | | |
| MBA total score | 49.7 ± 13.7 48.6 ± 13.8 $P = 0.487$ | 48.3 ± 10.7 48.8 ± 14.1 $P = 0.710$ | | |

Values are reported as mean ± standard deviation
CSS Clinical Severity Scale, First iteration Subjects enrolled 2006–2015, MBA Motor Behavioral Assessment, Second iteration Subjects enrolled 2015–2019

*P statistically significant

**Fig. 4 Frequency of anxiolytic use reported in medication logs by age group.** First iteration = subjects enrolled 2006–2015; second iteration = subjects enrolled 2015–2019
Frequency of SSRI use by medication logs

Based on medication logs, SSRI use for any indication was reported for 13.1% of participants in the first iteration and 11.0% in the second iteration ($P = 0.414$). The indication for use of an SSRI in the second iteration was primarily anxiety (87%) and clinical findings were similar to the anxiolytic treatment group defined by medication logs. SSRI use was similar between participants with typical and atypical RTT ($P = 0.383$). SSRI use was associated with older age in both iterations of the RNHS, and with lower clinical severity in the first iteration (Table 3). SSRI use was more common in participants with mild than severe $MECP2$ variants in the first (20.4% vs. 8.1%, $P < 0.001$) and second (15.9% vs. 8.0%, $P = 0.018$) iterations.

Comparison of direct report and medication logs

Looking only at individuals in both studies ($n = 352$), the number of participants receiving anxiolytic treatment is similar whether the data were collected from medication logs in the first iteration (18.8%) or from direct report in the second iteration (18.5%; $P < 0.000$). In all participants in the second iteration ($n = 662$), anxiolytic treatment according to the medication log and to the direct report do not overlap perfectly (14.5% and 16.6%, respectively), but their relationship is significant ($P < 0.001$); this significance holds for participants either in both studies ($n = 350, P < 0.001$) or only in cohort 2 ($n = 305, P < 0.001$). The frequency of anxious/nervous behavior was directly related to anxiolytic treatment based on direct report and medication log report of anxiolytic or SSRI use (all $P < 0.001$).

Discussion

Individuals with RTT display a wide variety of neurologic problems. Although the initial descriptions of the disorder highlighted autistic features, the current view is that other behavioral abnormalities are more significant and occur throughout the life of the individual. While several studies have reported on anxiety in RTT, these investigations have involved small numbers of participants and in general have not focused on this specific behavioral problem. The present study intended to provide the first large-scale examination of anxiety in RTT, utilizing the data collected by the multi-site RNHS. Because of the lack of records on psychiatric diagnoses in this study, we used a combination of parental report and medication logs from 1380 participants in two iterations of the RNHS. We confirmed the relatively high frequency of anxious or nervous behavior in RTT, which reached a level of clinical concern in at least 15% of participants as indicated by medication logs. We also identified frequency of anxiety-like behaviors, older age, and mild $MECP2$ variants as predictors of or associated factors to anxiolytic and SSRI use in RTT. Although it was reported that pharmacological treatment achieved good control of anxiety in most participants, this behavioral problem represented a major concern for approximately 10% of caregivers.
Previous attempts to quantify anxiety-like behavior in RTT have relied on rating scales administered to parents/caregivers or clinicians. The Rett Syndrome Behaviour Questionnaire (RSBQ) is a parent/caregiver scale addressing problem behaviors in RTT, with a fear/anxiety subscale consisting of 4 items. Anderson et al. [1] reviewed the RSBQ scores of an Australian cohort of individuals with RTT ($n = 137$) and found that anxiety occurred “often” in 11% of participants and “occasionally” in 58%. This study similarly shows that a majority of participants’ parents consider them to be at least occasionally anxious or nervous, with a minority having more frequent anxiety-like behavior.

Taking into consideration the challenges in identifying and quantifying anxiety-like behaviors in RTT, we combined two sources of information to determine the frequency and severity of anxiety-like behaviors and their associated features in this population. Data from medication logs in the first iteration and direct reports in the second iteration were comparable, as was anxiolytic treatment according to medication logs between the first and second iterations. Data consistency was supported by comparing findings from participants in both studies with those only in cohort 2. As suggested by previous studies [1, 8, 19, 27], our analyses demonstrated a relatively high frequency of anxiety-like behaviors in RTT. The figure (~ 15%) is higher than that reported in the general pediatric population (~ 6.5% worldwide) [24]. However, compared to other neurodevelopmental disorders, anxiety in RTT is less common: ~ 80% in fragile X syndrome [10] and ~ 25% in autism spectrum disorder [26].

We evaluated severity of anxiety-like behaviors in RTT using a combination of measures, including parental selection of the most troublesome medical and behavioral issues affecting their child’s quality of life, behavioral problems requiring treatment, and the level of symptom control with treatment. Anxiety was one of the top quality of life concerns for a substantial proportion of participants (10%) and was the most common behavioral problem reported and treated, with reasonable control achieved in most cases (approximately two-thirds). To our knowledge, only one study has examined anxiety severity in RTT. A cohort of girls with RTT ($n = 74$) had moderate severity scores using the RSBQ and two other parent/caregiver scales that measure anxiety-like behavior in intellectual disability and autism spectrum disorder: the Anxiety, Depression, and Mood Scale (ADAMS) and the Aberrant Behavior Checklist–Community (ABC-C) [3]. This study also found that anxiety scores were inversely related to clinical severity and quality of life scores. In general terms, our study confirms the association between more severe anxiety and milder clinical severity. Both RNHS iterations demonstrated higher frequency and severity of anxiety-like behaviors in participants with milder MECP2 pathogenic variants (R133C, R294X, R306C, and C-terminal truncations). However, the inverse relationship between anxiety scores and clinical severity was only present in the first iteration of the RNHS; it is unclear why this did not hold true in the second iteration.

In the RNHS, approximately 15% of participants used medications for anxiety. The present study was not designed to assess the efficacy or safety of these medications, but parent report indicates that most treated participants have good control. Associated factors to anxiolytic medication use in general, and SSRI use in particular, included increased frequency of anxiety-like behaviors, older age, and mild MECP2 variants. Increased treatment in older individuals may indicate greater impact of anxiety on functioning at older ages, greater clinician comfort with prescribing these medications in an older population, or both. Interestingly, RTT diagnosis type (typical or atypical) was not related to frequency or severity of anxiety-like behaviors.

This study is based on a large cohort of 1380 females with RTT, with 352 participating in both iterations of the RNHS, studied over many years. Therefore, the results are likely to be generalizable to the broad RTT community. Males and individuals with RTT-like disorders were excluded from the analyses, so results presented here may not apply to these populations. In addition to children, we included information collected from adult participants in this study. However, older individuals in neurodevelopmental disorder research studies may be a non-representative group because of ascertainment bias (due to severity, caregiver motivation, or other factors). Clear limitations in this study include the lack of standardized questionnaires and objective measures of anxiety-like behaviors. In a phase 1 clinical trial of IGF-1 in RTT, improved right-sided alpha band asymmetry on electroencephalogram (EEG), a biomarker of anxiety and mood disorders, was mildly associated with lowered ADAMS scores in 5 out of 6 participants [16]. These data support the notion that anxiety-like phenomena are a component of RTT; however, adequate instruments for assessing anxiety in RTT are still lacking. The complex study design with a partially overlapping cohort added analytical challenges and limited the conclusions. Consequently, future anxiety research in RTT should focus on developing standardized clinical measures, such as questionnaires, and identifying biomarkers of relevance to this abnormal behavior. Follow-up studies should integrate clinical data, as the one analyzed here, with novel and already established biomarkers of anxiety in the general population.
(e.g., electrodermal activity, heart rate variability, cortisol, catecholamines). Complementing this, efficacy and safety of specific SSRIs and other anxiolytics should be carefully documented in the RTT population. Non-pharmacological treatments of anxiety should also be considered and evaluated in RTT. Ultimately, future studies should be focused on supporting improvements in the clinical care of anxiety in RTT, including the accuracy of diagnosis and effectiveness of treatment.

Conclusions
This analysis demonstrates that most individuals with RTT display, at least occasionally, anxiety-like behavior and that anxiety is a significant parental concern. Older individuals and those with milder MECP2 variants are more likely to be treated, and pharmacological treatment can improve symptoms. Better diagnosis and treatment of anxiety in RTT should be a goal of both future studies and clinical care.

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Authors’ contributions
CBB, JLS, WKB, and AMP: drafting or revising manuscript for intellectual content, analysis or interpretation of data, design or conceptualization of study. AEJ, RED, and AES: drafting or revising manuscript for intellectual content, analysis, or interpretation of data. AAB, TAB, DKG, RHH, PTH, MDJ, JLN, SUP, RCR, SAS, and SMS: drafting or revising manuscript for intellectual content. Statistical analysis was conducted by Caroline B. Buchanan and Jennifer L. Stallworth at the Greenwood Genetic Center. All authors read and approved the final manuscript.

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Availability of data and materials
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.

Declarations
Ethics approval and consent to participate
Parental consent for study conduct and publication of results was obtained prior to entry into the study for all participants. Each participating institution retained institutional review board approval for the implementation of this study protocol and consent form (ClinicalTrials.gov identifiers: NCT00299312 and NCT02738281).

Consent for publication
Not applicable.

Competing interests
Caroline B. Buchanan received funding from Rett syndrome.org (Mentored Clinical Fellowship under US4 HD 061222 grant) and has done clinical trials with Acadia, Anavex, GW Pharmaceuticals, and Zynbena. Timothy A. Benke received funding from the NIH (U54 HD061222, R21 NS101288, R01 NS081248), International Foundation for CDKL5 Research, Loulou Foundation, and Children’s Hospital Colorado Foundation. Patricia F. Connolly received funding from the NIH and is a consultant for International Rett Syndrome Foundation, Takeda, and Marinus and has done clinical trials with Acadia, Ovid, GW Pharmaceuticals, Marinus, and Rett Syndrome Research Trust; all remuneration has been made to his department. Daniel G. Glaze received funding from the NIH, Rett syndrome.org, and the Blue Bird Circle. He has done clinical trials with Acadia, Neuren, and GW Pharmaceuticals. Richard H. Haas received funding from the NIH SU54HD061222 grant and has done clinical trials sponsored by Acadia, GW Pharmaceuticals, and Newron Pharmaceuticals. Jane B. Lane received funding from the NIH and is a consultant for International Rett Syndrome Foundation and GW Pharmaceuticals. David N. Lieberman has done clinical trials with Anavex, Acadia, GW Pharmaceuticals, and Rett Syndrome Research Trust. Eric D. Marsh received funding from Rett Syndrome Research Trust, NIH, State of Pennsylvania, Penn Orphan Disease Center, International Rett Syndrome Foundation, Loulou Foundation, and Eagles Autism Foundation. He is a consultant for Cipa Therapeutics and Stoke Therapeutics. He has done clinical trials with Zogenix, GW Pharmaceuticals, Acadia, Marinus, Stoke Therapeutics, and Rett Syndrome Research Trust. Jeffrey L. Neul received funding from the NIH and is a consultant for AveXis, Biohaven, Elxox Pharmaceuticals, Ovid, Takeda, and Teva Pharmaceuticals. He has done clinical trials with Acadia and Newron Pharmaceuticals. Sarika U. Peters received funding from NIH, Rett syndrome.org and is a consultant for Acadia Pharmaceuticals. Steve A. Skinner has done clinical trials with Acadia, Anavex, and GW Pharmaceuticals. Shannon M. Standridge is a speaker for Greenwuch Biosciences and Scientific Advisory Board of Acadia. Walter E. Kaufmann received funding from the NIH and is a consultant for Anavex, AveXis, Acadia, EryDel, Newron, GW Pharmaceuticals, Marinus, Biohaven, Zynbena, Ovid Therapeutics, and Stallia. Currently, he is Chief Medical Officer of AveXis Life Sciences Corp. Alan K. Percy received funding from the NIH and is a consultant for Anavex, AveXis, Acadia, and GW Pharmaceuticals. He has done clinical trials with Anavex, Acadia, GW Pharmaceuticals, and Rett Syndrome Research Trust. The rest of the authors, Jennifer L. Stallworth, Robin E. Joy, Rebekah E. Dixon, Alexandra E. Scott, Arthur A. Besang, Peter T. Heydemann, Mary D. Jones, and Robin C. Ryther, declare that they have no competing interests.

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