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Gastrointestinal Health Questionnaire (GHQ) for Rett Syndrome: Tool Development

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ABSTRACT (Word Count 220)

Purpose: We report the development and validation of a tool to assess gastrointestinal health in Rett syndrome (RTT). We hypothesized that the Gastrointestinal Health Questionnaire (GHQ) is a valid clinical outcomes measure of gastrointestinal health in RTT.

Methods: We used parent interviews, surveys, and literature review to generate a questionnaire related to gastrointestinal health and function, mood and behaviors, and parental concerns for individuals with RTT. Parents of affected and unaffected individuals provided responses to the GHQ, assessed the relevance and importance of statements, and completed five surveys related to gastrointestinal health, child-related mood and behaviors, and parent concerns. We used multivariate item analysis, two-sample t-tests, and correlations to assess the validity of the GHQ.

Results: We documented acceptable internal consistency of statements related to gastrointestinal health and function (Cronbach-□□= 0.91), RTT-related mood and behaviors (Cronbach-□□= 0.89), and parent concerns (Cronbach-□□= 0.95) in the GHQ. We documented favorable external validity, based on differences in response scores between parents of affected and unaffected individuals ($p<0.001$) and correlations in parental response scores between the GHQ and five validated questionnaires addressing similar issues ($p<0.001$).

Conclusion: The GHQ is a valid tool for the assessment of gastrointestinal health in RTT and offers the opportunity to field test the safety and efficacy of novel drug therapies in clinical trials for individuals affected with this disorder.

Key Words: *MECP2,* patient reported outcomes, drug trials, gastrointestinal health, gastrointestinal tool

What is Known

- Neurological outcomes and adverse events constitute the major efficacy and safety profiles of novel drug studies in Rett syndrome (RTT), but they may not capture the complete response in RTT.
- The clinical assessment of gastrointestinal outcomes is one area where the health response to novel drugs is overlooked.

What is New

- We developed the Gastrointestinal Health Questionnaire (GHQ) to assess gastrointestinal health and function, mood and behaviors, and parent concerns for individuals with RTT.
- We demonstrated that the GHQ is a valid tool to measure gastrointestinal outcomes and offers the opportunity to test efficacy and safety of novel drug therapies for RTT.

INTRODUCTION

Rett syndrome (RTT), an X-linked neurodevelopmental disorder caused by loss of function mutations in the methyl-CpG-binding protein (*MECP2)* gene, is a leading cause of developmental disability in children (1). The diagnosis of classic RTT is based on strict clinical criteria put forth by the Rett Syndrome Diagnostic Workgroup (2). The disorder is recognized between 6 and 18 months of age, primarily in girls who plateau in their developmental milestones and lose communication and purposeful hand skills coincident with the onset of hand stereotypies. Although neurological symptoms predominate, 95% of girls with RTT develop gastrointestinal problems that affect their health (3-6). Chewing and swallowing dysfunction, gastroesophageal reflux, gastroparesis, biliary tract disorders, gas bloating, and constipation complicate the clinical course of this disorder, predisposing girls with RTT to nutrient deficiencies, protein-energy malnutrition, and growth failure (3-6). For some individuals, the gastrointestinal manifestations may be more debilitating than the underlying neurological features of RTT.

Currently, no drugs have been approved for therapeutic use in RTT. However, multiple candidate drugs are being developed for clinical trials. We recently completed the Phase I/II trial of Trofinetide^{R}, an IGF-1 neuropeptide analogue that has favorable outcomes in RTT (7, 8). In the course of this trial, we identified gaps in outcome measures that would offer a broader scope essential for the assessment of health, response to a novel drug, and quality of life in these individuals. Although neurological outcomes and serious adverse events constitute the major efficacy and safety profiles of novel drug studies in RTT, they may not capture the full complexity of the clinical response from the perspective of the participant (9). The clinical assessment of gastrointestinal outcomes is one such area where the health and quality of life response to novel drugs is overlooked, but which could address an unmet need.

Herein we report the development and validation of a gastrointestinal system-specific tool, the Gastrointestinal Health Questionnaire (GHQ), for RTT. We hypothesized that the GHQ is a valid clinical outcomes measure of gastrointestinal health in RTT. The availability of this tool will broaden the opportunity to field test the safety and efficacy of novel drug therapies as they pertain to gastrointestinal issues in forthcoming clinical trials for RTT.

SUBJECTS AND METHODS

Subjects

Four parent groups of girls and women with RTT and one parent group of normally-developing, unaffected, age-matched girls, as well as adult women, were recruited for study. The parent groups of individuals with RTT were enrolled during local and national family support events or clinical research activities. The parent group of control girls and young adult women were enrolled during RTT-related clinical research activities. The parents of one RTT participant were unable to complete the questionnaire for health reasons and were excluded. Mothers comprised 86% of the parent groups; fathers or caregivers comprised the remainder. The affected individuals of the RTT parent groups represented more than 40 mutation. The distribution of mutations, based on their severity [mild (R133C, R294X, R306C, 3' truncations), moderate (T158M, all others except mild and severe), and severe (R106W, R255X, R270X, large deletions)] was 26%, 50%, and 24%, respectively, for the entire group of individuals (10).

Methods

Study design: We constructed a questionnaire for individuals with RTT based on three areas of focus: gastrointestinal health, function, medication use, and surgical interventions; RTT-related mood and behaviors; and parent concerns in relation to gastrointestinal issues. Tool development consisted of three phases: 1) identification of relevant gastrointestinal, behavioral, and parental issues, 2) conversion of gastrointestinal, behavioral, and parental issues into relevant and important statements, and 3) testing the gastrointestinal, behavioral, and parental statements for internal and external validity (11) (see Figure, Supplemental Digital Content 1, Study Design , http://links.lww.com/MPG/B998). The working language for tool development was English, with subsequent translation into Spanish.

Phase I: Identification of gastrointestinal issues: The aim of this phase was to compile a list of gastrointestinal, behavioral, and parental issues in RTT from parent interviews and responses to surveys, as well as a literature search (3, 4).

Patient Care Interviews: Parents of individuals with RTT were interviewed by one individual (KJM) during routine medical evaluations over a period of 10 years in the Gastroenterology, Hepatology, and Nutrition Clinic at Texas Children's Hospital, Houston, TX, to identify common gastrointestinal problems in RTT. Symptoms that described common gastrointestinal problems and approaches to medical and surgical interventions were assembled in a systematic fashion consistent with good clinical practice to generate the GHQ.

National Surveys: The results of nationwide surveys were abstracted to determine the prevalence of common gastrointestinal disorders in individuals with RTT (3, 4). In these surveys, parents identified symptoms associated with gastroesophageal reflux, gastroparesis, biliary tract disease, and constipation; feeding problems, including chewing and swallowing difficulties; and weight problems. The Rare Disease Clinical Research Network database for RTT [NCT 00296764] was interrogated to identify medications used to treat these disorders. This information provided additional source material for tool development.

Literature Search: A literature search was conducted on MEDLINE using the terms: "gastrointestinal", "irritable bowel", "Rett syndrome", "behavior", "genetic syndrome", and "parent" to identify relevant health-related quality of life questionnaires (12-41) . The search followed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (42). Questionnaires that included gastrointestinal symptoms, RTT-related emotional and social behaviors, and medically related concerns of parents whose children had genetic syndromes provided additional source material for tool development.

Phase II: Construction of Statements: The aim of this phase was to convert the list of gastrointestinal, behavioral, and parental issues into statements with acceptable formats consistent with the experience and perspective of the parents of individuals with RTT. The GHQ was divided into subsets that characterized gastrointestinal health, function, medication use, and surgical interventions; mood and behaviors of individuals with RTT; and parental concerns during the two weeks prior to testing. The language of individual statements was evaluated for readability at a 5th grade level.

Testing for Internal Consistency: Group I RTT parents (n=26) responded to each statement of the GHQ using a 5-point Likert scale to assess its internal consistency and rated each response for its importance, relevance, understandability, and acceptability using a 4-point

Likert scale. Parents provided feedback to ensure that the questionnaire reflected their concerns.

Phase IIIA: Preliminary Testing: This phase aimed to assess the ability of the GHQ to quantify the scope and magnitude of gastrointestinal problems in individuals with RTT. The purpose of this phase was to generate measures of internal and external validity and further refine the GHQ.

Testing for Discrimination between Groups: Group II RTT (n=49) and control (n=27) parents responded to each statement of the GHQ using a 5-point Likert scale to assess its internal consistency and determine the ability of the GHQ to discriminate between RTT and control groups.

Testing for External Validity: Group III RTT parents (n=18) responded to each statement of the GHQ using a 5-point Likert scale to assess its internal consistency and rated each statement for its relevance and importance using a 4-point Likert scale. Parents completed five validated surveys (permissions granted for each survey) related to gastrointestinal health and function, child-related mood and behaviors, and parental concerns to assess the external validity of the GHQ. The surveys included the Pediatric Quality of Life Inventory™ Gastrointestinal Symptoms Module (PedsQL™) (43), Gastrointestinal Quality of Life Index for Adults (GIQLI-A) (44), Rett Syndrome Behavioral Questionnaire (RSBQ) (38), Aberrant Behavior Checklist (ABC-C) (45), and Genetic Syndromes Stressors Scale (GSSS) (46).

Testing for Adequacy of Sample Size and Age Affect: The GHQ responses of RTT parent Groups I, II, and III (n=93) were combined to assess the adequacy of sample size, based on the consistency of response scores among the three small groups individually and the combined group as a whole. Differences in response scores between parents of younger and older age groups of individuals with RTT were evaluated.

Refinement: The GHQ was refined using predesigned rules for the retention of individual statements (11). At least 60% of responses for individual statements had scores of 3 or 4 on a four-point scale for relevance or importance. The mean score for each individual statement was greater than 1.5. The prevalence ratio, defined as the number of individuals reporting statement scores of 2, 3, or 4, divided by the total number of individuals that completed the statement, was >30%. The response range to individual statements was greater than 2 points for adequate variance. Floor or ceiling effects, defined as responses in categories 1 and 2 or 3 and 4, respectively, were not more than 10%. Parents did not express significant concerns for individual statements. The response rate for each statement was at least 95% to ensure compliance. The refined GHQ responses for Groups I, II, and III RTT parents were re-tested for their internal consistency and correlation with responses from the initial GHQ. The responses of Group II RTT and control parents were re-tested to determine their ability to discriminate between groups.

Phase IIIB: Final Testing: Group IV RTT parents (n=29) responded to each statement of the refined GHQ (see Questionnaire, Supplemental Digital Content 2 , http://links.lww.com/MPG/B999) using a 5-point Likert scale to assess its internal consistency and rated each statement for its importance and relevance using a 4-point Likert scale. Parents completed the five validated surveys related to gastrointestinal health and function, child-related mood and behaviors, and parental concern to assess the external validity of the refined GHQ.

Language Adaptation: The refined GHQ was adapted to account for language differences by translating the English version into Spanish. A certified Spanish translator used translation procedures that included forward and backward translation.

Statistical Analysis: In phases II , III, and IV, parents responded to each statement of the GHQ using a 5-point Likert scale with values ranging from 0 – 4 for ratings of never, almost never, sometimes, almost always, or always. Response scores for each individual statement item within the subsets for all participants were summed to generate their respective subset and total scores. Subset and total scores for all participants, as well as the median age and age ranges of the girls and women, were summarized using descriptive statistics (MiniTab, Version 18, MiniTab, Inc., State College, PA).

Multivariate item analysis was used to determine the Cronbach-□ value for each subset, as well as all subsets combined, to assess the internal validity of the GHQ. Statements with missing responses resulted in the exclusion of the individual's subset score from the statistical analysis. We considered a Cronbach-□ value□≥ 0.7 to be an acceptable measure of internal consistency for statements within each subset (10). Acuity scores were calculated as the mean subset or total score divided by the maximum possible subset or total score.

Parents evaluated the characteristics of each statement using a 4-point Likert scale with values ranging from 1 – 4 for ratings of not very to very relevant, important, understandable, and acceptable. These characteristics were summarized for each statement using descriptive statistics.

Two-sample t-tests were used to detect differences in subset and total response scores between RTT parents and controls and between parents of older and younger individuals with RTT. Correlations were used to determine the relation between subset or total scores of the initial and refined GHQ, as well as the PedsQL™, GIQLI-A, RSBQ, ABC-C, or GSSS and relevant subset or total scores of the GHQ.

RESULTS

Phase II: Group I parents of RTT individuals [median (range) age, 12 (2-43) y] completed 83 statement items in the GHQ. Item scores, acuity scores, and Cronbach-□ values for gastrointestinal health and function (□=0.91), health and pain (□=0.94), eat, chew, swallow (□=0.85), reflux, bloating, constipation (a=0.76), medication use (□=0.72), RTT-related mood and behaviors (□=0.86), parental concerns (□=0.94), and total items (□=0.95) were summarized. Subset scores for surgical intervention statements were removed from the multivariate item analysis (and all subsequent analyses) due to lack of variance. All remaining statements were retained based on acceptable Cronbach-□ values. Acuity scores for each subset were consistent with moderate severity of gastrointestinal function for individuals with RTT. Mean (SD) values and coefficients of variation for relevance, importance, understanding, and acceptable characteristics of 78 statements rated by parents were 3.1 ± 0.3 (10%), 3.4 ± 0.2 (7%), 3.9 ± 0.1 (2%), and 3.9 ± 0.1 (2%), respectively. All statements were retained based on acceptable ratings ≥ 2.00 and low coefficients of variation (11).

Phase IIIA: Group II parents of RTT individuals [median (range) age, 12 (5-36) y] and parents of unaffected girls or women [median (range) 10 (5-27) y] completed 78 statements in the GHQ. Item scores, acuity scores, and Cronbach-□ values for gastrointestinal health and

function (□=0.95), health and pain (□=0.94), eat, chew, swallow (□=0.91), reflux, bloating, constipation (□=0.87), RTT-related mood and behaviors (□=0.97), parental concerns (□=0.97), and total items (□=0.97) were summarized. Subset scores for medication statements were removed from the multivariate item analysis due to lack of variance. All item subset and total scores differed ($p<0.001$) between groups, providing evidence for the discriminatory capacity of the GHQ.

Group III parents of individuals with RTT [median (range) age, 13 (3-37) y] completed 78 statements in the GHQ. Item scores, acuity scores, and Cronbach-□ values for gastrointestinal health and function (□=0.94), health and pain (□=0.94), eat, chew, swallow (□=0.88), reflux, bloating, constipation (□=0.80), RTT-related mood and behaviors (□=0.96), parental concerns (□=0.95), and total items (□=0.95) were summarized. Subset scores for medication statements were removed from the multivariate item analysis due to lack of variance. All remaining statements were retained based on acceptable Cronbach-□ values. Mean (SD) values and coefficients of variation for relevance and importance characteristics of 69 statements rated by parents were 2.71 ± 0.27 (10%) and 2.88 ± 0.22 (8%), respectively. All items were retained based on acceptable ratings ≥ 2.00 and low coefficients of variation among responses (11). Subset and total scores for the PedsQL™, GIQLI-A, RSBQ, ABC-C, and GSSS surveys were calculated (data not shown). Correlations were detected between the PedsQL™ vs GHQ total scores ($p<0.11$); PedsQL™ vs GHQ gastrointestinal health and function subset score ($p<0.05$); GIQLI-A vs GHQ total scores ($p<0.02$); GIQLI-A vs GHQ gastrointestinal health and function subset score ($p<0.06$); GIQLI-A vs GHQ reflux, bloating, constipation subset score ($p<0.01$); RSBQ vs GHQ mood subset score ($p<0.001$); ABC-C vs GHQ mood subset score ($p<0.001$), and GSSS vs GHQ parent concerns subset score ($p<0.03$).

GHQ item scores and Cronbach-□ values, for gastrointestinal health and function (□=0.94), health and pain (□=0.94), eat, chew, swallow (□=0.88), reflux, bloating, constipation (□=0.86), RTT-related mood and behaviors (□=0.96), parental concerns (□=0.95), and total items (□=0.98) of Groups I, II and III parents combined whose daughters had RTT [median (range) 12 (2-43) y] were summarized. The sample size of each parent group was considered adequate based on the similarly acceptable Cronbach-□ value for the three groups combined. Differences in subset or total scores between parents of younger (9.1 ± 2.9 y) and older (22.8 ± 7.0 y) RTT individuals were not detected.

Refinement: The GHQ was refined based on the rules for statement retention: 1) 88% of statements (n=69) scored 3 or 4 for relevance, 100% of items scored 3 or 4 for importance; 2) 51% of statements had a mean score >1.5. 3) 83% of statements had a prevalence ratio >30%; 4) 100% of statements had a range > 2 points for adequate variance; 5) 75% of statements did not have a floor or ceiling effect; 6) no significant concerns about individual statements were expressed by parents; 7) 100% of individual statements had a response rate of at least 95% for compliance. After applying the retention rules, 41 statements were retained in the refined GHQ. Mean subset and total scores, as well as Cronbach-□ values, for gastrointestinal health and function, RTT-related mood and behaviors, and parental concerns from the refined GHQ for Groups I, II and III RTT parents were summarized (Table 1). Mean subset and total scores from the refined GHQ correlated significantly with those from the initial GHQ and were considered acceptable based on Cronbach-□ values when re-tested in Groups I, II, and III

RTT parents. Mean subset and total scores for the refined GHQ differed significantly between Group II RTT and control parents when retested (Table 2). Although not included in the analyses, the medication subset was retained in the refined GHQ, based on the assumption that novel drug candidates may change the need for medication use.

Phase IIIB: Group IV parents of individuals with RTT [median (range) 15 (2-53) y] completed the refined GHQ containing 50 statements. Mean subset and total item scores, acuity scores, and Cronbach-□ values for gastrointestinal health and function, RTT-related mood and behaviors, and parental concerns were summarized (Table 3). Subset scores for medication statements were removed from the multivariate item analysis due to lack of variance. All remaining subset statements were retained based on acceptable Cronbach-□ values. Mean relevance and importance scores of 41 statements rated by parents were 2.96 ± 0.32 (11%) and 3.11 ± 0.27 (9%), respectively. All statements were retained based on acceptable ratings ≥ 2.00 and low coefficients of variation among responses (11). Mean (SD) subset and total scores for the PedsQL™, GIQLI-A, RSBQ, ABC-C, and GSSS surveys were calculated. Significant correlations were detected between subset or total scores of the PedsQL™, GIQLI-A, RSBQ, ABC-C, or GSSS and respective subsets or total scores of the refined GHQ (Table 4).

DISCUSSION

Gastrointestinal problems complicate the clinical course and quality of life in girls and women with RTT (3, 4). Gastrointestinal problems are prevalent throughout life in individuals with RTT and pose a substantial medical burden for their caregivers. For some individuals, the gastrointestinal manifestations may be more debilitating than the underlying neurological features of RTT. The study described herein addressed the development and validation of a GHQ to assess gastrointestinal health in these individuals. The GHQ is the first instrument developed for this construct in individuals with RTT.

In the present study, we utilized a RTT gastrointestinal system-specific health-related framework for the development of the GHQ (47). We used multiple cross-sectional studies to develop a disease-specific tool for RTT, focusing on gastrointestinal health, function, medication use, and surgical interventions, RTT-related mood and behaviors, and parental concerns as the most relevant topics for study. We created the GHQ in three phases: 1) the identification of relevant gastrointestinal, behavioral, and parental issues, 2) the conversion of gastrointestinal, behavioral, and parental issues into relevant and important statements, and 3) testing the gastrointestinal, behavioral, and parental statements for internal and external validity (47). The purpose for the development of this tool was to fill a gap in clinical outcome measures for new drug development because existing tools have inadequate biometric and psychometric properties for RTT (48).

The generation and selection of suitable statements is central to the validity of a tool (49). We designed statements based on inductive (parent interviews or surveys) and deductive (literature review) methods to provide a comprehensive assessment of gastrointestinal health and function for RTT (47). The large number of individuals who responded to interviews or surveys provided assurances of a high level of content validity for tool development. The initial questionnaire was over-inclusive, containing nearly twice as many statements as the final questionnaire, providing a broad range of ways to evaluate gastrointestinal function and health (47, 48). Despite statement reduction, the final

questionnaire remained precise, demonstrating strong inter-item correlation within and between questionnaires (47) and retaining the ability to discriminate between test and control groups (49).

We evaluated the GHQ using the principles of item measurement theory (50, 51). In this process, we measured gastrointestinal health and function, mood and behavior, and parent concerns by a series of responses to groups of statements to which numerical values were assigned and subjected to statistical analysis. For parent responses, we used a 5-point Likert scale, rather than a scale with fewer points, for greater reliability in the interpretation of the tool (47, 48). Once the tool was developed, we performed multiple iterations of testing to demonstrate reproducibility of responses. Distinct, but representative, groups of parents with 18 to 49 individuals per group, provided responses similar to those obtained when the groups were combined as a whole, suggesting adequacy of sample size of the smaller groups (47, 48), and similarity of responses despite age differences.

Establishing internal and external validity is essential to tool development (52). We used patient care interviews for in-depth parental experiences and large survey groups for a range of parental experiences to document internal content validity of the GHQ (52). The participants represented a national group of RTT parents whose affected daughters reflected a range of age, ethnicity, and *MECP2* mutation type to demonstrate group variability (52). The initial iteration of the GHQ examined the physical, emotional, and social conditions associated with RTT and determined the relevance, importance, understandability, and acceptability of individual statements pertinent to the target population. We demonstrated adequate coverage of the GHQ for RTT, based on the high level of importance and relevance attributed to statement items by parents (50). In addition, all statement items used in the final questionnaire exceeded the threshold for inclusion based on previously established rules (11). The GHQ met conditions for internal content validity based on parental responses (47). We demonstrated the reliability of the GHQ by repeated testing in multiple groups of parents under separate settings, all of which provided similar results based on acceptable Cronbach-□□intra-class correlations (49). We demonstrated the sensitivity of the GHQ by including unaffected individuals to discriminate between groups, providing evidence for the external validity of the GHQ. We relied on five validated questionnaires related to gastrointestinal health and function, child-related mood and behaviors, and family concerns to provide additional evidence for the external validity of the GHQ. Although we initially identified 83 statements, after repetitive iterations, only 41 statements had sustained relevance and importance to participants. We empirically retained nine statements related to medication use because we have anecdotal evidence that novel drugs may alter medication use. The property of responsiveness will be tested when the GHQ is applied to clinical trials of novel drug therapies (49).

Potential limitations of the GHQ included those of social bias insofar as parents report what they think physicians want to hear, and acquiescence bias whereby parents tend to agree with positive responses regardless of subject matter (49). Parents served as proxies for their daughters, but this assignment was necessary given the loss of their expressive language function. Incomplete parental responses may contribute to potential error (9). Parents provided limited responses to items in the comparative instruments because they felt that the statements were irrelevant in the format presented. We did not validate the GHQ across

Spanish-speaking groups because comparative instruments were not available in other languages.

In summary, the GHQ is a valid instrument to assess gastrointestinal health in RTT. The content of the GHQ was relevant and important to parents of individuals with RTT within the realm of their experiences. The GHQ offers the opportunity to field test the safety and efficacy of novel drug therapies in clinical trials for individuals affected with this disorder. Additional field-testing is required to finalize tool development (47).

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Ethical approval: The Baylor College of Medicine Institutional Review Board (IRB) approved this study.

Informed consent: The IRB waived the requirement for written, informed consent from parents of affected and unaffected individuals, unaffected young adults, and health care professionals for completion of the questionnaires.

REFERENCES

1. Amir RE, Van den Veyver IB, Schultz R, et al. Influence of mutation type and X chromosome inactivation on Rett syndrome phenotypes. Ann Neurol. 2000;47:670-9.
2. Neul JL, Kaufmann WE, Glaze DG, et al. Rett syndrome: Revised diagnostic criteria and nomenclature. Ann Neurol. 2010;68:944-50.
3. Motil KJ, Caeg E, Barrish JO, et al. Gastrointestinal and nutritional problems occur frequently throughout life in girls and women with Rett syndrome. J Pediatr Gastroenterol Nutr. 2012;55:292-8.
4. Motil KJ, Lane JB, Barrish JO, et al. Biliary tract disease in girls and young women with Rett syndrome. J Pediatr Gastroenterol Nutr. 2019;68:799-805.
5. Motil KJ, Barrish JO, Lane J, et al. Vitamin D deficiency is prevalent in girls and women with Rett syndrome. J Pediatr Gastroenterol Nutr. 2011;53:569-74.
6. Tarquinio DC, Motil KJ, Hou W, et al. Growth failure and outcome in Rett syndrome: Specific growth references. Neurology. 2012;79:1653-61.
7. Glaze DG, Neul JL, Kaufmann WE, et al. Double-blind, randomized, placebo-controlled study of trofinetide in pediatric Rett syndrome. Neurology. 2019;92:e1912-e25.
8. Glaze DG, Neul JL, Percy A, et al. A double-blind, randomized, placebo-controlled clinical study of trofinetide in the treatment of Rett syndrome. Pediatr Neurol. 2017;76:37-46.
9. Lee L, Dumitra T, Fiore JF, Jr., et al. How well are we measuring postoperative "recovery" after abdominal surgery? Qual Life Res. 2015;24:2583-90.
10. 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;51:152-8.
11. Johnson C, Aaronson N, Blazeby JM, et al. EORTC Quality of Life Group: Guidelines for Developing Questionnaire Modules (4th ed). European Organisation for Research and Treatment of Cancer (EORTC). https://www.eortc.org/app/uploads/sites/2/2018/02/guidelines_for_developing_questionnaire-_final.pdf Published: 2011; Accessed: June 2, 2020.
12. Varni JW, Shulman RJ, Self MM, et al. Gastrointestinal symptoms predictors of health-related quality of life in patients with inflammatory bowel disease. J Pediatr Gastroenterol Nutr. 2016;63:e186-e92.
13. Varni JW, Bendo CB, Nurko S, et al. Health-related quality of life in pediatric patients with functional and organic gastrointestinal diseases. J Pediatr. 2015;166:85-90.
14. Tielemans MM, Jaspers Focks J, van Rossum LG, et al. Gastrointestinal symptoms are still prevalent and negatively impact health-related quality of life: A large cross-sectional population based study in The Netherlands. PLoS One. 2013;8:e69876.
15. Brunner HI, Johnson AL, Barron AC, et al. Gastrointestinal symptoms and their association with health-related quality of life of children with juvenile rheumatoid arthritis: Validation of a gastrointestinal symptom questionnaire. J Clin Rheumatol. 2005;11:194-204.
16. Varni JW, Lane MM, Burwinkle TM, et al. Health-related quality of life in pediatric patients with irritable bowel syndrome: A comparative analysis. J Dev Behav Pediatr. 2006;27:451-8.

17. Varni JW, Shulman RJ, Self MM, et al. Symptom profiles in patients With irritable bowel syndrome or functional abdominal pain compared with healthy controls. J Pediatr Gastroenterol Nutr. 2015;61:323-9.
18. Varni JW, Nurko S, Shulman RJ, et al. Pediatric functional constipation gastrointestinal symptom profile compared with healthy controls. J Pediatr Gastroenterol Nutr. 2015;61:424-30.
19. Varni JW, Franciosi JP, Shulman RJ, et al. PedsQL gastrointestinal symptoms scales and gastrointestinal worry scales in pediatric patients with inflammatory bowel disease in comparison with healthy controls. Inflamm Bowel Dis. 2015;21:1115-24.
20. Rutten JM, Benninga MA, Vlieger AM. IBS and FAPS in children: A comparison of psychological and clinical characteristics. J Pediatr Gastroenterol Nutr. 2014;59:493-9.
21. Varni JW, Bendo CB, Denham J, et al. PedsQL gastrointestinal symptoms module: Feasibility, reliability, and validity. J Pediatr Gastroenterol Nutr. 2014;59:347-55.
22. Varni JW, Kay MT, Limbers CA, et al. PedsQL gastrointestinal symptoms module item development: Qualitative methods. J Pediatr Gastroenterol Nutr. 2012;54:664-71.
23. Guandalini S, Magazzu G, Chiaro A, et al. VSL#3 improves symptoms in children with irritable bowel syndrome: A multicenter, randomized, placebo-controlled, double-blind, crossover study. J Pediatr Gastroenterol Nutr. 2010;51:24-30.
24. Khan S, Campo J, Bridge JA, et al. Long-term outcome of functional childhood constipation. Dig Dis Sci. 2007;52:64-9.
25. Muller-Lissner S, Koch G, Talley NJ, et al. Subject's Global Assessment of Relief: An appropriate method to assess the impact of treatment on irritable bowel syndrome-related symptoms in clinical trials. J Clin Epidemiol. 2003;56:310-6.
26. Hubbard CS, Becerra L, Heinz N, et al. Abdominal pain, the adolescent and altered brain structure and function. PLoS One. 2016;11:e0156545.
27. Grigoleit HG, Grigoleit P. Peppermint oil in irritable bowel syndrome. Phytomedicine. 2005;12:601-6.
28. Epstein A, Leonard H, Davis E, et al. Conceptualizing a quality of life framework for girls with Rett syndrome using qualitative methods. Am J Med Genet A. 2016;170:645-53.
29. Parisi L, Di Filippo T, Roccella M. The quality of life in girls with Rett syndrome. Ment Illn. 2016;8:6302.
30. Killian JT, Jr., Lane JB, Lee HS, et al. Caretaker quality of life in Rett syndrome: Disorder features and psychological predictors. Pediatr Neurol. 2016;58:67-74.
31. Lane JB, Lee HS, Smith LW, et al. Clinical severity and quality of life in children and adolescents with Rett syndrome. Neurology. 2011;77:1812-8.
32. Lamb AE, Biesecker BB, Umstead KL, et al. Family functioning mediates adaptation in caregivers of individuals with Rett syndrome. Patient Educ Couns. 2016;99:1873-9.
33. Barnes KV, Coughlin FR, O'Leary HM, et al. Anxiety-like behavior in Rett syndrome: Characteristics and assessment by anxiety scales. J Neurodev Disord. 2015;7:30.
34. Robertson L, Hall SE, Jacoby P, et al. The association between behavior and genotype in Rett syndrome using the Australian Rett Syndrome Database. Am J Med Genet B Neuropsychiatr Genet. 2006;141b:177-83.
35. Einspieler C, Freilinger M, Marschik PB. Behavioural biomarkers of typical Rett syndrome: Moving towards early identification. Wien Med Wochenschr. 2016;166:333-7.

36. Cianfaglione R, Clarke A, Kerr M, et al. A national survey of Rett syndrome: Behavioural characteristics. J Neurodev Disord. 2015;7:11.
37. Mount RH, Hastings RP, Reilly S, et al. Behavioural and emotional features in Rett syndrome. Disabil Rehabil. 2001;23:129-38.
38. Mount RH, Charman T, Hastings RP, et al. The Rett Syndrome Behaviour Questionnaire (RSBQ): Refining the behavioural phenotype of Rett syndrome. J Child Psychol Psychiatry. 2002;43:1099-110.
39. Lingen M, Albers L, Borchers M, et al. Obtaining a genetic diagnosis in a child with disability: Impact on parental quality of life. Clin Genet. 2016;89:258-66.
40. Marchal JP, Maurice-Stam H, Hatzmann J, et al. Health related quality of life in parents of six to eight year old children with Down syndrome. Res Dev Disabil. 2013;34:4239-47.
41. Mazaheri MM, Rae-Seebach RD, Preston HE, et al. The impact of Prader-Willi syndrome on the family's quality of life and caregiving, and the unaffected siblings' psychosocial adjustment. J Intellect Disabil Res. 2013;57:861-73.
42. Moher D, Liberati A, Tetzlaff J, et al. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. PLoS Med. 2009;6:e1000097.
43. Varni JW. PedsQL™ Gastrointestinal Symptoms Module. Version 3. https://eprovide.mapi-trust.org/instruments/pediatric-quality-of-life-inventory-gastrointestinal-symptoms-scales. Published: 1998; Accessed: May 20, 2020.
44. Eypasch E, Williams JI, Wood-Dauphinee S, et al. Gastrointestinal Quality of Life Index: Development, validation and application of a new instrument. Br J Surg. 1995;82:216-22.
45. Marshburn EC, Aman MG. Factor validity and norms for the Aberrant Behavior Checklist in a community sample of children with mental retardation. J Autism Dev Disord. 1992;22:357-73.
46. Griffith GM, Hastings RP, Nash S, et al. "You have to sit and explain it all, and explain yourself." Mothers' experiences of support services for their offspring with a rare genetic intellectual disability syndrome. J Genet Couns. 2011;20:165-77.
47. Boateng GO, Neilands TB, Frongillo EA, et al. Best practices for developing and validating scales for health, social, and behavioral research: A primer. Front Public Health. 2018;6:149.
48. Reise SP, Waller NG, Comrey AL. Factor analysis and scale revision. Psychol Assess. 2000;12:287-97.
49. Trajanovska M, Catto-Smith AG. Quality of life measures for fecal incontinence and their use in children. J Gastroenterol Hepatol. 2005;20:919-28.
50. Testa MA, Simonson DC. Assessment of quality-of-life outcomes. N Engl J Med. 1996;334:835-40.
51. Lord FM. Applications of Item Response Theory to Practical Testing Problems. Mahwah, NJ: Lawrence Erlbaum Associates; 1980.
52. Brod M, Tesler LE, Christensen TL. Qualitative research and content validity: developing best practices based on science and experience. Qual Life Res. 2009;18:1263-78.

Table 1. Refined Gastrointestinal Health Questionnaire for girls and young women with Rett syndrome (RTT): Groups I (n=26), II (n=49), and III (n=18) RTT parent Likert scale scores, Cronbach-□ values, and correlations between the initial and refined GHQ

Domain	Group I			Group II			Group III			Pearson Correlation[c]		
	Number	Likert Scale[a]	Cronbach-□[b]	Number	Likert Scale[a]	Cronbach-□[b]	Number	Likert Scale[a]	Cronbach-□[b]	Group I	Group II	Group III
Gastrointestinal	25	50 ± 16	0.85	48	41 ± 19	0.91	17	44 ± 13	0.81	0.94	0.98	0.93
Health/Pain	26	9 ± 4	0.77	48	6 ± 5	0.84	18	6 ± 4	0.76	0.86	0.97	0.87
Eat/Chew/Swallow	26	17 ± 9	0.78	49	16 ± 10	0.83	18	19 ± 7	0.75	0.95	0.96	0.97
GER/BLT/C[d]	26	24 ± 7	0.74	49	20 ± 9	0.84	17	19 ± 8	0.75	0.82	0.92	0.93
Mood/Behavior	24	7 ± 4	0.76	48	6 ± 5	0.96	18	7 ± 4	0.91	0.94	0.97	0.92
Parent Concerns	26	14 ± 9	0.90	49	10 ± 10	0.96	18	15 ± 10	0.93	0.98	0.99	0.98
Total	23	72 ± 24	0.91	47	58 ± 31	0.95	17	66 ± 24	0.92	0.97	0.99	0.98

[a]Values expressed as mean ± SD

[b]Statements considered acceptable if Cronbach-□ ≥ 0.70

[c]Correlation between initial and revised GHQ subset and total scores; all values significant at $P < 0.001$

[d]GER/BLT/C, Gastroesophageal reflux/bloating/constipation

Table 2. Refined Gastrointestinal Health Questionnaire for girls and young women with Rett syndrome (RTT) and unaffected individuals: Group II RTT (n=49) and control (n=27) parent Likert scale scores, acuity scores, and Cronbach–□ measure of internal consistency

	Rett				Unaffected			
Domain	Number	Likert Scale Score[a]	Acuity Score[b]	Cronbach-□[c]	Number	Likert Scale Score[a]	Acuity Score[b]	P-Value[d]
Gastrointestinal	48	41 ± 19	0.37	0.91	27	3 ± 5	0.03	0.001
Health/Pain	48	6 ± 5	0.30	0.84	27	0.3 ± 1	0.01	0.001
Eat/Chew/Swallow	49	16 ± 10	0.44	0.83	27	0.3 ± 1	0.01	0.001
GER/BLT/C[e]	49	20 ± 9	0.36	0.84	27	2 ± 4	0.04	0.001
Mood/Behavior	48	6 ± 5	0.30	0.96	27	0.7 ± 2	0.03	0.001
Parent Concerns	49	10 ± 10	0.25	0.96	27	0.2 ± 1	0.01	0.001
Total	47	58 ± 31	0.34	0.95	27	4 ± 7	0.02	0.001

[a]Values expressed as mean ± SD
[b]Values expressed as subset or total score divided by maximum subset or total score
[c]Statements considered acceptable if Cronbach-□ ≥ 0.70
[d]Significant differences in Likert scale scores between RTT and control groups
[e]GER/BLT/C, Gastroesophageal reflux/bloating/constipation

Table 3. Refined Gastrointestinal Health Questionnaire for girls and young women with Rett syndrome (RTT): Group IV (n=29) parent Likert scale scores, acuity scores, and Cronbach-□ measures of internal consistency

Domain	Number[a]	Likert Scale Score[b]	Acuity Score[c]	Cronbach-□[d]
Gastrointestinal	23	43 ± 19	0.38	0.91
Health/Pain	26	7 ± 5	0.35	0.89
Eat/Chew/Swallow	26	15 ± 8	0.42	0.77
GER/BLT/C[e]	26	22 ± 9	0.39	0.86
Mood/Behavior	29	7 ± 5	0.35	0.89
Parent Concerns	29	14 ± 10	0.44	0.95
Total	23	65 ± 30	0.40	0.95

[a]Number of participants included in multivariate item analysis

[b]Values expressed as mean ± SD

[c]Values expressed as subset or total score divided by maximum subset or total score

[d]Statements considered acceptable if Cronbach-□ ≥ 0.70

[e]GER/BLT/C: Gastroesophageal reflux/bloating/constipation

Table 4. Gastrointestinal Health Questionnaire (GHQ) for girls and young women with Rett Syndrome (RTT): Correlations between GHQ subset or total scores and standardized survey scores for Group IV (n=28) RTT parents as measures of external validity

Questionnaire Comparisons[a]	Pearson Correlation	P-value[b]
GHQ vs PedsQL™ total scores	0.87	0.001
GHQ vs PedsQL™ Health/Pain subset scores	0.65	0.001
GHQ vs PedsQL™ Eat/Chew/Swallow subset scores	0.55	0.01
GHQ vs PedsQL™ GER/BLT/C[b] subset scores	0.89	0.001
GHQ vs PedsQL™ Mood/Worry subset scores	0.57	0.01
GHQ GI subset score vs PedsQL™ total scores	0.88	0.001
GHQ vs GIQLI-A total scores	0.90	0.001
GHQ GI subset vs GIQLI-A total score	0.84	0.001
GHQ GER/BLT/C[c] subset vs GIQLI-A total score	0.78	0.001
GHQ vs RSBQ total score	0.54	0.01
GHQ Mood subset vs RSBQ total score	0.63	0.001
GHQ vs ABC-C total score	0.36	0.10
GHQ Mood subset vs ABC-C total score	0.60	0.001
GHQ vs GSSS total scores	0.70	0.001
GHQ Parent Concerns subset vs GSSS total score	0.70	0.001

[a]Pediatric Quality of Life Inventory™ Gastrointestinal Symptoms Module (PedsQL™) (42), the Gastrointestinal Quality of Life Index for Adults (GIQLI-A) (43), the Rett Syndrome Behavioral Questionnaire (RSBQ) (37), the Aberrant Behavior Checklist (ABC-C) (44), and the Genetic Syndromes Stressors Scale (GSSS) (45)

[b]Significance for correlation coefficient between GHQ scores vs validated questionnaire scores

[c]GER/BLT/C: Gastroesophageal reflux/bloating/constipation