PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Development of the Tailored Rett Intervention and Assessment Longitudinal (TRIAL) database and the Rett Evaluation of Symptoms and Treatments (REST) Questionnaire
AUTHORS	Singh, Jatinder; Lievesley, Kate; Fiori, Federico; Santosh, Paramala

VERSION 1 - REVIEW

REVIEWER	Jeffrey Neul UCSD
REVIEW RETURNED	19-Dec-2016

GENERAL COMMENTS	This manuscript outlined a plan to develop a symptom questionnaire for and perform physiological assessments on people with Rett syndrome. In general, the manuscript presents a reasonable outline of this plan, but my main issue with the manuscript is that because it is a plan there are no results and the need to publish the plan alone is not clear to me. It reads like a grant application rather than a primary scientific manuscript. Aside from this general issue, here are some specific issues that should be addressed: 1. There are a small number of boys who have Rett syndrome, but the report cited in line 16 (Reichow 2015) that there are 57 reported cases is not true. Whilst there are exceptional cases of males who truly meet the clinical criteria for Rett syndrome, all have either Kleinfelters (XXY) or are somatic mosaics for MECP2 mutations, and the number of reported cases is much less than 57. The manuscript cited includes males with MECP2 duplication disorder (a distinct disorder) and boys with congenital encephalopathy due to MECP2 mutations (again, a distinct disorder). This reference should be removed, as should the reference to the number of cases of boys with Rett syndrome unless the genetic nuances described above are included. Honestly, the entire aspect of discussing Rett syndrome in boys is actually unnecessary and can be removed without any problem at all. 2. I am concerned that the sample size estimation for the number of people queried during the initial development stage and the validation stage is far too small given the variability of clinical phenotypes in Rett syndrome. I think it is absolutely too small for Stage 3 given the wide variance both inter and intra-individual in physiological features. 3. There is a fundamental concern about comparisons between Rett syndrome and ASD because in Rett all participants will be girls, and in ASD the majority will be boys. Some consideration of these gender differences and how to address them should be considered and discussed. 4.In Table 1, what does “TBC” mean?

REVIEWER	Daniel C. Tarquinio Department of Pediatrics, Division of Child Neurology Children's Healthcare of Atlanta, USA
REVIEW RETURNED	13-Jan-2017

GENERAL COMMENTS	Overall: 1) Major concerns: The authors consistently mingle the concepts of treatment and recording outcome measures. Statistical plan is untenable and founded on incorrect premises. Abstract: The “generic treatment plan” has not been relevant since the 1980’s when disorder specific care began to evolve. The statement about the questionnaire needed doesn’t follow from this claim, even if pediatric neurologists were still treating these patients “generically”. The FDA PROM document is a comprehensive document, but the methods they stipulate for development are general and can be interpreted in many ways. Introduction: Pathophysiology of disorder is detailed, but predominantly irrelevant in terms of PROM. Although both are understood deeply, as the authors themselves point out very little follows from what we know about the function of MECP2 in terms of clinical phenotype. The summary of existing measures is cursory, demonstrates near complete ignorance of the topic, and is frankly insulting to the efforts of those who have developed those measures over the past 20 years. I refer the authors to the Rett Syndrome Behavioural Questionnaire, Motor Behavioral Assessment, and Clinical Severity Scale to start. Certainly none is “perfect” and I challenge the authors to find a single disease with a “complete all-embracing instrument”. However, ignoring what has been done is a poor start. The idea of using wearable technology is laudable. However, absolutely no explanation is provided as to how this noisy, problematic data will be integrated with the PROM. Methods: 6-months is an unreasonable starting point. The average age of diagnosis is older than 2 years, and the authors don’t propose a simultaneous screening technique to capture young children. Focus groups are useful, however determining whether to use a likert scale or VAS should be left to the statisticians. Parents won’t be able to “rate” their reliability. Moreover, focus groups cannot be used to estimate statistical sample size. P. 11, line 37. RTT is NOT associated with a high penetrance of ASD, and the paper cited doesn’t support this. Rather some of the behaviors present in both disorders overlap. The sample size projections are clearly inadequate for the projected, based on the number of variables and domains involved. This number would be sufficient for a survey of 3-4 items. Results: None. Discussion: None.

REVIEWER	A-M Bisgaard Centre for Rett syndrome, Department of Clinical Genetics, Kennedy Centre, Rigshospitalet.
REVIEW RETURNED	25-Jan-2017

GENERAL COMMENTS	The authors describe an area where there is lack of tools and outcome measures to follow individuals with Rett syndrome over time. It is also an area without consensus among researchers. It is interesting that the authors combine questionnaires with physiological measures and that the families are involved in the process. The aim is also to develop an alert system that is useful to families and clinicians in order to discover possible clinical issues at an early stage. I have some issues and comments; however not on the statistical work since it is outside my skills. It might be interesting to known what the authors think about the international perspectives. Title The title is difficult to read as I get the impression that there are two questionnaires. The authors describe development and validation of REST and not a questionnaire to assess REST. I suggest a reformulation – maybe just erase ..a questionnaire to assess… Abstract gives a good overview. Strengths and limitations Another limitation could be that participation might be very time consuming for families. Introduction The authors write about the genetic background but they lack information on the clinical aspects and symptoms. Please include a paragraph about that. P5, l16: the incidence is reported to be 1:9000 in an Australian study from 2011. P5, l 19: I suggest to write reported instead of documented (I guess that the figure is higher; not all are published) P5, l 25: ….mutations in CDKL5 and FOXG1: I suggest to write " leading to atypical RTT" afterwards. P7, l 12: the natural history study is ongoing and please mention number of included patients. P7, l 25: ref 2: I suggest to include more references. …certain deletions: I think it is more correct to write some mutations or variants. P 7, l 54-56: I do not understand the sentence. P 8, l 30-44: there are some repetitions of words. –suggest to rewrite it. p 11, l 14-21: Cognitive interviews? P 11, l 28 and 48: these diagnoses are rarely made in children as young as 6 months. Why has the authors chosen this and not for instance 1½ year? P 12, l 50: It might be valuable with a photo of the Wearable Sensor Tecnology. P 13, l 39: 10-20 participants will be included. –why so few? Genes as MECP2 should be in italic.

VERSION 1 – AUTHOR RESPONSE

Reviewer 1: Comments to authors:
Comments:
This manuscript outlined a plan to develop a symptom questionnaire for and perform physiological assessments on people with Rett syndrome. In general, the manuscript presents a reasonable outline of this plan, but my main issue with the manuscript is that because it is a plan there are no results and the need to publish the plan alone is not clear to me. It reads like a grant application rather than a primary scientific manuscript.
Response: Thank you for your comment. Per the guidelines of BMJ Open manuscript submissions, BMJ Open Protocol manuscripts should only report planned or ongoing research studies. If data collection is complete, as stated by the editorial guidelines, the manuscript will not be considered. We had already mentioned this to the editor prior to submission i.e. at present we have no results and it is anticipated that the REST Questionnaire will be developed by April 2017 and validated thereafter. The approximate end date for the study will be April 2019.

Aside from this general issue, here are some specific issues that should be addressed:
1. There are a small number of boys who have Rett syndrome, but the report cited in line 16 (Reichow 2015) that there are 57 reported cases is not true. Whilst there are exceptional cases of males who truly meet the clinical criteria for Rett syndrome, all have either Kleinfelters (XXY) or are somatic mosaics for MECP2 mutations, and the number of reported cases is much less than 57. The manuscript cited includes males with MECP2 duplication disorder (a distinct disorder) and boys with congenital encephalopathy due to MECP2 mutations (again, a distinct disorder). This reference should be removed, as should the reference to the number of cases of boys with Rett syndrome unless the genetic nuances described above are included. Honestly, the entire aspect of discussing Rett syndrome in boys is actually unnecessary and can be removed without any problem at all.
Response: References to Reichow et al. (2015) and MECP2 duplication (Sztainberg et al., 2015) have been removed. Were we have mentioned congenital encephalopathy, this text has also been deleted and the wording updated

2. I am concerned that the sample size estimation for the number of people queried during the initial development stage and the validation stage is far too small given the variability of clinical phenotypes in Rett syndrome. I think it is absolutely too small for Stage 3 given the wide variance both inter and intra-individual in physiological features.
Response: The sample size (between 10-20 participants) required for stage 1 (Questionnaire Development Stage) should be sufficient for the purpose of conducting focus groups and developing the initial REST questionnaire. Small sample sizes are an unavoidable consequence of studying a rare disease. In our experience, focus groups involving families with children with rare diseases are well versed with the problems associated with the condition in question. Often, the themes that need to be addressed get saturated after a couple of focus groups, leading to us getting the basic structure of the items needed to be tested in Stage 2. However, we do share your concerns for the sample sizes required for Stage 2 (Questionnaire Validation Stage) and Stage 3 (Wearable Technology Stage), and are submitting a substantial amendment to the Research Ethics Committee (REC) to increase the sample size. Accordingly, we have increased the sample size to 150 (n=100 RTT cohort and n=50 ASD cohort) in Stage 2. The number in the ASD cohort will be split so that 25 parents/carers will have a female diagnosed with ASD. Stage 3 will have 100 participants (n=50 RTT and n=50 ASD [25 male and 25 female]).

3. There is a fundamental concern about comparisons between Rett syndrome and ASD because in Rett all participants will be girls, and in ASD the majority will be boys. Some consideration of these gender differences and how to address them should be considered and discussed.
Response: Thank you for raising this concern. We have included the following text in the manuscript on page 19 to address gender differences:

In Stage 3 (Wearable Sensor Technologies Stage), if the data meet the requirements for parametric testing, the general linear model (GLM) (ANOVA) covaried for gender will be applied to the RTT and ASD cohorts.

4. In Table 1, what does “TBC” mean?
Response: This has been rectified and text added to Table 1.

Reviewer 2: Comments to authors:
Comments:
Overall:
1. Major concerns: The authors consistently mingle the concepts of treatment and recording outcome measures. Statistical plan is untenable and founded on incorrect premises.
Response: Thank you for your comments. The manuscript has now been restructured based on your comments made by yourself and the other reviewers. The analysis plan has been developed on the methods described in Santosh et al. (2016)1 detailing the plan required for the development and validation of a questionnaire to assess concerning behaviours and mental health in individuals with autism spectrum disorder and by Mount et al. (2002) for the Rett Syndrome Behaviour Questionnaire (RSBQ)2. For this protocol, the Statistical Analysis Plan (SAP) has been reviewed by KCL R&D (King’s College London, Research and Development) and by the resident statistician. If we need to adjust the Statistical Analysis Plan (SAP), through guidance from KCL R&D and our resident statistician, we will amend the SAP accordingly. There are two aspects that are being explored – i) being able to profile symptoms in Rett syndrome, and ii) exploring whether the measure is able to capture change appropriately. Validation of both these aspects will be achieved during the project. The team has experience in having developed and validated over 30 different patient / carer reported outcome measures used in neurodisability.
References
1. Santosh P, Tarver J, Gibbons F. et al. (2016). Protocol for the development and validation of a questionnaire to assess concerning behaviours and mental health in individuals with autism spectrum disorders: the Assessment of Concerning Behaviour (ACB) scale. BMJ Open. 6: e010693.
2. Mount RH, Charman T, Hastings RP, Reilly S, Cass H (2002). The Rett Syndrome Behaviour Questionnaire (RSBQ): Refining the behavioural phenotype of Rett syndrome. J. Child Psychol. Psychiatry 43: 1099–1110.

2. Abstract: The “generic treatment plan” has not been relevant since the 1980’s when disorder specific care began to evolve. The statement about the questionnaire needed doesn’t follow from this claim, even if pediatric neurologists were still treating these patients “generically”. The FDA PROM document is a comprehensive document, but the methods they stipulate for development are general and can be interpreted in many ways.
Response: Any text relating to generic treatment plan has been removed.

3. Introduction: Pathophysiology of disorder is detailed, but predominantly irrelevant in terms of PROM. Although both are understood deeply, as the authors themselves point out very little follows from what we know about the function of MECP2 in terms of clinical phenotype. The summary of existing measures is cursory, demonstrates near complete ignorance of the topic, and is frankly insulting to the efforts of those who have developed those measures over the past 20 years. I refer the authors to the Rett Syndrome Behavioural Questionnaire, Motor Behavioral Assessment, and Clinical Severity Scale to start. Certainly none is “perfect” and I challenge the authors to find a single disease with a “complete all-embracing instrument”. However, ignoring what has been done is a poor start.
Response: We had alluded to some of the relevant outcome measures such as RSBQ and Gross Motor Scale elsewhere in the manuscript; however, we have now included a section that provides a salient overview on the development of measures in RTT syndrome. The text is also included below:

Added text:

Capture of disease severity and sensitivity to change throughout the lifespan in patients are important elements that need to be considered when developing clinically meaningful outcome measures. The Unified Parkinson Disease Rating Scale, (UPDRS) provides a good example of an outcome measure that is effective and can capture disease severity and clinically meaningful change of symptoms of Parkinson's disease34. With rare diseases, the Sanfilippo Behaviour Rating Scale (SBRS), a 68 item questionnaire developed using 44 families, is also effective and can map the behavioural phenotype of children with Sanfilippo syndrome to disease progression and/or results from treatment across the lifespan35. In RTT, the current outcome measures are inadequate in their ability to capture disease severity across the lifespan, although others have made significant headway in this area. The 37 item motor–behavioral assessment (MBA) incorporates historical items with items from direct clinician evaluations and has been used to describe clinical severity in RTT36,37, whilst the Rett Syndrome Behavioral Questionnaire (RSBQ), a validated checklist, was designed to differentiate individuals with RTT compared to those with severe intellectual disability38. Other measures tested in RTT include the Anxiety Depression and Mood Scale (ADAMS)39, the clinician based International Scoring System (ISS)40,41 to evaluate the disease severity, Vineland Adaptive Behaviour scale42, the 13 item Rett Clinical Severity Scale (RCSS)37,43 and its modified version42. Others have developed RTT specific anchors such as for the Clinical Global Impression Severity (CGI-S) scale based on scores from the RCSS for improved outcome measures in clinical trials44. Quality of Life (QOL) measures such as the Child Health Questionnaire-P50 have also been used in RTT45 including a recent Phase II open label clinical trial using glatiramer acetate46. Some of these measures such as the MBA, RSBQ, ADAMS and RCSS have been implemented into clinical trials to evaluate the effect of Insulin-like Growth Factor (IGF-1)46,47 or Sarizotan48 in individuals with RTT, or to develop a novel scoring tool (Rett Severity Score [RSS]) to assess the impact of IGF-1 treatment in RTT41. Other scales such as the Mullen Scales for Early Learning used in other rare disorders49, have also been adapted for use in RTT47. These measures are not without their faults. Some have suggested that the MPA can be difficult to use with some items that describe disease regression having not been validated24. This is important given that in some RTT patients, disease regression has been described as being transient or often goes unrecognised50. Others such as the RSBQ although are suitable to measure some aspects of behaviour such as mood and anxiety51 might not be able to capture the salient features of behaviour as an outcome measure in a clinical trial in RTT patients. Furthermore, there is differing reliability of anxiety scales in RTT, with ADAMS especially its Social Avoidance subscale having the best psychometric properties in comparison to the RSBQ52. Whilst no outcome measure will be perfect, these studies have paved the way for more sensitive outcome measures to be developed such as the validated 15 item Gross Motor Scale for individuals with RTT53

References

34. Lang AE, Eberly S, Goetz CG et al. (2013). Movement disorder society unified Parkinson disease rating scale experiences in daily living: longitudinal changes and correlation with other assessments. Mov. Disord. 28: 1980-1986.

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36. FitzGerald PM, Jankovic J. & Percy AK. (1990). Rett syndrome and associated movement disorders. Mov. Disord. 5: 195–202.

37. Tarquinio DC, Motil KJ, Hou W. et al. (2012). Growth failure and outcome in Rett syndrome: specific growth references. Neurology 79: 1653–61.

38. Mount RH, Charman T, Hastings RP, Reilly S, Cass H (2002). The Rett Syndrome Behaviour Questionnaire (RSBQ): Refining the behavioural phenotype of Rett syndrome. J. Child. Psychol. Psychiatry 43: 1099–1110.

39. Esbensen AJ, Rojahn J, Aman MG, Ruedrich S (2003). Reliability and validity of an assessment instrument for anxiety, depression, and mood among individuals with mental retardation. J. Autism Dev. Disord. 33: 617–629.

40. Kerr A. M., Nomura Y., Armstrong D., et al. (2001). Guidelines for reporting clinical features in cases with MECP2 mutations. Brain & Development. 23: 208–211.
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42. Kaufmann WE, Tierney E, Rohde CA. et al. (2012). Social impairments in Rett syndrome: Characteristics and relationship with clinical severity. J. Intellect. Disabil. Res. 56: 233–247.
43. Tarquinio DC, Hou W, Berg et al. (2017). Longitudinal course of epilepsy in Rett syndrome and related disorders. Brain. 140: 306-318.
44. Neul JL, Glaze DG, Percy AK. et al. (2015). Improving Treatment Trial Outcomes for Rett Syndrome: The Development of Rett-specific Anchors for the Clinical Global Impression Scale. J. Child Neurol. 30: 1743-1748.
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46. Djukic A, Holtzer R, Shinnar S. et al. (2016). Pharmacologic Treatment of Rett Syndrome With Glatiramer Acetate. Pediatr. Neurol. 61: 51-57.
47. Khwaja OS, Ho E, Barnes KV. et al. (2014). Safety, pharmacokinetics, and preliminary assessment of efficacy of mecasermin (recombinant human IGF-1) for the treatment of Rett syndrome. Proc. Natl. Acad. Sci. U S A. 111:4596-4601.
48. https://www.clinicaltrials.gov/ct2/show/NCT02790034?term=newron&rank=2 (accessed 25 February 2017).
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50. Naidu S, Johnston MV. (2011). Neurodevelopmental disorders: Clinical criteria for Rett syndrome. Nat. Rev. Neurol. 7: 312-314
51. Robertson L, Hall SE, Jacoby P. et al. (2006). The association between behavior and genotype in Rett syndrome using the Australian Rett Syndrome Database. Am. J. Med. Genet. B. Neuropsychiatr Genet. 141B (2): 177-183.
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4. The idea of using wearable technology is laudable. However, absolutely no explanation is provided as to how this noisy, problematic data will be integrated with the PROM.
Response: The manuscript has now been restructured and we have included a paragraph in ‘Stage 3: Wearable Sensor Technology’ explaining this (below):
Added text:
Using wearable sensor technology as a PROM is not without its challenges. In RTT, wearable technology has been used to explore respiratory and cardiac function in observational studies79,78 and in two recent clinical trials46,47, however, inherently captured biometric data can be noisy especially from quasi-periodic oscillations from cardiac rhythms. Wrist worn devices might be particularly susceptible to this type of noise. To mitigate these issues, we have applied the methods described previously81,82 to analyse heart rate variability and electrodermal activity as metrics when evaluating wrist sensor biometric data and autonomic function in a 15 year old girl with RTT. We were able to demonstrate a recalibration of the autonomic equilibrium from pre-treatment to post-treatment using buspirone (30 mg/day)58, and subsequent improvement in EBAD in this girl. Quasi-periodic oscillations cannot be easily quantified using conventional methods. To manage this phase-rectified signal averaging (PRSA)83 may be used in conjunction with spectral factorization and applied to the beat-to-beat RR interval data, which is particularly prone to extraneous noise. This methodology coupled with EDA assessment will provide more sensitive methods to capture changes in autonomic physiology in patients with RTT.

References

46. Djukic A, Holtzer R, Shinnar S. et al. (2016). Pharmacologic Treatment of Rett Syndrome With Glatiramer Acetate. Pediatr. Neurol. 61: 51-57.
47. Khwaja OS, Ho E, Barnes KV. et al. (2014). Safety, pharmacokinetics, and preliminary assessment of efficacy of mecasermin (recombinant human IGF-1) for the treatment of Rett syndrome. Proc. Natl. Acad. Sci. U S A. 111:4596-4601.
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5. Methods: 6-months is an unreasonable starting point. The average age of diagnosis is older than 2 years, and the authors don't propose a simultaneous screening technique to capture young children.
Response: We have adjusted the starting point to 6 years. This is based on our recent experiences managing RTT patients with EBAD and is a reasonable starting point based on the evidence available from studies done on clinical phenotypes and when the cardinal features of autonomic dysfunction are first thought to appear as indicated by Zogbi, H (2016).

Focus groups are useful, however determining whether to use a likert scale or VAS should be left to the statisticians. Parents won't be able to "rate" their reliability.
Response: We have removed the mention of the Likert scale and slider bar.

6. P. 11, line 37. RTT is NOT associated with a high penetrance of ASD, and the paper cited doesn't support this. Rather some of the behaviors present in both disorders overlap.
Response: The text has been amended.

7. The sample size projections are clearly inadequate for the projected, based on the number of variables and domains involved. This number would be sufficient for a survey of 3-4 items.
Response: We share your concerns and agree that small sample sizes are an unavoidable consequence of studying a rare disease. However, we have adjusted the sample sizes required for Stage 2 (Questionnaire Validation Stage) and Stage 3 (Wearable Technology Stage), and are submitting a substantial amendment to the Research Ethics Committee (REC) to increase the sample size. Accordingly, we have increased the sample size to 100 RTT and 50 ASD for Stage 2 and 50 RTT and 50 ASD in Stage 3 of the protocol.

Reviewer 3: Comments to authors:
The authors describe an area where there is lack of tools and outcome measures to follow individuals with Rett syndrome over time. It is also an area without consensus among researchers. It is interesting that the authors combine questionnaires with physiological measures and that the families are involved in the process. The aim is also to develop an alert system that is useful to families and clinicians in order to discover possible clinical issues at an early stage.

I have some issues and comments; however not on the statistical work since it is outside my skills. It might be interesting to know what the authors think about the international perspectives.
1. Title
The title is difficult to read as I get the impression that there are two questionnaires. The authors describe development and validation of REST and not a questionnaire to assess REST. I suggest a reformulation – maybe just erase .a questionnaire to assess…
Response: Thank you for your comment. The title has been amended.

2. Abstract
gives a good overview.
Strengths and limitations
Another limitation could be that participation might be very time consuming for families.
Response: Added to the limitations.

3. Introduction
The authors write about the genetic background but they lack information on the clinical aspects and symptoms.
Please include a paragraph about that.
Response: We have now added a short paragraph on the clinical aspects:
Added text:
Although there have been considerable advances in understanding the genetics and into the genetic testing of RTT, the diagnosis of RTT is based on the 2010 revised consensus clinical criteria3 (see Table 1 in Neul et al., 2010) and recommends that all individuals with RTT should be first be assessed according to the revised clinical criteria followed by a thorough genetic test for MECP2. Given that about 3-5% of RTT individuals who fulfil the diagnostic clinical criteria do not have MECP2 mutations, and this is even higher for atypical RTT cases25, more recently clinical predictors that can facilitate a clinician's decision making to order genetic testing for RTT have been provided26. This showed that the likelihood of a having a positive MECP2 test was greatest in patients with partial or complete attenuation of hand skills. Impairments in gait and hand stereotypies were also strong predictors. Of interest was that loss of speech did not discriminate whether an individual was MECP2+ or MECP2-.
References
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25. Dolce A, Ben-Zeev B, Naidu S, Kossoff EH. (2013). Rett syndrome and epilepsy: an update for child neurologists. Pediatr. Neurol. 48: 337-345.
26. Knight VM, Horn PS, Gilbert DL, Standridge SM. (2016). The Clinical Predictors That Facilitate a Clinician's Decision to Order Genetic Testing for Rett Syndrome. Pediatr. Neurol. 63: 66-70.

4. P5, l16: the incidence is reported to be 1:9000 in an Australian study from 2011.
Response: Text has been updated to reflect the geographical variations.

5. P5, l 19: I suggest to write reported instead of documented (I guess that the figure is higher; not all are published)
Response: No longer applicable as based on the suggestions by Reviewer 1, we have now removed the text.

6. P5, l 25: ….mutations in CDKL5 and FOXG1: I suggest to write " leading to atypical RTT" afterwards.
Response: Added

7. P7, l 12: the natural history study is ongoing and please mention number of included patients.

Response: Text added

8. P7, l 25: ref 2: I suggest to include more references. …certain deletions: I think it is more correct to write some mutations or variants.
Response: The text has been updated – 'certain deletions' have been removed and replaced with 'some mutations or variants.' We have also added references to the two most seminal studies of adequate sample size i.e. that used data from InterRett (Bebbington et al., 2008)54 and the US Natural History (Neul et al., 2008)55. These studies provided definitive information about genotype–phenotype relationships in RTT and are described elegantly by Leonard et al. (2017)24.
References
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54. Bebbington A, Anderson A, Ravine D. et al. (2008). Investigating genotype-phenotype relationships in Rett syndrome using an international data set. Neurology. 70: 868–75.
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9. P 7, l 54-56: I do not understand the sentence.
Response: We have modified the text to improve clarity to state that based on our clinical experience of managing patients in the Centre for Interventional Paediatric Psychopharmacology and Rare Diseases (CIPPRD), autonomic dysfunction is often found in patients who do not respond to treatment and those with significant functional disability.

10. P 8, l 30-44: there are some repetitions of words. –suggest to rewrite it.
Response: Text has been modified and we have removed the repetitive statements.

11. p 11, l 14-21: Cognitive interviews?
Response: Heading has been removed

12. P 11, l 28 and 48: these diagnoses are rarely made in children as young as 6 months. Why has the authors chosen this and not for instance 1½ year?
Response: We have adjusted the age group between 6 to 40 years old. This is based on three factors (I) our clinical experience, (II) the evidence available from studies done on clinical phenotypes and (III) when the cardinal features of autonomic dysfunction are first thought to appear as indicated by Zogbi, H (2016).

13. P 12, l 50: It might be valuable with a photo of the Wearable Sensor Technology.
Response: We had also considered this but decided against showing photos due to copyright and trademark issues. We will be testing a variety of wearable sensor devices and decided that for the purposes of this protocol paper to avoid showing example photos of these devices.

14. P 13, l 39: 10-20 participants will be included. –why so few?
Response: The sample size (between 10-20 participants) required for stage 1 (Questionnaire Development Stage) should be sufficient for the purpose of conducting focus groups and developing the initial REST questionnaire. As a guide in the development of the RSBQ (Mount et al., 2002), after evaluating about 40 case reports that contained descriptions of behavioural features, the descriptors were extracted and reduced to 110 items. These 110 items were presented to five clinicians: a speech and language therapist, a paediatrician, an occupational therapist, a music therapist and a physiotherapist, who were all experienced in the assessment and management of children and adults with RTT. Following review these items were reduced to 100 and seven parents were asked to comment on them, which led to further refinement and modification of a few of the items. As stated above, we have significantly increased the numbers taking part in Stage 2 of the validation.

14. Genes as MECP2 should be in italic.
Response: Updated

VERSION 2 – REVIEW

REVIEWER	Daniel Tarquinio Emory Univ. USA
REVIEW RETURNED	13-Mar-2017

GENERAL COMMENTS	Much improved

REVIEWER	Anne-Marie Bisgard Department of Clinical Genetics Rigshospitalet Copenhagen Denmark
REVIEW RETURNED	22-Mar-2017

GENERAL COMMENTS	The authors have responded satisfactorily to my questions. However, I am still curious on what the authors think about the international perspectives.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 2
Reviewer Name: Daniel Tarquinio
Institution and Country: Emory Univ, USA
Please state any competing interests: None declared
Please leave your comments for the authors below
Much improved
Response: Thank you.
4. Reviewer: 3
Reviewer Name: Anne-Marie Bisgard
Institution and Country: Department of Clinical Genetics, Rigshospitalet, Copenhagen, Denmark
Please state any competing interests: None declared
Please leave your comments for the authors below
The authors have responded satisfactorily to my questions. However, I am still curious on what the authors think about the international perspectives.
Response: Thank you for your comment. We have added the following text to the manuscript ('Strengths and Limitations of the Study' and the 'Dissemination' sections) that emphasizes the global perspectives of this study:
Added text:
As the HealthTrackerTM-based TRIAL database is web-based, with appropriate funding, it has the potential to be used globally, allowing for quicker development of decision-support analytics and personalized care.

VERSION 3 – REVIEW

REVIEWER	Anne-Marie Bisgaard Department of Clinical Genetics, Rigshospitalet, Copenhagen, Denmark
REVIEW RETURNED	13-Apr-2017

GENERAL COMMENTS	Thanks for the reply.