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## Protocol for the Development and Validation of a Questionnaire to assess the Rett Evaluation of Symptoms and Treatments (REST) Questionnaire

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Manuscripts

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3 **Protocol for the Development and Validation of a Questionnaire**  
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6 **to assess the Rett Evaluation of Symptoms and Treatments**  
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8  
9 **(REST) Questionnaire**  
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49 **Key Words**  
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51 Biomarkers; HealthTracker<sup>TM</sup>; Questionnaire Development and Validation; Rett Syndrome;  
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53 Tailored Rett Intervention and Assessment Longitudinal (TRIAL) database; Wearable Sensor  
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55 Technology  
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## Abstract

**Introduction:** Rett Syndrome (RTT) is a pervasive neurodevelopmental disorder that presents with deficits in brain functioning leading to language and learning regression, characteristic hand stereotypies and developmental delay. RTT has divergent symptomatology and its clinical heterogeneity belies the premise that individuals with RTT should be treated using a generic treatment plan. What is needed is a single, multidimensional questionnaire that embraces all symptoms, and the relationships between them, that can capture clinically meaningful data across the lifespan in individuals with RTT. This standalone questionnaire will be used for signposting areas for better practice. The new outcome measure will also be able to marry with the physiological aspects of the disease obtained using wearable sensor technology as well as genetic and psychosocial data to stratify patients. Taken together, this will empower clinicians and researchers with the confidence to delineate between different aspects of disorder symptomatology in order to streamline treatment pathways for individuals or for those patients entering clinical trials. This protocol describes the anticipated development and validation of the Rett Evaluation of Symptoms and Treatments (REST) questionnaire, which when linked with the outcomes of the wearable sensor technology, will serve as a barometer for longitudinal patient monitoring and improve treatment pathways in patients with RTT.

**Methods and Analysis:** The United States (US) Food and Drug Administration (FDA) Guidance for Patient-reported Outcome Measures (PROM) will be used as a template to inform the methodology of the study. It will follow an iterative framework that will include item/concept identification, item/concept elicitation in parent/carer mediated focus groups, expert clinician feedback, web based presentation of questionnaires, initial scale development, cognitive interviews, instrument refinement and instrument validation.

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3 **Ethics and dissemination:** The study has received favourable opinion from the NHS  
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5 Research Ethics Committee (REC): NHS Research Ethics Committee (REC) – London,  
6  
7 Bromley Research Ethics Committee (reference: 15/LO/1772).  
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For peer review only

### Strengths and Limitations of the Study:

Strengths of this Study are:

- It will follow the FDA framework for Patient Reported Outcome Measures.
- The new questionnaire will correlate behavioural data with the physiological aspects of the disease.
- It will gather feedback from parent-based charities such as Reverse Rett UK.
- The new questionnaire will capture clinically meaningful change of symptomatology in individuals with RTT across the lifespan.
- It will enable the streamlining of treatment and expedite triaging of care by signposting patients to correct specialists sooner to enable timely intervention.
- The use of HealthTracker™, a multi-modal eHealth web-based monitoring platform, will make the questionnaire as user friendly as possible and allows it to be tailored to individual participant.

Limitation of this Study:

- A limitation is that the questionnaire battery will be completed remotely so the extent to which participants feel comfortable using the Internet and computers may affect completion rates.

## Introduction

Rett Syndrome (RTT) can trace its genesis to a clinical waiting room in Vienna (1965)<sup>1</sup> where Dr. Rett first observed the clinical signs and symptoms of RTT. Symptoms usually appear between 6 to 18 months after birth<sup>2</sup> and clinically RTT presents with impairments in brain functioning leading to language and learning regression, hand stereotypies (hand washing/wringing) and developmental stagnation. RTT is predominantly found in young females with an incidence of about 1: 10,000 live births<sup>3</sup> and so far 57 cases of RTT have been documented in males<sup>4</sup>. The prevalence is underscored by the high clinical variability of the disease and hence the frequency could be underestimated. Loss of function in the methyl-CpG binding protein 2 (*MeCP2*) gene is responsible for the disorder in the vast majority of cases<sup>5</sup>, with rarer cases being attributed to mutations in *CDKL5* and *FOXP1* gene<sup>6,7</sup>. More recently, mutations in other less known candidate genes have emerged such as those encoding ankyrin repeat proteins and neuronal acetylcholine receptor subunits<sup>8</sup>. *MeCP2* is a highly conserved nuclear protein in the mammalian brain<sup>9</sup> and notably the disorder is reversible in mice models of RTT<sup>10</sup> and in *MECP2* duplication syndrome<sup>11</sup>.

*MeCP2* acts as a critical epigenetic modulator in the mammalian brain, controlling overlapping mechanisms such as DNA methylation and post-translational mechanisms<sup>12</sup>. Through differential post-translational modification at serine 164<sup>13</sup>, *MeCP2* may help in limiting transcriptional noise<sup>14</sup> of other genes. For example, mutations in the gene *switch-insensitive 3 family member A (SIN3A)*, a *MeCP2* interactor and transcriptional repressor – crucial for cortical integrity, causes intellectual disability and ASD<sup>15</sup> and the *MeCP2*<sup>R306C</sup> mutation prevents *MeCP2* from interacting with the NCoR/histone deacetylase 3 (HDAC3) complex resulting in impediments in social and cognitive functioning in animal models<sup>16</sup>. *MeCP2* has complex genome level modalities, and the general opinion is that loss of *MeCP2* impacts other genes crucial for post-natal neuronal development. This seems to be the

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2  
3 significant driver for the classical RTT clinical phenotype. Genes in neuronal development  
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5 tend to be long (100 kb or larger)<sup>17</sup> and as the transcriptional repression function of MeCP2 is  
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7 biased towards longer genes<sup>18</sup>, it is likely that impairments of long genes associated with  
8  
9 neuronal development dictates the functional and developmental versatility of MeCP2 seen in  
10  
11 RTT. This has a knock-on effect on the homeostasis of excitatory and inhibitory  
12  
13 pathways<sup>19,20</sup> in RTT brains leading to the clinical versatility that is commonly observed.

14  
15 MECP2 being an X-linked gene, has an impact on the phenotype of RTT patients and the  
16  
17 clinical severity. The X chromosome inactivation can cause uneven expression of wild type  
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19 and mutant alleles resulting in skewed patterns of RTT phenotype severity<sup>21,22</sup>, and the degree  
20  
21 of DNA-methylation-dependent long gene repression<sup>18</sup>. The range of severity in RTT is  
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23 therefore broad and ranges from lethal neonatal encephalopathy to milder forms (where  
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25 symptoms are less severe); hence assessment and treatment must be individually tailored to  
26  
27 each affected person.  
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### 31 *Pre-existing measures in RTT*

32  
33 As far as we are aware, no complete all-embracing instrument has been developed for  
34  
35 individuals with RTT that has the ability to capture longitudinal pharmacological,  
36  
37 behavioural, genetic and psychosocial information, as well as an ability to correlate this with  
38  
39 the physiological aspects of the disease. At present there are no datasets or instruments that  
40  
41 provide such information. Previous datasets/instruments have been inconsistent and provide  
42  
43 limited information on the behavioural and physiological facets of the disease. Whilst, some  
44  
45 might provide information on the genetic diagnoses of individuals with RTT, there is a lack  
46  
47 of consistency. First, RettBase, collected mainly molecular genetic data from the Australian  
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49 cohort of RTT patients<sup>23</sup>. Some other instruments have included both genetic and clinical  
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51 data, although the clinical data was limited. InterRETT, an Australian Rett syndrome  
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53 database, was based on data collection by distributing a questionnaire to families<sup>24</sup>. The  
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3 Italian Rett Database and Biobank consisted of 357 patients and had 20 structured and seven  
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5 descriptive clinical items along with 17 structured genetic items<sup>25</sup>. The British Isles Rett  
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7 Syndrome Survey, included 275 British Rett patients and had 271 structured and 94  
8  
9 descriptive clinical items, and six structured genetic items<sup>26</sup>. An American survey collected  
10  
11 data on the natural history of the disease that allowed researchers and physicians to access  
12  
13 comprehensive patient data<sup>27</sup>. These datasets were preserved and integrated into the Rett  
14  
15 Networked Database<sup>26</sup> and offers an amalgamated data repository for researchers to access  
16  
17 anonymized patient information. Elsewhere, the Japanese RTT database (JRDB) includes the  
18  
19 clinical data from 102 females with a median age of 11 years old<sup>28</sup>.

22  
23 Large cross-sectional studies investigating the genotype-phenotype relationships have  
24  
25 revealed divergence in the phenotype seen in individuals with RTT<sup>29</sup>. Certain deletions  
26  
27 dictate a more severe phenotype when it comes to motor abilities<sup>29,30</sup> and cardio-respiratory  
28  
29 phenotypes<sup>31</sup>. This has paved the way for specific questionnaires to be developed and  
30  
31 validated such as the Rett Syndrome Gross Motor Scale<sup>32</sup> to measure the gross motor abilities  
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33 in individuals with RTT and a modified version of the International Scoring System<sup>33</sup> in RTT  
34  
35 to assess the clinical impact of the common symptoms. Whilst these instruments have their  
36  
37 merits and would be invaluable for pinpointing specific outcomes in RTT such as gross motor  
38  
39 deficits and cardio-respiratory function, they are limited in their scope and have not been  
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41 evaluated for capturing clinically meaningful change of key symptomatology longitudinally.  
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43 Moreover, as autonomic dysfunction does not appear to be governed by any specific mutation  
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45 in RTT<sup>31</sup>, assessing the autonomic dysfunction in individuals in RTT is a pressing clinical  
46  
47 concern.  
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51  
52 Autonomic dysfunction is a pivotal factor that requires consideration when managing patients  
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54 with rare disorders such as RTT. From a clinical perspective, this situation is often found in  
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56 treatment non-responders and those with significant functional disability. Autonomic  
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3 dysfunction co-occurs in the context of emotional and behavioural dysregulation, and  
4  
5 recently using wearable sensor technology, we have shown that Emotional, Behavioural and  
6  
7 Autonomic Dysregulation (EBAD) is a crucial factor that needs to be considered when  
8  
9 managing patients with RTT<sup>34,35</sup>. Although autonomic dysfunction has been investigated in  
10  
11 individuals with RTT<sup>36-39</sup>, the progression of autonomic dysfunction and the developmental  
12  
13 trajectory of EBAD has never been researched. Moreover, the components of EBAD in an all  
14  
15 encompassing questionnaire that can map across other symptomatology longitudinally in  
16  
17 individuals with RTT has not previously been shown.  
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## 20 21 **Aim**

22  
23 The objective of this protocol is to develop and validate a comprehensive multi-system  
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25 questionnaire (Rett Evaluation of Symptoms and Treatments – REST questionnaire) that can  
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27 profile the symptomatology of patients with RTT by capturing clinically meaningful data  
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29 across the lifespan allowing better understanding of patient needs. In parallel, information  
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31 collected using wearable sensor technology<sup>34,35</sup> will be linked to data obtained from the  
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33 REST questionnaire, genetic data and also information about available psychosocial support  
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35 from the patient and their family, to form a comprehensive Tailored Rett Intervention and  
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37 Assessment Longitudinal (TRIAL) database. Using the data obtained from the new REST  
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39 questionnaire, physiological measurements (using wearable sensor technology), genetic and  
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41 clinically derived psychosocial support data, the TRIAL database will streamline treatment  
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43 approaches to expedite triaging of care by signposting patients to correct specialists earlier  
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45 than is currently happening (Figure 1). Specifically, the functionality of the multi-modal  
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47 HealthTracker™ platform will be exploited so that the data from the TRIAL database can be  
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49 used to develop a parent/carer alert system to signal when it may be useful to request  
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51 unscheduled clinician appointments. Using this functionality, the TRIAL database will also  
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3 be able to stratify patients to inform adaptive clinical trial design, by allowing pre-existing  
4 datasets to be used so that rare disease trials can be done in a more cost-effective manner.  
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### 7 **Methods and Analyses**

8  
9 The title of this questionnaire was based on the feedback of the focus groups involving  
10 parents and carers of children with RTT from the parent based charities such as Reverse Rett  
11 UK, and clinician feedback. It will incorporate elements from previous scales<sup>32,33</sup> and  
12 standardised RTT questionnaires – data from the Natural History Study<sup>40</sup>, Rett Syndrome  
13 Behaviour Questionnaire (RSBQ)<sup>41</sup> and the modified version of the Rett Syndrome Severity  
14 Scale (RSSS)<sup>42</sup>. It is anticipated that the questionnaire will not take more than 30 minutes to  
15 complete.  
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24 The United States (US) Food and Drug Administration (FDA) Guidance for Patient-reported  
25 Outcome Measures (PROM)<sup>43</sup> will be used as a template to guide the methodology in the  
26 study. It was described in Santosh *et al.* (2016)<sup>44</sup> and will follow an iterative framework that  
27 will involve item/concept identification, item/concept elicitation in parent/carer mediated  
28 focus groups, clinician feedback, web based presentation of questionnaires initial scale  
29 development, cognitive interviews, instrument refinement and instrument validation.  
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### 38 ***Stage 1: Qualitative Development of the Rett Evaluation of Symptoms and Treatment*** 39 ***(REST) questionnaire***

#### 40 ***Concept identification***

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42 For this initial phase, a systematic literature review will be conducted according to the  
43 “Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)<sup>45</sup> to  
44 identify signs and symptoms that are deemed to be problematic in RTT. A draft version will  
45 be reviewed by expert clinicians who have substantial experience in RTT and Autism  
46 Spectrum Disorder (ASD). Common themes will be identified and draft version of the  
47 questionnaire will be prepared based on their feedback.  
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### *Concept elicitation*

This stage will involve parents/carers of individuals aged 6 months to 40 years with RTT. A series of focus groups anticipated to last about 1.5 hours will be conducted as part of the concept elicitation stage. These focus groups will include parents/carers of individuals with RTT from the parent based charities such as Reverse Rett, UK, and clinicians who see RTT patients. The groups will follow a semi-structured format using open-ended questions to allow participants to discuss their experiences and views. Some of the focus groups will be on item generation whilst others may centre on reviewing draft versions of the questionnaire identifying pertinent themes. Focus groups will be audio recorded and each group will include approximately 4-6 parents of children with RTT. Up to two researchers may be present for the focus groups, which will be led by a Consultant Child and Adolescent Psychiatrist/Specialist. All participants will also be asked to complete a demographic questionnaire.

### *Web based presentation of questionnaires on the HealthTracker™ platform*

HealthTracker™, a web-based health monitoring platform<sup>46</sup>, has been successfully trialed in multi-centric European Union FP7 studies<sup>47,48</sup> and also in a questionnaire development and validation study<sup>49</sup>. Parents and carers will be shown how the REST questionnaire might appear on the HealthTracker™ platform, how the response options to the questionnaire could be presented (i.e. Likert scale or slider bar) and whether a choice of single or multiple choice questions would be appropriate. The various views of the focus groups will be used to choose the most optimal web-based visualization of the questionnaire.

### *Tool review*

As far as the authors are aware, no questionnaire exists that not only is RTT focused but can capture a broad range of problematic themes, for example, the developmental trajectory of EBAD. Nor do these existing questionnaires/scales attempt to marry this with the

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3 physiological measurements from the wearable sensor technology. At this stage, a further  
4 literature review will be conducted to identify any themes that may have been missed during  
5 the focus groups and whether any further areas of RTT symptomatology that were not  
6 highlighted in the focus groups needs to be addressed. In addition, parents/carers from  
7 Reverse Rett, UK, will be consulted and any feedback incorporated into the tool review stage.

#### 14 *Cognitive interviews*

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16 Following the focus groups, study participants will be sent a copy of the draft version of the  
17 questionnaire (via email or post). Once this part has been completed, a draft operating beta  
18 version of the questionnaire will be finalised.

#### 22 ***Stage 2: Validation of the Rett Evaluation of Symptoms and Treatments (REST)*** 23 ***questionnaire***

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27 This stage of the study will involve parents/carers of individuals aged 6 months to 40 years  
28 with RTT. Questionnaires to assess the longitudinal trajectory of symptomatology in rare  
29 diseases have proven to be difficult to validate<sup>50,51</sup>. To broach this conundrum, it is important  
30 to focus on the symptoms and not just the clinical diagnosis, and be able to evaluate the  
31 symptom level across other comparable patient groups. RTT is associated with a high  
32 penetrance of ASD and at the neuronal level share many salient features<sup>52</sup>. Both RTT and  
33 ASD exhibit deficits in social behaviour and speech and in both cases individuals may share  
34 common stereotypical behaviours<sup>53</sup>. Due to these similarities and based on consultation with  
35 clinicians with expertise in ASD, as a comparator group, this stage of the study will also  
36 include parents/carers/partners of individuals aged 6 months to 40 years with ASD with  
37 significant intellectual disability. It will also involve clinicians who see patients with RTT  
38 and ASD who will test the clinician version of the questionnaire. Participants (parents/carers  
39 and clinicians) will be recruited to complete the respective versions of the Rett Evaluation of  
40 Symptoms and Treatments (REST) questionnaire as well as other standardised questionnaires  
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3 – namely the RSSS and the RSBQ (Table 1). The RSSS<sup>42,54</sup> and the RSBQ<sup>41,42,55</sup> have  
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5 previously been used in studies with RTT patients. Pertinent information will also be taken  
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7 from the RTT Natural History Study<sup>40</sup>. It is anticipated that 25 participants in the RTT cohort  
8  
9 and 25 in the ASD with significant intellectual disability cohort will complete the  
10  
11 questionnaire battery. Although there is significant symptom overlap in patients with RTT  
12  
13 and ASD, participants in the ASD cohort will be asked to complete only the relevant  
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15 questions in the questionnaire battery that would be applicable and relevant to them.  
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18 The questionnaire battery will be presented to study participants in HealthTracker<sup>TM</sup>, a multi-  
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20 modal web-based portal for remote online completion using developmentally appropriate  
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22 interfaces. Participants will be given a unique ID number and log-in information and will be  
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24 asked to complete the questionnaires independently. The research team will be able to  
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26 support participants with questionnaire completion should they need it. Where applicable,  
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28 participants will also be able to complete paper versions of the questionnaires if they request  
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30 them. Participant medical records will be accessed only by members of the study team to  
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32 validate the questionnaire against details of diagnoses obtained from patient case notes as  
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34 well as against the Development and Well-being Assessment (DAWBA)<sup>56</sup>, and  
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36 treatment/medication status if they are available in case notes. Patient records will also be  
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38 used to gain genetic information on the specific mutation and diagnosis. Consent will be  
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40 obtained to access medical notes.  
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45 All participants will be asked to complete the questionnaire battery, at baseline, again after 1  
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47 week and then between 4 and 6 months after first completion to assess questionnaire stability.  
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### 49 ***Stage 3: Wearable Sensor Technology***

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51 The use of wearable sensor technology to improve treatment outcomes has gathered  
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53 momentum in recent years<sup>57</sup> and is currently being used to develop new outcome measures in  
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55 patients with complex neurodisability such as Amyotrophic Lateral Sclerosis<sup>58</sup>. We have  
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3 shown that wearable sensor technology can assist in the management of EBAD in individuals  
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5 with rare and complex genetic disorders<sup>59</sup>, such as RTT<sup>34,35</sup>, to assist in improving treatment  
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7 outcomes. In the context of this study, the outcomes of the wearable sensor technology will  
8  
9 marry into the outcomes of the newly developed questionnaire (REST), with psychosocial  
10  
11 and genetic data to create the TRIAL database. The technology will be evaluated in  
12  
13 individuals with RTT, ASD and healthy controls.  
14

### 15 ***Sample size***

#### 16 *Justification for Sample Size*

17  
18 Owing to the small sample population of individuals with rare and complex genetic disorders,  
19  
20 formal modelling to obtain sample size estimates will not be readily applicable. In this view,  
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22 focus groups will be used to understand the nature of the problem and the acceptability of  
23  
24 various measures and their presentation to potential research participants. The qualitative  
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26 modification and new development of the questionnaire will be done by running focus groups  
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28 and the sample size was calculated based on the requirements needed for field testing.  
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#### 34 *Stage 1: Questionnaire Development Stage*

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36 It is anticipated that the total number of participants for the questionnaire development stage  
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38 of the study will be between 10-20 (including participants and clinicians).  
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#### 41 *Stage 2: Questionnaire Validation Stage*

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43 The total number of participants for the questionnaire validation stage of the study will be 50  
44  
45 participants (25 RTT cohort and 25 ASD cohort).  
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#### 48 *Stage 3: Wearable Sensor Technologies Stage*

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50 The total number of participants for the wearable technology stage of the study is expected to  
51  
52 be similar as those who participated in the validation phase of the study. This part of the  
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54 study will also include a matched healthy control group.  
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#### 56 *Stage 4: Longitudinal monitoring in RTT patients*

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3 Longitudinal data capture on a 3 monthly basis from 80 - 100 parents/carers of individuals  
4  
5 with RTT will be undertaken using the REST questionnaire over a 12-18 month period.  
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7 Ethics submission for stage 4 of the study will be done after stages 1-3 have been completed.  
8

### 9 10 ***Recruitment***

11 Information sheets (and age appropriate information sheets where relevant) will be provided  
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13 for all participants, in addition to consent forms. Information sheets will emphasise that  
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15 participant involvement in the research is voluntary and they have the right to withdraw from  
16  
17 the research at any time, without giving a reason. In addition, participants will be advised that  
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19 participating or withdrawing from the research will have no impact on their usual care that  
20  
21 they are currently receiving, or will receive in the future. A minimum of 24 hours will be  
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23 given between providing study information and recruitment of participants into the study.  
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### 27 ***Stage 1: Questionnaire Development Stage Recruitment***

28  
29 Parents/carers of individuals with RTT and clinicians who work with individuals with RTT  
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31 will be recruited. Due to the group-based nature of the focus groups, parents/carers or  
32  
33 clinicians who have not provided consent will not be able to partake in focus groups and will  
34  
35 be excluded. The focus groups will comprise of parents/carers of individuals with RTT and  
36  
37 clinicians working with patients with RTT. Depending on the nature of the focus groups  
38  
39 about 4-6 participants will take part in each focus group.  
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### 43 ***Questionnaire Development Stage - Inclusion Criteria***

- 44
- 45 • Parents/carers/partners/relatives of individuals aged 6 months to 40 years with  
46  
47 RTT.
  - 48  
49 • Clinicians who work within healthcare settings in South London and Maudsley  
50  
51 (SLaM) NHS Foundation Trust that see children and/or adults with RTT and  
52  
53 associated developmental conditions.
  - 54  
55 • Without any exclusion for concurrent stable medication.  
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3 *Questionnaire Development Stage - Exclusion Criteria*  
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- 5 • Parents/carers whom do not have a reasonable level of English. This is because a  
6 reasonable level of English will be required to engage in the focus groups.  
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10 ***Stage 2: Questionnaire Validation Stage Recruitment***

11 For this stage of the study, parents/carers of individuals with RTT and those with ASD will  
12 be recruited via clinician/researcher invite. Study participants will be under the care of a  
13 service within SLaM NHS Foundation Trust. Where relevant,  
14 parents/carers/partners/relatives of individuals with RTT and ASD will be asked to provide  
15 details of their clinician at the time of consent so that they can also be contacted by the  
16 research team and invited to take part.  
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25 *Questionnaire Validation Stage - Inclusion Criteria*  
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- 27 • Parents/carers of individuals aged 6 months to 40 years with RTT or ASD.  
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29

30 *Questionnaire Validation Stage - Exclusion Criteria*  
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- 32 • If parents/carers of individuals aged 6 months to 40 years with RTT or ASD are not  
33 able to (or expected to not be able to) complete questionnaires they will be excluded  
34 from the study.  
35  
36  
37  
38 • Parents/carers who do not have a reasonable level of English will be excluded from  
39 the validation stage of the study. This is because a reasonable level will be required to  
40 complete questionnaires which will only be available in English at the validation  
41 stage. A research assistant may assist the Parent/carer in completion.  
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48 ***Stage 3: Wearable Sensor Technologies Stage Recruitment***

49 Individuals aged between 5 to 40 years with RTT and ASD, and  
50 parents/carers/partners/relatives of individuals with RTT and ASD will be recruited via  
51 clinician/researcher invite. Information sheets (and age appropriate information sheets where  
52 relevant) will be provided for all participants, in addition to consent forms and where  
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3 applicable assent forms. Healthy controls will be recruited via clinician/researcher invite  
4  
5 using widely used and appropriate advertising channels.  
6

7 *Wearable Sensor Technologies Stage - Inclusion Criteria*  
8

9  
10 RTT

- 11 • Females aged 5 - 40 years with confirmed diagnosis of RTT (via  
12 clinician/researcher invite).  
13
- 14 • Parents/carers/partners/relatives of individuals aged 5 - 40 years with RTT.  
15  
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18 ASD

- 19 • Males and females aged 5 - 40 years with ASD (via clinician/researcher invite).  
20  
21
- 22 • Parents/carers/partners/relatives of individuals aged 5 - 40 years with ASD.  
23  
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27 Healthy Controls

- 28 • Males and females aged 5 - 40 years considered to be healthy for their age (via  
29 clinician/researcher invite).  
30  
31
- 32 • Are capable of understanding and complying with the requirements of the  
33 protocol.  
34  
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37

38 *Wearable Sensor Technologies Stage - Exclusion Criteria*  
39

40 RTT

- 41 • Individuals aged 5 - 40 years with RTT who are not able to (or expected to not be  
42 able to) wear the wearable sensor technology will be excluded from the study.  
43  
44
- 45 • Parents/carers/partners/relatives of individuals aged 5 - 40 years with RTT who do  
46 not have a reasonable level of English.  
47  
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51 ASD

- 52 • Individuals aged 5 - 40 years with ASD who are not able to (or expected to not be  
53 able to) wear the sensor technology will be excluded from the study.  
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- Parents/carers/partners/relatives of individuals aged 5 - 40 years with ASD who do not have a reasonable level of English.

#### Healthy Controls

- Individuals who are not able to (or expected to not be able to) wear the sensor technology will be excluded from the study.
- Individuals who do not have a reasonable level of English.

### **Analyses Plan**

#### ***Questionnaire Development***

Data obtained from the focus groups will be recorded securely and transcribed accurately, paying close attention to the identified themes and issues. The analysis will be performed as described previously<sup>44</sup>. In brief, the focus group data will be organised into clinically meaningful themes using thematic and content analysis. Following this, to manage the qualitative data generated from the focus groups, NVivo software will be used and the data analysis will be guided by the framework for thematic analysis<sup>60</sup>.

#### ***Questionnaire Validation***

The quantitative data will be analysed using the latest version of the SPSS statistical package (IBM SPSS Statistics for Windows. Armonk, NY: IBM Corp.).

#### ***Internal Consistency***

Internal consistency of the measures will be reported using Cronbach's alpha. Alpha coefficients  $\geq 0.85$  will be indicative of reasonable evidence of internal reliability<sup>61</sup>. Where applicable, 'alpha if deleted analyses' will be performed to see if omitting any item(s) from the (subthemes of the) questionnaire would strengthen the measure.

#### ***Test-retest reliability***

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2  
3 Intra-class correlation (ICC) will be used to assess test-retest reliability on subscale and total  
4 scores as described<sup>62</sup>. Given the exploratory nature of this study, weighted Cohen's kappa  
5 values will also be determined to assess test-retest reliability at the item level. The ICC will  
6 also be performed after 4 to 6 months after initial completion of the questionnaire to assess  
7 the long term stability of the new questionnaire.  
8  
9

### 10 11 12 13 *Validity*

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15  
16 Validity (discriminative power) of the new questionnaire will be assessed using Receiver  
17 Operating Characteristic (ROC) analyses as described in Santosh et al. (2016)<sup>44</sup>. As there are  
18 no gold standard questionnaires for patients with RTT, where applicable the ROC analyses  
19 will also be performed on the scores on the RTT Natural History Study<sup>40</sup>, the RSBQ<sup>41</sup> and the  
20 RSSS<sup>42</sup>. Where necessary and if data are available, ANOVA (general linear model) will be  
21 performed with grouping variable DAWBA diagnoses (coded in 1 for positive and coded 0  
22 for negative diagnosis) so that the differences in REST scoring can be assessed.  
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### 32 33 *Factor analysis*

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35 Studies involving small sample sizes have often been plagued by the inappropriate use of  
36 Exploratory Factor Analysis (EFA) or Principal Component Analysis (PCA) to identify  
37 clinically meaningful factor items<sup>63</sup>. Many recommendations have been put forward  
38 regarding sample sizes but there does not seem to be an overall consensus<sup>64</sup>. Some have  
39 suggested improbable sample sizes that would not be feasible for studies of rare and complex  
40 genetic diseases<sup>65</sup>. In these instances, methods to reveal the multi-dimensional aspects of  
41 factor structure are not as straightforward. Recently, the Regularized Exploratory Factor  
42 Analysis (REFA) was introduced<sup>66</sup> that is recommended over EFA and PCA, when samples  
43 sizes are less than 50. Despite this new approach, it is unclear whether the REFA would be  
44 applicable for a multidimensional questionnaire in a condition with many variables. In the  
45 context of the new questionnaire, the robustness of the REST will be evaluated using tools  
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3 applicable for smaller samples sizes<sup>66</sup> and those used in exploratory studies as described  
4  
5 recently<sup>67</sup>.  
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7

## 8 9 **Dissemination**

10  
11 The goal of this study is to develop and validate a new RTT questionnaire. The REST  
12  
13 questionnaire will reduce the burden and improve the overall healthcare delivery of  
14  
15 individuals with RTT and when coupled with the data from the wearable sensor technology  
16  
17 as well as psychosocial and genetic information will be used to construct the TRIAL  
18  
19 database. Using the functionality of the HealthTracker<sup>TM</sup> platform the TRIAL database will  
20  
21 provide all the necessary information to clinicians and researchers about different aspects of  
22  
23 the disease and serve as a barometer for improving treatment pathways in individuals. This  
24  
25 will allow algorithms to be developed alerting parent/carers to request unscheduled clinician  
26  
27 appointments when symptoms deviate significantly from one another thereby streamlining  
28  
29 the patient care pathway. Rare disorders such as RTT have a limited patient population and it  
30  
31 is therefore crucial for patients to be stratified using phenotype and biomarkers (such as those  
32  
33 obtained through wearable sensor monitoring). Adaptive clinical trial design using Bayesian  
34  
35 methodology has been suggested to augment the statistical power and decrease the number of  
36  
37 patients required for a rare disease trial<sup>51</sup>. In this view, the TRIAL database will serve for the  
38  
39 recruitment of patients into clinical trials as baseline information would already be available  
40  
41 so the clinical trial can be conducted with fewer patients and in a more cost-effective manner.  
42  
43 Results stemming from this study will be disclosed unreservedly and the findings published  
44  
45 in scientific journals and will also be presented in meetings and conferences for professionals,  
46  
47 patients as well as carers and families. Reverse Rett UK will lead on wider dissemination of  
48  
49 the applied research findings to engage policy-makers, key professional groups and service  
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51 managers, and parents/carers of children with RTT.  
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## Author Contributions

JS drafted, wrote the manuscript, and wrote the documentation required for ethical approval of the study. KL provided important intellectual review of the manuscript and reviewed the documentation required for ethical approval of the study. FF reviewed the statistical components and reviewed the manuscript. PS secured funding and conceived the study, and revised the manuscript critically for important intellectual content.

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## Conflicts of Interest

PS is the co-inventor of the HealthTracker™ and is a Director and shareholder in HealthTracker Ltd. FF is a Data Analyst and KL is a Project Manager employed by HealthTracker Ltd respectively.

JS is on the Professional Advisory Board for Reverse Rett UK and acts as a Scientific Advisor.

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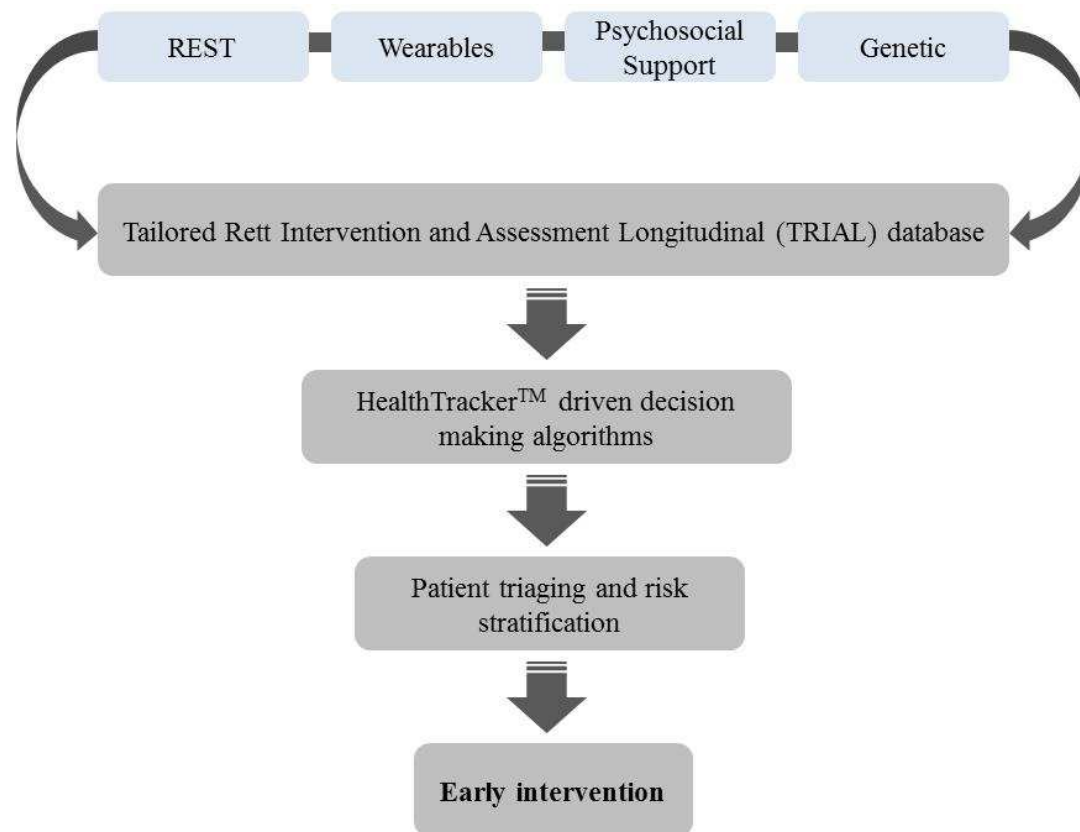
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Figure 1: Flow diagram illustrating the sequence of steps showing how data obtained from the TRIAL database can be used to provide timely intervention to streamline treatment outcomes in patients with RTT syndrome



Abbreviations: REST (Rett Evaluation of Symptoms and Treatments) questionnaire; RTT (Rett Syndrome); TRIAL (Tailored Rett Intervention and Assessment Longitudinal) database

Notes:

The term 'wearables' has been coined for wearable sensor technology

Genetic and psychosocial information will be captured as part of the REST questionnaire

Table 1: Measures to be administered during Stage 2 (Validation) and Stage 3 (Wearable Sensor Technology) of the Study

Measure	Key Information	Administered to:					
		Individual with RTT	Individual with ASD	Healthy Subjects	Parent/Carer of Child with RTT/*ASD	Parent/Carer/Partner of Adult with RTT/*ASD	Clinician/Researcher
Rett Natural History study <sup>40</sup>	542 individuals with classical RTT and 96 with atypical RTT provide information on clinical severity of RTT						X
Rett Syndrome Behavioural Questionnaire (RSBQ) <sup>41</sup>	Provides an accurate measure of the behavioural features of RTT				X	X	
Rett Syndrome Severity Score (RSSS) <sup>42</sup>	Provides information on the overall clinical severity and severity across individual parameters: <ul style="list-style-type: none"> <li>• frequency and manageability of seizures;</li> <li>• respiratory abnormalities</li> <li>• scoliosis;</li> <li>• ability to walk;</li> <li>• hand use;</li> <li>• speech;</li> <li>• sleep hygiene</li> </ul>				X	X	
Rett Evaluation of Symptoms and Treatments (REST) questionnaire	TBC				X	X	X
Wearable Sensor Technology	TBC	X	X	X			
Anticipated Administration Time (minutes)		30	30	30	~60	~60	~60

\* Participants in the ASD cohort will be asked to complete only the relevant questions in the questionnaire battery that would be applicable and relevant to them  
Abbreviations: ASD (Autism Spectrum Disorder); REST (Rett Evaluation of Symptoms and Treatments) questionnaire; RSBQ (Rett Syndrome Behavioural Questionnaire); RSSS (Rett Syndrome Severity Score); RTT (Rett Syndrome)

# BMJ Open

## Protocol for the Development and Validation of the Rett Evaluation of Symptoms and Treatments (REST) Questionnaire

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# Protocol for the Development and Validation of the Rett Evaluation of Symptoms and Treatments (REST) Questionnaire

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## Key Words

Biomarkers; HealthTracker<sup>TM</sup>; Questionnaire Development and Validation; Rett Syndrome;  
Tailored Rett Intervention and Assessment Longitudinal (TRIAL) database; Wearable Sensor  
Technology



## Abstract

**Introduction:** Rett Syndrome (RTT) is a pervasive neurodevelopmental disorder that presents with deficits in brain functioning leading to language and learning regression, characteristic hand stereotypies and developmental delay. Different mutations in the gene implicated in RTT - methyl-CpG-binding protein 2 (*MECP2*) establishes RTT as a disorder with divergent symptomology ranging from individuals with severe to milder phenotypes. A reliable and single multidimensional questionnaire is needed that can embrace all symptoms, and the relationships between them, and can map clinically meaningful data to symptomatology across the lifespan in RTT patients. This standalone questionnaire will be used for signposting areas for better practice. The questionnaire will also be able to marry with the physiological aspects of the disease obtained using wearable sensor technology as well as genetic and psychosocial data to stratify patients. Taken together, this will empower clinicians and researchers with the confidence to delineate between different aspects of disorder symptomatology in order to streamline care pathways for individuals or for those patients entering clinical trials. This protocol describes the anticipated development and validation of the Rett Evaluation of Symptoms and Treatments (REST) questionnaire, which when linked with the outcomes of the wearable sensor technology, will serve as a barometer for longitudinal patient monitoring in patients with RTT.

**Methods and Analysis:** The United States (US) Food and Drug Administration (FDA) Guidance for Patient-reported Outcome Measures (PROM) will be used as a template to inform the methodology of the study. It will follow an iterative framework that will include item/concept identification, item/concept elicitation in parent/carer mediated focus groups, expert clinician feedback, web based presentation of questionnaires, initial scale development, instrument refinement and instrument validation.

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**Ethics and dissemination:** The study has received favourable opinion from the NHS Research Ethics Committee (REC): NHS Research Ethics Committee (REC) – London, Bromley Research Ethics Committee (reference: 15/LO/1772).

For peer review only

## Strengths and Limitations of the Study:

Strengths of this Study are:

- It will follow the FDA framework for Patient Reported Outcome Measures.
- The new questionnaire will correlate behavioural data with the physiological aspects of the disease.
- It will gather feedback from parent-based charities such as Reverse Rett UK.
- The new questionnaire will capture clinically meaningful change of symptomatology in individuals with RTT across the lifespan.
- It will enable the streamlining of treatment and expedite triaging of care by signposting patients to correct specialists sooner to enable timely intervention.
- The use of HealthTracker™, a multi-modal eHealth web-based monitoring platform, will make the questionnaire as user friendly as possible and allows it to be tailored to individual participant.

Limitation of this Study:

- A limitation is that the questionnaire battery will be completed remotely so the extent to which participants feel comfortable using the Internet and computers may affect completion rates.
- Participation might be time consuming for families.

## Introduction

Rett Syndrome (RTT) can trace its genesis to a clinical waiting room in Vienna (1965)<sup>1</sup> where Dr. Rett first observed the clinical signs and symptoms of RTT. Symptoms usually appear between 6 to 18 months after birth<sup>2</sup> and clinically RTT presents with impairments in brain functioning leading to language and learning regression, hand stereotypies (hand washing/wringing) and developmental stagnation. RTT is predominantly found in young females with an incidence of about 1: 10,000 live births<sup>3</sup>. There are geographical variations<sup>4</sup> with one Australian study indicating a prevalence of about 1:9000<sup>5</sup>. The prevalence is probably underscored by the high clinical variability of the disease and hence the frequency could be underestimated. Loss of function in the methyl-CpG binding protein 2 (*MECP2*) gene is responsible for the disorder in the vast majority of cases<sup>6</sup>, with rarer cases being attributed to mutations in *CDKL5* and *FOXG1* gene<sup>7,8</sup> leading to atypical or variant RTT. More recently, mutations in other less known candidate genes have emerged such as those encoding ankyrin repeat proteins and neuronal acetylcholine receptor subunits<sup>9</sup>. MeCP2 is a highly conserved nuclear protein abundant in the mammalian brain<sup>10</sup> and notably the disorder is reversible in mice models of RTT<sup>11</sup>.

MeCP2 acts as a critical epigenetic modulator in the mammalian brain, controlling overlapping mechanisms such as DNA methylation and post-translational mechanisms<sup>12</sup>. Through differential post-translational modification at serine 164<sup>13</sup>, MeCP2 may help in limiting transcriptional noise<sup>14</sup> of other genes. For example, mutations in the gene *switch-insensitive 3 family member A (SIN3A)*, a *MECP2* interactor and transcriptional repressor – crucial for cortical integrity, causes intellectual disability and ASD<sup>15</sup> and the *MECP2*<sup>R306C</sup> mutation prevents MeCP2 from interacting with the NCoR/histone deacetylase 3 (HDAC3) complex resulting in impediments in social and cognitive functioning in animal models<sup>16</sup>. *MeCP2* has complex genome level modalities, and the general opinion is that loss of the

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3 transcriptional repressor function of *MECP2* impacts other genes crucial for post-natal  
4 neuronal development and has led others to suggest that this leads to a sub-optimal brain<sup>17</sup>.  
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6 This seems to be the significant driver for the classical RTT clinical phenotype. Genes in  
7 neuronal development tend to be long (100 kb or larger)<sup>18</sup> and as the transcriptional  
8 repression function of *MECP2* is biased towards longer genes<sup>19</sup>, it is likely that impairments  
9 of long genes associated with neuronal development dictates the functional and  
10 developmental versatility of the MeCP2 protein seen in RTT. This has a knock-on effect on  
11 the homeostasis of excitatory and inhibitory pathways<sup>20,21</sup> in RTT brains leading to the  
12 clinical versatility that is commonly observed.  
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22 *MECP2* being an X-linked gene, has an impact on the phenotype of RTT patients and the  
23 clinical severity. The X chromosome inactivation can cause uneven expression of wild type  
24 and mutant alleles resulting in skewed patterns of RTT phenotype severity<sup>22,23</sup>, and the degree  
25 of DNA-methylation-dependent long gene repression<sup>19</sup>. The range of functional ability in  
26 RTT patients is therefore broad and depending upon the type of genetic mutation ranges from  
27 patients with severe functional impairments to those with milder symptoms<sup>24</sup>; hence  
28 assessment and care pathways must be individually tailored to each affected person.  
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37 Although there have been considerable advances in understanding the genetics and into the  
38 genetic testing of RTT, the diagnosis of RTT is based on the 2010 revised consensus clinical  
39 criteria<sup>3</sup> (see Table 1 in Neul et al., 2010) and recommends that all individuals with RTT  
40 should be first be assessed according to the revised clinical criteria followed by a thorough  
41 genetic test for *MECP2*. Given that about 3-5% of RTT individuals who fulfil the diagnostic  
42 clinical criteria do not have *MECP2* mutations, and this is even higher for atypical RTT  
43 cases<sup>25</sup>, more recently clinical predictors that can facilitate a clinician's decision making to  
44 order genetic testing for RTT have been provided<sup>26</sup>. This showed that the likelihood of a  
45 having a positive *MECP2* test was greatest in patients with partial or complete attenuation of  
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3 hand skills. Impairments in gait and hand stereotypies were also strong predictors. Of interest  
4 was that loss of speech did not discriminate whether an individual was *MECP2+* or *MECP2-*.

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7 *Pre-existing measures in RTT*

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9 As far as we are aware, no complete instrument has been developed for individuals with RTT  
10 that has the ability to capture longitudinal pharmacological, behavioural, genetic and  
11 psychosocial information, as well as an ability to correlate this with the physiological aspects  
12 of the disease. Previous datasets/instruments have been inconsistent and provide limited  
13 information on the behavioural and physiological facets of the disease. Whilst, some might  
14 provide information on the genetic diagnoses of individuals with RTT, there is a lack of  
15 consistency. First, RettBase, collected mainly molecular genetic data from the Australian  
16 cohort of RTT patients<sup>27</sup>. Some other instruments have included both genetic and clinical  
17 data, although the clinical data was limited. InterRETT, an Australian Rett syndrome  
18 database, was based on data collection by distributing a questionnaire to families<sup>28</sup>. The  
19 Italian Rett Database and Biobank consisted of 357 patients and had 20 structured and seven  
20 descriptive clinical items along with 17 structured genetic items<sup>29</sup>. The British Isles Rett  
21 Syndrome Survey, included 275 British Rett patients and had 271 structured and 94  
22 descriptive clinical items, and six structured genetic items<sup>30</sup>. An American survey collected  
23 data on the natural history of the disease that allowed researchers and physicians to access  
24 comprehensive patient data on more than 1000 individuals with RTT<sup>31,32</sup>. These datasets were  
25 preserved and integrated into the Rett Networked Database<sup>30</sup> and offers an amalgamated data  
26 repository for researchers to access anonymized patient information. Elsewhere, the Japanese  
27 RTT database (JRDB) includes the clinical data from 102 females with a median age of 11  
28 years old<sup>33</sup>.

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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

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3 outcome measures. The Unified Parkinson Disease Rating Scale, (UPDRS) is a good example  
4  
5 of an outcome measure that is effective and can capture disease severity and clinically  
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7 meaningful change of symptoms of Parkinson's disease<sup>34</sup>. With rare diseases, the Sanfilippo  
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9 Behaviour Rating Scale (SBRS), a 68 item questionnaire developed using 44 families, is also  
10  
11 effective and can map the behavioural phenotype of children with Sanfilippo syndrome to  
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13 disease progression and/or results from treatment across the lifespan<sup>35</sup>. In RTT, the current  
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15 outcome measures are inadequate in their ability to capture disease severity across the  
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17 lifespan, although others have made significant headway in this area. The 37 item motor-  
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19 behavioral assessment (MBA) incorporates historical items with items from direct clinician  
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21 evaluations and has been used to describe clinical severity in RTT<sup>36,37</sup>, whilst the Rett  
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23 Syndrome Behavioral Questionnaire (RSBQ), a validated checklist, was designed to  
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25 differentiate individuals with RTT compared to those with severe intellectual disability<sup>38</sup>.  
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27 Other measures tested in RTT include the Anxiety Depression and Mood Scale (ADAMS)<sup>39</sup>,  
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29 the clinician based International Scoring System (ISS)<sup>40,41</sup> to evaluate the disease severity,  
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31 Vineland Adaptive Behaviour scale<sup>42</sup>, the 13 item Rett Clinical Severity Scale (RCSS)<sup>37,43</sup>  
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33 and its modified version<sup>42</sup>. Others have developed RTT specific anchors such as for the  
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35 Clinical Global Impression Severity (CGI-S) scale based on scores from the RCSS for  
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37 improved outcome measures in clinical trials<sup>44</sup>. Quality of Life (QOL) measures such as the  
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39 Child Health Questionnaire-P50 have also been used in RTT<sup>45</sup> including a recent Phase II  
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41 open label clinical trial using glatiramer acetate<sup>46</sup>. Some of these measures such as the MBA,  
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43 RSBQ, ADAMS and RCSS have been implemented into clinical trials to evaluate the effect  
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45 of Insulin-like Growth Factor (IGF-1)<sup>47</sup> or Sarizotan<sup>48</sup> in individuals with RTT, or to develop  
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47 a novel scoring tool (Rett Severity Score [RSS]) to assess the impact of IGF-1 treatment in  
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49 RTT<sup>41</sup>. Other scales such as the Mullen Scales for Early Learning used in other rare  
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51 disorders<sup>49</sup>, have also been adapted for use in RTT<sup>47</sup>. These measures are not without their  
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3 faults. Some have suggested that the MPA can be difficult to use with some items that  
4 describe disease regression having not been validated<sup>24</sup>. This is important given that in some  
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faults. Some have suggested that the MPA can be difficult to use with some items that describe disease regression having not been validated<sup>24</sup>. This is important given that in some RTT patients, disease regression has been described as being transient or often goes unrecognised<sup>50</sup>. Others such as the RSBQ although are suitable to measure some aspects of behaviour such as mood and anxiety<sup>51</sup> 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 RSBQ<sup>52</sup>. 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 RTT<sup>53</sup>.

#### *Autonomic Function in RTT*

Large cross-sectional studies investigating the genotype-phenotype relationships have revealed divergence in the phenotype seen in individuals with RTT<sup>54,55</sup>. These were the first studies of sufficient sample size that bestowed important information on the genotype and phenotype relationships in RTT, and have been elegantly summarised elsewhere<sup>24</sup>. Some mutations or variants dictate a more severe phenotype when it comes to motor abilities<sup>24,54,55</sup> and cardio-respiratory phenotypes<sup>24,56</sup>. Moreover, at present it is unknown whether autonomic dysfunction is governed by any specific mutation in RTT<sup>24,56</sup>. Assessing the autonomic dysfunction in individuals in RTT is therefore a pressing clinical concern.

Autonomic dysfunction is a pivotal factor that requires consideration when managing patients with rare disorders such as RTT. From our clinical experience when managing patients in the Centre for Interventional Paediatric Psychopharmacology and Rare Diseases (CIPPRD)<sup>57</sup>, autonomic dysfunction is often found in patients who do not respond to treatment and those with significant functional disability. Autonomic dysfunction co-occurs in the context of



1  
2  
3 emotional and behavioural dysregulation, and recently using wearable sensor technology, we  
4  
5 have shown that Emotional, Behavioural and Autonomic Dysregulation (EBAD) is a crucial  
6  
7 factor that needs to be considered when managing patients with RTT<sup>58,59</sup>. Although  
8  
9 autonomic dysfunction has been investigated in individuals with RTT<sup>60-63</sup>, the progression of  
10  
11 autonomic dysfunction and the developmental trajectory of EBAD has never been researched.  
12  
13 Moreover, the components of EBAD in a questionnaire that can map across other  
14  
15 symptomatology longitudinally in individuals with RTT has not previously been shown.

### 17 18 **Aim**

19  
20 The objective of this protocol is to develop and validate a comprehensive multi-system  
21  
22 questionnaire (Rett Evaluation of Symptoms and Treatments – REST) that can profile the  
23  
24 symptomatology of patients with RTT and is sensitive to change across the lifespan allowing  
25  
26 better understanding of patient needs. In parallel, information collected using wearable sensor  
27  
28 technology<sup>58,59</sup> will be linked to data obtained from the REST questionnaire, genetic data and  
29  
30 also information about available psychosocial support from the patient and their family, to  
31  
32 form a comprehensive Tailored Rett Intervention and Assessment Longitudinal (TRIAL)  
33  
34 database. The TRIAL database will streamline treatment approaches to expedite triaging of  
35  
36 care by signposting patients to correct specialists earlier than is currently happening (Figure  
37  
38 1). Specifically, the functionality of the multi-modal HealthTracker™ platform will be  
39  
40 exploited so that anonymised data from the TRIAL database can be used to develop a  
41  
42 parent/carer alert system to signal when it may be useful to request unscheduled clinician  
43  
44 appointments. Using this functionality, the TRIAL database will also be able to stratify  
45  
46 patients to inform adaptive clinical trial design, by allowing pre-existing datasets to be used  
47  
48 so that rare disease trials can be done in a more cost-effective manner.  
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### 52 53 **Methods and Analyses**

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3 The title of this questionnaire was based on the feedback of the focus groups involving  
4 parents and carers of children with RTT from the parent based charities such as Reverse Rett  
5 UK, and clinician feedback. It will incorporate elements from previous scales<sup>40,52</sup> and  
6 standardised RTT questionnaires – data from the Natural History Study<sup>3</sup>, Rett Syndrome  
7 Behaviour Questionnaire (RSBQ)<sup>38</sup> and the modified version of the Rett Syndrome Severity  
8 Scale (RSSS)<sup>42</sup>. It is anticipated that the questionnaire will not take more than 30 minutes to  
9 complete.  
10

11  
12 The United States (US) Food and Drug Administration (FDA) Guidance for Patient-reported  
13 Outcome Measures (PROM)<sup>64</sup> will be used as a template to guide the methodology in the  
14 study. It was described in Santosh *et al.* (2016)<sup>65</sup> and will follow an iterative framework that  
15 will involve item/concept identification, item/concept elicitation in parent/carer mediated  
16 focus groups, clinician feedback, web based presentation of questionnaires initial scale  
17 development, instrument refinement and instrument validation.  
18  
19

### 20 ***Stage 1: Qualitative Development of the Rett Evaluation of Symptoms and Treatment*** 21 ***(REST) questionnaire***

#### 22 *Concept identification*

23 For this initial phase, a systematic literature review will be conducted according to the  
24 “Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)<sup>66</sup> to  
25 identify signs and symptoms that are deemed to be problematic in RTT. A draft version will  
26 be reviewed by expert clinicians who have substantial experience in RTT and Autism  
27 Spectrum Disorder (ASD). Common themes will be identified and draft version of the  
28 questionnaire will be prepared based on their feedback.  
29

#### 30 *Concept elicitation*

31 This stage will involve parents/carers of individuals aged between 6 to 40 years with RTT. A  
32 series of focus groups anticipated to last about 1.5 hours will be conducted as part of the  
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3 concept elicitation stage. These focus groups will include parents/carers of individuals with  
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5 RTT from the parent based charities such as Reverse Rett, UK, and clinicians who see RTT  
6  
7 patients. The groups will follow a semi-structured format using open-ended questions to  
8  
9 allow participants to discuss their experiences and views. Some of the focus groups will be on  
10  
11 item generation whilst others may centre on reviewing draft versions of the questionnaire  
12  
13 identifying pertinent themes. Focus groups will be audio recorded and each group will  
14  
15 include approximately 4-6 parents of children with RTT. Up to two researchers may be  
16  
17 present for the focus groups, which will be led by a Consultant Child and Adolescent  
18  
19 Psychiatrist/Specialist. All participants will also be asked to complete a demographic  
20  
21 questionnaire.  
22  
23

#### 24 *Web based presentation of questionnaires on the HealthTracker™ platform*

25  
26 HealthTracker™, a web-based health monitoring platform<sup>67</sup>, has been successfully trialled in  
27  
28 multi-centric European Union FP7 studies<sup>68,69</sup> and also in a questionnaire development and  
29  
30 validation study<sup>70</sup>. Parents and carers will be shown how the REST questionnaire might  
31  
32 appear on the HealthTracker™ platform, how the response options to the questionnaire could  
33  
34 be presented and whether a choice of single or multiple choice questions would be  
35  
36 appropriate. The various views of the focus groups will be used to choose the most optimal  
37  
38 web-based visualization of the questionnaire.  
39  
40

#### 41 *Tool review*

42  
43 As far as the authors are aware, no questionnaire exists that not only is RTT focused but can  
44  
45 capture a broad range of problematic themes, in particular, the developmental trajectory of  
46  
47 EBAD. Nor do these existing questionnaires/scales attempt to marry this with the  
48  
49 physiological measurements from the wearable sensor technology. At this stage, a further  
50  
51 literature review will be conducted to identify any themes that may have been missed during  
52  
53 the focus groups and whether any further areas of RTT symptomatology that were not  
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3 highlighted in the focus groups needs to be addressed. In addition, parents/carers from  
4 Reverse Rett, UK, will be consulted and any feedback incorporated into the tool review stage.  
5  
6 Following the focus groups, study participants will be sent a copy of the draft version of the  
7 questionnaire (via email or post). Once this part has been completed, a draft operating beta  
8 version of the questionnaire will be finalised.  
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13 ***Stage 2: Validation of the Rett Evaluation of Symptoms and Treatments (REST)***  
14 ***questionnaire***  
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16  
17 This stage of the study will involve parents/carers of individuals aged between 6 to 40 years  
18 with RTT. Questionnaires to assess the longitudinal trajectory of symptomatology in rare  
19 diseases have proven to be difficult to validate<sup>71,72</sup>. To broach this conundrum, it is important  
20 to focus on the symptoms and not just the clinical diagnosis, and be able to evaluate the  
21 symptom level across other comparable patient groups. At the neuronal level, the brains of  
22 RTT and ASD patients share many core features<sup>73</sup>. Both RTT and ASD exhibit behaviours  
23 that might overlap i.e. there are deficits in social behaviour and speech and in both cases  
24 individuals may share common stereotypical behaviours<sup>74</sup>. Due to these similarities and based  
25 on consultation with clinicians with expertise in ASD, as a comparator group, this stage of  
26 the study will also include parents/carers/partners of individuals aged between 6 to 40 years  
27 with ASD with significant intellectual disability. It will also involve clinicians who see  
28 patients with RTT and ASD who will test the clinician version of the questionnaire.  
29  
30 Participants (parents/carers and clinicians) will be recruited to complete the respective  
31 versions of the Rett Evaluation of Symptoms and Treatments (REST) questionnaire as well as  
32 other standardised questionnaires – namely the RSSS and the RSBQ (Table 1). The  
33 RSSS<sup>37,42,43,75</sup> and the RSBQ<sup>38,51</sup> have previously been used in studies with RTT patients.  
34  
35 Pertinent information will also be taken from the RTT Natural History Study<sup>31,32</sup>. It is  
36 anticipated that 50 participants in the RTT cohort and 50 in the ASD with significant  
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3 intellectual disability cohort will complete the questionnaire battery. Although there is  
4 significant symptom overlap in patients with RTT and ASD, participants in the ASD cohort  
5 will be asked to complete only the relevant questions in the questionnaire battery that would  
6 be applicable and relevant to them.  
7  
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10  
11 The questionnaire battery will be presented to study participants in HealthTracker™, a multi-  
12 modal web-based portal for remote online completion using developmentally appropriate  
13 interfaces. Participants will be given a unique ID number and log-in information and will be  
14 asked to complete the questionnaires independently. The research team will be able to  
15 support participants with questionnaire completion should they need it. Where applicable,  
16 participants will also be able to complete paper versions of the questionnaires if they request  
17 them. Participant medical records will be accessed only by members of the study team to  
18 validate the questionnaire against details of diagnoses obtained from patient case notes as  
19 well as against the Development and Well-being Assessment (DAWBA)<sup>76</sup>, and  
20 treatment/medication status if they are available in case notes. Patient records will also be  
21 used to gain genetic information on the specific mutation and diagnosis. Consent will be  
22 obtained to access medical notes.  
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37 All participants will be asked to complete the questionnaire battery, at baseline, again after 1  
38 week and then between 4 and 6 months after first completion to assess questionnaire stability.  
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### 41 ***Stage 3: Wearable Sensor Technology***

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43 The use of wearable sensor technology to improve treatment outcomes has gathered  
44 momentum in recent years<sup>77</sup> and is currently being used to develop new outcome measures in  
45 patients with complex neurodisability such as Amyotrophic Lateral Sclerosis<sup>78</sup>. Using  
46 wearable sensor technology as a PROM is not without its challenges. In RTT, wearable  
47 technology has been used to explore respiratory and cardiac function in observational  
48 studies<sup>79,80</sup> and in two recent clinical trials<sup>46,47</sup>, however, inherently captured biometric data  
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3 can be noisy especially from quasi-periodic oscillations from cardiac rhythms. Wrist worn  
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5 devices might be particularly susceptible to this type of noise. To mitigate these issues, we  
6  
7 have applied the methods described previously<sup>81,82</sup> to analyse heart rate variability and  
8  
9 electrodermal activity as metrics when evaluating wrist sensor biometric data and autonomic  
10  
11 function in a 15 year old girl with RTT. We were able to demonstrate a recalibration of the  
12  
13 autonomic equilibrium from pre-treatment to post-treatment using buspirone (30 mg/day)<sup>58</sup>,  
14  
15 and subsequent improvement in EBAD in this girl. Quasi-periodic oscillations cannot be  
16  
17 easily quantified using conventional methods. To manage this phase-rectified signal  
18  
19 averaging (PRSA)<sup>83</sup> may be used in conjunction with spectral factorization and applied to the  
20  
21 beat-to-beat RR interval data, which is particularly prone to extraneous noise. This  
22  
23 methodology coupled with EDA assessment will provide more sensitive methods to capture  
24  
25 changes in autonomic physiology in patients with RTT. In the context of this study, the  
26  
27 outcomes of the wearable sensor technology will marry into the outcomes of the newly  
28  
29 developed questionnaire (REST), with psychosocial and genetic data to create the TRIAL  
30  
31 database. The technology will be evaluated in individuals with RTT, ASD and healthy  
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33 controls.  
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### 36 ***Sample size***

#### 37 *Justification for Sample Size*

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40 Owing to the small sample population of individuals with rare and complex genetic disorders,  
41  
42 formal modelling to obtain sample size estimates will not be readily applicable.  
43  
44

#### 45 *Stage 1: Questionnaire Development Stage*

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47  
48 It is anticipated that the total number of participants for the questionnaire development stage  
49  
50 of the study will be between 10-20 (including participants and clinicians). In our experience,  
51  
52 focus groups involving families with children with rare diseases are well versed with the  
53  
54 problems associated with the condition in question. Often, the themes that need to be  
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3 addressed get saturated after a couple of focus groups, leading to us getting the basic structure  
4  
5 of the items needed to be tested in Stage 2.

#### 6 7 *Stage 2: Questionnaire Validation Stage*

8  
9 The total number of participants for the questionnaire validation stage of the study will be  
10  
11 150 participants (n=100 RTT cohort and n=50 ASD cohort). The number in the ASD cohort  
12  
13 will be split so that 25 parents/carers will either have a male or female diagnosed with ASD.  
14

#### 15 16 *Stage 3: Wearable Sensor Technologies Stage*

17  
18 The total number of participants for the wearable technology stage of the study is expected to  
19  
20 be 100 participants (n=50 RTT cohort and n=50 [25 male and 25 female] ASD cohort). This  
21  
22 part of the study will also include a matched healthy control group.  
23

#### 24 25 *Stage 4: Longitudinal monitoring in RTT patients*

26  
27 Longitudinal data capture on a 3 monthly basis from 80 - 100 parents/carers of individuals  
28  
29 with RTT will be undertaken using the REST questionnaire over a 12-18 month period.  
30  
31 Ethics submission for stage 4 of the study will be done after stages 1-3 have been completed.  
32

#### 33 34 ***Recruitment***

35  
36 Information sheets (and age appropriate information sheets where relevant) will be provided  
37  
38 for all participants, in addition to consent forms. Information sheets will emphasise that  
39  
40 participant involvement in the research is voluntary and they have the right to withdraw from  
41  
42 the research at any time, without giving a reason. In addition, participants will be advised that  
43  
44 participating or withdrawing from the research will have no impact on their usual care that  
45  
46 they are currently receiving, or will receive in the future. A minimum of 24 hours will be  
47  
48 given between providing study information and recruitment of participants into the study.  
49

#### 50 51 ***Stage 1: Questionnaire Development Stage Recruitment***

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53 Parents/carers of individuals with RTT and clinicians who work with individuals with RTT  
54  
55 will be recruited. Due to the group-based nature of the focus groups, parents/carers or  
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57

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2  
3 clinicians who have not provided consent will not be able to partake in focus groups and will  
4  
5 be excluded. The focus groups will comprise of parents/carers of individuals with RTT and  
6  
7 clinicians working with patients with RTT. Depending on the nature of the focus groups  
8  
9 about 4-6 participants will take part in each focus group.  
10

#### 11 *Questionnaire Development Stage - Inclusion Criteria*

- 12
- 13
- 14 • Parents/carers/partners/relatives of individuals aged between 6 to 40 years with RTT.
- 15
- 16 • Clinicians who work within healthcare settings in South London and Maudsley
- 17 (SLaM) NHS Foundation Trust that see children and/or adults with RTT and
- 18 associated developmental conditions.
- 19
- 20
- 21
- 22 • Without any exclusion for concurrent stable medication.
- 23

#### 24 *Questionnaire Development Stage - Exclusion Criteria*

- 25
- 26
- 27 • Parents/carers whom do not have a reasonable level of English. This is because a
- 28 reasonable level of English will be required to engage in the focus groups.
- 29
- 30

#### 31 ***Stage 2: Questionnaire Validation Stage Recruitment***

32  
33 For this stage of the study, parents/carers of individuals with RTT and those with ASD will  
34  
35 be recruited via clinician/researcher invite. Study participants will be under the care of a  
36  
37 service within SLaM NHS Foundation Trust. Where relevant,  
38  
39 parents/carers/partners/relatives of individuals with RTT and ASD will be asked to provide  
40  
41 details of their clinician at the time of consent so that they can also be contacted by the  
42  
43 research team and invited to take part.  
44

#### 45 *Questionnaire Validation Stage - Inclusion Criteria*

- 46
- 47
- 48 • Parents/carers of individuals aged between 6 to 40 years with RTT or ASD.
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### *Questionnaire Validation Stage - Exclusion Criteria*

- If parents/carers of individuals aged between 6 to 40 years with RTT or ASD are not able to (or expected to not be able to) complete questionnaires they will be excluded from the study.
- Parents/carers who do not have a reasonable level of English will be excluded from the validation stage of the study. This is because a reasonable level will be required to complete questionnaires which will only be available in English at the validation stage. A research assistant may assist the Parent/carer in completion.

### ***Stage 3: Wearable Sensor Technologies Stage Recruitment***

Individuals aged between 6 to 40 years with RTT and ASD, and parents/carers/partners/relatives of individuals with RTT and ASD will be recruited via clinician/researcher invite. Information sheets (and age appropriate information sheets where relevant) will be provided for all participants, in addition to consent forms and where applicable assent forms. Healthy controls will be recruited via clinician/researcher invite using widely used and appropriate advertising channels.

### *Wearable Sensor Technologies Stage - Inclusion Criteria*

#### RTT

- Females aged 6 - 40 years with confirmed diagnosis of RTT (via clinician/researcher invite).
- Parents/carers/partners/relatives of individuals aged 6 - 40 years with RTT.

#### ASD

- Males and females aged 6 - 40 years with ASD (via clinician/researcher invite).
- Parents/carers/partners/relatives of individuals aged 6 - 40 years with ASD.

#### Healthy Controls

- Males and females aged 6 - 40 years considered to be healthy for their age (via clinician/researcher invite).
- Are capable of understanding and complying with the requirements of the protocol.

#### *Wearable Sensor Technologies Stage - Exclusion Criteria*

##### RTT

- Individuals aged 6 - 40 years with RTT who are not able to (or expected to not be able to) wear the wearable sensor technology will be excluded from the study.
- Parents/carers/partners/relatives of individuals aged 6 - 40 years with RTT who do not have a reasonable level of English.

##### ASD

- Individuals (aged 6 - 40 years with ASD who are not able to (or expected to not be able to) wear the sensor technology will be excluded from the study.
- Parents/carers/partners/relatives of individuals aged 6 - 40 years with ASD who do not have a reasonable level of English.

##### Healthy Controls

- Individuals who are not able to (or expected to not be able to) wear the sensor technology will be excluded from the study.
- Individuals who do not have a reasonable level of English.

#### **Analyses Plan**

##### ***Questionnaire Development***

Data obtained from the focus groups will be recorded securely and transcribed accurately, paying close attention to the identified themes and issues. The analysis will be performed as described previously<sup>65</sup>. In brief, the focus group data will be organised into clinically

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2  
3 meaningful themes using thematic and content analysis. Following this, to manage the  
4 qualitative data generated from the focus groups, NVivo software will be used and the data  
5 analysis will be guided by the framework for thematic analysis<sup>84</sup>.  
6  
7

### 8 9 ***Questionnaire Validation***

10  
11 The quantitative data will be analysed using the latest version of the SPSS statistical package  
12 (IBM SPSS Statistics for Windows. Armonk, NY: IBM Corp.).  
13

#### 14 15 *Internal Consistency*

16  
17 Internal consistency of the measures will be reported using Cronbach's alpha. Alpha  
18 coefficients  $\geq 0.85$  will be indicative of reasonable evidence of internal reliability<sup>85</sup>. Where  
19 applicable, 'alpha if deleted analyses' will be performed to see if omitting any item(s) from  
20 the (subthemes of the) questionnaire would strengthen the measure.  
21  
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#### 26 27 *Test-retest reliability*

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29 Intra-class correlation (ICC) will be used to assess test-retest reliability on subscale and total  
30 scores as described<sup>86</sup>. Given the exploratory nature of this study, weighted Cohen's kappa  
31 values will also be determined to assess test-retest reliability at the item level. The ICC will  
32 also be performed after 4 to 6 months after initial completion of the questionnaire to assess  
33 the long term stability of the new questionnaire.  
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#### 39 40 *Validity*

41  
42 Validity (discriminative power) of the new questionnaire will be assessed using Receiver  
43 Operating Characteristic (ROC) analyses as described in Santosh et al. (2016)<sup>65</sup>. As there are  
44 no gold standard questionnaires for patients with RTT, where applicable the ROC analyses  
45 will also be performed on the scores on the RTT Natural History Study, the RSBQ and the  
46 RSSS. Where necessary and if data are available, ANOVA (general linear model) will be  
47 performed with grouping variable DAWBA diagnoses (coded in 1 for positive and coded 0  
48 for negative diagnosis) so that the differences in REST scoring can be assessed.  
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### *Factor analysis*

Studies involving small sample sizes have often been plagued by the inappropriate use of Exploratory Factor Analysis (EFA) or Principal Component Analysis (PCA) to identify clinically meaningful factor items<sup>87</sup>. Many recommendations have been put forward regarding sample sizes but there does not seem to be an overall consensus<sup>88</sup>. Some have suggested improbable sample sizes that would not be feasible for studies of rare and complex genetic diseases<sup>89</sup>. In these instances, methods to reveal the multi-dimensional aspects of factor structure are not as straightforward. Recently, the Regularized Exploratory Factor Analysis (REFA) was introduced<sup>90</sup> that is recommended over EFA and PCA, when samples sizes are less than 50. Despite this new approach, it is unclear whether the REFA would be applicable for a multidimensional questionnaire in a condition with many variables. In the context of the new questionnaire, the robustness of the REST will be evaluated using tools applicable for smaller samples sizes<sup>90</sup> and those used in exploratory studies as described recently<sup>91</sup>.

### ***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.

### **Dissemination**

The goal of this study is to develop and validate a new RTT questionnaire. The REST questionnaire will reduce the burden and improve the overall healthcare delivery of individuals with RTT and when coupled with the data from the wearable sensor technology as well as psychosocial and genetic information will be used to construct the TRIAL database. Using the functionality of the HealthTracker<sup>TM</sup> platform the TRIAL database will provide all the necessary information to clinicians and researchers about different aspects of

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2  
3 the disease and serve as a barometer for improving treatment pathways in individuals. This  
4 will allow algorithms to be developed alerting parent/carers to request unscheduled clinician  
5 appointments when symptoms deviate significantly from one another thereby streamlining  
6 the patient care pathway. Rare disorders such as RTT have a limited patient population and it  
7 is therefore crucial for patients to be stratified using phenotype and biomarkers (such as those  
8 obtained through wearable sensor monitoring). Adaptive clinical trial design using Bayesian  
9 methodology has been suggested to augment the statistical power and decrease the number of  
10 patients required for a rare disease trial<sup>72</sup>. In this view, the TRIAL database will serve for the  
11 recruitment of patients into clinical trials as baseline information would already be available  
12 so the clinical trial can be conducted with fewer patients and in a more cost-effective manner.  
13 Results stemming from this study will be disclosed unreservedly and the findings published  
14 in scientific journals and will also be presented in meetings and conferences for professionals,  
15 patients as well as carers and families. Reverse Rett UK will lead on wider dissemination of  
16 the applied research findings to engage policy-makers, key professional groups and service  
17 managers, and parents/carers of children with RTT.

### 35 **Funding**

36 We thank Reverse Rett UK for funding this research study (Ref No: PCCTABR)

### 41 **Author Contributions**

42 JS drafted, wrote and revised the manuscript, and wrote the documentation required for  
43 ethical approval of the study. KL provided important intellectual review of the manuscript  
44 and reviewed the documentation required for ethical approval of the study. FF reviewed the  
45 statistical components and reviewed the manuscript. PS secured funding and conceived the  
46 study, and revised the manuscript critically for important intellectual content.

### **Acknowledgements**

We are indebted to Reverse Rett UK for their helpful comments and suggestions on the study design.

### **Conflicts of Interest**

PS is the co-inventor of the HealthTracker™ and is a Director and shareholder in HealthTracker Ltd. FF is a Data Analyst and KL is a Project Manager employed by HealthTracker Ltd respectively.

JS is on the Professional Advisory Board for Reverse Rett UK and acts as a Scientific Advisor.

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3 Figure 1: Flow diagram illustrating the sequence of steps showing how data obtained from  
4 the TRIAL database can be used to provide timely intervention to streamline treatment  
5 outcomes in patients with RTT syndrome  
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10 **INSERT FIGURE 1 HERE**  
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13 Abbreviations: REST (Rett Evaluation of Symptoms and Treatments) questionnaire; RTT (Rett Syndrome);  
14 TRIAL (Tailored Rett Intervention and Assessment Longitudinal) database  
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16 Notes:

17 The term 'wearables' has been coined for wearable sensor technology

18 Genetic and psychosocial information will be captured as part of the REST questionnaire  
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Table 1: Measures to be administered during Stage 2 (Validation) and Stage 3 (Wearable Sensor Technology) of the Study

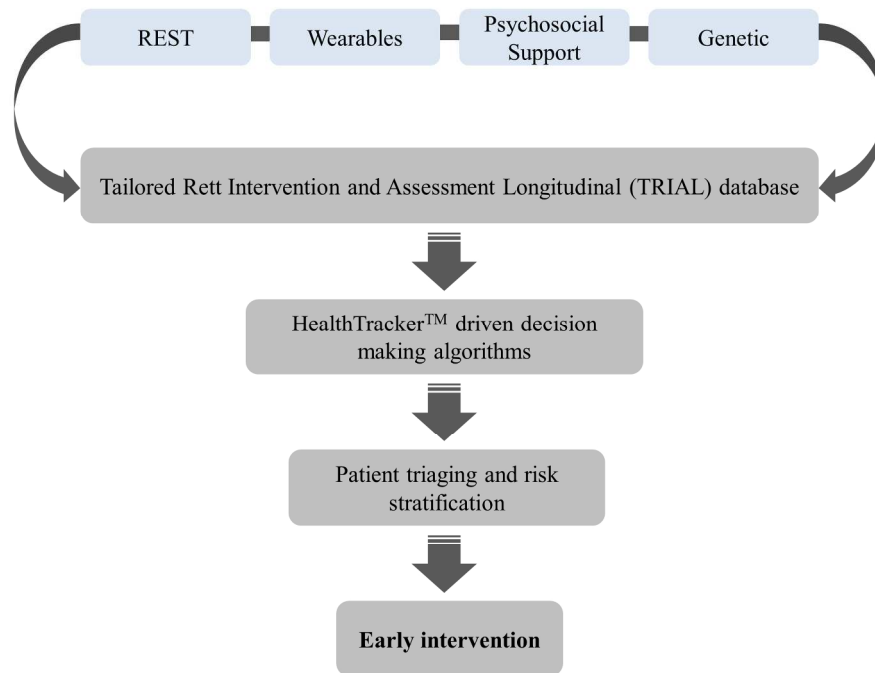
Measure	Key Information	Administered to:					
		Individual with RTT	Individual with ASD	Healthy Subjects	Parent/Carer of Child with RTT/*ASD	Parent/Carer/Partner of Adult with RTT/*ASD	Clinician/Researcher
Rett Natural History study <sup>32</sup>	More than 1000 participants with RTT providing information on important aspects of disorder symptomatology						X
Rett Syndrome Behavioural Questionnaire (RSBQ) <sup>38</sup>	Provides an accurate measure of the behavioural features of RTT				X	X	
Rett Syndrome Severity Score (RSSS) <sup>42</sup>	Provides information on the overall clinical severity and severity across individual parameters: <ul style="list-style-type: none"> <li>• frequency and manageability of seizures;</li> <li>• respiratory abnormalities</li> <li>• scoliosis;</li> <li>• ability to walk;</li> <li>• hand use;</li> <li>• speech;</li> <li>• sleep hygiene</li> </ul>						X
Rett Evaluation of Symptoms and Treatments (REST) questionnaire	A multidimensional questionnaire that can capture clinically meaningful data across the lifespan in individuals with RTT and improve treatment pathways				X	X	X
Wearable Sensor Technology	Captures real-time biometric physiological data (heart rate variability, skin conductance, blood volume pressure, perspiration and temperature)	X	X	X			
Anticipated		30	30	30	~60	~60	~60

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Administration Time (minutes)							
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\* Participants in the ASD cohort will be asked to complete only the relevant questions in the questionnaire battery that would be applicable and relevant to them  
 Abbreviations: ASD (Autism Spectrum Disorder); REST (Rett Evaluation of Symptoms and Treatments) questionnaire; RSBQ (Rett Syndrome Behavioural Questionnaire); RSSS (Rett Syndrome Severity Score); RTT (Rett Syndrome)

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Figure 1: Flow diagram illustrating the sequence of steps showing how data obtained from the TRIAL database can be used to provide timely intervention to streamline treatment outcomes in patients with RTT syndrome

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Abbreviations: REST (Rett Evaluation of Symptoms and Treatments) questionnaire; RTT (Rett Syndrome); TRIAL (Tailored Rett Intervention and Assessment Longitudinal) database

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Notes:

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The term 'wearables' has been coined for wearable sensor technology  
Genetic and psychosocial information will be captured as part of the REST questionnaire

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# BMJ Open

## Development of the Tailored Rett Intervention and Assessment Longitudinal (TRIAL) database and the Rett Evaluation of Symptoms and Treatments (REST) Questionnaire

Journal:	<i>BMJ Open</i>
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<b>Primary Subject Heading</b>:	Mental health
Secondary Subject Heading:	Paediatrics, Patient-centred medicine
Keywords:	Child & adolescent psychiatry < PSYCHIATRY, Protocols & guidelines < HEALTH SERVICES ADMINISTRATION & MANAGEMENT, MENTAL HEALTH

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Manuscripts

# Development of the Tailored Rett Intervention and Assessment Longitudinal (TRIAL) database and the Rett Evaluation of Symptoms and Treatments (REST) Questionnaire

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## Key Words

Biomarkers; HealthTracker<sup>TM</sup>; Questionnaire Development and Validation; Rett Syndrome; Tailored Rett Intervention and Assessment Longitudinal (TRIAL) database; Wearable Sensor Technology

## Abstract

**Introduction:** Rett Syndrome (RTT) is a pervasive neurodevelopmental disorder that presents with deficits in brain functioning leading to language and learning regression, characteristic hand stereotypies and developmental delay. Different mutations in the gene implicated in RTT - methyl-CpG-binding protein 2 (*MECP2*) establishes RTT as a disorder with divergent symptomology ranging from individuals with severe to milder phenotypes. A reliable and single multidimensional questionnaire is needed that can embrace all symptoms, and the relationships between them, and can map clinically meaningful data to symptomatology across the lifespan in RTT patients. As part of the HealthTracker™-based Tailored Rett Intervention and Assessment Longitudinal (TRIAL) database, the Rett Evaluation of Symptoms and Treatments (REST) Questionnaire will be able to marry with the physiological aspects of the disease obtained using wearable sensor technology, along with genetic and psychosocial data to stratify patients. Taken together, the web-based TRIAL database will empower clinicians and researchers with the confidence to delineate between different aspects of disorder symptomatology to streamline care pathways for individuals or for those patients entering clinical trials. This protocol describes the anticipated development of the REST questionnaire and the TRIAL database which links with the outcomes of the wearable sensor technology, and will serve as a barometer for longitudinal patient monitoring in patients with RTT.

**Methods and Analysis:** The US FDA Guidance for Patient-reported Outcome Measures (PROM) will be used as a template to inform the methodology of the study. It will follow an iterative framework that will include item/concept identification, item/concept elicitation in parent/carer mediated focus groups, expert clinician feedback, web based presentation of questionnaires, initial scale development, instrument refinement and instrument validation.

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**Ethics and dissemination:** The study has received favourable opinion from the NHS Research Ethics Committee (REC): NHS Research Ethics Committee (REC) – London, Bromley Research Ethics Committee (reference: 15/LO/1772).

For peer review only



## Strengths and Limitations of the Study:

Strengths of this Study are:

- The development of the Rett Evaluation of Symptoms and Treatments (REST) questionnaire will follow the FDA framework for Patient Reported Outcome Measures.
- The new questionnaire will capture clinically meaningful change of symptomatology in individuals with Rett Syndrome (RTT) across the lifespan.
- The HealthTracker™-based TRIAL database will link the behavioural data with the physiological aspects of the disease.
- It will gather feedback from parent-based charities such as Reverse Rett UK.
- The TRIAL database will enable the streamlining of treatment and expedite triaging of care by signposting patients to correct specialists sooner to enable timely intervention.
- The use of HealthTracker™, a multi-modal eHealth web-based monitoring platform, will make the TRIAL database as user friendly as possible and allows it to be tailored to the individual participant.
- The TRIAL database has the potential to be used globally, allowing for quicker development of decision-support analytics and personalized care.

Limitation of this Study:

- The questionnaire battery will be completed remotely so the extent to which participants feel comfortable using the Internet and computers may affect completion rates.
- Participation might be time consuming for families.

## Introduction

Rett Syndrome (RTT) can trace its genesis to a clinical waiting room in Vienna (1965)<sup>1</sup> where Dr. Rett first observed the clinical signs and symptoms of RTT. Symptoms usually appear between 6 to 18 months after birth<sup>2</sup> and clinically RTT presents with impairments in brain functioning leading to language and learning regression, hand stereotypies (hand washing/wringing) and developmental stagnation. RTT is predominantly found in young females with an incidence of about 1: 10,000 live births<sup>3</sup>. There are geographical variations<sup>4</sup> with one Australian study indicating a prevalence of about 1:9000<sup>5</sup>. The prevalence is probably underscored by the high clinical variability of the disease and hence the frequency could be underestimated. Loss of function in the methyl-CpG binding protein 2 (*MECP2*) gene is responsible for the disorder in the vast majority of cases<sup>6</sup>, with rarer cases being attributed to mutations in *CDKL5* and *FOXG1* gene<sup>7,8</sup> leading to atypical or variant RTT. More recently, mutations in other less known candidate genes have emerged such as those encoding ankyrin repeat proteins and neuronal acetylcholine receptor subunits<sup>9</sup>. MeCP2 is a highly conserved nuclear protein abundant in the mammalian brain<sup>10</sup> and notably the disorder is reversible in mice models of RTT<sup>11</sup>.

MeCP2 acts as a critical epigenetic modulator in the mammalian brain, controlling overlapping mechanisms such as DNA methylation and post-translational mechanisms<sup>12</sup>. Through differential post-translational modification at serine 164<sup>13</sup>, MeCP2 may help in limiting transcriptional noise<sup>14</sup> of other genes. For example, mutations in the gene *switch-insensitive 3 family member A* (*SIN3A*), a *MECP2* interactor and transcriptional repressor – crucial for cortical integrity, causes intellectual disability and ASD<sup>15</sup> and the *MECP2*<sup>R306C</sup> mutation prevents MeCP2 from interacting with the NCoR/histone deacetylase 3 (HDAC3) complex resulting in impediments in social and cognitive functioning in animal models<sup>16</sup>. *MeCP2* has complex genome level modalities, and the general opinion is that loss of the

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3 transcriptional repressor function of *MECP2* impacts other genes crucial for post-natal  
4 neuronal development and has led others to suggest that this leads to a sub-optimal brain<sup>17</sup>.  
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6 This seems to be the significant driver for the classical RTT clinical phenotype. Genes in  
7 neuronal development tend to be long (100 kb or larger)<sup>18</sup> and as the transcriptional  
8 repression function of *MECP2* is biased towards longer genes<sup>19</sup>, it is likely that impairments  
9 of long genes associated with neuronal development dictates the functional and  
10 developmental versatility of the MeCP2 protein seen in RTT. This has a knock-on effect on  
11 the homeostasis of excitatory and inhibitory pathways<sup>20,21</sup> in RTT brains leading to the  
12 clinical versatility that is commonly observed.

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22 *MECP2* being an X-linked gene, has an impact on the phenotype of RTT patients and the  
23 clinical severity. The X chromosome inactivation can cause uneven expression of wild type  
24 and mutant alleles resulting in skewed patterns of RTT phenotype severity<sup>22,23</sup>, and the degree  
25 of DNA-methylation-dependent long gene repression<sup>19</sup>. The range of functional ability in  
26 RTT patients is therefore broad and depending upon the type of genetic mutation ranges from  
27 patients with severe functional impairments to those with milder symptoms<sup>24</sup>; hence  
28 assessment and care pathways must be individually tailored to each affected person.

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37 Although there have been considerable advances in understanding the genetics and into the  
38 genetic testing of RTT, the diagnosis of RTT is based on the 2010 revised consensus clinical  
39 criteria<sup>3</sup> (see Table 1 in Neul *et al.*, 2010) and recommends that all individuals with RTT  
40 should be first be assessed according to the revised clinical criteria followed by a thorough  
41 genetic test for *MECP2*. Given that about 3-5% of RTT individuals who fulfil the diagnostic  
42 clinical criteria do not have *MECP2* mutations, and this is even higher for atypical RTT  
43 cases<sup>25</sup>, more recently clinical predictors that can facilitate a clinician's decision making to  
44 order genetic testing for RTT have been provided<sup>26</sup>. This showed that the likelihood of a  
45 having a positive *MECP2* test was greatest in patients with partial or complete attenuation of  
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3 hand skills. Impairments in gait and hand stereotypies were also strong predictors. Of interest  
4 was that loss of speech did not discriminate whether an individual was *MECP2+* or *MECP2-*.

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7 *Pre-existing measures in RTT*

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9 As far as we are aware, no complete instrument has been developed for individuals with RTT  
10 that can capture longitudinal pharmacological, behavioural, genetic and psychosocial  
11 information, as well as an ability to correlate this with the physiological aspects of the  
12 disease. Previous datasets/instruments have been inconsistent and provide limited information  
13 on the behavioural and physiological facets of the disease. Whilst, some might provide  
14 information on the genetic diagnoses of individuals with RTT, there is a lack of consistency.  
15 First, RettBase, collected mainly molecular genetic data from the Australian cohort of RTT  
16 patients<sup>27</sup>. Some other instruments have included both genetic and clinical data, although the  
17 clinical data was limited. InterRETT, an Australian Rett syndrome database, was based on  
18 data collection by distributing a questionnaire to families<sup>28</sup>. The Italian Rett Database and  
19 Biobank consisted of 357 patients and had 20 structured and seven descriptive clinical items  
20 along with 17 structured genetic items<sup>29</sup>. The British Isles Rett Syndrome Survey, included  
21 275 British Rett patients and had 271 structured and 94 descriptive clinical items, and six  
22 structured genetic items<sup>30</sup>. An American survey collected data on the natural history of the  
23 disease that allowed researchers and physicians to access comprehensive patient data on more  
24 than 1000 individuals with RTT<sup>31,32</sup>. These datasets were preserved and integrated into the  
25 Rett Networked Database<sup>30</sup> and offers an amalgamated data repository for researchers to  
26 access anonymized patient information. Elsewhere, the Japanese RTT database (JRDB)  
27 includes the clinical data from 102 females with a median age of 11 years old<sup>33</sup>.

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29 Capture of disease severity and sensitivity to change throughout the lifespan in patients are  
30 important elements that need to be considered when developing clinically meaningful  
31 outcome measures. The Unified Parkinson Disease Rating Scale, (UPDRS) is a good example  
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3 of an outcome measure that is effective and can capture disease severity and clinically  
4 meaningful change of symptoms of Parkinson's disease<sup>34</sup>. With rare diseases, the Sanfilippo  
5 Behaviour Rating Scale (SBRS), a 68-item questionnaire developed using 44 families, is also  
6 effective and can map the behavioural phenotype of children with Sanfilippo syndrome to  
7 disease progression and/or results from treatment across the lifespan<sup>35</sup>. In RTT, the current  
8 outcome measures are inadequate in their ability to capture disease severity across the  
9 lifespan, although others have made significant headway in this area. The 37-item motor-  
10 behavioural assessment (MBA) incorporates historical items with items from direct clinician  
11 evaluations and has been used to describe clinical severity in RTT<sup>36,37</sup>, whilst the Rett  
12 Syndrome Behavioral Questionnaire (RSBQ), a validated checklist, was designed to  
13 differentiate individuals with RTT compared to those with severe intellectual disability<sup>38</sup>.  
14 Other measures tested in RTT include the Anxiety Depression and Mood Scale (ADAMS)<sup>39</sup>,  
15 the clinician based International Scoring System (ISS)<sup>40,41</sup> to evaluate the disease severity,  
16 Vineland Adaptive Behaviour scale<sup>42</sup>, the 13 item Rett Clinical Severity Scale (RCSS)<sup>37,43</sup>  
17 and its modified version<sup>42</sup>. Others have developed RTT specific anchors such as for the  
18 Clinical Global Impression Severity (CGI-S) scale based on scores from the RCSS for  
19 improved outcome measures in clinical trials<sup>44</sup>. Quality of Life (QOL) measures such as the  
20 Child Health Questionnaire-P50 have also been used in RTT<sup>45</sup> including a recent Phase II  
21 open label clinical trial using glatiramer acetate<sup>46</sup>. Some of these measures such as the MBA,  
22 RSBQ, ADAMS and RCSS have been implemented into clinical trials to evaluate the effect  
23 of Insulin-like Growth Factor (IGF-1)<sup>47</sup> or Sarizotan<sup>48</sup> in individuals with RTT, or to develop  
24 a novel scoring tool (Rett Severity Score [RSS]) to assess the impact of IGF-1 treatment in  
25 RTT<sup>41</sup>. Other scales such as the Mullen Scales for Early Learning used in other rare  
26 disorders<sup>49</sup>, have also been adapted for use in RTT<sup>47</sup>. These measures are not without their  
27 faults. Some have suggested that the MBA can be difficult to use with some items that

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3 describe disease regression having not been validated<sup>24</sup>. This is important given that in some  
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5 RTT patients, disease regression has been described as transient or often goes unrecognised<sup>50</sup>.  
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7 Others such as the RSBQ although are suitable to measure some aspects of behaviour such as  
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9 mood and anxiety<sup>51</sup> might not be able to capture the salient features of behaviour as an  
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11 outcome measure in a clinical trial in RTT patients. Furthermore, there is differing reliability  
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13 of anxiety scales in RTT, with ADAMS especially its Social Avoidance subscale having the  
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15 best psychometric properties in comparison to the RSBQ<sup>52</sup>. Whilst no outcome measure will  
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17 be perfect, these studies have paved the way for more sensitive outcome measures to be  
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19 developed such as the validated 15 item Gross Motor Scale for individuals with RTT<sup>53</sup>.

### 22 *Autonomic Function in RTT*

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24 Large cross-sectional studies investigating the genotype-phenotype relationships have  
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26 revealed divergence in the phenotype seen in individuals with RTT<sup>54,55</sup>. These were the first  
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28 studies of sufficient sample size that bestowed important information on the genotype and  
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30 phenotype relationships in RTT, and have been elegantly summarised elsewhere<sup>24</sup>. Some  
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32 mutations or variants dictate a more severe phenotype when it comes to motor abilities<sup>24,54,55</sup>  
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34 and cardio-respiratory phenotypes<sup>24,56</sup>. Moreover, at present it is unknown whether autonomic  
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36 dysfunction is governed by any specific mutation in RTT<sup>24,56</sup>. Assessing the autonomic  
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38 dysfunction in individuals in RTT is therefore a pressing clinical concern.  
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42 Autonomic dysfunction is a pivotal factor that requires consideration when managing patients  
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44 with rare disorders such as RTT. From our clinical experience when managing patients in the  
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46 Centre for Interventional Paediatric Psychopharmacology and Rare Diseases (CIPPRD)<sup>57</sup>,  
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48 autonomic dysfunction is often found in patients who do not respond to treatment and those  
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50 with significant functional disability. Autonomic dysfunction co-occurs in the context of  
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52 emotional and behavioural dysregulation, and recently using wearable sensor technology, we  
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54 have shown that Emotional, Behavioural and Autonomic Dysregulation (EBAD) is a crucial  
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3 factor that needs to be considered when managing patients with RTT<sup>58,59</sup>. Although  
4 autonomic dysfunction has been investigated in individuals with RTT<sup>60-63</sup>, the progression of  
5 autonomic dysfunction and the developmental trajectory of EBAD has never been researched.  
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7 Moreover, the components of EBAD in a questionnaire that can map across other  
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### **Aim**

The objective of this study is to develop and validate a comprehensive multi-system questionnaire (Rett Evaluation of Symptoms and Treatments – REST) that can profile the symptomatology of patients with RTT and is sensitive to change across the lifespan allowing better understanding of patient needs. In parallel, information collected using wearable sensor technology<sup>58,59</sup> will be linked to data obtained from the REST questionnaire, genetic data and information about available psychosocial support from the patient and their family, to form a comprehensive Tailored Rett Intervention and Assessment Longitudinal (TRIAL) database. The TRIAL database will streamline treatment approaches to expedite triaging of care by signposting patients to correct specialists earlier than is currently happening (Figure 1). Specifically, the functionality of the multi-modal HealthTracker™ platform will be exploited so that anonymised data from the TRIAL database can be used to develop a parent/carer alert system to signal when it may be useful to request unscheduled clinician appointments. Using this functionality, the TRIAL database will also be able to stratify patients to inform adaptive clinical trial design, by allowing pre-existing datasets to be used so that rare disease trials can be done in a more cost-effective manner.

### **Methods and Analyses**

The title of this questionnaire was based on the feedback of the focus groups involving parents and carers of children with RTT from the parent based charities such as Reverse Rett UK, and clinician feedback. It will incorporate elements from previous scales<sup>40,52</sup> and

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3 standardised RTT questionnaires – data from the Natural History Study<sup>3</sup>, Rett Syndrome  
4 Behaviour Questionnaire (RSBQ)<sup>38</sup> and the modified version of the Rett Syndrome Severity  
5 Scale (RSSS)<sup>42</sup>. It is anticipated that the questionnaire will not take more than 30 minutes to  
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7 complete.  
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11 The United States (US) Food and Drug Administration (FDA) Guidance for Patient-reported  
12 Outcome Measures (PROM)<sup>64</sup> will be used as a template to guide the methodology in the  
13 study. It was described in Santosh *et al.* (2016)<sup>65</sup> and will follow an iterative framework that  
14 will involve item/concept identification, item/concept elicitation in parent/carer mediated  
15 focus groups, clinician feedback, web based presentation of questionnaires, initial scale  
16 development, instrument refinement and instrument validation.  
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24 ***Stage 1: Qualitative Development of the Rett Evaluation of Symptoms and Treatment***  
25 ***(REST) questionnaire***  
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27 ***Concept identification***  
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30 For this initial phase, a systematic literature review will be conducted according to the  
31 “Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)<sup>66</sup> to  
32 identify signs and symptoms that are deemed to be problematic in RTT. A draft version will  
33 be reviewed by expert clinicians who have substantial experience in RTT and Autism  
34 Spectrum Disorder (ASD). Common themes will be identified and draft version of the  
35 questionnaire will be prepared based on their feedback.  
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44 ***Concept elicitation***  
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46 This stage will involve parents/carers of individuals aged between 6 to 40 years with RTT. A  
47 series of focus groups anticipated to last about 1.5 hours will be conducted as part of the  
48 concept elicitation stage. These focus groups will include parents/carers of individuals with  
49 RTT from the parent-based charities such as Reverse Rett, UK, and clinicians who see RTT  
50 patients. The groups will follow a semi-structured format using open-ended questions to  
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3 allow participants to discuss their experiences and views. Some of the focus groups will be on  
4 item generation whilst others may centre on reviewing draft versions of the questionnaire  
5 identifying pertinent themes. Focus groups will be audio recorded and each group will  
6 include approximately 4-6 parents/carers of children with RTT. Up to two researchers may be  
7 present for the focus groups, which will be led by a Consultant Child and Adolescent  
8 Psychiatrist/Specialist. All participants will also be asked to complete a demographic  
9 questionnaire.

#### 10 11 12 *Web based presentation of questionnaires on the HealthTracker™ platform*

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14 HealthTracker™, a web-based health monitoring platform<sup>67</sup>, has been successfully trialed in  
15 multi-centric European Union FP7 studies<sup>68,69</sup> and in a questionnaire development and  
16 validation study<sup>70</sup>. Parents and carers will be shown how the REST questionnaire might  
17 appear on the HealthTracker™ platform, how the response options to the questionnaire could  
18 be presented and whether a choice of single or multiple-choice questions would be  
19 appropriate. The various views of the focus groups will be used to choose the most optimal  
20 web-based visualization of the questionnaire.

#### 21 22 23 *Tool review*

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25 As far as the authors are aware, no questionnaire exists that not only is RTT focused but can  
26 capture a broad range of problematic themes, in particular, the developmental trajectory of  
27 EBAD. Nor do these existing questionnaires/scales attempt to marry this with the  
28 physiological measurements from wearable sensor technology. At this stage, a further  
29 literature review will be conducted to identify any themes that may have been missed during  
30 the focus groups and whether any further areas of RTT symptomatology that were not  
31 highlighted in the focus groups needs to be addressed. In addition, parents/carers from  
32 Reverse Rett, UK, will be consulted and any feedback incorporated into the tool review stage.

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3 Following the focus groups, study participants will be sent a copy of the draft version of the  
4 questionnaire (via email or post). Once this part has been completed, a draft operating beta  
5 version of the questionnaire will be finalised.  
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9 ***Stage 2: Validation of the Rett Evaluation of Symptoms and Treatments (REST)***  
10 ***questionnaire***  
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13 This stage of the study will involve parents/carers of individuals aged between 6 to 40 years  
14 with RTT. Questionnaires to assess the longitudinal trajectory of symptomatology in rare  
15 diseases have proven to be difficult to validate<sup>71,72</sup>. To broach this conundrum, it is important  
16 to focus on the symptoms and not just the clinical diagnosis, and be able to evaluate the  
17 symptom level across other comparable patient groups. At the neuronal level, the brains of  
18 RTT and ASD patients share many core features<sup>73</sup>. Both RTT and ASD exhibit behaviours  
19 that might overlap i.e. there are deficits in social behaviour and speech and in both cases  
20 individuals may share common stereotypical behaviours<sup>74</sup>. Due to these similarities and based  
21 on consultation with clinicians with expertise in ASD, as a comparator group, this stage of  
22 the study will also include parents/carers/partners of individuals aged between 6 to 40 years  
23 with ASD with significant intellectual disability. It will also involve clinicians who see  
24 patients with RTT and ASD who will test the clinician version of the questionnaire.  
25 Participants (parents/carers and clinicians) will be recruited to complete the respective  
26 versions of the Rett Evaluation of Symptoms and Treatments (REST) questionnaire as well as  
27 other standardised questionnaires – namely the RSSS and the RSBQ (Table 1). The  
28 RSSS<sup>37,42,43,75</sup> and the RSBQ<sup>38,51</sup> have previously been used in studies with RTT patients.  
29 Pertinent information will also be taken from the RTT Natural History Study<sup>31,32</sup>. It is  
30 anticipated that 50 participants in the RTT cohort and 50 in the ASD with significant  
31 intellectual disability cohort will complete the questionnaire battery. Although there is  
32 significant symptom overlap in patients with RTT and ASD, participants in the ASD cohort  
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3 will be asked to complete only the relevant questions in the questionnaire battery that would  
4 be applicable and relevant to them.  
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7 The questionnaire battery will be presented to study participants in HealthTracker™, a multi-  
8 modal web-based portal for remote online completion using developmentally appropriate  
9 interfaces. Participants will be given a unique ID number and log-in information and will be  
10 asked to complete the questionnaires independently. The research team will be able to  
11 support participants with questionnaire completion should they need it. Where applicable,  
12 participants will also be able to complete paper versions of the questionnaires if they request  
13 them. Participant medical records will be accessed only by members of the study team to  
14 validate the questionnaire against details of diagnoses obtained from patient case notes as  
15 well as against the Development and Well-being Assessment (DAWBA)<sup>76</sup>, and  
16 treatment/medication status if they are available in case notes. Patient records will also be  
17 used to gain genetic information on the specific mutation and diagnosis. Consent will be  
18 obtained to access medical notes.  
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33 All participants will be asked to complete the questionnaire battery, at baseline, again after 1  
34 week and then between 4 and 6 months after first completion to assess questionnaire stability.  
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### 37 ***Stage 3: Wearable Sensor Technology***

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39 The use of wearable sensor technology to improve treatment outcomes has gathered  
40 momentum in recent years<sup>77</sup> and is currently being used to develop new outcome measures in  
41 patients with complex neurodisability such as Amyotrophic Lateral Sclerosis<sup>78</sup>. Using  
42 wearable sensor technology as a PROM is not without its challenges. In RTT, wearable  
43 technology has been used to explore respiratory and cardiac function in observational  
44 studies<sup>79,80</sup> and in two recent clinical trials<sup>46,47</sup>, however, inherently captured biometric data  
45 can be noisy especially from quasi-periodic oscillations from cardiac rhythms. Wrist worn  
46 devices might be particularly susceptible to this type of noise. To mitigate these issues, we  
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3 have applied the methods described previously<sup>81,82</sup> to analyse heart rate variability and  
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5 electrodermal activity as metrics when evaluating wrist sensor biometric data and autonomic  
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7 function in a 15-year-old girl with RTT. We were able to demonstrate a recalibration of the  
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9 autonomic equilibrium from pre-treatment to post-treatment using buspirone (30 mg/day)<sup>58</sup>,  
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11 and subsequent improvement in EBAD in this girl. Quasi-periodic oscillations cannot be  
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13 easily quantified using conventional methods. To manage this, phase-rectified signal  
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15 averaging (PRSA)<sup>83</sup> may be used in conjunction with spectral factorization and applied to the  
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17 beat-to-beat RR interval data, which is particularly prone to extraneous noise. This  
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19 methodology coupled with EDA assessment will provide more sensitive methods to capture  
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21 changes in autonomic physiology in patients with RTT. In the context of this study, the  
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23 outcomes of the wearable sensor technology will marry into the outcomes of the newly  
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25 developed questionnaire (REST), with psychosocial and genetic data to create the TRIAL  
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27 database. The technology will be evaluated in individuals with RTT, ASD and healthy  
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29 controls.  
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### 32 ***Sample size***

#### 33 *Justification for Sample Size*

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35 Owing to the small sample population of individuals with rare and complex genetic disorders,  
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37 formal modelling to obtain sample size estimates will not be readily applicable.  
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#### 40 *Stage 1: Questionnaire Development Stage*

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42 It is anticipated that the total number of participants for the questionnaire development stage  
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44 of the study will be between 10-20 (including participants and clinicians). In our experience,  
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46 focus groups involving families with children with rare diseases are well versed with the  
47  
48 problems associated with the condition in question. Often, the themes that need to be  
49  
50 addressed get saturated after a couple of focus groups, leading to us getting the basic structure  
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52 of the items needed to be tested in Stage 2.  
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### *Stage 2: Questionnaire Validation Stage*

The total number of participants for the questionnaire validation stage of the study will be 150 participants (n=100 RTT cohort and n=50 ASD cohort). The number in the ASD cohort will be split so that 25 parents/carers will either have a male or female diagnosed with ASD.

### *Stage 3: Wearable Sensor Technologies Stage*

The total number of participants for the wearable technology stage of the study is expected to be 100 participants (n=50 RTT cohort and n=50 [25 male and 25 female]) ASD cohort). This part of the study will also include a matched healthy control group.

### *Stage 4: Longitudinal monitoring in RTT patients*

Longitudinal data capture on a 3-monthly basis from 80 - 100 parents/carers of individuals with RTT will be undertaken using the REST questionnaire over a 12-18 month period. Ethics submission for stage 4 of the study will be done after stages 1-3 have been completed.

### ***Recruitment***

Information sheets (and age appropriate information sheets where relevant) will be provided for all participants, in addition to consent forms. Information sheets will emphasise that participant involvement in the research is voluntary and they have the right to withdraw from the research at any time, without giving a reason. In addition, participants will be advised that participating or withdrawing from the research will have no impact on their usual care that they are currently receiving, or will receive in the future. A minimum of 24 hours will be given between providing study information and recruitment of participants into the study.

### ***Stage 1: Questionnaire Development Stage Recruitment***

Parents/carers of individuals with RTT and clinicians who work with individuals with RTT will be recruited. Due to the group-based nature of the focus groups, parents/carers or clinicians who have not provided consent will not be able to partake in focus groups and will be excluded. The focus groups will comprise of parents/carers of individuals with RTT and

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3 clinicians working with patients with RTT. Depending on the nature of the focus groups  
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5 about 4-6 participants will take part in each focus group.  
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7 *Questionnaire Development Stage - Inclusion Criteria*

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- Parents/carers/partners/relatives of individuals aged between 6 to 40 years with RTT.
  - Clinicians who work within healthcare settings in South London and Maudsley (SLaM) NHS Foundation Trust that see children and/or adults with RTT and associated developmental conditions.
  - Without any exclusion for concurrent stable medication.

20 *Questionnaire Development Stage - Exclusion Criteria*

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- Parents/carers whom do not have a reasonable level of English. This is because a reasonable level of English will be required to engage in the focus groups.

27 ***Stage 2: Questionnaire Validation Stage Recruitment***

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29 For this stage of the study, parents/carers of individuals with RTT and those with ASD will  
30  
31 be recruited via clinician/researcher invite. Study participants will be under the care of a  
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33 service within SLaM NHS Foundation Trust. Where relevant,  
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35 parents/carers/partners/relatives of individuals with RTT and ASD will be asked to provide  
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37 details of their clinician at the time of consent so that they can also be contacted by the  
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39 research team and invited to take part.  
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42 *Questionnaire Validation Stage - Inclusion Criteria*

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- Parents/carers of individuals aged between 6 to 40 years with RTT or ASD.

46 *Questionnaire Validation Stage - Exclusion Criteria*

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- If parents/carers of individuals aged between 6 to 40 years with RTT or ASD are not able to (or expected to not be able to) complete questionnaires they will be excluded from the study.

- Parents/carers who do not have a reasonable level of English will be excluded from the validation stage of the study. This is because a reasonable level will be required to complete questionnaires, which will only be available in English at the validation stage. A research assistant may assist the Parent/carer in completion.

### ***Stage 3: Wearable Sensor Technologies Stage Recruitment***

Individuals aged between 6 to 40 years with RTT and ASD, and parents/carers/partners/relatives of individuals with RTT and ASD will be recruited via clinician/researcher invite. Information sheets (and age appropriate information sheets where relevant) will be provided for all participants, in addition to consent forms and where applicable assent forms. Healthy controls will be recruited via clinician/researcher invite using widely used and appropriate advertising channels.

#### *Wearable Sensor Technologies Stage - Inclusion Criteria*

##### RTT

- Females aged 6 - 40 years with confirmed diagnosis of RTT (via clinician/researcher invite).
- Parents/carers/partners/relatives of individuals aged 6 - 40 years with RTT.

##### ASD

- Males and females aged 6 - 40 years with ASD (via clinician/researcher invite).
- Parents/carers/partners/relatives of individuals aged 6 - 40 years with ASD.

##### Healthy Controls

- Males and females aged 6 - 40 years considered to be healthy for their age (via clinician/researcher invite).
- Are capable of understanding and complying with the requirements of the protocol.

### *Wearable Sensor Technologies Stage - Exclusion Criteria*

#### RTT

- Individuals aged 6 - 40 years with RTT who are not able to (or expected to not be able to) wear the wearable sensor technology will be excluded from the study.
- Parents/carers/partners/relatives of individuals aged 6 - 40 years with RTT who do not have a reasonable level of English.

#### ASD

- Individuals (aged 6 - 40 years with ASD who are not able to (or expected to not be able to) wear the sensor technology will be excluded from the study.
- Parents/carers/partners/relatives of individuals aged 6 - 40 years with ASD who do not have a reasonable level of English.

#### Healthy Controls

- Individuals who are not able to (or expected to not be able to) wear the sensor technology will be excluded from the study.
- Individuals who do not have a reasonable level of English.

### **Analyses Plan**

#### ***Questionnaire Development***

Data obtained from the focus groups will be recorded securely and transcribed accurately, paying close attention to the identified themes and issues. The analysis will be performed as described previously<sup>65</sup>. In brief, the focus group data will be organised into clinically meaningful themes using thematic and content analysis. Following this, to manage the qualitative data generated from the focus groups, NVivo software will be used and the data analysis will be guided by the framework for thematic analysis<sup>84</sup>.

#### ***Questionnaire Validation***



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3 The quantitative data will be analysed using the latest version of the SPSS statistical package  
4 (IBM SPSS Statistics for Windows. Armonk, NY: IBM Corp.).

#### 5 6 7 *Internal Consistency*

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9 Internal consistency of the measures will be reported using Cronbach's alpha. Alpha  
10 coefficients  $\geq 0.85$  will be indicative of reasonable evidence of internal reliability<sup>85</sup>. Where  
11 applicable, 'alpha if deleted analyses' will be performed to see if omitting any item(s) from  
12 the (subthemes of the) questionnaire would strengthen the measure.

#### 13 14 15 16 17 *Test-retest reliability*

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19 Intra-class correlation (ICC) will be used to assess test-retest reliability on subscale and total  
20 scores as described<sup>86</sup>. Given the exploratory nature of this study, weighted Cohen's kappa  
21 values will also be determined to assess test-retest reliability at the item level. The ICC will  
22 also be performed after 4 to 6 months after initial completion of the questionnaire to assess  
23 the long-term stability of the new questionnaire.

#### 24 25 26 27 28 29 30 31 *Validity*

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33 Validity (discriminative power) of the new questionnaire will be assessed using Receiver  
34 Operating Characteristic (ROC) analyses as described in Santosh et al. (2016)<sup>65</sup>. As there are  
35 no gold standard questionnaires for patients with RTT, where applicable the ROC analyses  
36 will also be performed on the scores on the RTT Natural History Study, the RSBQ and the  
37 RSSS. Where necessary and if data are available, ANOVA (general linear model) will be  
38 performed with grouping variable DAWBA diagnoses (coded in 1 for positive and coded 0  
39 for negative diagnosis) so that the differences in REST scoring can be assessed.

#### 40 41 42 43 44 45 46 47 48 *Factor analysis*

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50 Studies involving small sample sizes have often been plagued by the inappropriate use of  
51 Exploratory Factor Analysis (EFA) or Principal Component Analysis (PCA) to identify  
52 clinically meaningful factor items<sup>87</sup>. Many recommendations have been put forward  
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3 regarding sample sizes but there does not seem to be an overall consensus<sup>88</sup>. Some have  
4 suggested improbable sample sizes that would not be feasible for studies of rare and complex  
5 genetic diseases<sup>89</sup>. In these instances, methods to reveal the multi-dimensional aspects of  
6 factor structure are not as straightforward. Recently, the Regularized Exploratory Factor  
7 Analysis (REFA) was introduced<sup>90</sup> that is recommended over EFA and PCA, when samples  
8 sizes are less than 50. Despite this new approach, it is unclear whether the REFA would be  
9 applicable for a multidimensional questionnaire in a condition with many variables. In the  
10 context of the new questionnaire, the robustness of the REST will be evaluated using tools  
11 applicable for smaller samples sizes<sup>90</sup> and those used in exploratory studies as described  
12 recently<sup>91</sup>.

### 23 ***Gender Differences***

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

### 32 **Study dates**

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34 The study is expected to complete by January 2019.

### 37 **Dissemination**

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39 The goal of this study is to develop and validate a new RTT questionnaire. Data from the  
40 REST questionnaire will be linked with the data from the wearable sensor technology as well  
41 as psychosocial and genetic information to construct the TRIAL database, which will  
42 improve the overall healthcare delivery for individuals with RTT. Using the functionality of  
43 the HealthTracker<sup>TM</sup> platform, the TRIAL database will provide all the necessary information  
44 to clinicians and researchers about different aspects of the disease and serve as a barometer  
45 for improving treatment pathways in individuals. This will allow algorithms to be developed  
46 alerting parent/carers to request unscheduled clinician appointments when symptoms deviate  
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3 significantly from one another thereby streamlining the patient care pathway. As the  
4 HealthTracker™-based TRIAL database is web-based, with appropriate funding, it has the  
5 potential to be used globally, allowing for quicker development of decision-support analytics  
6 and personalized care.  
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11 Rare disorders such as RTT have a limited patient population and it is therefore crucial for  
12 patients to be stratified using phenotype and biomarkers (such as those obtained through  
13 wearable sensor monitoring). Adaptive clinical trial design using Bayesian methodology has  
14 been suggested to augment the statistical power and decrease the number of patients required  
15 for a rare disease trial<sup>72</sup>. In this view, the TRIAL database will serve for the recruitment of  
16 patients into clinical trials, as baseline information would already be available so the clinical  
17 trial can be conducted with fewer patients and in a more cost-effective manner.  
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21 Results stemming from this study will be disclosed unreservedly and the findings published  
22 in scientific journals and will also be presented in meetings and conferences for professionals,  
23 patients as well as carers and families. Reverse Rett UK will lead on wider dissemination of  
24 the applied research findings to engage policy-makers, key professional groups and service  
25 managers, and parents/carers of children with RTT.  
26  
27

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29  
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31 HealthTracker Ltd for significantly subsidising the costs for this study.  
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### 37 **Author Contributions**

38  
39 JS drafted, wrote and revised the manuscript, and wrote the documentation required for  
40 ethical approval of the study. KL provided important intellectual review of the manuscript  
41 and reviewed the documentation required for ethical approval of the study. FF reviewed the  
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3 statistical components and reviewed the manuscript. PS secured funding and conceived the  
4  
5 study, and revised the manuscript critically for important intellectual content.  
6  
7

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10  
11 We are indebted to Reverse Rett UK for their helpful comments and suggestions on the study  
12  
13 design.  
14

### 15 16 **Conflicts of Interest**

17  
18 PS is the co-inventor of the HealthTracker™ and is the Chief Executive Officer and  
19  
20 shareholder in HealthTracker Ltd. FF is a Data Analyst and KL is a Project Manager  
21  
22 employed by HealthTracker Ltd respectively.  
23

24 JS is on the Professional Advisory Board for Reverse Rett UK and acts as a Scientific  
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26 Advisor.  
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3 Figure 1: Flow diagram illustrating the sequence of steps showing how data obtained from  
4 the TRIAL database can be used to provide timely intervention to streamline treatment  
5 outcomes in patients with RTT syndrome  
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10 **INSERT FIGURE 1 HERE**  
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13 Abbreviations: REST (Rett Evaluation of Symptoms and Treatments) questionnaire; RTT (Rett Syndrome);  
14 TRIAL (Tailored Rett Intervention and Assessment Longitudinal) database  
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16 Notes:

17 The term 'wearables' has been coined for wearable sensor technology

18 Genetic and psychosocial information will be captured as part of the REST questionnaire  
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Table 1: Measures to be administered during Stage 2 (Validation) and Stage 3 (Wearable Sensor Technology) of the Study

Measure	Key Information	Administered to:					
		Individual with RTT	Individual with ASD	Healthy Subjects	Parent/Carer of Child with RTT/*ASD	Parent/Carer/Partner of Adult with RTT/ASD*	Clinician/Researcher
Rett Natural History study <sup>32</sup>	More than 1000 participants with RTT providing information on important aspects of disorder symptomatology						X
Rett Syndrome Behavioural Questionnaire (RSBQ) <sup>38</sup>	Provides an accurate measure of the behavioural features of RTT				X	X	
Rett Syndrome Severity Score (RSSS) <sup>42</sup>	Provides information on the overall clinical severity and severity across individual parameters: <ul style="list-style-type: none"> <li>• frequency and manageability of seizures;</li> <li>• respiratory abnormalities</li> <li>• scoliosis;</li> <li>• ability to walk;</li> <li>• hand use;</li> <li>• speech;</li> <li>• sleep hygiene</li> </ul>						X
Rett Evaluation of Symptoms and Treatments (REST) questionnaire	A multidimensional questionnaire that can capture clinically meaningful data across the lifespan in individuals with RTT and improve treatment pathways				X	X	X
Wearable Sensor Technology	Captures real-time biometric physiological data (heart rate variability, skin conductance, blood volume pressure, perspiration and temperature)	X	X	X			
Anticipated		30	30	30	~60	~60	~60

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Administration Time (minutes)							
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\* Participants in the ASD cohort will be asked to complete only the relevant questions in the questionnaire battery that would be applicable and relevant to them  
 Abbreviations: ASD (Autism Spectrum Disorder); REST (Rett Evaluation of Symptoms and Treatments) questionnaire; RSBQ (Rett Syndrome Behavioural Questionnaire); RSSS (Rett Syndrome Severity Score); RTT (Rett Syndrome)

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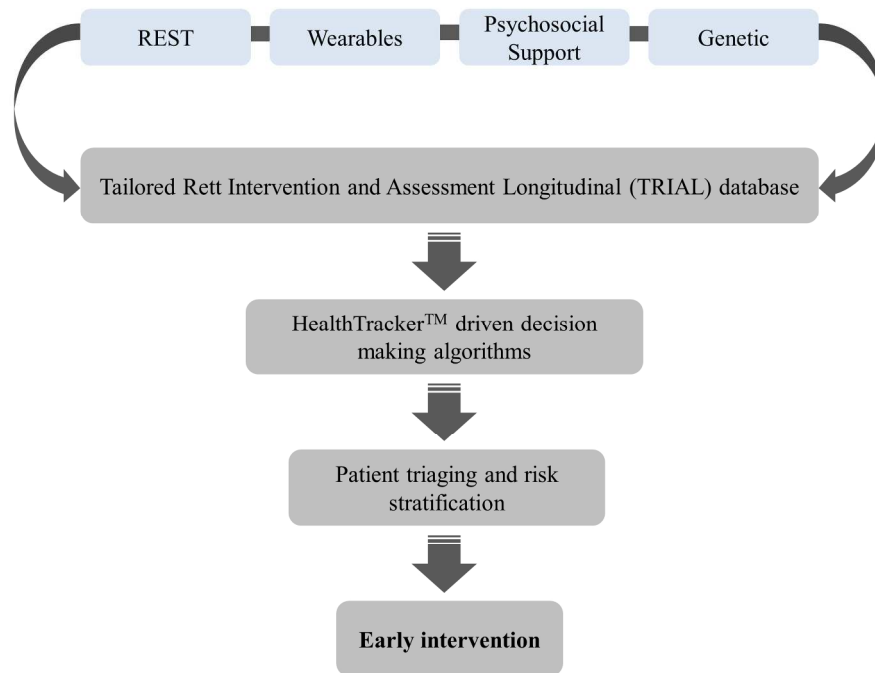


Figure 1: Flow diagram illustrating the sequence of steps showing how data obtained from the TRIAL database can be used to provide timely intervention to streamline treatment outcomes in patients with RTT syndrome

Abbreviations: REST (Rett Evaluation of Symptoms and Treatments) questionnaire; RTT (Rett Syndrome); TRIAL (Tailored Rett Intervention and Assessment Longitudinal) database

Notes:

The term 'wearables' has been coined for wearable sensor technology  
Genetic and psychosocial information will be captured as part of the REST questionnaire

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