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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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Key Words

Biomarkers; HealthTracker™; 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. RTT has divergent symptomology 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.

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).

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)[1] where Dr. Rett first observed the clinical signs and symptoms of RTT. Symptoms usually appear between 6 to 18 months after birth[2] 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[3] and so far 57 cases of RTT have been documented in males[4]. 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[5], with rarer cases being attributed to mutations in CDKL5 and FOXG1 gene[6,7]. More recently, mutations in other less known candidate genes have emerged such as those encoding ankyrin repeat proteins and neuronal acetylcholine receptor subunits[8]. MeCP2 is a highly conserved nuclear protein in the mammalian brain[9] and notably the disorder is reversible in mice models of RTT[10] and in *MECP2* duplication syndrome[11].

MeCP2 acts as a critical epigenetic modulator in the mammalian brain, controlling overlapping mechanisms such as DNA methylation and post-translational mechanisms[12]. Through differential post-translational modification at serine 164[13], MeCP2 may help in limiting transcriptional noise[14] 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[15] and the $MeCP2^{R306C}$ mutation prevents MeCP2 from interacting with the NCoR/histone deacetylase 3 (HDAC3) complex resulting in impediments in social and cognitive functioning in animal models[16]. 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

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

MECP2 being an X-linked gene, has an impact on the phenotype of RTT patients and the clinical severity. The X chromosome inactivation can cause uneven expression of wild type and mutant alleles resulting in skewed patterns of RTT phenotype severity[21,22], and the degree of DNA-methylation-dependent long gene repression[18]. The range of severity in RTT is therefore broad and ranges from lethal neonatal encephalopathy to milder forms (where symptoms are less severe); hence assessment and treatment must be individually tailored to each affected person.

Pre-existing measures in RTT

As far as we are aware, no complete all-embracing instrument has been developed for individuals with RTT that has the ability to capture longitudinal pharmacological, behavioural, genetic and psychosocial information, as well as an ability to correlate this with the physiological aspects of the disease. At present there are no datasets or instruments that provide such information. Previous datasets/instruments have been inconsistent and provide limited information on the behavioural and physiological facets of the disease. Whilst, some might provide information on the genetic diagnoses of individuals with RTT, there is a lack of consistency. First, RettBase, collected mainly molecular genetic data from the Australian cohort of RTT patients[23]. Some other instruments have included both genetic and clinical data, although the clinical data was limited. InterRETT, an Australian Rett syndrome database, was based on data collection by distributing a questionnaire to families[24]. The

Italian Rett Database and Biobank consisted of 357 patients and had 20 structured and seven descriptive clinical items along with 17 structured genetic items[25]. The British Isles Rett Syndrome Survey, included 275 British Rett patients and had 271 structured and 94 descriptive clinical items, and six structured genetic items[26]. An American survey collected data on the natural history of the disease that allowed researchers and physicians to access comprehensive patient data[27]. These datasets were preserved and integrated into the Rett Networked Database[26] and offers an amalgamated data repository for researchers to access anonymized patient information. Elsewhere, the Japanese RTT database (JRDB) includes the clinical data from 102 females with a median age of 11 years old[28].

Large cross-sectional studies investigating the genotype-phenotype relationships have revealed divergence in the phenotype seen in individuals with RTT[29]. Certain deletions dictate a more severe phenotype when it comes to motor abilities[29,30] and cardio-respiratory phenotypes[31]. This has paved the way for specific questionnaires to be developed and validated such as the Rett Syndrome Gross Motor Scale[32] to measure the gross motor abilities in individuals with RTT and a modified version of the International Scoring System[33] in RTT to assess the clinical impact of the common symptoms. Whilst these instruments have their merits and would be invaluable for pinpointing specific outcomes in RTT such as gross motor deficits and cardio-respiratory function, they are limited in their scope and have not been evaluated for capturing clinically meaningful change of key symptomatology longitudinally. Moreover, as autonomic dysfunction does not appear to be governed by any specific mutation in RTT[31], assessing the autonomic dysfunction in individuals in RTT is a pressing clinical concern.

Autonomic dysfunction is a pivotal factor that requires consideration when managing patients with rare disorders such as RTT. From a clinical perspective, this situation is often found in treatment non-responders and those with significant functional disability. Autonomic

dysfunction co-occurs in the context of emotional and behavioural dysregulation, and recently using wearable sensor technology, we have shown that Emotional, Behavioural and Autonomic Dysregulation (EBAD) is a crucial factor that needs to be considered when managing patients with RTT[34,35]. Although autonomic dysfunction has been investigated in individuals with RTT[36-39], the progression of autonomic dysfunction and the developmental trajectory of EBAD has never been researched. Moreover, the components of EBAD in an all encompassing questionnaire that can map across other symptomatology longitudinally in individuals with RTT has not previously been shown.

Aim

The objective of this protocol is to develop and validate a comprehensive multi-system questionnaire (Rett Evaluation of Symptoms and Treatments – REST questionnaire) that can profile the symptomatology of patients with RTT by capturing clinically meaningful data across the lifespan allowing better understanding of patient needs. In parallel, information collected using wearable sensor technology[34,35] will be linked to data obtained from the REST questionnaire, genetic data and also information about available psychosocial support from the patient and their family, to form a comprehensive Tailored Rett Intervention and Assessment Longitudinal (TRIAL) database. Using the data obtained from the new REST questionnaire, physiological measurements (using wearable sensor technology), genetic and clinically derived psychosocial support data, 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 the 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[32,33] and standardised RTT questionnaires – data from the Natural History Study[40], Rett Syndrome Behaviour Questionnaire (RSBQ)[41] and the modified version of the Rett Syndrome Severity Scale (RSSS)[42]. It is anticipated that the questionnaire will not take more than 30 minutes to complete.

The United States (US) Food and Drug Administration (FDA) Guidance for Patient-reported Outcome Measures (PROM)[43] will be used as a template to guide the methodology in the study. It was described in Santosh *et al*. (2016)[44] and will follow an iterative framework that will involve item/concept identification, item/concept elicitation in parent/carer mediated focus groups, clinician feedback, web based presentation of questionnaires initial scale development, cognitive interviews, instrument refinement and instrument validation.

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

Concept identification

For this initial phase, a systematic literature review will be conducted according to the "Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)[45] to identify signs and symptoms that are deemed to be problematic in RTT. A draft version will be reviewed by expert clinicians who have substantial experience in RTT and Autism Spectrum Disorder (ASD). Common themes will be identified and draft version of the questionnaire will be prepared based on their feedback.

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[46], has been successfully trialled in multi-centric European Union FP7 studies[47,48] and also in a questionnaire development and validation study[49]. 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

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

Cognitive interviews

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

Stage 2: Validation of the Rett Evaluation of Symptoms and Treatments (REST) questionnaire

This stage of the study will involve parents/carers of individuals aged 6 months to 40 years with RTT. Questionnaires to assess the longitudinal trajectory of symptomatology in rare diseases have proven to be difficult to validate[50,51]. To broach this conundrum, it is important to focus on the symptoms and not just the clinical diagnosis, and be able to evaluate the symptom level across other comparable patient groups. RTT is associated with a high penetrance of ASD and at the neuronal level share many salient features[52]. Both RTT and ASD exhibit deficits in social behaviour and speech and in both cases individuals may share common stereotypical behaviours[53]. Due to these similarities and based on consultation with clinicians with expertise in ASD, as a comparator group, this stage of the study will also include parents/carers/partners of individuals aged 6 months to 40 years with ASD with significant intellectual disability. It will also involve clinicians who see patients with RTT and ASD who will test the clinician version of the questionnaire. Participants (parents/carers and clinicians) will be recruited to complete the respective versions of the Rett Evaluation of Symptoms and Treatments (REST) questionnaire as well as other standardised questionnaires

– namely the RSSS and the RSBQ (Table 1). The RSSS[42,54] and the RSBQ[41,42,55] have previously been used in studies with RTT patients. Pertinent information will also be taken from the RTT Natural History Study[40]. It is anticipated that 25 participants in the RTT cohort and 25 in the ASD with significant intellectual disability cohort will complete the questionnaire battery. Although there is significant symptom overlap in patients with RTT and ASD, 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.

The questionnaire battery will be presented to study participants in HealthTracker™, a multi-modal web-based portal for remote online completion using developmentally appropriate interfaces. Participants will be given a unique ID number and log-in information and will be asked to complete the questionnaires independently. The research team will be able to support participants with questionnaire completion should they need it. Where applicable, participants will also be able to complete paper versions of the questionnaires if they request them. Participant medical records will be accessed only by members of the study team to validate the questionnaire against details of diagnoses obtained from patient case notes as well as against the Development and Well-being Assessment (DAWBA)[56], and treatment/medication status if they are available in case notes. Patient records will also be used to gain genetic information on the specific mutation and diagnosis. Consent will be obtained to access medical notes.

All participants will be asked to complete the questionnaire battery, at baseline, again after 1 week and then between 4 and 6 months after first completion to assess questionnaire stability.

Stage 3: Wearable Sensor Technology

The use of wearable sensor technology to improve treatment outcomes has gathered momentum in recent years[57] and is currently being used to develop new outcome measures in patients with complex neurodisability such as Amyotrophic Lateral Sclerosis[58]. We have

shown that wearable sensor technology can assist in the management of EBAD in individuals with rare and complex genetic disorders[59], such as RTT[34,35], to assist in improving treatment outcomes. In the context of this study, the outcomes of the wearable sensor technology will marry into the outcomes of the newly developed questionnaire (REST), with psychosocial and genetic data to create the TRIAL database. The technology will be evaluated in individuals with RTT, ASD and healthy controls.

Sample size

Justification for Sample Size

Owing to the small sample population of individuals with rare and complex genetic disorders, formal modelling to obtain sample size estimates will not be readily applicable. In this view, focus groups will be used to understand the nature of the problem and the acceptability of various measures and their presentation to potential research participants. The qualitative modification and new development of the questionnaire will be done by running focus groups and the sample size was calculated based on the requirements needed for field testing.

Stage 1: Questionnaire Development Stage

It is anticipated that the total number of participants for the questionnaire development stage of the study will be between 10-20 (including participants and clinicians).

Stage 2: Questionnaire Validation Stage

The total number of participants for the questionnaire validation stage of the study will be 50 participants (25 RTT cohort and 25 ASD cohort).

Stage 3: Wearable Sensor Technologies Stage

The total number of participants for the wearable technology stage of the study is expected to be similar as those who participated in the validation phase of the study. 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 clinicians working with patients with RTT. Depending on the nature of the focus groups about 4-6 participants will take part in each focus group.

Questionnaire Development Stage - Inclusion Criteria

- Parents/carers/partners/relatives of individuals aged 6 months 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.

Questionnaire Development Stage - Exclusion Criteria

- 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.

Stage 2: Questionnaire Validation Stage Recruitment

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

Questionnaire Validation Stage - Inclusion Criteria

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

Questionnaire Validation Stage - Exclusion Criteria

- If parents/carers of individuals aged 6 months 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 my assist the Parent/carer in completion.

Stage 3: Wearable Sensor Technologies Stage Recruitment

Individuals aged between 5 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 5 - 40 years with confirmed diagnosis of RTT (via clinician/researcher invite).
- Parents/carers/partners/relatives of individuals aged 5 - 40 years with RTT.

ASD

- Males and females aged 5 - 40 years with ASD (via clinician/researcher invite).
- Parents/carers/partners/relatives of individuals aged 5 - 40 years with ASD.

Healthy Controls

- Males and females aged 5 - 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 5 - 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 5 - 40 years with RTT who do not have a reasonable level of English.

ASD

- Individuals aged 5 - 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 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[44]. 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[60].

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 ≥ 0.85 will be indicative of reasonable evidence of internal reliability[61]. Where applicable, 'alpha if deleted analyses' will be perfomed to see if omitting any item(s) from the (subthemes of the) questionnaire would strengthen the measure.

Test-retest reliability

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

Validity

Validity (discriminative power) of the new questionnaire will be assessed using Receiver Operating Characteristic (ROC) analyses as described in Santosh et al. (2016)[44]. As there are no gold standard questionnaires for patients with RTT, where applicable the ROC analyses will also be performed on the scores on the RTT Natural History Study[40], the RSBQ[41] and the RSSS[42]. Where necessary and if data are available, ANOVA (general linear model) will be performed with grouping variable DAWBA diagnoses (coded in 1 for positive and coded 0 for negative diagnosis) so that the differences in REST scoring can be assessed.

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[63]. Many recommendations have been put forward regarding sample sizes but there does not seem to be an overall consensus[64]. Some have suggested improbable sample sizes that would not be feasible for studies of rare and complex genetic diseases[65]. 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[66] 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[66] and those used in exploratory studies as described recently[67].

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™ platform the TRIAL database will provide all the necessary information to clinicians and researchers about different aspects of the disease and serve as a barometer for improving treatment pathways in individuals. This will allow algorithms to be developed alerting parent/carers to request unscheduled clinician appointments when symptoms deviate significantly from one another thereby streamlining the patient care pathway. Rare disorders such as RTT have a limited patient population and it is therefore crucial for patients to be stratified using phenotype and biomarkers (such as those obtained through wearable sensor monitoring). Adaptive clinical trial design using Bayesian methodology has been suggested to augment the statistical power and decrease the number of patients required for a rare disease trial[51]. In this view, the TRIAL database will serve for the recruitment of patients into clinical trials as baseline information would already be available so the clinical trial can be conducted with fewer patients and in a more cost-effective manner. Results stemming from this study will be disclosed unreservedly and the findings published in scientific journals and will also be presented in meetings and conferences for professionals, patients as well as carers and families. Reverse Rett UK will lead on wider dissemination of the applied research findings to engage policy-makers, key professional groups and service 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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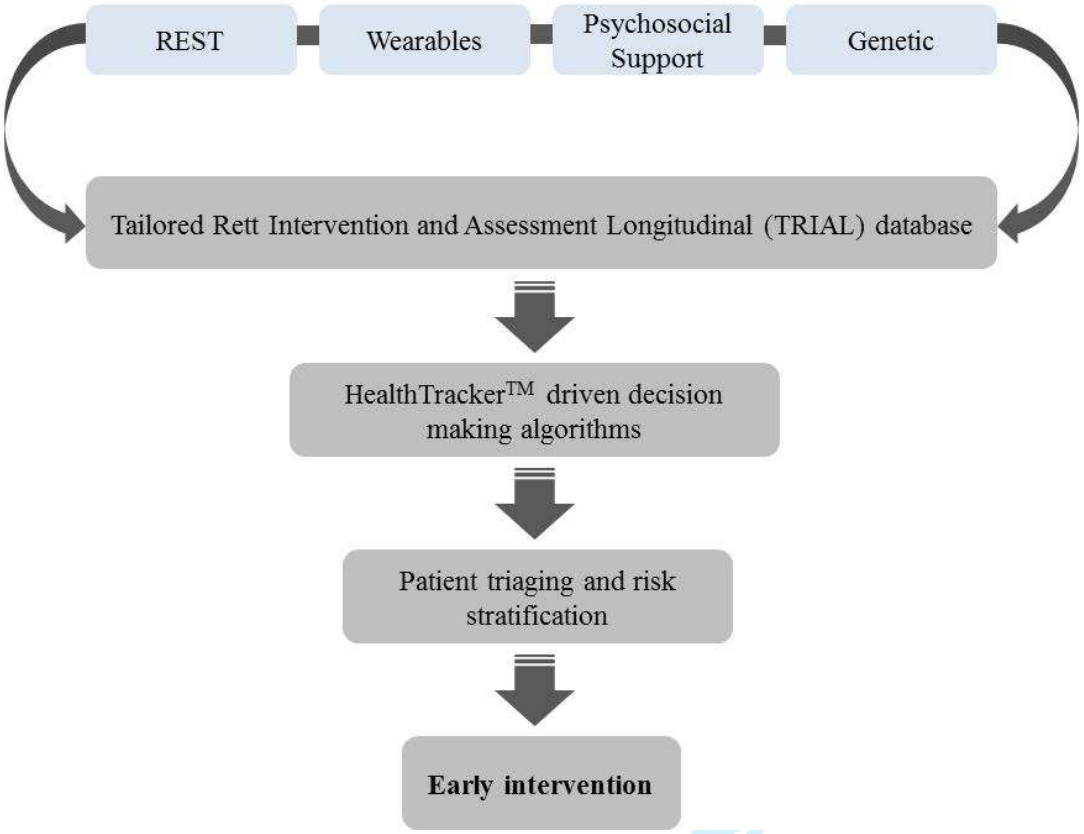
Figure 1: Flow diagram illustrating the sequence of steps showing how data obtained from the TRIAL database can be used to provide timely intervention to streamline treatment outcomes in patients with RTT syndrome



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

		Administered to:					
Measure	**Key Information**	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[40]	542 individuals with classical RTT and 96 with atypical RTT provide information on clinical severity of RTT						**X**
Rett Syndrome Behavioural Questionnaire (RSBQ)[41].	Provides an accurate measure of the behavioural features of RTT				**X**	**X**	
Rett Syndrome Severity Score (RSSS)[42]	Provides information on the overall clinical severity and severity across individual parameters: • frequency and manageability of seizures; • respiratory abnormalities • scoliosis; • ability to walk; • hand use; • speech; • sleep hygiene				**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)

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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™; 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.

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).

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)[1] where Dr. Rett first observed the clinical signs and symptoms of RTT. Symptoms usually appear between 6 to 18 months after birth[2] 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[3]. There are geographical variations[4] with one Australian study indicating a prevalence of about 1:9000[5]. 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[6], with rarer cases being attributed to mutations in CDKL5 and FOXG1 gene[7,8] 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[9]. MeCP2 is a highly conserved nuclear protein abundent in the mammalian brain[10] and notably the disorder is reversible in mice models of RTT[11].

MeCP2 acts as a critical epigenetic modulator in the mammalian brain, controlling overlapping mechanisms such as DNA methylation and post-translational mechanisms[12]. Through differential post-translational modification at serine 164[13], MeCP2 may help in limiting transcriptional noise[14] 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[15] and the $MECP2^{R306C}$ mutation prevents MeCP2 from interacting with the NCoR/histone deacetylase 3 (HDAC3) complex resulting in impediments in social and cognitive functioning in animal models[16]. *MeCP2* has complex genome level modalities, and the general opinion is that loss of the

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

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

Although there have been considerable advances in understanding the genetics and into the genetic testing of RTT, the diagnosis of RTT is based on the 2010 revised consensus clinical criteria[3] (see Table 1 in Neul et al., 2010) and recommends that all individuals with RTT should be first be assessed according to the revised clinical criteria followed by a thorough genetic test for *MECP2*. Given that about 3-5% of RTT individuals who fulfil the diagnostic clinical criteria do not have *MECP2* mutations, and this is even higher for atypical RTT cases[25], more recently clinical predictors that can facilitate a clinician's decision making to order genetic testing for RTT have been provided[26]. This showed that the likelihood of a having a positive *MECP2* test was greatest in patients with partial or complete attenuation of

hand skills. Impairments in gait and hand stereotypies were also strong predictors. Of interest was that loss of speech did not discriminate whether an individual was *MECP2+* or *MECP2-*.

Pre-existing measures in RTT

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

Capture of disease severity and sensitivity to change throughout the lifespan in patients are important elements that need to be considered when developing clinically meaningful

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

faults. Some have suggested that the MPA can be difficult to use with some items that describe disease regression having not been validated[24]. This is important given that in some RTT patients, disease regression has been described as being transient or often goes unrecognised[50]. Others such as the RSBQ although are suitable to measure some aspects of behaviour such as mood and anxiety[51] 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[52]. 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[53].

Autonomic Function in RTT

Large cross-sectional studies investigating the genotype-phenotype relationships have revealed divergence in the phenotype seen in individuals with RTT[54,55]. 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[24]. Some mutations or variants dictate a more severe phenotype when it comes to motor abilities[24,54,55] and cardio-respiratory phenotypes[24,56]. Moreover, at present it is unknown whether autonomic dysfunction is governed by any specific mutation in RTT[24,56], 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)[57], 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

emotional and behavioural dysregulation, and recently using wearable sensor technology, we have shown that Emotional, Behavioural and Autonomic Dysregulation (EBAD) is a crucial factor that needs to be considered when managing patients with RTT[58,59]. Although autonomic dysfunction has been investigated in individuals with RTT[60-63], the progression of autonomic dysfunction and the developmental trajectory of EBAD has never been researched. Moreover, the components of EBAD in a questionnaire that can map across other symptomatology longitudinally in individuals with RTT has not previously been shown.

Aim

The objective of this protocol 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[58,59] will be linked to data obtained from the REST questionnaire, genetic data and also 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[40,52] and standardised RTT questionnaires – data from the Natural History Study[3], Rett Syndrome Behaviour Questionnaire (RSBQ)[38] and the modified version of the Rett Syndrome Severity Scale (RSSS)[42]. It is anticipated that the questionnaire will not take more than 30 minutes to complete.

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

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

Concept identification

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

Concept elicitation

This stage will involve parents/carers of individuals aged between 6 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[67], has been successfully trialled in multi-centric European Union FP7 studies[68,69] and also in a questionnaire development and validation study[70]. 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 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, in particular, the developmental trajectory of EBAD. Nor do these existing questionnaires/scales attempt to marry this with the physiological measurements from the wearable sensor technology. At this stage, a further literature review will be conducted to identify any themes that may have been missed during the focus groups and whether any further areas of RTT symptomatology that were not

highlighted in the focus groups needs to be addressed. In addition, parents/carers from Reverse Rett, UK, will be consulted and any feedback incorporated into the tool review stage. Following the focus groups, study participants will be sent a copy of the draft version of the questionnaire (via email or post). Once this part has been completed, a draft operating beta version of the questionnaire will be finalised.

Stage 2: Validation of the Rett Evaluation of Symptoms and Treatments (REST) questionnaire

This stage of the study will involve parents/carers of individuals aged between 6 to 40 years with RTT. Questionnaires to assess the longitudinal trajectory of symptomatology in rare diseases have proven to be difficult to validate[71,72]. To broach this conundrum, it is important to focus on the symptoms and not just the clinical diagnosis, and be able to evaluate the symptom level across other comparable patient groups. At the neuronal level, the brains of RTT and ASD patients share many core features[73]. Both RTT and ASD exhibit behaviours that might overlap i.e. there are deficits in social behaviour and speech and in both cases individuals may share common stereotypical behaviours[74]. Due to these similarities and based on consultation with clinicians with expertise in ASD, as a comparator group, this stage of the study will also include parents/carers/partners of individuals aged between 6 to 40 years with ASD with significant intellectual disability. It will also involve clinicians who see patients with RTT and ASD who will test the clinician version of the questionnaire. Participants (parents/carers and clinicians) will be recruited to complete the respective versions of the Rett Evaluation of Symptoms and Treatments (REST) questionnaire as well as other standardised questionnaires – namely the RSSS and the RSBQ (Table 1). The RSSS[37,42,43,75] and the RSBQ[38,51] have previously been used in studies with RTT patients. Pertinent information will also be taken from the RTT Natural History Study[31,32]. It is anticipated that 50 participants in the RTT cohort and 50 in the ASD with significant

intellectual disability cohort will complete the questionnaire battery. Although there is significant symptom overlap in patients with RTT and ASD, 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.

The questionnaire battery will be presented to study participants in HealthTracker™, a multi-modal web-based portal for remote online completion using developmentally appropriate interfaces. Participants will be given a unique ID number and log-in information and will be asked to complete the questionnaires independently. The research team will be able to support participants with questionnaire completion should they need it. Where applicable, participants will also be able to complete paper versions of the questionnaires if they request them. Participant medical records will be accessed only by members of the study team to validate the questionnaire against details of diagnoses obtained from patient case notes as well as against the Development and Well-being Assessment (DAWBA)[76], and treatment/medication status if they are available in case notes. Patient records will also be used to gain genetic information on the specific mutation and diagnosis. Consent will be obtained to access medical notes.

All participants will be asked to complete the questionnaire battery, at baseline, again after 1 week and then between 4 and 6 months after first completion to assess questionnaire stability.

Stage 3: Wearable Sensor Technology

The use of wearable sensor technology to improve treatment outcomes has gathered momentum in recent years[77] and is currently being used to develop new outcome measures in patients with complex neurodisability such as Amyotrophic Lateral Sclerosis[78]. Using wearable sensor technology as a PROM is not without its challenges. In RTT, wearable technology has been used to explore respiratory and cardiac function in observational studies[79,80] and in two recent clinical trials[46,47], however, inherently captured biometric data

can be noisy especially from quasi-periodic oscillations from cardiac rhythms. Wrist worn devices might be particularly susceptible to this type of noise. To mitigate these issues, we have applied the methods described previously[81,82] to analyse heart rate variability and electrodermal activity as metrics when evaluating wrist sensor biometric data and autonomic function in a 15 year old girl with RTT. We were able to demonstrate a recalibration of the autonomic equilibrium from pre-treatment to post-treatment using buspirone (30 mg/day)[58], and subsequent improvement in EBAD in this girl. Quasi-periodic oscillations cannot be easily quantified using conventional methods. To manage this phase-rectified signal averaging (PRSA)[83] may be used in conjunction with spectral factorization and applied to the beat-to-beat RR interval data, which is particularly prone to extraneous noise. This methodology coupled with EDA assessment will provide more sensitive methods to capture changes in autonomic physiology in patients with RTT. In the context of this study, the outcomes of the wearable sensor technology will marry into the outcomes of the newly developed questionnaire (REST), with psychosocial and genetic data to create the TRIAL database. The technology will be evaluated in individuals with RTT, ASD and healthy controls.

Sample size

Justification for Sample Size

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

Stage 1: Questionnaire Development Stage

It is anticipated that the total number of participants for the questionnaire development stage of the study will be between 10-20 (including participants and clinicians). In our experience, focus groups involving families with children with rare diseases are well versed with the problems associated with the condition in question. Often, the themes that need to be

addressed get saturated after a couple of focus groups, leading to us getting the basic structure of the items needed to be tested in Stage 2.

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 clinicians working with patients with RTT. Depending on the nature of the focus groups about 4-6 participants will take part in each focus group.

Questionnaire Development Stage - Inclusion Criteria

- 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.

Questionnaire Development Stage - Exclusion Criteria

- 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.

Stage 2: Questionnaire Validation Stage Recruitment

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

Questionnaire Validation Stage - Inclusion Criteria

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

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 my 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[65]. 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[84].

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 ≥ 0.85 will be indicative of reasonable evidence of internal reliability[85]. Where applicable, 'alpha if deleted analyses' will be perfomed to see if omitting any item(s) from the (subthemes of the) questionnaire would strengthen the measure.

Test-retest reliability

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

Validity

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

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[87]. Many recommendations have been put forward regarding sample sizes but there does not seem to be an overall consensus[88]. Some have suggested improbable sample sizes that would not be feasible for studies of rare and complex genetic diseases[89]. 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[90] 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[90] and those used in exploratory studies as described recently[91].

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™ platform the TRIAL database will provide all the necessary information to clinicians and researchers about different aspects of

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

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Author Contributions

JS drafted, wrote and revised 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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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

INSERT FIGURE 1 HERE

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

		Administered to:					
Measure	**Key Information**	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[32]	More than 1000 participants with RTT providing information on important aspects of disorder symptomatology						**X**
Rett Syndrome Behavioural Questionnaire (RSBQ)[38].	Provides an accurate measure of the behavioural features of RTT				**X**	**X**	
Rett Syndrome Severity Score (RSSS)[42]	Provides information on the overall clinical severity and severity across individual parameters: • frequency and manageability of seizures; • respiratory abnormalities • scoliosis; • ability to walk; • hand use; • speech; • sleep hygiene						**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

Administration Time (minutes)							

* 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)

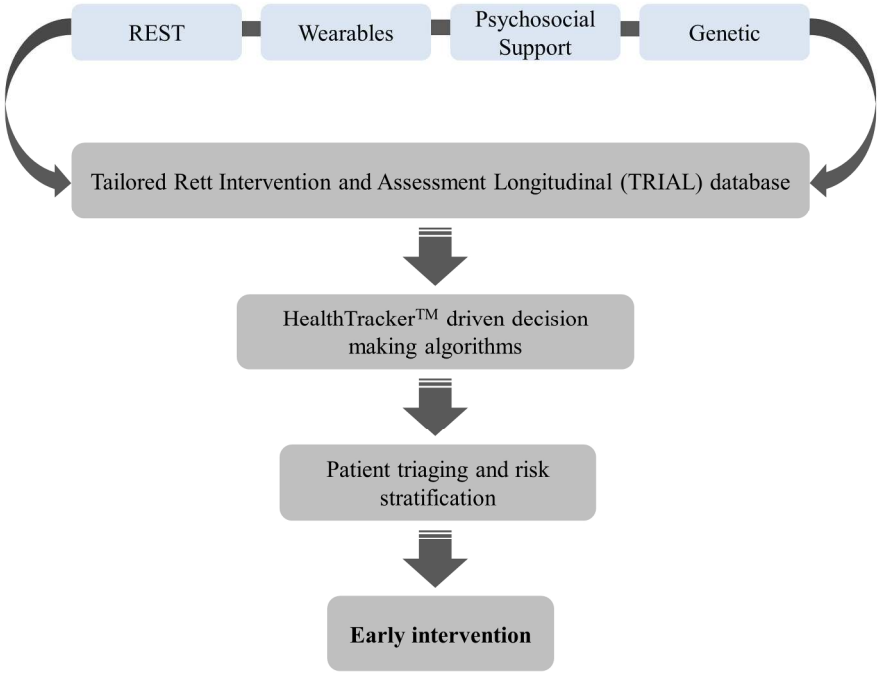

Figure 1: Flow diagram illustrating the sequence of steps showing how data obtained from the TRIAL database can be used to provide timely intervention to streamline treatment outcomes in patients with RTT syndrome

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

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The term 'wearables' has been coined for wearable sensor technology
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190x254mm (300 x 300 DPI)

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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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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™; 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.

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).

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)[1] where Dr. Rett first observed the clinical signs and symptoms of RTT. Symptoms usually appear between 6 to 18 months after birth[2] 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[3]. There are geographical variations[4] with one Australian study indicating a prevalence of about 1:9000[5]. 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[6], with rarer cases being attributed to mutations in CDKL5 and FOXG1 gene[7,8] 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[9]. MeCP2 is a highly conserved nuclear protein abundant in the mammalian brain[10] and notably the disorder is reversible in mice models of RTT[11].

MeCP2 acts as a critical epigenetic modulator in the mammalian brain, controlling overlapping mechanisms such as DNA methylation and post-translational mechanisms[12]. Through differential post-translational modification at serine 164[13], MeCP2 may help in limiting transcriptional noise[14] 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[15] and the $MECP2^{R306C}$ mutation prevents MeCP2 from interacting with the NCoR/histone deacetylase 3 (HDAC3) complex resulting in impediments in social and cognitive functioning in animal models[16]. *MeCP2* has complex genome level modalities, and the general opinion is that loss of the

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

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

Although there have been considerable advances in understanding the genetics and into the genetic testing of RTT, the diagnosis of RTT is based on the 2010 revised consensus clinical criteria[3] (see Table 1 in Neul *et al*., 2010) and recommends that all individuals with RTT should be first be assessed according to the revised clinical criteria followed by a thorough genetic test for *MECP2*. Given that about 3-5% of RTT individuals who fulfil the diagnostic clinical criteria do not have *MECP2* mutations, and this is even higher for atypical RTT cases[25], more recently clinical predictors that can facilitate a clinician's decision making to order genetic testing for RTT have been provided[26]. This showed that the likelihood of a having a positive *MECP2* test was greatest in patients with partial or complete attenuation of

hand skills. Impairments in gait and hand stereotypies were also strong predictors. Of interest was that loss of speech did not discriminate whether an individual was *MECP2+* or *MECP2-*.

Pre-existing measures in RTT

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

Capture of disease severity and sensitivity to change throughout the lifespan in patients are important elements that need to be considered when developing clinically meaningful outcome measures. The Unified Parkinson Disease Rating Scale, (UPDRS) is a good example

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

describe disease regression having not been validated[24]. This is important given that in some RTT patients, disease regression has been described as transient or often goes unrecognised[50]. Others such as the RSBQ although are suitable to measure some aspects of behaviour such as mood and anxiety[51] 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[52]. 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[53].

Autonomic Function in RTT

Large cross-sectional studies investigating the genotype-phenotype relationships have revealed divergence in the phenotype seen in individuals with RTT[54,55]. 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[24]. Some mutations or variants dictate a more severe phenotype when it comes to motor abilities[24,54,55] and cardio-respiratory phenotypes[24,56]. Moreover, at present it is unknown whether autonomic dysfunction is governed by any specific mutation in RTT[24,56]. 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)[57], 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 emotional and behavioural dysregulation, and recently using wearable sensor technology, we have shown that Emotional, Behavioural and Autonomic Dysregulation (EBAD) is a crucial

factor that needs to be considered when managing patients with RTT[58,59]. Although autonomic dysfunction has been investigated in individuals with RTT[60-63], the progression of autonomic dysfunction and the developmental trajectory of EBAD has never been researched. Moreover, the components of EBAD in a questionnaire that can map across other symptomatology longitudinally in individuals with RTT has not previously been shown.

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[58,59] 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[40,52] and

standardised RTT questionnaires – data from the Natural History Study[3], Rett Syndrome Behaviour Questionnaire (RSBQ)[38] and the modified version of the Rett Syndrome Severity Scale (RSSS)[42]. It is anticipated that the questionnaire will not take more than 30 minutes to complete.

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

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

Concept identification

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

Concept elicitation

This stage will involve parents/carers of individuals aged between 6 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/carers 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[67], has been successfully trialled in multi-centric European Union FP7 studies[68,69] and in a questionnaire development and validation study[70]. 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 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, in particular, the developmental trajectory of EBAD. Nor do these existing questionnaires/scales attempt to marry this with the physiological measurements from wearable sensor technology. At this stage, a further literature review will be conducted to identify any themes that may have been missed during the focus groups and whether any further areas of RTT symptomatology that were not highlighted in the focus groups needs to be addressed. In addition, parents/carers from Reverse Rett, UK, will be consulted and any feedback incorporated into the tool review stage.

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

Stage 2: Validation of the Rett Evaluation of Symptoms and Treatments (REST) questionnaire

This stage of the study will involve parents/carers of individuals aged between 6 to 40 years with RTT. Questionnaires to assess the longitudinal trajectory of symptomatology in rare diseases have proven to be difficult to validate[71,72]. To broach this conundrum, it is important to focus on the symptoms and not just the clinical diagnosis, and be able to evaluate the symptom level across other comparable patient groups. At the neuronal level, the brains of RTT and ASD patients share many core features[73]. Both RTT and ASD exhibit behaviours that might overlap i.e. there are deficits in social behaviour and speech and in both cases individuals may share common stereotypical behaviours[74]. Due to these similarities and based on consultation with clinicians with expertise in ASD, as a comparator group, this stage of the study will also include parents/carers/partners of individuals aged between 6 to 40 years with ASD with significant intellectual disability. It will also involve clinicians who see patients with RTT and ASD who will test the clinician version of the questionnaire. Participants (parents/carers and clinicians) will be recruited to complete the respective versions of the Rett Evaluation of Symptoms and Treatments (REST) questionnaire as well as other standardised questionnaires – namely the RSSS and the RSBQ (Table 1). The RSSS[37,42,43,75] and the RSBQ[38,51] have previously been used in studies with RTT patients. Pertinent information will also be taken from the RTT Natural History Study[31,32]. It is anticipated that 50 participants in the RTT cohort and 50 in the ASD with significant intellectual disability cohort will complete the questionnaire battery. Although there is significant symptom overlap in patients with RTT and ASD, 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.

The questionnaire battery will be presented to study participants in HealthTracker™, a multi-modal web-based portal for remote online completion using developmentally appropriate interfaces. Participants will be given a unique ID number and log-in information and will be asked to complete the questionnaires independently. The research team will be able to support participants with questionnaire completion should they need it. Where applicable, participants will also be able to complete paper versions of the questionnaires if they request them. Participant medical records will be accessed only by members of the study team to validate the questionnaire against details of diagnoses obtained from patient case notes as well as against the Development and Well-being Assessment (DAWBA)[76], and treatment/medication status if they are available in case notes. Patient records will also be used to gain genetic information on the specific mutation and diagnosis. Consent will be obtained to access medical notes.

All participants will be asked to complete the questionnaire battery, at baseline, again after 1 week and then between 4 and 6 months after first completion to assess questionnaire stability.

Stage 3: Wearable Sensor Technology

The use of wearable sensor technology to improve treatment outcomes has gathered momentum in recent years[77] and is currently being used to develop new outcome measures in patients with complex neurodisability such as Amyotrophic Lateral Sclerosis[78]. Using wearable sensor technology as a PROM is not without its challenges. In RTT, wearable technology has been used to explore respiratory and cardiac function in observational studies[79,80] and in two recent clinical trials[46,47], however, inherently captured biometric data can be noisy especially from quasi-periodic oscillations from cardiac rhythms. Wrist worn devices might be particularly susceptible to this type of noise. To mitigate these issues, we

have applied the methods described previously[81,82] to analyse heart rate variability and electrodermal activity as metrics when evaluating wrist sensor biometric data and autonomic function in a 15-year-old girl with RTT. We were able to demonstrate a recalibration of the autonomic equilibrium from pre-treatment to post-treatment using buspirone (30 mg/day)[58], and subsequent improvement in EBAD in this girl. Quasi-periodic oscillations cannot be easily quantified using conventional methods. To manage this, phase-rectified signal averaging (PRSA)[83] may be used in conjunction with spectral factorization and applied to the beat-to-beat RR interval data, which is particularly prone to extraneous noise. This methodology coupled with EDA assessment will provide more sensitive methods to capture changes in autonomic physiology in patients with RTT. In the context of this study, the outcomes of the wearable sensor technology will marry into the outcomes of the newly developed questionnaire (REST), with psychosocial and genetic data to create the TRIAL database. The technology will be evaluated in individuals with RTT, ASD and healthy controls.

Sample size

Justification for Sample Size

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

Stage 1: Questionnaire Development Stage

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

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

clinicians working with patients with RTT. Depending on the nature of the focus groups about 4-6 participants will take part in each focus group.

Questionnaire Development Stage - Inclusion Criteria

- 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.

Questionnaire Development Stage - Exclusion Criteria

- 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.

Stage 2: Questionnaire Validation Stage Recruitment

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

Questionnaire Validation Stage - Inclusion Criteria

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

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 my 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[65]. 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[84].

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 ≥ 0.85 will be indicative of reasonable evidence of internal reliability[85]. Where applicable, 'alpha if deleted analyses' will be perfomed to see if omitting any item(s) from the (subthemes of the) questionnaire would strengthen the measure.

Test-retest reliability

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

Validity

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

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[87]. Many recommendations have been put forward

regarding sample sizes but there does not seem to be an overall consensus[88]. Some have suggested improbable sample sizes that would not be feasible for studies of rare and complex genetic diseases[89]. 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[90] 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[90] and those used in exploratory studies as described recently[91].

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.

Study dates

The study is expected to complete by January 2019.

Dissemination

The goal of this study is to develop and validate a new RTT questionnaire. Data from the REST questionnaire will be linked with the data from the wearable sensor technology as well as psychosocial and genetic information to construct the TRIAL database, which will improve the overall healthcare delivery for individuals with RTT. Using the functionality of the HealthTracker™ platform, the TRIAL database will provide all the necessary information to clinicians and researchers about different aspects of the disease and serve as a barometer for improving treatment pathways in individuals. This will allow algorithms to be developed alerting parent/carers to request unscheduled clinician appointments when symptoms deviate

significantly from one another thereby streamlining the patient care pathway. As the HealthTracker™-based TRIAL database is web-based, with appropriate funding, it has the potential to be used globally, allowing for quicker development of decision-support analytics and personalized care.

Rare disorders such as RTT have a limited patient population and it is therefore crucial for patients to be stratified using phenotype and biomarkers (such as those obtained through wearable sensor monitoring). Adaptive clinical trial design using Bayesian methodology has been suggested to augment the statistical power and decrease the number of patients required for a rare disease trial[72]. In this view, the TRIAL database will serve for the recruitment of patients into clinical trials, as baseline information would already be available so the clinical trial can be conducted with fewer patients and in a more cost-effective manner.

Results stemming from this study will be disclosed unreservedly and the findings published in scientific journals and will also be presented in meetings and conferences for professionals, patients as well as carers and families. Reverse Rett UK will lead on wider dissemination of the applied research findings to engage policy-makers, key professional groups and service managers, and parents/carers of children with RTT.

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Author Contributions

JS drafted, wrote and revised 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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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 the Chief Executive Officer 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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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

INSERT FIGURE 1 HERE

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

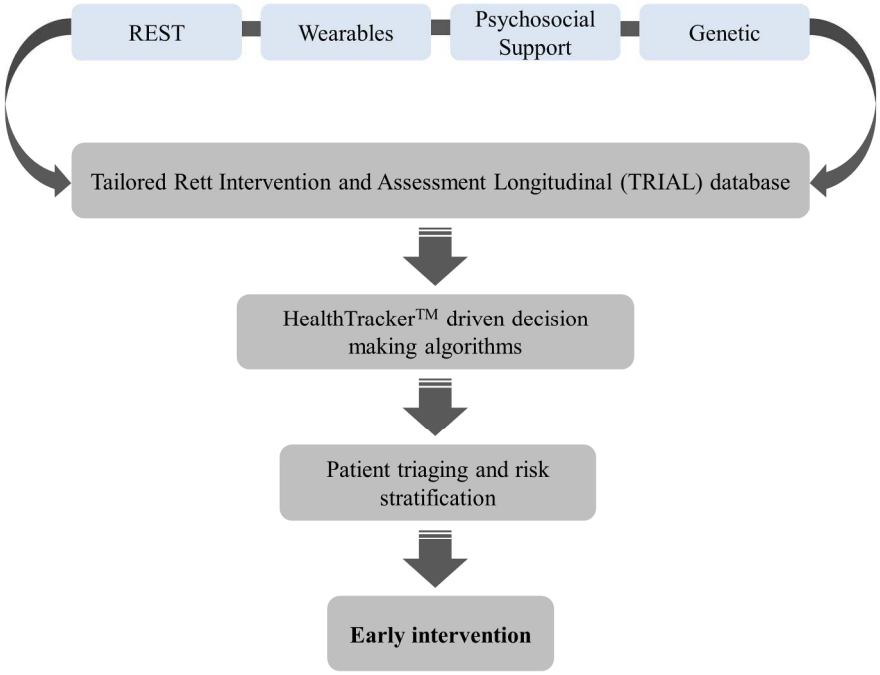
Table 1: Measures to be administered during Stage 2 (Validation) and Stage 3 (Wearable Sensor Technology) of the Study

		Administered to:					
Measure	**Key Information**	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[32]	More than 1000 participants with RTT providing information on important aspects of disorder symptomatology						**X**
Rett Syndrome Behavioural Questionnaire (RSBQ)[38].	Provides an accurate measure of the behavioural features of RTT				**X**	**X**	
Rett Syndrome Severity Score (RSSS)[42]	Provides information on the overall clinical severity and severity across individual parameters: • frequency and manageability of seizures; • respiratory abnormalities • scoliosis; • ability to walk; • hand use; • speech; • sleep hygiene						**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

Administration Time (minutes)							

* 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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190x254mm (300 x 300 DPI)