Review Paper

Autonomic dysfunction and sudden death in patients with Rett syndrome: a systematic review

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Background: Rett syndrome (RTT), a debilitating neuropsychiatric disorder that begins in early childhood, is characterized by impairments in the autonomic nervous system that can lead to sudden unexpected death. This study explores the mechanisms of autonomic dysfunction to identify potential risk factors for sudden death in patients with RTT. **Methods:** Following the Reporting Items for Systematic Review and Meta-Analyses (PRISMA) criteria, we undertook comprehensive systematic reviews using the PubMed, Scopus, Cochrane, PsycINFO, Embase and Web of Science databases. **Results:** We identified and critically appraised 39 articles for autonomic dysfunction and 5 for sudden death that satisfied the eligibility criteria. Following thematic analysis, we identified 7 themes: breathing irregularities, abnormal spontaneous brainstem activations, heart rate variability metrics, QTc changes, vagal imbalance, fluctuation in peptides and serotonergic neurotransmission. We grouped these 7 themes into 3 final themes: (A) brainstem modulation of breathing, (B) electrical instability of the cardiovascular system and (C) neurochemical changes contributing to autonomic decline. We described key evidence relating to each theme and identified important areas that could improve the clinical management of patients with RTT. **Limitations:** The heterogeneity of the methods used to assess autonomic function increased the difficulty of making inferences from the different studies. **Conclusion:** This study identified the important mediators of autonomic dysfunction and sudden death in patients with RTT. We proposed brainstem mechanisms and emphasized risk factors that increase brainstem vulnerability. We discussed clinical management to reduce sudden death and future directions for this vulnerable population.

Introduction

Rett syndrome (RTT) is a childhood neurologic disorder that occurs in about 1 in 10 000 of the population.[1] In most cases, a sporadic de novo mutation in the gene responsible for global modulation of chromatin architecture, methyl-CpG binding protein 2 (*MECP2*),[2,3] has been recognized as the main cause. By interacting with more than a few dozen other proteins,[4] *MECP2* represents the molecular ground zero for driving the development of neuronal genes. Defects in this process are largely in part due to the reduced affinity of MeCP2 for linking DNA to the NCoR/SMRT complex.[5,6] This impairment leads to excessive transcriptional noise[7] that inadvertently has a negative impact on the regulation of neuronal gene length. The failure of *MECP2* to dampen this "noise" ultimately compromises neurogenesis and neuronal migration, and the disrupted neuronal integrity can manifest clinically as the deleterious motor, gastrointestinal and cardiorespiratory phenotypes that are frequently observed. In RTT, the expression of normal and mutated *MECP2* varies widely from patient to patient.[8,9] Recent studies in animal models have further suggested that a precision medicine approach is required to stratify patients into subgroups by type of *MECP2* mutation.[10] The mutational profile of *MECP2* and the underpinning clinical symptoms have proven to be significant obstacles for developing viable therapeutic strategies. And yet, the severity of clinical symptoms is variable even when the genotype–phenotype relationship has been determined.[11] Thus, a truly holistic multimodal approach is warranted to manage clinical outcomes in patients with RTT.

The range of emotional, behavioural and autonomic dysregulation (EBAD) seen in patients with RTT remains a clinical challenge for treating and improving the quality of life of these patients. The EBAD can emerge with a multitude of symptoms on the emotional, behavioural and autonomic spectrum. These can include repetitive rocking, screaming, agitation, anxiety, low mood, breathing impairment, cardiac issues, temperature abnormalities and gastrointestinal disturbances. Autonomic dysfunction is likely to have a pivotal functional impact on these symptoms. Despite this, of the 34 clinical trials done in patients with

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RTT, very few evaluated the impact of autonomic dysfunction,[12] and none assessed the effect it might have on EBAD and patient outcomes.

Given that the developmental symptomatology of RTT changes across the lifespan of the disorder, mapping the trajectory of EBAD longitudinally would be beneficial in terms of funnelling patients to the correct specialists sooner, so that treatment can be initiated more quickly. This factor is critical, because in a recent web-based questionnaire survey involving 413 families with a family member that had RTT, only 31 patients had received some form of treatment for autonomic disturbances, such as medications, rebreathing apparatus or noninvasive ventilation.[13] New measures might be able to assist in optimizing treatment strategies and stratifying patients for clinical trials.[14]

At present, no studies to our knowledge have focused on investigating the quality of evidence for autonomic dysregulation in patients with RTT. Autonomic dysregulation is likely to be a critical driver for the diverse symptoms exhibited by patients with RTT. The autonomic component can be exploited and targeted therapeutically using biometric-guided therapy to manage EBAD in patients with RTT[15,16] and in those with multi-comorbid neurodevelopmental disorders.[17] This is important given that the autonomic component of EBAD is pervasive and has widespread effects on multiple overlapping systems. In RTT, the magnitude of behavioural and emotional dysregulation is large,[18] and nearly all patients with the classical RTT phenotype have some degree of cardiorespiratory impairment.[19,20] Further evidence has indicated that cardiac abnormalities are caused by MeCP2 dysfunction in the autonomic nervous system rather than a direct reflection of mutations in cardiomyocytes themselves,[21] and as the disease progresses in patients with RTT, there is subclinical biventricular impairment that is thought to be caused in part by neuroinflammation.[22] These findings underscore the premise that the autonomic component of EBAD might play a key role in initiating the sudden unexpected cardiac death that affects about 25% of patients.[23] Some evidence has also suggested a link between sudden cardiac death from ventricular arrhythmias and cardiac autonomic imbalance in patients with RTT.[24] More recent evidence has pointed out that more than half of the deaths documented in the United States natural history study, which represents more than 10% of the United States population with RTT, were unexpected and mostly due to some form of cardiorespiratory distress.[19,25] Other data have suggested that there has been little change in survival rates in RTT over the last 30 years,[26] and given that about 18% of patients with RTT show abnormalities in cardiac repolarization (QT),[21] understanding the pathophysiological mechanisms of autonomic dysfunction might help in managing the abnormal cardiac problems seen clinically that can lead to sudden death.

The number of reviews exploring changes in the autonomic nervous system in patients with RTT are limited,[27,28] and as far as we are aware, no study has systematically assessed the quality of research in this area. Therefore, the aim of this review was to identify studies that investigated autonomic issues and the possible causes of sudden death in patients with RTT, to evaluate the quality of the research, to critically appraise the extant literature and identify emerging themes, and to identify important brainstem mechanisms to provide clinical insight and reduce sudden death in this vulnerable population. Understanding the neural mechanisms of autonomic dysfunction and sudden death is critical for the clinical management of these patients.

Methods

We performed 2 separate systematic reviews using the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) criteria.[29] The first review examined autonomic dysfunction, and the second focused on the causes of sudden death in patients with RTT.

Article selection

The selection of articles for each systematic review was performed by the first (J.S.) and the second author (E.L.) independently. Database searches were done in a blinded manner using the same search terms. Once the eligible articles had been identified independently by J.S. and E.L., any doubts were resolved during a meeting to review the queried article(s) against the inclusion and exclusion criteria. The final selection of eligible articles included in the critical appraisals was made based on agreement between J.S. and E.L.

Autonomic dysfunction in RTT: systematic review

The electronic search to identify and capture pertinent literature regarding autonomic dysfunction in RTT was performed in November and December 2018. We used the following search terms: Rett syndrome OR MECP2 AND autonomic dysfunction OR autonomic dysregulation.

We searched the PubMed, Scopus, Cochrane, PsycINFO, Embase and Web of Science databases. No year restrictions were applied. To make the search as comprehensive as possible, we used the Boolean operators "AND" and "OR" to link the search terms.

Studies in which patients had a diagnosis of RTT (genetically or clinically) were included. All studies that reported autonomic dysfunction were included.

Sudden death in RTT: systematic review

We followed a similar procedure for the second systematic review, which we performed in January and February 2019. To capture electronic records related to causes of sudden death in patients with RTT, we used the following search terms: Rett syndrome OR MECP2 AND sudden death AND cause.

We searched the same databases as in the first systematic review: PubMed, Scopus, Cochrane, PsycINFO, Embase and Web of Science. No year restrictions were applied, and we used the Boolean operators "OR" and "AND" to link the search terms.

Studies in which patients had a diagnosis of RTT (genetically or clinically) were included. All studies that reported sudden death and cause were included.

Eligibility criteria

Inclusion criteria were as follows: English-language articles in peer-reviewed academic/scientific journals, full-text articles available electronically in English.

Exclusion criteria were as follows: studies done in animal models; studies using *CDKL5* variants; conference abstracts, case reports/summaries, review articles, articles in press and book chapters.

Data extraction

We extracted information relating to autonomic dysfunction and sudden death from the included articles, as well as key evidence relating to the methods used, the number of study participants and the core findings from the applicable studies.

Quality assessment of eligible articles

We critically appraised the eligible articles using a checklist based on the appraisal criteria developed by the Joanna Briggs Institute[30] for the evaluation of studies included in systematic reviews.[31] This is a valid instrument consisting of 10 criteria. It has been used recently for the quality assessment of studies in a systematic review assessing the quality of life in families affected by RTT and to provide a comprehensive analysis of the quality-of-life subdomain that was deemed to be most affected in RTT.[32] For the quality appraisal, we added an item relating to conflicts of interest (i.e., factors such as industry sponsorship, professional conflicts, intellectual conflicts or others that might unduly affect the design, analysis or reporting of the study). Conflict of interest is an important consideration when critically appraising evidence, but its presence does not necessarily mean that a study has a high risk of bias. Other factors also need to be taken into consideration when determining whether studies have a low, moderate or high risk of bias.[33] The 11 criteria used to assess the quality of eligible articles for each systematic review are presented in Appendix 1, Figure S1, available at jpn.ca/190033-a1.

Identification of themes

Following quality appraisal of the articles, we used a grounded-theory approach to look for interconnections and relationships from the data in the eligible articles. This approach to thematic analysis is a recognized method of generating themes directly from data[34] and uses colour to code for words and terms as a way of identifying themes. The first and second author performed coding manually; they then reviewed the key findings from the eligible articles for themes and organized the themes into categories. The final theme categories that emerged were based on consensus among all authors. The frequency of each theme identified was presented using Excel (Microsoft).

Results

Autonomic dysfunction in RTT: study characteristics and quality assessment

The PRISMA flow-chart for the systematic review of autonomic dysfunction in RTT is presented in Appendix 1, Figure S2. The database search revealed 876 records, and we identified 3 additional records[20,35,36] from other sources. After duplicates were removed (n = 150), we screened the titles and abstracts of 729 records and excluded 670. We then assessed the abstracts and titles of the remaining 59 full-text articles for eligibility against the inclusion and exclusion criteria, and excluded a further 20 full-text articles. We included 39 full-text articles for analysis; their characteristics, including relevant commentary pertinent to autonomic physiology in patients with RTT, are provided in Table 1.

Characteristics of the eligible articles

Studies exploring autonomic physiology in patients with RTT were multi-dimensional (Table 1). The methods used varied, and numerous topics were explored in patients across different age ranges. The studies were quite diverse in nature and ranged from investigations of breathing disturbances in 10% to 20% of the United States RTT population[19] to analyzing genotype–phenotype relationships in 4 European countries between 1999 and 2012,[54] to numerous physiology-based studies that focused on comparing metrics of autonomic function in patients with RTT and typically developed participants or controls. Given the multifaceted role of the autonomic nervous system, providing an accurate measure of autonomic physiology and autonomic dysfunction requires that measurement methods capture a variety of outputs from the system's sympathetic and parasympathetic arms. This requirement was reflected in the 39 articles. A variety of methods were used to assess autonomic physiology in patients with RTT. More than 60% of the studies (24/39) used some type of cardiac and/or respiratory metric in patients.[20,35,37,38,40,41,43,47–52,54,56,57,59–61,63,65,66,68,69]

Quality assessment of articles

A full assessment of each article is presented in Table 2. Important findings relevant to the criteria are described below.

Appropriate patient recruitment

Many of the studies recruited patients appropriately, basing inclusion on a clinical or genetic diagnosis (or both) of RTT. In 1 study, the sample was mixed, consisting of people with *MECP2* (n = 118), *CDKL5* (n = 12), *FOXG1* (n = 1) and *MEF2C* (n = 1) mutations, and 19 who were mutation-negative.[20] In another study, it was uncertain whether the sample was characteristic of the sample population, because participants were X-linked with intellectual disability.[48] Five studies recruited 10 or fewer participants with RTT.[44,47,48,63,70] These studies met the eligibility criteria and were therefore included.

Table 1: Sample characteristics, assessment methods and key findings of the eligible studies for systematic review: autonomic dysfunction (part 1 of 6)

Study	*N*	Participant age	Mutation/diagnosis	Assessment methods*	Key findings†
Acampa et al.[37] (2008)	32 RTT (all F)	Mean ± SD: 12.1 ± 6.3 yr	Diagnosis confirmed using clinical criteria and *MECP2* molecular analysis	12-lead ECG with spectral components (VLF, LF, HF) Plasma leptin levels	Sympathovagal balance (LF/HF) was correlated with plasma leptin levels ($r = 0.44$; $p = 0.001$) Vagal activity (HF) was negatively correlated with plasma leptin levels ($r = –0.037$, $p = 0.03$) Plasma leptin levels were positively correlated with the LF component ($r = 0.047$, $p = 0.01$) The findings suggest that patients with RTT have increased plasma leptin levels, and this is coupled with a desynchronization of the ANS
Amir et al.[38] (2000)	71 sporadic, 7 familial	Mean ± SD: 13.0 ± 7.43 yr	Confirmed using diagnostic criteria	ECG, EEG and severity scale such as the CCSS	Clinical evaluation revealed that 41/44 patients had some type of breathing abnormality, and 36/42 patients presented with clinical findings akin to autonomic dysfunction (skin or temperature changes) A higher frequency of awake respiratory dysfunction was observed in patients with truncating mutations
Anderson et al.[39] (2014)	423‡ (all F)	≥ 18 yr	RTT confirmed by the presence of mutation or by consensus clinical criteria	Clinical comorbidities, such as breathing patterns, were used as an indication of autonomic dysfunction	Information on breathing patterns from the Australian RTT cohort showed disordered breathing patterns in 66.4% of participants, including 74.2% who hyperventilated and 88.7% with breath-holding or apneic episodes. In this group, 62.9% showed both hyperventilation and breath-holding Breathing patterns showed little variations in terms of the mutation present.
Bergström-Isacsson et al.[40] (2013)	29 RTT (27 F, 2 M) 11 non-clinical	Mean ± SD: RTT 16.58 ± 9.57 yr Non-clinical 3.36 ± 1.96 yr	23 classic RTT, 1 congenital, 3 variants, 2 male variants	Autonomic measurement using NeuroScope, along with EEG	ASBAs were observed in 28/29 patients with RTT, and in none of the non-clinical control group It was suggested that some facial expressions, such as false smiles, were the result of abnormal brainstem activation Facial expressions in patients with RTT caused by abnormal brainstem functioning can be differentiated from those originating from emotions
Bergström-Isacsson et al.[41] (2014)	29 RTT (27 F, 2 M) 11 non-clinical	Mean ± SD: RTT 16.58 ± 9.57 yr Non-clinical 3.36 ± 1.96 yr	23 classic RTT, 1 congenital, 3 variants, 2 male variants	NeuroScope, EEG and video recordings; CVT, CSB, MAP and coefficient of variation of MAP-CV were used to characterize brainstem responses to different musical stimuli	Mean baseline values of CVT, MAP-CV and CSB values were lower for patients with RTT compared to the non-clinical control group Mean ± SD MAP values were 58.36 ± 14.81 mm Hg in patients with RTT v. 47.74 ± 5.83 mm Hg in the non-clinical group Brainstem responses to music stimuli were diverse in patients with RTT; authors suggested that in RTT patients the responses to music (as assessed by brainstem assessment) were intact Study underscored the importance of music in patients with RTT
Cass et al.[42] (2003)	87	2–4 yr ($n = 26$) 5–9 yr ($n = 28$) 10–19 yr ($n = 13$) 20–44 yr ($n = 20$)	Classic RTT ($n = 76$), atypical or variant ($n = 11$)	Assessment scale that evaluated a wide range of areas, including breathing abnormalities cognitive skills, epilepsy etc.	Most common breathing dysregulations were hyperventilation and breath-holding The prevalence of breathing disturbances was highest in patients 5 to 9 yr and 10 to 19 yr Trend (albeit slight) for improvements in autonomic disturbances into adulthood
Crosson et al.[43] (2017)	100 (98 F; 2 M)	6.19 ± 3.60 yr (range 1–17 yr)	All patients positive for *MECP2* mutation	ECG for detailed QT measurements	Mean QTc (422.6 ms) did not exceed prolonged QTc values (defined as > 450 ms) QTc tended to increase with age and clinical severity ($p = 0.09$) QTc prolongation was not associated with the type of mutation ($p = 0.52$)
d'Orsi et al.[44] (2012)	8 (F)	Median (range): 14.5 yr (7–20 yr)	All patients had confirmed *MECP2* mutations	Video polygraphy to assess differences in epileptic and non-epileptic paroxysmal events	Video polygraphs were useful in pinpointing paroxysmal events that caused breathing and autonomic dysregulation in patients with RTT
Deguchi et al.[45] (2000)	14 RTT (F) 10 case controls§	RTT 6–35 yr Case controls 2 mo–29 yr	Diagnosis confirmed by pediatric neurologists	Immunohistochemical examination for substance P immunoreactivity	Substance P immunoreactivity was decreased in several anatomic brain and spinal cord regions in samples of patients with RTT but not in samples of controls Authors suggested that reduced substance P expression in these regions, particularly the solitary tracts and reticular formation, could be associated with autonomic dysfunction in patients with RTT Decreases in substance P in RTT could be secondary to defects in the serotoninergic neurotransmitter system

Table 1: Sample characteristics, assessment methods and key findings of the eligible studies for systematic review: autonomic dysfunction (part 2 of 6)

Study	*N*	Participant age	Mutation/diagnosis	Assessment methods*	Key findings†
Deguchi et al.[46] (2001)	14 RTT (F) 22 case controls§	RTT 5–41 yr Case controls 14 wk–31 yr	Molecular analysis of *MECP2* mutation was not available for each sample	Immunohistochemical examination of substance P, tyrosine hydroxylase and vasoactive intestinal peptide	Findings from immunohistochemical examination of substance P were not statistically different in the RTT and case control samples Authors suggested that gastrointestinal dysfunction (feeding impairment and constipation) in RTT was due to autonomic dysfunction originating from the brainstem
Djukic et al.[47] (2016)	10 F	Median (range): 14 yr (10–21 yr)	Genotype confirmed	Gait velocity, EEG and respiratory measurements	Following treatment with glatiramer acetate, EEG events decreased in all patients (4) who presented with these events at baseline Statistically significant improvement ($p \leq 0.03$) in memory and breath-holding index
Dotti et al.[48] (2004)	3 (1 F, 2 M)	F 44 yr M1 34 yr M2 29 yr	Family of siblings, all with X-linked intellectual disability due to *MECP2* mutation	12-lead ECG, spectral components (VLF, LF and HF) and QTc analyses	LF/HF values all elevated in patients compared to literature norms Anomalies in QTc and QTc dispersion also noted in patients
Guideri et al.[49] (1999)	54 RTT (F) 28 healthy controls (F)	Mean ± SD: RTT 10 ± 5.5 yr Healthy controls 9.7 ± 4.6 yr	Diagnosis confirmed by authors and clinical stage determined based on Hagberg and Witt-Engerstrom[71]	12-lead ECG, spectral components (VLF, LF and HF) and QTc analyses	LF/HF ratio significantly higher in patients with RTT compared to healthy controls (4 ± 2.9 v. 0.9 ± 0.6; $p < 0.0001$) HR variability (R-R interval) significantly lower in patients with RTT compared with healthy controls (616 ± 101 ms^2 v. 725 ± 120 ms^2; $p < 0.0001$) QTc elevated in patients with RTT compared to healthy controls (0.441 ± 0.02 s v. 0.40 ± 0.01 s; $p < 0.0001$) Decreased HR variability suggested to be a risk factor for sudden death
Guideri et al.[50] (2001)	74 RTT (F) 10 RTT with preserved speech 40 age-matched healthy controls (F)	Mean ± SD: RTT 10.2 ± 5.3 yr RTT with preserved speech 9.4 ± 6.8 yr Age-matched healthy controls 10.2 ± 4.9 yr	Diagnosis confirmed by authors and clinical stage determined based on Hagberg and Witt-Engerstrom[71]	12-lead ECG, spectral component (VLF, LF and HF) and QTc analyses	Patients with RTT: 31 were in stage II, 27 in stage III and 16 in stage IV LF/HF ratio significantly elevated in girls with classic RTT compared to healthy controls (4.1 ± 3.6 v. 0.9 ± 0.48; $p < 0.001$) Patients with RTT and preserved speech also showed increased LF/HF ratios compared to healthy controls (1.79 ± 0.9 v. 0.9 ± 0.48; $p < 0.02$) Prolonged QTc in 55% of patients with classic RTT and 20% of patients with preserved speech
Guideri et al.[51] (2004)	28 RTT (F)	Mean ± SD: 7.25 ± 3.4 yr¶	Diagnosis independently confirmed by 2 child neuropsychiatrists	12-lead ECG, spectral components (VLF, LF and HF) and QTc analyses Measurement of plasma serotonin levels	In treatment-naïve participants (no anticonvulsant therapy), plasma serotonin levels were negatively correlated with LF/HF ratio ($r = –0.629$; $p < 0.02$)
Guideri et al.[52] (2005)	22 RTT (F) (10 active treatment, 12 age-matched controls [placebo])	Mean ± SD: Active treatment 6.3 ± 4.3 yr Controls 6.3 ± 4.0 yr	Diagnosis independently confirmed by 2 child neuropsychiatrists	12-lead ECG, spectral components (VLF, LF and HF) and QTc analyses	In the control group, total power and LF were reduced and QTcD was significantly increased from baseline after 6–18 mo After active treatment for 6 mo using acetyl-L-carnitine, total power ($p = 0.01$), VLF ($p = 0.01$) and LF ($p = 0.009$) increased significantly Although the LH/HF ratio decreased in the active treatment group, this was not statistically significant compared to the control arm
Halbach et al.[53] (2008)	53**	> 16 yr	Clinical diagnosis of RTT	Questionnaire assessment	Autonomic manifestations (e.g., cold feet) identified in 96% of patients with RTT 50% of patients with RTT had some form of sleep disturbance. Although not statistically significant, the frequency of night-time unrest was more prevalent in participants aged 20–30 yr and 30+ yr 38% of patients had apnea, 39% had hyperventilation, 73% had breath-holding and 41% swallowed air. The frequency of apnea was lower in participants aged 30+ yr (23%; χ^2 test $p = 0.01$). Hyperventilation, breath-holding and air swallowing were not statistically different for the other age groups 39% of patients had night-time screaming, and 66% had frequency of mood changes Agitation was found in 54%

Table 1: Sample characteristics, assessment methods and key findings of the eligible studies for systematic review: autonomic dysfunction (part 3 of 6)

Study	*N*	Participant age	Mutation/diagnosis	Assessment methods*	Key findings†
Halbach et al.[11] (2012)	137 (F)	Mean (range): 14.9 yr (2–49 yr)	137 molecularly confirmed (73 with mutations in TRD, 41 with mutations in MBD, 15 with mutations in CTS and 8 with other mutations	Autonomic features assessed using the ISS autonomic function domain items Q–T (Q = disturbed awake breathing rhythm; R = peripheral circulation of extremities; S = mood disturbance; T = sleep disturbance)	Mean ± SD (range) score in autonomic domain: 3.28 ± 1.7 (0–8) Location of the mutation was not associated with mean ± SE scores in this ISS domain (MBD 3.0 ± 0.27; TRD 3.5 ± 0.20; CTS 2.7 ± 0.44; ANCOVA with Bonferroni correction: $p = 0.21$) No statistical significance in ISS item Q–T scores between mutations ($p = 0.47$) 81% of females had breathing problems (item Q), and genotype–phenotype analysis related to breathing revealed that participants were not different with respect to scores or across mutations (χ^2 test $p > 0.26$). Age at examination was not a confounder (χ^2 test $p = 0.18$) Poor peripheral circulation (item R) was evident in 56% of participants. Genotype–phenotype analysis revealed that participants were not different with respect to scores or across mutations (χ^2 test $p > 0.16$). Age at examination could be a confounder (χ^2 test $p = 0.004$) About 50% of participants had mood disturbances (item S). From this, 12% had agitation and crying spells. Genotype–phenotype analysis revealed that participants were not different with respect to scores or across mutations (χ^2 test $p > 0.06$). Age at examination was not a confounder (χ^2 test $p = 0.88$) Sleep disturbances (item T) were found in 50% of participants. Genotype–phenotype analysis revealed that participants were not different with respect to scores or across mutations (χ^2 test $p > 0.21$). Age at examination was not a confounder (χ^2 test $p = 0.25$).
Halbach et al.[54] (2016)	132 (F; from 4 EU countries, 1999–2012)	Mean ± SD: 12.46 ± 9.36 yr	Inclusion based on molecular confirmation; diagnosis based on consensus clinical criteria from Hagberg et al.[72] and Neul et al.[1]	Autonomic measurement using NeuroScope synchronized with EEG	Autonomic dysfunction was observed in all patients Heart rate values were within the normal range, suggesting a normal resting sympathetic tone Mean CVT was lower in patients compared with the normal mean value in young adults in previous studies The extent of autonomic dysfunction was not limited to a certain group or specific mutation
Hara et al.[55] (2011)	27 RTT (F) 53 typically developed controls	Mean ± SD: RTT 15.8 ± 8.3 yr Control 16.9 ± 10.2 yr	Genetic analysis for *MECP2* performed on all patients with RTT; diagnosis confirmed using clinical consensus criteria	Assays to measure plasma ghrelin, serum growth hormone and IGF-1	In patients with RTT, plasma levels of ghrelin were significantly lower than those in typically developing controls Ghrelin levels were also lower in RTT patients with eating difficulties and constipation compared to patients without these symptoms
Julu et al.[56] (2001)	56 RTT (F) 11 controls	RTT 2–35 yr Case controls 5–28 yr	RTT participants had classic criteria, except 6 with no deterioration in occipitofrontal circumference, 4 with stalled development (but without regression) and 4 with seizures pre-regression	NeuroScope and synchronized with EEG and video measurements	Mean HR and MAP at baseline were not different between RTT and controls Mean CSB was significantly lower in RTT patients than in controls ($p < 0.01$) Mean CVT was significantly lower in RTT patients than in controls ($p < 0.002$) Increased neuronal firing of brainstem neurons, denoted as “brainstem epilepsy,” was observed
Julu and Witt-Engerström[35] (2005)	72 (71 F, 1 M)	Mean (range): 17.3 yr (1–45 yr)	67 classic RTT, 3 atypical-like, 2 variants	NeuroScope plus other measures, such as head circumference and BMI	Parasympathetic tone (CVT and CSB) was statistically lower in the feeble phenotype compared to the forceful and apneustic phenotypes ($p < 0.05$). No difference in baseline sympathetic tone (HR and MAP) for different phenotypes ASBAs reflected by spikes in HR metrics were documented in 1 patient

Table 1: Sample characteristics, assessment methods and key findings of the eligible studies for systematic review: autonomic dysfunction (part 4 of 6)

Study	*N*	Participant age	Mutation/diagnosis	Assessment methods*	Key findings†
Kumar et al.[57] (2017)	24 RTT 24 healthy controls	Mean ± SD: RTT 9.06 ± 3.4 yr Healthy controls 9.75 ± 3.13 yr	Participants were positive for *MECP2* mutation; diagnosis based on revised criteria in Neul et al.[1]	Heart rate variability, head-up tilt test and cold pressor test to evaluate physiologic parameters of autonomic function in patients	A statistically significant difference was noted in sympathovagal imbalance (LF/HF ratio), with concomitant sympathetic overactivity in patients with RTT compared to healthy controls. Heart rate variability was also decreased in patients with RTT, and the mean of longest QTc interval (590.1 ms) was longer than that of the control group (489.35 ms)
Larsson et al.[58] (2005)	NA (questionnaire 125/178)††	Mean (range): 19.6 yr (2.5–54 yr)	Questionnaire sent to families that had a female member diagnosed with RTT	Questionnaire	Signs of autonomic dysfunction were documented: cold feet (n = 113), small feet (n = 97), bluish red feet (n = 78) and swollen feet (n = 38) Breath-holding, hyperventilation and aerophagia reported for 51%, 55% and 44% of cases, respectively For those who reported breathing abnormalities, 85% (35/41) started at ≤ 5 yr
Larsson et al.[59] (2013)	21 RTT (F) 14 typically developed, age-matched controls	Median (min– max): RTT 20 yr (5–46 yr) Typically developed 24 yr (5–43 yr)	21 participants with RTT (18 classic, 3 variants) diagnosed according to consensus clinical criteria from Hagberg et al.[72] and Kerr and Witt Engerstrom;[73] mutations confirmed in 16/18 participants with classic RTT phenotype	Autonomic measurement using the NeuroScope and EEG	No statistically significant differences between patients with RTT and typically developed controls in terms of orthostatic responses (HR, SBP, DBP and MAP) when getting up from a seated position or when standing up for 3 min ASBAs observed in 17/21 patients with RTT At baseline, patients with RTT had lower SBP and higher HR than typically developed controls Autonomic function was not compromised in patients with RTT when getting up from a sitting to standing position compared to typically developed controls
Larsson et al.[60] (2018)	12 RTT 14 healthy controls	Median (min– max): RTT 22 yr (5–46 yr) Healthy controls 24 yr (5–43 yr)	10 patients with typical/classic RTT and 2 with atypical/variant RTT mutations; mutations confirmed in 9/10 patients with typical RTT Mutations not confirmed in 1 patient with classic RTT (patient 22) and 2 patients with variant RTT (patients 11 and 14)	NeuroScope to measure autonomic responses while walking on a treadmill for 3 and 6 min Finger photoplethysmography to measure blood pressure Video recordings	Study based on only patients with RTT who were able to walk ASBAs present in 10/12 patients with RTT Patients with RTT could walk on a treadmill continuously for up to 6 min No statistically significant differences for CVT, MAP or CSB between the RTT and control groups at the end of the walking period Suggested that walking could foster regular breathing in patients with RTT
Mackay et al.[13] (2017)	NA (questionnaire; 413/482 families [85.7%] returned the questionnaire)	0–6 yr (n = 51) 7–12 yr (n = 133) 13–19 yr (n = 122) > 20 yr (n = 107)	All families had a member with a clinical diagnosis of RTT and a *MECP2* mutation (411 F, 2 M)	Web-based questionnaire through a research electronic data capture software program	Autonomic dysregulation was highly prevalent in patients with RTT. Breath-holding, hyperventilation and abdominal bloating reported in 68.8%, 46.4% and 42.4% of cases, respectively Abdominal bloating (44.1%), followed by breath-holding (35.8%) and hyperventilation (35.1%), considerably affected caregiver perception of quality of life Patients with the p.Arg294* mutation (n = 30) were most affected by breath-holding, hyperventilation and abdominal bloating
Mancini et al.[61] (2017)	26 RTT (34 randomized, 26 completed)	Median 10.5 yr (of total n = 34)	Clinical diagnosis and confirmation by *MECP2* genotyping	Primary outcome was change from baseline in AHI; ECG, respiration rate and oxygen saturation also evaluated	No statistically significant difference for AHI between the desipramine (high and low dose) and placebo treatment groups (p = 0.78)
Matsuishi et al.[62] (1997)	20 RTT 28 controls‡‡	Mean ± SD: RTT 5.1 ± 2.8 yr (n = 16); 27.8 ± 5.0 yr (n = 4) Controls 5.9 ± 2.6 yr (n = 11); 33.2 ± 8.4 yr (n = 17)	Confirmed through clinical diagnosis	Measurement of substance P and CSF levels	Substance P levels in the CSF were significantly lower in patients with RTT compared to controls Levels did not differ significantly by clinical stage

Table 1: Sample characteristics, assessment methods and key findings of the eligible studies for the systematic review: autonomic dysfunction (part 5 of 6)

Study	*N*	Participant age	Mutation/diagnosis	Assessment methods*	Key findings†
O'Leary et al.[63] (2017)	5 F	Mean (range): 6.56 yr (4.1–10.9 yr)	Clinical diagnosis (classical RTT); all in Hagberg III (post-regression) stage	EDA, HR, CSS and FLACC assessments	Autonomic responses (HR and EDA) to venipuncture was higher at baseline compared to vital-sign collection HR and EDA responses were not significantly correlated with CSS or FLACC scores
Paterson et al.[64] (2005)	11 RTT§ 7 controls§	Median (range): RTT samples 12 yr (5–24 yr) Case control samples 14 yr (3–26 yr)	For the serotonin transport binding study, mutations confirmed in 7 RTT samples; diagnosis in 3 samples was unknown—these were deemed RTT-like	Immunohistochemical and radioligand binding methodology	No differences in 5-HT cell number between RTT and case control samples Serotonin transporter binding (125I-RTI-55) decreased significantly in the dorsal motor nucleus of the vagus DMX across time ($p = 0.049$) in the control samples but not in the RTT samples ($p = 0.51$) Findings indicated a disordered serotoninergic neurotransmitter system in patients with RTT
Percy et al.[65] (1994)	25	Mean ± SD: Active group 5.8 ± 2.7 yr Placebo group 8.1 ± 4.6 yr	Diagnosis based on clinical criteria	Assessment scale and neurophysiological parameters such as EEG	Opiate antagonist (naltrexone) was suggested to modify some of the parameters related to respiratory dysfunction in patients with RTT
Pini et al.[20] (2016)	151 (149 F, 2 M)	Mean (range): 12 yr (1–49 yr)	Clinical diagnosis as described by Neul et al.[1] made by 2 independent experts in child neuropsychiatry; molecular analysis negative in 19 that had a clinical diagnosis	NeuroScope and severity scales (ISS and PBZ)	Differences were observed for the cardiorespiratory phenotypes among age groups. Breathing abnormalities such as hyperpnea, apnea and breath-holding were more important in younger girls (0–5 and 6–14 yr); feeble breathers were more predominant in older participants (14+ yr) Cardiac activity was significantly different between forceful and feeble breathers ($F = 3.08$; $p < 0.005$) CVT was significantly different in feeble and apneustic v. forced breathers ($F = 5.80$; $p < 0.005$)
Rohdin et al.[66] (2007)	12 RTT (F)	7–20 yr	Of the 12 patients, 8 were classic RTT, 3 congenital and 1 variant	Noninvasive cardiorespiratory monitoring system	Mean brainstem/autonomic score (0–10) was 4.6 based on ISS§§ Patients with frequent apnea did not also have increased cardiac events (tachycardia, bradycardia and stable heart rate; $p = 0.34$) Frequency of apneas and heart events increased with age (apnea index during wakefulness increased with age; $p = 0.02$) Dysregulated breathing and HR patterns evident during sleep
Tarquinio et al.[19] (2018)	1205 (recruited through RTT natural history study 2006–-2015) 1185 analyzed sample	Median (min–max): 10.2 yr (0.7–66.5 yr)	Consensus diagnostic criteria used to categorize participants with classic RTT, atypical RTT or *MECP2* mutation without RTT	Kaplan–Meier estimator used to determine the lifetime frequency of breathing abnormalities Logistic regression used to evaluate risk factors	778 female patients with classic RTT were followed over 9 yr Breathing dysfunction was highly prevalent (nearly 100%) in participants with classic or atypical severe RTT v. those with atypical mild RTT (60%–70%) At recruitment, breath-holding, hyperventilation and aerophagia were reported by 67.1%, 51.6% and 47.2% of parents, respectively Autonomic dysfunction (OR 1.4) alongside poor hand use (OR 2.0), impaired mobility (OR 1.7), prevalence of stereotypies (OR 1.6), severity of dystonia (OR 1.5), hyperreflexia (OR 1.4), axial hypertonia (OR 1.2) and seizure frequency (OR 1.2), were risk factors linked to higher odds of severe breathing dysregulation Age (OR 1.0), number of joint contractures (OR 0.8) and scoliosis severity (OR 0.7) were all linked to lower odds of severe breathing dysregulation
Vignoli et al.[67] (2012)	NA (questionnaire 84/130 families)	≥ 14 yr (Group 1: 14–20 yr; Group 2: 21–29 yr; Group 3 > 29 yr)	Families were part of the Italian Association for Rett Syndrome Severity of RTT for each patient was assessed using the modified Kerr score (Kerr et al.[74])	Questionnaire based on the modified Kerr score (Kerr et al.[74])	Breathing abnormalities, abnormal heart rhythms and vasculocutaneous problems were reported in 70%, 18% and 92% of females participants, respectively. Of the 70% that reported breathing dysregulation, 57% had apnea and 36% had hyperventilation Autonomic disturbances propagated into adulthood

Table 1: Sample characteristics, assessment methods and key findings of the eligible studies for the systematic review: autonomic dysfunction (part 6 of 6)

Study	*N*	Participant age	Mutation/diagnosis	Assessment methods*	Key findings†
Weese-Mayer et al.[68] (2006)	47 RTT (F) 47 age- and sex-matched typically developing controls	2–7 yr	Clinical diagnosis and identified mutation in *MECP2*	Cardiorespiratory and waveform monitoring	A significant increase in HR during breath-holding with a concomitant reduction in R-R interval in the RTT group during wakefulness Cardiorespiratory dysregulation was evident during breath-holding and during relatively normal breathing in RTT patients
Weese-Mayer et al.[69] (2008)	47 RTT (F) 47 age- and sex-matched typically developing controls	2–7 yr	Clinical diagnosis and identified mutation in *MECP2*	Cardiorespiratory and waveform monitoring	Young patients with RTT had more regular breathing and HR patterns at night versus during the day, but they did experience night-time irregularities with respect to breathing and HR A desynchronization of autonomic control is present during the day as well as at night in patients with RTT
Yuge et al.[70] (2017)	4	Mean ± SD: 21.75 ± 8.18 yr	3 patients had typical RTT mutation; 1 patient (patient 2) had an atypical RTT mutation	Clinical improvement after ghrelin treatment based on changes in rating scales (BFMDRS, SDCF and VAS)	Following ghrelin treatment, autonomic dysfunction was improved in 2 patients (patient 1 and 2) that had severe dystonia and head tremor

5-HT = 5-hydroxytryptamine; AHI = Apnea Hypopnea Index; ANCOVA = analysis of covariance; ANS = autonomic nervous system; ASBA = abnormal spontaneous brainstem activation; BFMDRS = Burke–Fahn–Marsden Dystonia Rating Scale; BMI = body mass index; CSB = cardiac sensitivity to baroreflex; CSF = cerebrospinal fluid; CCSS = Composite Clinical Severity Score; CTS = C-terminal segment; CVT = cardiac vagal tone; DBP = diastolic blood pressure; DMX = dorsal motor nucleus of cranial nerve X; ECG = electrocardiogram; EDA = electrodermal activity; EEG = electroencephalography; EU = European Union; FLACC = Face Legs Activity Cry Consolability; HF = high frequency; HR = heart rate; IGF-1 = insulin-like growth factor 1; ISS = International Scoring System; LF = low frequency; MAP = mean arterial pressure; MAP-CV = coefficient of variation of mean arterial pressure; MBD = methyl-CpG-binding domain; NA = not applicable; OR = odds ratio; PBZ scale = Pini Bonuccelli Zappella scale; QT = Q and T waves on electrocardiogram; QTc = corrected QT; QTcD = QTc dispersion; R-R = inter-beat interval; RTT = Rett syndrome; SBP = systolic blood pressure; SD = standard deviation; SDCF = scoring for different clinical features; SE = standard error; TRD = transcription repression domain; VAS = visual analog scale; VLF = very low frequency.

*When applicable, these relate to assessment methods that aim to measure autonomic dysfunction.

†Are related to the key autonomic findings of the study.

‡Patients were sourced from the Australian RTT database (*n* = 150) and the InterRett database (*n* = 273).

§Brainstem (Paterson et al.[64]), intestinal (colon, bowel; Deguchi et al.[46]) or brain (Deguchi et al.[45]) tissue samples from autopsied patients and/or case controls.

¶Female participants with RTT (*n* = 28) were split into 2 treatment groups depending upon medication used for seizures. Group 1 (without therapy) had 10 participants (7.3 ± 3.5 yr) and group 2 (with therapy) had 18 participants (7.2 ± 3.4 yr).

**In this study, a questionnaire was sent out to 70 participants (predominantly parents) of which 76% were returned. Data were derived for 53 female patients with RTT aged > 16 yr.

††Of the 125 surveyed, 94 were answered by families, 24 by family and staff, 6 by staff only, and 1 in which it was not reported who answered the questionnaire.

‡‡These included childhood controls with neurologic disease (*n* = 11) and adult controls with gynecological disease (*n* = 7), urological disease (*n* = 6) and orthopaedic disease (*n* = 4).

§§Scores were based on the slightly modified ISS developed by Kerr et al.[74]

Sufficient sample size

This criterion was met by 3 studies.[38,41,57] In these studies, a sample size or power calculation was indicated[41,57] or the authors mentioned elsewhere that the study was sufficiently powered to detect differences.[38] In other studies, this criterion remained unclear: for example, in 1 study, the small sample size was used to correct for baseline values,[65] but no power calculation was provided. Another study had used nonparametric tests to account for small sample sizes.[60] Halbach and colleagues[53] considered the power element and indicated that certain elements of their study (such as exploring the genotype–phenotype relationship) were underpowered to detect differences. Other studies analyzed data from a large number of patients (≥ 100) recruited from different patient databases or centres, such as the Australian Rett Syndrome database,[39] the United States natural history study,[19,43] 6 participating centres in 4 European Union countries,[54] the Tuscany Rett Centre[20] and the Maastricht–Leuven Rett syndrome database.[11] These studies did not provide power calculations, because data from ≥ 100 patients were analyzed in each study and probably precluded requirements for formal sample-size calculations. It is likely that other studies were underpowered to detect a discernible effect, or specific subgroups within studies might have been underpowered, as shown for the association between QTc prolongation and age.[43] Another study suggested that the scarcity of evidence from human studies using desipramine prevented a formal sample-size estimate in their study.[61]

Objective and standard criteria for measurements

A significant proportion of the studies used a variety of methods to assess autonomic physiology. At present, there is no gold-standard measurement of autonomic function. For this criterion, 7 studies had used techniques of spectral analysis, such as fast Fourier transform algorithms.[37,48–52,57] One of these studies had also used more traditional methods to evaluate autonomic function, such as the head-up tilt and cold pressor tests.[57] Eight studies used the NeuroScope device to measure autonomic physiology under different conditions.[20,35,40,41,54,56,59,60] This device assesses cardiac vagal tone (CVT), cardiac sensitivity to baroreflex (CSB) and mean arterial pressure (MAP).[56] Three studies[49,50,52] used objective measures to track autonomic metrics as the disorder advanced. These studies were valuable, because they could illuminate patterns of abnormal rectification of vagus tone in patients with RTT and how this leads to

Table 2: Quality assessment of eligible studies as rated by the critical appraisal tool and related commentary for systematic review: autonomic dysfunction (part 1 of 9)*

	Criteria†										
Study	1. Was the sample characteristic of the specific population?	2. Were patients recruited in an appropriate way?	3. Was the sample size sufficient to power the study?	4. Were the study participants described in detail and in a way that fostered comparison with other relevant studies?	5. Was the data analysis undertaken with adequate description of the identified sample?	6. Were objective and standard criteria used for the measurements?‡	7. Were the assessment and measurement methods used reliably?	8. Were the statistical analyses used appropriate?	9. Were relevant confounding factors described and accounted for?	10. If sub-populations were identified, were they done according to objective criteria?	11. Was there a conflict of interest?§
Acampa et al.[37] (2008)	Yes	Yes	Unclear; power calculation not provided	Yes	Yes	Yes; EEG and spectral waveform methods using the Cardioline system	Yes	Yes; parameters were analyzed using FFT	No	NA	Unclear
Amir et al.[38] (2000)	Yes	Yes	Yes; power to detect a difference described	Yes	Yes	Yes; clinical evaluation (including ECG and EEG) and mutational analysis	Yes	Yes; genotype–phenotype correlation analysis described in detail	No	NA	Unclear
Anderson et al.[39] (2014)	Yes (from the Australian Rett Syndrome Database and InterRett)	Yes	NA	Yes	Yes	No	NA	Yes; Kaplan–Meier methods used to determine survival probability	Unclear	NA	No
Bergström-Isacsson et al.[40] (2013)	Yes	Yes	Unclear; power calculation not provided	Yes	Yes	Yes; using EEG and video recordings; NeuroScope used to assess autonomic parameters	Yes; using NeuroScope	Yes; provided in sufficient detail	No; limitations were described, but not confounding factors	NA	Unclear
Bergström-Isacsson et al.[41] (2014)	Yes	Yes	Yes; power calculation described	Yes	Yes	Yes; using EEG and video recordings; NeuroScope used to assess autonomic parameters	Yes; using NeuroScope, EEG and video recordings	Yes	Yes; related to the effect of music on emotional responses and the influence of blood gases	NA	Unclear

Table 2: Quality assessment of eligible studies as rated by the critical appraisal tool and related commentary for systematic review: autonomic dysfunction (part 2 of 9)*

	Criteria†										
Study	1. Was the sample characteristic of the specific population?	2. Were patients recruited in an appropriate way?	3. Was the sample size sufficient to power the study?	4. Were the study participants described in detail and in a way that fostered comparison with other relevant studies?	5. Was the data analysis undertaken with adequate description of the identified sample?	6. Were objective and standard criteria used for the measurements?‡	7. Were the assessment and measurement methods used reliably?	8. Were the statistical analyses used appropriate?	9. Were relevant confounding factors described and accounted for?	10. If sub-populations were identified, were they done according to objective criteria?	11. Was there a conflict of interest?§
Cass et al.[42] (2003)	Yes	Yes	Unclear; power calculation not provided	Yes	Yes	Unclear; a wide range of assessment tools used (assessment scales, parent interviews, clinical observations and assessments)	Yes	Yes	Yes; factors such as the lack of standardized measures were discussed	Yes	Unclear
Crosson et al.[43] (2017)	Yes	Yes	Unclear; numbers in certain subgroups may have been under-powered	Yes	Yes	Yes; standard ECG measurements used	Yes	Yes	Yes	Yes; subgroups were defined by age, clinical severity and genotype	No
d'Orsi et al.[44] (2012)	Yes	Yes	Unclear; power calculation not provided	Yes	Yes	Yes; video-polygraphy	Yes	Yes; video, EEG and EMG analysis described in detail	No	NA	Unclear; authors provided a statement on ethical publication that conformed with article guidelines
Deguchi et al.[45] (2000)	Yes	NA	NA	Yes	Yes	No; methodology used to evaluate substance P immunoreactivity	Yes	NA	Yes; in terms of primary and secondary defects	NA	Unclear
Deguchi et al.[46] (2001)	Yes	NA	NA	Yes	Yes	No; methodology used to evaluate substance P immunoreactivity	Yes	NA	Yes; study was critiqued	NA	Unclear

Table 2: Quality assessment of eligible studies as rated by the critical appraisal tool and related commentary for systematic review: autonomic dysfunction (part 3 of 9)*

	Criteria†										
Study	1. Was the sample characteristic of the specific population?	2. Were patients recruited in an appropriate way?	3. Was the sample size sufficient to power the study?	4. Were the study participants described in detail and in a way that fostered comparison with other relevant studies?	5. Was the data analysis undertaken with adequate description of the identified sample?	6. Were objective and standard criteria used for the measurements?‡	7. Were the assessment and measurement methods used reliably?	8. Were the statistical analyses used appropriate?	9. Were relevant confounding factors described and accounted for?	10. If sub-populations were identified, were they done according to objective criteria?	11. Was there a conflict of interest?§
Djukic et al.[47] (2016)	Yes	Yes	NA	Yes	Yes	Yes; cognitive function assessed using Tobii eye-tracking technology; other assessment methods included EEG and QOL using Child Health Questionnaire P50	Yes	Yes; small sample size taken into consideration	Yes; lack of placebo control and blinded assessments mentioned	NA	Unclear
Dotti et al.[48] (2004)	Unclear; individuals had X-linked intellectual disability with *MECP2* mutation	Yes	NA	Yes	Yes	Yes; HR variability assessed using a commercially available system	Yes	Yes; analysis of parameters used the spectral method (FFT)	No	NA	Unclear
Guideri et al.[49] (1999)	Yes	Yes	Unclear; power calculation not provided	Yes	Yes	Yes; HR parameters assessed using a commercially available system	Yes	Yes; using FFT	No	NA	Unclear
Guideri et al.[50] (2001)	Yes	Yes	Unclear; power calculation not provided	Yes	Yes	Yes; HR parameters assessed using a commercially available system	Yes	Yes; spectral waveform analysis using FFT	No	NA	Yes; study was financially supported by the Sigma Tau Chemical Company
Guideri et al.[51] (2004)	Yes	Yes	Unclear; power calculation not provided	Yes	Yes	Yes; HR variability assessed using a commercially available system	Yes	Yes; analysis of parameters using the spectral method (FFT)	No	Unclear; groups split based on anti-convulsant status	Unclear

Table 2: Quality assessment of eligible studies as rated by the critical appraisal tool and related commentary for systematic review: autonomic dysfunction (part 4 of 9)*

	Criteria†										
Study	1. Was the sample characteristic of the specific population?	2. Were patients recruited in an appropriate way?	3. Was the sample size sufficient to power the study?	4. Were the study participants described in detail and in a way that fostered comparison with other relevant studies?	5. Was the data analysis undertaken with adequate description of the identified sample?	6. Were objective and standard criteria used for the measurements?‡	7. Were the assessment and measurement methods used reliably?	8. Were the statistical analyses used appropriate?	9. Were relevant confounding factors described and accounted for?	10. If sub-populations were identified, were they done according to objective criteria?	11. Was there a conflict of interest?§
Guideri et al.[52] (2005)	Yes	Yes	Unclear; power calculation not provided	Yes	Yes	Yes; ECG and spectral waveform methods using an imaging system	Yes	Yes, spectral waveform analysis using FFT	No	NA	Unclear
Halbach et al.[53] (2008)	Yes (Dutch Rett parent organization)	NA	Unclear; statistical power to study the effect on genotype and anticonvulsant treatment was limited, but no power calculation was provided	Yes; but prevalence of autonomic dysfunction varied across populations	Yes	No; modified questionnaire assessment	Yes	Yes	Yes; the cross-sectional nature of the study was discussed	Yes; split into 3 age groups	Unclear
Halbach et al.[11] (2012)	Yes (the Maastricht–Leuven Rett Syndrome database)	Yes	Unclear; no power calculation given, but limitations in small sample size were considered	Yes	Yes	No; ISS and DNA analysis	Yes	Yes; including considerations for age at examination	Yes; authors indicates issues related to the small sample size	Yes; age at examination was split into 3 subgroups	Unclear

Table 2: Quality assessment of eligible studies as rated by the critical appraisal tool and related commentary for systematic review: autonomic dysfunction (part 5 of 9)*

	Criteria†										
Study	1. Was the sample characteristic of the specific population?	2. Were patients recruited in an appropriate way?	3. Was the sample size sufficient to power the study?	4. Were the study participants described in detail and in a way that fostered comparison with other relevant studies?	5. Was the data analysis undertaken with adequate description of the identified sample?	6. Were objective and standard criteria used for the measurements?‡	7. Were the assessment and measurement methods used reliably?	8. Were the statistical analyses used appropriate?	9. Were relevant confounding factors described and accounted for?	10. If sub-populations were identified, were they done according to objective criteria?	11. Was there a conflict of interest?§
Halbach et al.[54] (2016)	Yes	Yes	NA	Yes; can be compared with other studies in which the NeuroScope was used	Yes	Yes; NeuroScope; autonomic index and respiratory analyses done using VaguSoft and LARS respiratory analysis software, respectively; ISS used to assess severity of clinical features with EEG and video recording	Yes; using modified ISS score and NeuroScope alongside other physiological parameters	Yes	Yes	NA	Yes; 1 of the authors was an inventor of the NeuroScope
Hara et al.[55] (2011)	Yes	Yes	Unclear; power calculation not provided	Yes	Yes	No; measurement of plasma levels of ghrelin and other growth factors	Yes	Yes	No	NA	Unclear
Julu et al.[56] (2001)	Yes	Yes	Unclear; power calculation not provided	Yes	Yes	Yes; using EEG, video and NeuroScope running VaguSoft software	Yes	Yes	No	NA	Yes; first author invented the NeuroScope
Julu and Witt-Engerström[35] (2005)	Yes	Yes	Unclear; power calculation not provided	Yes	Yes	Yes; using EEG and NeuroScope running VaguSoft software	Yes	Yes	Yes	NA	Yes; first author invented the NeuroScope
Kumar et al.[57] (2017)	Yes	Yes	Yes; sample size calculation provided	Yes	Yes	Yes; standard measurements of autonomic function (head-up tilt test and the cold pressor test) used	Yes	Yes; spectral waveform densities calculated using FFT	Yes	NA	No
Larsson et al.[58] (2005)	Yes	Yes	NA	Yes	Yes	No; questionnaire used	NA	Yes	Yes; limitations considered	NA	Unclear

Table 2: Quality assessment of eligible studies as rated by the critical appraisal tool and related commentary for systematic review: autonomic dysfunction (part 6 of 9)*

	Criteria†										
Study	1. Was the sample characteristic of the specific population?	2. Were patients recruited in an appropriate way?	3. Was the sample size sufficient to power the study?	4. Were the study participants described in detail and in a way that fostered comparison with other relevant studies?	5. Was the data analysis undertaken with adequate description of the identified sample?	6. Were objective and standard criteria used for the measurements?‡	7. Were the assessment and measurement methods used reliably?	8. Were the statistical analyses used appropriate?	9. Were relevant confounding factors described and accounted for?	10. If sub-populations were identified, were they done according to objective criteria?	11. Was there a conflict of interest?§
Larsson et al.[59] (2013)	Yes	Yes	Unclear; power calculation not provided	Yes	Yes	Yes; NeuroScope with synchronized EEG	Yes; NeuroScope running VaguSoft software	Yes; linear mixed model described in sufficient detail	Yes; findings were applicable only to RTT patients who could stand	NA	Yes; 1 of the authors was an inventor of the NeuroScope
Larsson et al.[60] (2018)	Yes	Yes	NA	Yes; can be compared with other studies in which the NeuroScope was used	Yes	Yes; standard criteria included finger photoplethysmography and video recordings; NeuroScope	Yes; NeuroScope to measure autonomic responses	Yes; sample size was small, so authors chose to perform non-parametric (Mann–Whitney) statistical tests to evaluate differences in autonomic responses between RTT and controls	Yes; findings were applicable only to RTT patients who were still able to walk	NA	Yes; 1 of the authors was an inventor of the NeuroScope
Mackay et al.[13] (2017)	NA (family-based questionnaire)	NA	NA	Yes	Yes	No; web-based questionnaire	Yes	Yes	Yes; authors accounted for risk of recall bias and that parent-reported outcomes were likely to differ from clinician-reported outcomes when assessing differences in autonomic dysregulation	Yes; age sub-populations were described	No

Table 2: Quality assessment of eligible studies as rated by the critical appraisal tool and related commentary for systematic review: autonomic dysfunction (part 7 of 9)*

	Criteria†										
Study	1. Was the sample characteristic of the specific population?	2. Were patients recruited in an appropriate way?	3. Was the sample size sufficient to power the study?	4. Were the study participants described in detail and in a way that fostered comparison with other relevant studies?	5. Was the data analysis undertaken with adequate description of the identified sample?	6. Were objective and standard criteria used for the measurements?‡	7. Were the assessment and measurement methods used reliably?	8. Were the statistical analyses used appropriate?	9. Were relevant confounding factors described and accounted for?	10. If sub-populations were identified, were they done according to objective criteria?	11. Was there a conflict of interest?§
Mancini et al.[61] (2017)	Yes	Yes	No; study might have been under-powered to detect an effect	Yes; participants described, but findings could not be compared with other studies because no previous data exists on the effect of the drug on women/girls with RTT	Yes	Yes; ApneaLink device used for respiratory measurements, alongside a parent-based severity scale (SSI)	Yes	Yes	Yes	NA	No; sponsor had no role in the study design, analysis or interpretation
Matsuishi et al.[62] (1997)	Yes	Yes	Unclear; power calculation not provided	Yes	Yes	No; methodology for substance P radio-immunoassay	Yes	NA	No	NA	Unclear
O'Leary et al.[63] (2017)	Yes	Yes	NA	Yes	Yes	Yes; using physiologic methods (EDA and HR) alongside the FLACC scale	Yes	Yes	Yes	NA	Yes; 3 authors had a conflict of interest
Paterson et al.[64] (2005)	Yes	NA	NA	NA; autopsied RTT and case control brainstem samples	NA	No; conventional radio-immunoassay methods	Yes	Yes; if an age interaction was present, this was taken into account using linear regression analysis	Yes; factors that could affect serotonin transporter binding values were discussed	NA	Unclear

Table 2: Quality assessment of eligible studies as rated by the critical appraisal tool and related commentary for systematic review: autonomic dysfunction (part 8 of 9)*

	Criteria†										
Study	1. Was the sample characteristic of the specific population?	2. Were patients recruited in an appropriate way?	3. Was the sample size sufficient to power the study?	4. Were the study participants described in detail and in a way that fostered comparison with other relevant studies?	5. Was the data analysis undertaken with adequate description of the identified sample?	6. Were objective and standard criteria used for the measurements?‡	7. Were the assessment and measurement methods used reliably?	8. Were the statistical analyses used appropriate?	9. Were relevant confounding factors described and accounted for?	10. If sub-populations were identified, were they done according to objective criteria?	11. Was there a conflict of interest?§
Percy et al.[65] (1994)	Yes	Yes	Unclear; small sample size used to correct for baseline values	Yes	Yes	Yes; EEG and assessment scales	Yes	Yes	Yes	Yes; influence on the crossover design was discussed	Unclear
Pini et al.[20] (2016)	Yes; however, the sample also consisted of those with *CDKL5* (*n* = 12), *FOXG1* (*n* = 1) and *MEF2C* (*n* = 1) mutations; 19 were mutation-negative	Yes	NA	Yes	Yes	Yes; NeuroScope used to assess autonomic parameters, with severity (ISS) and PBZ scale	Yes	Yes	Unclear	Yes, 3 age groups clearly defined	No
Rohdin et al.[66] (2007)	Yes	Yes	Unclear; power calculation not provided	Yes	Yes	Yes; cardiorespiratory monitoring done using a noninvasive cardiac event measurement system	Yes	Yes	Yes; confounding factors for the methodological aspects of the study were discussed	NA	Unclear
Tarquinio et al.[19] (2018)	Yes; via the RTT natural history study)	NA	Yes; analyzed sample was 1185; of these, 922 had classic RTT, 778 of whom were longitudinally followed for 9 yr	Yes	Yes	No; assessment scales	NA	Yes; standard statistical tests used to measure the lifetime frequency of breathing problems and assess risk factors	NA	NA	No

Table 2: Quality assessment of eligible studies as rated by the critical appraisal tool and related commentary for systematic review: autonomic dysfunction (part 9 of 9)*

	Criteria†										
Study	1. Was the sample characteristic of the specific population?	2. Were patients recruited in an appropriate way?	3. Was the sample size sufficient to power the study?	4. Were the study participants described in detail and in a way that fostered comparison with other relevant studies?	5. Was the data analysis undertaken with adequate description of the identified sample?	6. Were objective and standard criteria used for the measurements?‡	7. Were the assessment and measurement methods used reliably?	8. Were the statistical analyses used appropriate?	9. Were relevant confounding factors described and accounted for?	10. If sub-populations were identified, were they done according to objective criteria?	11. Was there a conflict of interest?§
Vignoli et al.[67] (2012)	Yes (families from Italian Association for Rett Syndrome)	NA	NA	Yes	Yes	No; modified questionnaire based on the Kerr severity score[74]	Yes	Yes	Unclear to a certain extent; difficulty assessing statistically significant differences in specific mutational profiles and severity scores	Yes; age at examination was split into 3 subgroups	Unclear
Weese-Mayer et al.[68] (2006)	Yes	Yes	Unclear; power calculation not provided	Yes	Yes	Yes; cardio-respiratory parameters assessed using LifeShirt technology	Yes	Yes	Yes; caveats applicable to the study findings	NA	Yes; LifeShirt technology provided by VivoMetrics
Weese-Mayer et al.[69] (2008)	Yes	Yes	Unclear; power calculation not provided	Yes	Yes	Yes; cardio-respiratory parameters assessed using LifeShirt technology	Yes	Yes	Yes; confounding factors considered when taking into account the cross-correlation analysis	NA	Yes; sponsor was industry (VivoMetrics)
Yuge et al.[70] (2017)	Yes	Yes	No	Yes	Yes	Unclear; clinical evaluations based on assessment scales (BFMDRS, SDCF and VAS)	Yes	NA	Yes; small sample size and lack of a control group	NA	No

BFMDRS = Burke–Fahn–Marsden Dystonia Rating Scale; ECG = electrocardiogram; EDA = electrodermal activity; EEG = electroencephalography; EMG = electromyogram; FFT = fast Fourier transform; FLAAC scale = Face Legs Activity Cry Consolability scale; HR = heart rate; ISS = International Scoring System; NA = not applicable; PBZ scale = Pini Bonuccelli Zappella scale; QOL = quality of life; SDCF = scoring for different clinical features; RTT = Rett syndrome; SSI = Symptom Severity Index; VAS = visual analog scale.

*Adapted from Table 2 and Appendix 1 from Munn et al,[31] and from Corchón et al.[32]

†Ratings were defined as Yes (fully meeting the criterion), No (not meeting the criterion), Unclear (unclear to whether the criterion was met) and NA (criterion was not applicable).

‡There is no gold standard method for assessing autonomic function. In this instance, objective measures were those that measured autonomic function directly and provided a physiologic assessment. Nonobjective measures were defined as patient-reported outcome measures such as questionnaires.

§Conflict of interest is defined as factors that might raise the risk of bias in study design, analysis or reporting. This can include industry sponsorship and/or intellectual conflict. Studies rated "Yes" had a conflict of interest from industry sponsorship or intellectual conflict. When no conflict of interest statements were mentioned, we rated these studies as "Unclear"; "No" indicates no conflict of interest.

changes that could imbalance the autonomic nervous system over the lifespan of the disease.

Nonobjective measures of autonomic function (questionnaires or scales) were found in 6 studies.[13,19,42,53,58,67] Some of these used web-based[13] or paper-based questionnaires,[58,67] or other sources (such as medical history and direct observations[42]) to identify autonomic manifestations. These studies were useful in providing a caregiver's perspective on autonomic features and how they can affect quality of life.[13,53] One study used the modified Kerr severity score to probe for autonomic and clinical features across mutations,[67] and another used an assessment schedule to evaluate a wide range of symptoms in patients with RTT.[42] In some instances, the autonomic function domain of the International Scoring System was used alongside objective measures of autonomic function[20,66]

Conflict of interest

Nine studies had some conflict of interest, defined as sponsorship by industry or some form of intellectual conflict.[35,50,54,56,59,60,63,68,69] In other studies, the extent of conflicting interests could not be evaluated, because no statement of conflict was provided. Three of the authors in 1 study had conflicting interests, and this was detailed in the declaration of interests section.[63] Two studies[68,69] had used LifeShirt technology to assess cardiorespiratory parameters in patients with RTT. Although both studies were conducted using the same sample, in one[69] the manufacturer of LifeShirt technology (VivoMetrics) was a cosponsor, and in the other had provided the LifeShirt vest.[68] Five studies had used a commercially available cardiorespiratory system (Cardioline) to assess autonomic function.[48–52] One of these studies was also funded by an industry sponsor.[50]

Double-counting of participants

Some studies had used or had recruited from the same sample. In Larsson and colleagues,[60] 12 female participants with RTT were recruited from 21 that had taken part in the previous study.[59] In 2 other studies, physiologic responses to stimuli such as music and facial expressions were taken from a pooled sample of 29 patients with RTT.[40,41] Other studies had used the same RTT and control samples to compare night-time breathing data[69] with daytime breathing data.[68] In some instances, information was gathered from the same database, such as the International Rett Syndrome Phenotype Database (InterRett), which was used to assess the health status of 273 patients[39] and in a follow-up questionnaire study sent to 413 families who had been recruited from InterRett.[13] Similarly, 1185 patients from the RTT natural history study were used to assess breathing abnormalities,[19] and patient data from the same study were used to assess QTc changes according to age and clinical severity in 100 patients.[43]

Sudden death in RTT: study characteristics and quality assessment

The PRISMA flow-chart for the systematic review of sudden death in RTT is shown in Appendix 1, Figure S3. The database search revealed 521 records. After duplicates were removed, 474 records remained. After screening of the title and abstract, we excluded 459. We assessed the remaining 15 full-text articles for eligibility against the inclusion and exclusion criteria and excluded a further 10 articles. We included 5 articles for analysis; their characteristics are provided in Table 3.

Characteristics of the eligible articles

The characteristics of the 5 eligible articles revealed a considerable overlap between sudden death and autonomic dysfunction. Three of the studies originated from the same group (University of Siena, Italy).[49,52,75] These studies focused largely on sympathovagal balance, looking for changes in heart rate variability that could be potentiating factors for sudden death. One other study had used different metrics of autonomic physiology, such as CVT and MAP,[76] and another focused on QT parameters to explore risk factors associated with sudden death.[77]

Quality assessment of articles

A full assessment of each article is presented in Table 4. Important findings relevant to the criteria are described below.

Appropriate patient recruitment

Three studies[49,52,77] fulfilled this criterion, but for the remainder of the studies it was unclear whether patient recruitment had been appropriate. Although 1 study mentioned that patients with RTT had been recruited from the Department of Child Neurology at the University of Siena, it provided no information about the mutation or diagnosis of patients with RTT, or how the age-matched controls were recruited.[75] Another study provided no information about diagnostic criteria or how the patients or age-matched controls were recruited.[76]

Sufficient sample size

It was unclear whether any of the studies were sufficiently powered to detect an effect. None of the studies provided power calculations, although in 1 study the authors did consider that the effect of T-wave abnormalities could not be adjusted for age because the sample size was too small.[77]

Objective and standard criteria for measurements

Given the pervasive network of physiologic systems that contribute to the deleterious end point of sudden death in RTT, there is no standardized way to measure the risk factors that lead to this outcome. All of the studies used objective measures that assessed some form of autonomic metric. Three of the studies had used traditional techniques of spectral analysis, such as fast Fourier transform.[49,52,75] One of these used echocardiograms to investigate whether changes in cardiac structure might be linked to sudden death.[75] Another looked at heart rate dynamics using the NeuroScope device together with chest plethysmography,[76] or used electrocardiograms to assess whether abnormal findings might elevate the risk of sudden cardiac death.[77]

Table 3: Sample characteristics, assessment methods and key findings of the eligible studies for systematic review: sudden death

Study	*N*	Participant age	Mutation/diagnosis	Assessment methods	Key findings
Guideri et al.[49] (1999)	54 RTT (F) 28 healthy controls (F)	Mean ± SD: RTT 10 ± 5.5 yr Healthy controls 9.71 ± 4.6 yr	RTT diagnosis confirmed in each patient, alongside clinical stage based on Hagberg and Witt-Engerstrom[71]	12-lead ECG along with spectral component measurements (VLF, LF and HF) and QTc analyses	LF/HF ratio was significantly higher in the RTT group across all 4 stages compared with healthy controls HR variability (R-R interval) was significantly lower in the RTT group compared with healthy controls Decreased HR variability was suggested to be a risk factor for sudden death in RTT
Guideri et al.[75] (2004)	32 RTT (F) 30 age-matched healthy controls (F)	RTT 4 ± 4.1 yr Healthy controls 6.8 ± 2.1 yr	Patients recruited from the Department of Child Neurology, University of Siena	Echocardiography to examine structural abnormalities and blood flow of the heart ECG and spectral component measurements (VLF, LF and HF) and QTc	LF/HF was significantly higher in the RTT group compared with healthy controls (3.8 ± 2.9 v. 1 ± 0.5; $p < 0.001$) QTc interval was significantly longer in the RTT group (0.44 ± 0.02) compared to healthy controls (0.40 ± 0.01; $p < 0.001$) No difference in echocardiography findings between the RTT group and healthy controls
Guideri et al.[52] (2005)	22 RTT (10 active treatment; 12 untreated)*	Mean ± SD: Active treatment 6.3 ± 4.3 yr Untreated 6.3 ± 4.0 yr	RTT diagnosis confirmed by 2 child neuropsychiatrists	Spectral waveform 12-lead ECG, along with spectral components (VLF, LF and HF) and QTc analyses	Examined the premises that reduced HR variability in RTT causes sudden death and that acetyl-L-carnitine might increase HR variability and have a protective affect on the cardiac system In the untreated group, total power and LF decreased significantly from baseline values after 6–18 mo; QTcD was elevated After 6 mo of acetyl-L-carnitine treatment, total power ($p = 0.01$), VLF ($p = 0.01$) and LF ($p = 0.009$) increased significantly compared to basal values No statistically significant difference ($p = 0.6$) in R-R interval between the active treatment and untreated groups at baseline or at follow-up Acetyl-L-carnitine might have a cardioprotective effect on the ANS and might decrease the frequency of sudden cardiac death in patients with RTT
Julu et al.[76] (1997)	6 RTT (F) 8 controls (F)	RTT 4–11 yr Controls 4–11 yr	Information not available	NeuroScope alongside measures such as chest plethysmograph and MAP measurements	In the RTT group, mean ± SEM CVT was 3.6 ± 0.7 units v. 10.5 ± 0.9 units in the control group ($p < 0.001$) MAP was 94.6 ± 6.4 mmHg in controls v. 78 ± 4.33 mmHg in patients with RTT Vagal tone was disrupted during hyperventilation Immaturity of medullary cardiorespiratory neurons contribute to autonomic disturbances that could result in sudden death in people with RTT
Sekul et al.[77] (1994)	34 RTT† 41 controls	RTT 2–22 yr Controls 2–18 yr	RTT diagnosis confirmed in each patient, alongside clinical stage based on Hagberg and Witt-Engerstrom[71] by 1 or more of the investigators	ECG parameters	Patients with RTT had longer QTc intervals ($p < 0.001$) and T-wave abnormalities ($p < 0.001$) compared to age-matched controls In patients with RTT with T-wave abnormalities, the frequency tended to increase from Stage II (36%) to Stage III (69%) and Stage IV (70%) Changes in T-waves and QTc were highlighted as risk factors that might contribute to sudden death patients with RTT

ANS = autonomic nervous system; CVT = cardiac vagal tone; ECG = electrocardiogram; HF = high frequency; HR = heart rate; LF = low frequency; MAP = mean arterial pressure; QT = Q and T waves on electrocardiogram; QTc = corrected QT; QTcD = QTc dispersion; R-R = inter-beat interval; RTT = Rett syndrome; SD = standard deviation; SEM = standard error of the mean; VLF = very low frequency.
*Ten girls with RTT were randomized to receive active treatment and 12 girls with RTT were age-matched untreated controls.
†Data from 61 ECGs (12-lead) were obtained from 34 patients with RTT.

Conflict of interest

One study had some conflict of interest.[76] For the other studies, it remained unclear whether a conflict of interest existed, because they did not provide a conflict of interest statement. One study provided funding information, but no conflict or declaration of interest statement.[77]

Identification of themes

Autonomic dysfunction in RTT

Upon reviewing the eligible studies shown in Table 1, we identified 7 themes (Fig. 1A and B). The theme with the highest frequency was breathing irregularities, appearing in

Table 4: Quality assessment of eligible studies as rated by the critical appraisal tool and related commentary for systematic review: sudden death* (part 1 of 2)

	Criteria†										
Study	1. Was the sample characteristic of the specific population?	2. Were patients recruited in an appropriate way?	3. Was the sample size sufficient to power the study?	4. Were the study participants described in detail and in a way that fostered comparison with other relevant studies?	5. Was the data analysis undertaken with adequate description of the identified sample?	6. Were objective and standard criteria used for the measurements?‡	7. Were the assessment and measurement methods used reliably?	8. Were the statistical analyses used appropriate?	9. Were relevant confounding factors described and accounted for?	10. If sub-populations were identified, were they done according to objective criteria?	11. Was there a conflict of interest?§
Guideri et al.[49] (1999)¶	Yes	Yes	Unclear; power calculation not provided	Yes	Yes	Yes; HR parameters were assessed using a commercially available system	Yes	Yes, using FFT	No	NA	Unclear
Guideri et al.[75] (2004)	Yes	Unclear; patients with RTT were recruited from the University of Siena (Department of Child Neurology), but no other information was provided about the recruitment of aged-matched controls	Unclear; power calculation not provided	Yes	Yes	Yes; ECG and HR parameters were measured using a commercially available system	Yes	Yes, spectral waveform analysis using FFT	No	No	Unclear
Guideri et al.[52] (2005)¶	Yes	Yes	Unclear; power calculation not provided	Yes	Yes	Yes; ECG and spectral waveform methods were measured using an imaging system	Yes	Yes, spectral waveform analysis using FFT	No	NA	Unclear
Julu et al.[76] (1997)	Yes	Unclear; no information about recruitment was provided	Unclear; power calculation not provided	Yes	Unclear; limited information was provided on data analysis	Yes; using chest plethysmography and NeuroScope	Yes	Yes	No	NA	Yes; first author invented the NeuroScope

Table 4: Quality assessment of eligible studies as rated by the critical appraisal tool and related commentary for systematic review: sudden death* (part 2 of 2)

	Criteria†										
Study	1. Was the sample characteristic of the specific population?	2. Were patients recruited in an appropriate way?	3. Was the sample size sufficient to power the study?	4. Were the study participants described in detail and in a way that fostered comparison with other relevant studies?	5. Was the data analysis undertaken with adequate description of the identified sample?	6. Were objective and standard criteria used for the measurements?‡	7. Were the assessment and measurement methods used reliably?	8. Were the statistical analyses used appropriate?	9. Were relevant confounding factors described and accounted for?	10. If sub-populations were identified, were they done according to objective criteria?	11. Was there a conflict of interest?§
Sekul et al.[77] (1994)	Yes	Yes	Unclear; authors said that the statistical power to study the effect on T-wave abnormalities adjusted for age was too small, but no power calculation was provided	Yes	Yes	Yes; using ECG parameters	Yes	Yes	Yes; limitations in terms of the number of patients receiving medication were discussed	NA	Unclear; funding information provided but no conflict of interest information

ECG = electrocardiogram; FFT = fast Fourier transform; HR = heart rate; NA = not applicable; RTT = Rett syndrome.
*Adapted from Table 2 and Appendix 1 from Munn et al,[31] and from Corchón et al.[32]
†Ratings were defined as Yes (fully meeting the criterion), No (not meeting the criterion), Unclear (unclear to whether the criterion was met) and NA (criterion was not applicable).
‡There is no gold standard method for assessing sudden death in people with RTT. In this instance, objective measures were those that measured autonomic function directly and could provide insights into the potential mechanisms for sudden death in patients with RTT.
§Conflict of interest is defined as factors that might raise the risk of bias in study design, analysis or reporting. This can include industry sponsorship and/or intellectual conflict. Studies rated "Yes" had a conflict of interest from industry sponsorship or intellectual conflict. When no conflict of interest statements were mentioned, we rated these studies as "Unclear"; "No" indicates no conflict of interest.
¶Also presented in Table 1.

13 studies that included 2120 participants (Fig. 2). This was followed by themes concerned with heart rate variability metrics such as CVT, CSB and MAP (7 studies in 461 participants), QT changes (6 studies in 286 participants) and vagal imbalance reflected by the LF/HF ratio (7 studies in 246 participants). Changes in peptide levels (ghrelin, leptin and substance P) also emerged as a theme from 6 studies, as did studies showing ASBAs (5 studies in 56 participants). Serotonergic neurotransmission was the theme with the lowest frequency, identified in 2 studies that included 28 participants[51] and another study that used post-mortem brainstem tissue samples from 11 patients with RTT.[64]

The initial 7 themes shared similarities, and based on consensus agreement, we condensed them into 3 final themes (Fig. 1C): (A) brainstem modulation of breathing, (B) electrical instability of the cardiovascular system and (C) neurochemical changes contribute to autonomic decline. The total frequency scores for the 3 themes are presented in Figure 2.

We present evidence for each theme below, and describe supporting adjunct studies. If applicable, conflicting evidence related to each theme is also described.

Theme A: Brainstem modulation of breathing

Breathing irregularities and abnormal spontaneous brainstem activations (ASBAs) were merged into Theme A. Among the 39 included studies, a few measured brainstem paroxysms. We know that ASBAs are characterized by spontaneous depolarization of brainstem neurons[40] that leads to aberrant misfiring of neural circuits, such as within the cardiorespiratory dyad. However, ASBAs were measured in only about 13% (5/39) of the included studies. One study showed that ASBAs were frequent or very frequent during a 5-minute period in 79% (23/29) of patients.[40] Similar events were observed in other studies in patients with RTT.[35,56,59,60]

Another study had used video polygraphy to assess paroxysmal events — especially those that can be differentiated from epileptic and nonepileptic events — and was able to marry this with breathing disturbances.[44] Impaired brainstem modulation of breathing could have serious consequences for patients with RTT. Two studies[35,56] had noted brainstem shutdown in patients with RTT. In these studies, CVT and CSB approached zero (reflective of brainstem shutdown); it was suggested that brainstem shutdown lasting

for a few minutes might be a consequence of brainstem hyperactivation bought on by forceful breathing[56] or gasping.

Theme B: Electrical instability of the cardiovascular system

Autonomic dysfunction in RTT is reflected in imbalances between the sympathetic and parasympathetic arms of the autonomic nervous system. This impairment of the autonomic system causes electrical instability of the cardiovascular system that can be measured by metrics of heart rate variability. Several of the included studies used various metrics to assess autonomic dysfunction in patients with RTT, and following consensus agreement, we merged themes relating to vagal imbalance and other heart rate metrics such as QT changes, CSB, MAP and CVT into a single theme. A summary of baseline autonomic metrics in patients with RTT (and, where relevant, comparison to healthy or typically developing controls) is presented in Appendix 1, Figure S4.

The baseline findings from the included studies showed that the sympathovagal balance suggested to reflect the LF/HF metric was higher in patients with RTT than in typically developing or healthy controls.[49,50,57] An important finding related to the LF/HF ratio was that it persisted and did not dramatically decrease during the progression of disease.[49,50] Other metrics, such as CVT and CSB, were lower in patients with RTT compared with controls. The CVT and CSB are reflections of parasympathetic responses, and because CVT represents the major inhibitory output to the heart, reduced CVT and CSB responses may be indicative of reduced inhibitory output from the nucleus tractus solitarius and the nucleus ambiguus.[35,56] These findings point to a sympathetic–parasympathetic imbalance.

As far as we are aware, only 1 study[57] has used the time-domain metrics standard deviation of all NN intervals (SDNN) and percentage of successive R-R intervals that differ by more than 50 ms (pNN50) to assess autonomic function in patients with RTT compared to typically developing controls. Both the parasympathetic and sympathetic components of the autonomic nervous system contribute to SDNN,[78] and over a 24-hour recording period, SDNN is likened to the gold standard of cardiac risk stratification.[79] On the other hand, pNN50 is more representative of parasympathetic activity.[78]

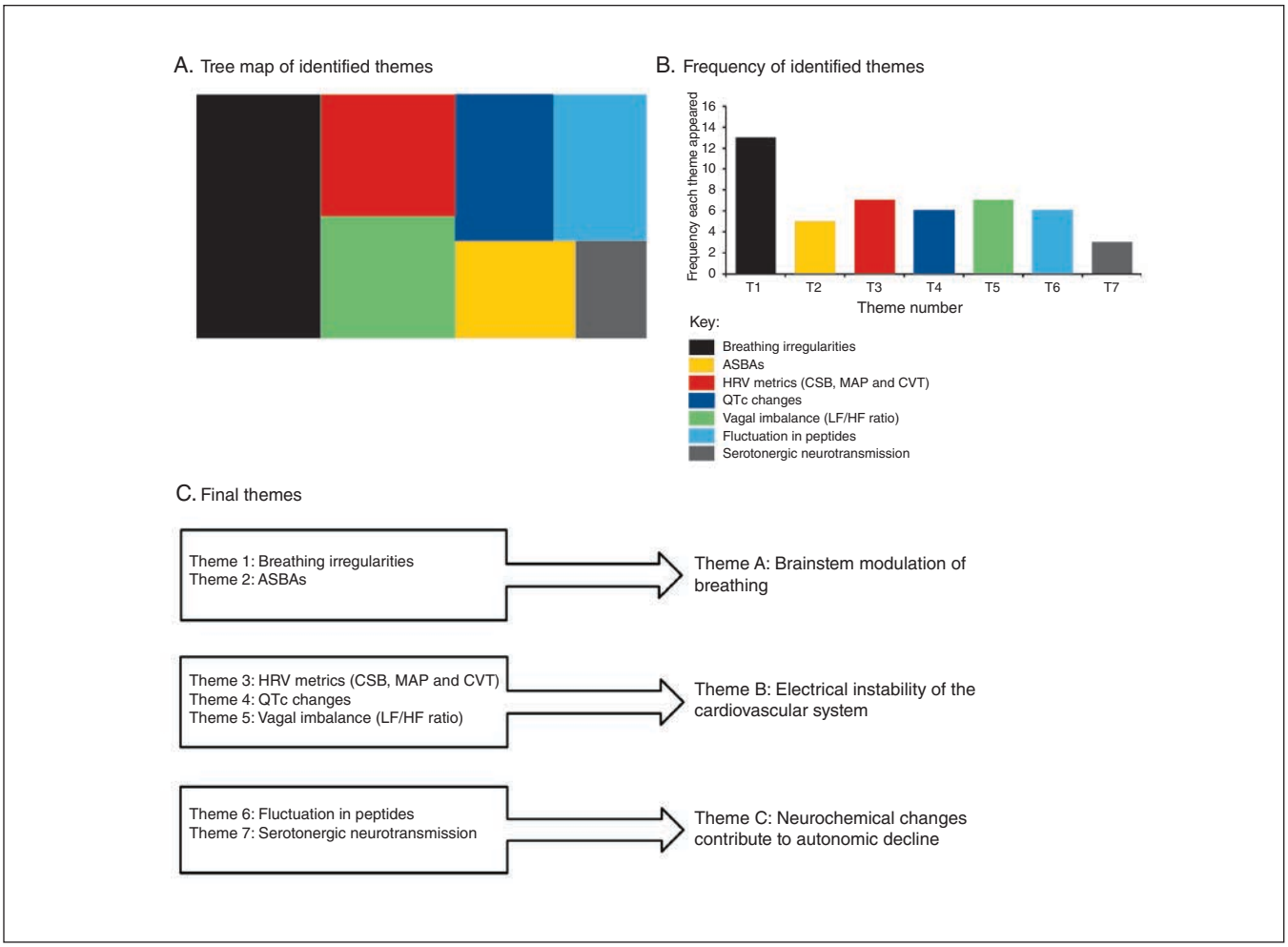

Fig. 1: Identification of themes. ASBA = abnormal spontaneous brainstem activation; CSB = cardiac sensitivity to baroreflex; CVT = cardiac vagal tone; HF = high frequency; HRV = heart rate variability; LF = low frequency; MAP = mean arterial pressure; QT = Q and T waves on electrocardiogram; QTc = corrected QT.

In 23 patients with RTT, both SDNN and pNN50 values were lower than in the control group. The median SDNN value in the RTT group was 39.3 ms, compared with 62.5 ms in the control group.[57] It has been suggested that values < 50 ms for SDNN may be associated with higher cardiac risk,[79] but how this translates to the broader RTT population remains unclear.

A sub-theme that emerged from the eligible studies was heart rate variability (the time between successive beats of the heart, denoted by the R-R interval) and QT prolongation. Data from studies in patients with RTT (and, where relevant, healthy or typically developed controls) is presented in Appendix 1, Figure S5. These studies showed that the R-R interval was lower and the mean longest QTc interval was

Colour code	Theme number	Theme	Participants for each theme, *n*		Frequency of each theme, *n*
	1	Breathing irregularities*	2120		13
	2	ASBAs†	56		5
	3	HRV metrics (CSB, MAP and CVT)‡	461		7
	4	QTc changes§	286		6
	5	Vagal imbalance (LF/HF ratio)¶	246		7
	6	Fluctuation in peptides**	Ghrelin	31	6
			Leptin	32	
			Substance P	48	
			Total	111	
	7	Serotonergic neurotransmission††	39		3

Fig. 2: Frequency of themes identified from key findings of eligible studies.

*Information relating to breathing irregularities emerged from 13 studies with a total of 2120 participants (questionnaires not counted as patient studies): Tarquinio et al.[19] (2018), 1185 participants with RTT; Mackay et al.[13] (2017), 413 families returned questionnaires; Pini et al.[20] (2016), 151 participants with a clinical diagnosis of RTT; Anderson et al.[39] (2014), 423 participants with RTT; Halbach et al.[11] (2012), 137 participants with RTT; Vignoli et al.[67] (2012), 84 families returned questionnaires; Weese-Mayer et al.[69] (2008) and Weese-Mayer et al.[68] (2006), 47 participants with RTT; Halbach et al.[53] (2008), 70 postal questionnaires; Rohdin et al.[66] (2007), 12 participants with RTT; Larsson et al.[58] (2005), 125 questionnaires; Cass et al.[42] (2003), 87 participants with RTT; Amir et al.[38] (2000), 78 participants with RTT.

†ASBAs were observed in 5 studies including 56 participants. Larsson et al.[60] (2018) and Larsson et al.[59] (2013), 21 participants with RTT; Bergström-Isacsson et al.[40] (2013), 29 participants with RTT; Julu and Witt-Engerström[35] (2005), 1 participant with RTT; Julu et al.[56] (2001), 5 participants with RTT.

‡Heart rate metrics such as CSB, MAP and/or CVT emerged from 7 studies including 461 participants. Larsson et al.[60] (2018) and Larsson et al.[59] (2013), 21 participants with RTT; Pini et al.[20] (2016), 151 participants with a clinical diagnosis of RTT; Halbach et al.[54] (2016), 132 participants with RTT; Bergström-Isacsson et al.[41] (2014), 29 participants with RTT; Julu and Witt-Engerström[35] (2005), 72 participants with RTT; Julu et al.[56] (2001), 56 participants with RTT.

§QTc changes, whether no change or prolongations, emerged from 6 studies including 286 participants. Crosson et al.[43] (2017), 100 participants with RTT; Kumar et al.[57] (2017), 23 participants with RTT; Guideri et al.[24] (2005), 22 participants with RTT; Dotti et al.[48] (2004), 3 participants with *MECP2* mutation and X-linked intellectual disability; Guideri et al.[50] (2001), 84 participants with RTT (74 with RTT and 10 with RTT preserved speech variant); Guideri et al.[49] (1999), 54 participants with RTT.

¶Vagal imbalance (LF/HF) ratio was explored in 7 studies including 246 participants. Kumar et al.[57] (2017), 23 participants with RTT; Acampa et al.[37] (2008), 32 participants with RTT; Guideri et al.[24] (2005), 22 participants with RTT; Dotti et al.[48] (2004), 3 participants with *MECP2* mutation and X-linked intellectual disability; Guideri et al.[71] (2004), 28 participants with RTT; Guideri et al.[50] (2001), 84 participants with RTT (74 with RTT and 10 with RTT preserved speech variant); Guideri et al.[49] (1999), 54 participants with RTT.

**Fluctuations of peptides were explored in 6 studies including 111 participants. Substance P came from RTT autopsy tissue samples[45,46] and cerebrospinal fluid[62]. Ghrelin: Yuge et al.[70] (2017), 4 participants with RTT; Hara et al.[55] (2011), 27 participants with RTT. Leptin: Acampa et al.[37] (2008), 32 participants with RTT. Substance P: Deguchi et al.[45] (2000), 14 participants with RTT; Deguchi et al.[46] (2001), 14 participants with RTT; Matsuishi et al.[62] (1997), 20 participants with RTT.

††Serotonin or serotoninergic neurotransmission emerged in 2 studies including 39 participants. Guideri et al.[51] (2004), 28 participants with RTT; Paterson et al.[64] (2005), 11 brainstem tissue samples from participants with RTT.

ASBA = abnormal spontaneous brainstem activation; CSB = cardiac sensitivity to baroreflex; CVT = cardiac vagal tone; HF = high frequency; HRV = heart rate variability; LF = low frequency; MAP = mean arterial pressure; QT = Q and T waves on electrocardiogram; QTc = corrected QT; RTT = Rett syndrome.

prolonged in patients with RTT compared with controls. Lower values for R-R can lead to abnormal cardiac changes, because heart rate tends to increase during breath-holding in RTT.[68] Specifically, during breath-holding the heart rate response is said to be exaggerated, and it overshoots,[68] which can cause oscillations in blood pressure.[56] The amplitude of the beat-to-beat variance as reflected by the root mean square is also lower in patients with RTT compared with controls.[68] This finding suggests that the vagally mediated changes to heart rate are significantly impaired during breath-holding in patients with RTT.

Although CVT and CSB were consistently lower across studies, values for MAP that reflected sympathetic responses were unclear. In 1 study, values for MAP were higher in controls compared to patients with RTT.[60] This study was conducted in patients with RTT who were ambulatory, and because the MAP values were not significantly different during walking in the control and RTT groups, the authors reasoned that there was no indication of sympathetic overdrive in patients with RTT. Two other studies[35,59] have shown reduced MAP values in patients with RTT compared to controls. These findings were in contrast with another study[41] that showed higher MAP values for the RTT group compared to controls.

The mean longest QTc interval was prolonged in 23 patients with RTT compared to age-matched controls.[57] This finding was in agreement with previous studies that showed increases in the QT interval in people with RTT.[48–50,52] Conversely, a study in 100 patients with RTT showed that average QTc values were within the normal range for patients with RTT of similar age.[43]

Theme C: Neurochemical changes contribute to autonomic decline

Changes in neurochemicals (ghrelin, leptin, substance P and serotonin) all seemed to contribute to autonomic decline. Plasma levels of ghrelin were lower in patients with RTT in comparison to typically developing controls.[55] Perturbations in plasma ghrelin levels might be associated with autonomic dysfunction in RTT. A recent small pilot study showed that treatment with ghrelin reduced the symptoms of autonomic decline in 2 patients.[70] Ghrelin has been suggested to play a key role in maintaining synaptic integrity in higher brain centres,[80] and it rarely functions in isolation. Ghrelin has an inverse relationship with leptin, in that it inhibits the transcription of ghrelin and reduces its circulating levels in a dose-dependent manner.[81] In line with this finding, a study of 32 patients with RTT[37] showed that leptin levels were elevated. It demonstrated a significant correlation between plasma leptin and the sympathovagal balance. Importantly, the correlation was said to be independent of anthropometric characteristics, suggesting that elevated leptin levels caused hyperactivation of the autonomic nervous system, and the patient's weight had minimal influence.

In RTT, plasma serotonin was lower in patients with RTT and was negatively correlated with the sympathovagal balance.[51] This finding was observed only in patients not treated with anticonvulsants. Reduced serotonergic tone is likely to increase sympathetic activity to the myocardium, and the breakdown in this restraint seems to be a critical element of autonomic dysfunction in RTT. In an exploration of higher brainstem regions in RTT, it was suggested that this phenomenon was localized in the dorsal motor nucleus of cranial nerve X (DMX).[64] A key finding from this study was that unlike control samples in which serotonin transporter binding in the DMX decreased over time, in RTT brainstem samples this binding was maintained and remained unchanged.

Levels of the peptide substance P in the cerebrospinal fluid of patients with RTT were lower compared with controls.[62] This finding was in alignment with another study using autopsied samples from patients with RTT, showing that the level of substance P expression was significantly reduced in areas of the brainstem responsible for the integration of cardiorespiratory responses.[45] A follow-up study showed that gastrointestinal dysfunction (feeding impairment and constipation) reflected by decreased substance P expression resided outside the enteric nervous system and most likely originated from specific networks in the brainstem.[46]

Sudden death in RTT

Given the limited number of eligible articles identified as part of the systematic review of sudden death, it was not possible to draw meaningful interconnections and relationships from the data, and thematic analysis was not possible. Still, the quality appraisal of the articles showed that autonomic dysfunction and sudden death should not be considered unique entities, but should be viewed holistically from the perspective of an aberrant cardiovascular manifestation that has its origins in the brainstem. We discuss evidence relating to risk factors for sudden death in RTT below.

There is an elevated risk of sudden cardiac death in RTT[82] that is higher than in the healthy population of the same age.[83] Our systematic review identified 5 studies that provided evidence related to risk factors for sudden death in RTT. First, heart rate variability is lower in patients with RTT.[49] This finding is reflected in the sympathovagal balance that is significantly higher in patients with RTT across all 4 stages of the disorder compared with healthy controls. Second, the mechanism of reduced heart rate variability as a risk factor was independent of structural and functional abnormalities of the heart.[75] Echocardiographic investigations showed no abnormal changes to cardiac function or architecture. A follow-up study consisting of 10 patients on active treatment showed that heart rate variability could be increased by using acetyl-L-carnitine.[52] Third, immaturity of medullary cardiorespiratory neurones has also been suggested as a risk factor for sudden death in patients with RTT.[76] Fourth, another study has suggested that increases in QT prolongation and T-wave changes could lead to sudden death of cardiac origin.[77] Interestingly, T-wave abnormalities showed a tendency to increase with advancing stages of the disorder.

Reduced heart rate variability coupled with abnormal changes in cardiac repolarization contribute to elevated risk in patients with RTT. Some further evidence indicates that low plasma levels of nerve growth factor could be associated with QT prolongation.[84] This study, conducted in 23 patients

with RTT, showed that levels of nerve growth factor were significantly reduced in patients with QT prolongation compared with those with a normal QT interval (p = 0.02). This finding suggests a possible relationship between reduced plasma nerve growth factor levels and abnormal ventricular repolarization in RTT.

Although studies indicate the presence of QT prolongation as a risk factor for sudden death in RTT, 1 study[43] showed that the QT interval was within the normal range for patients with RTT. Another study using echocardiography[22] revealed a significant impairment in biventricular function with a reduced pre-load in 92 patients with RTT, pointing to a possible cardiac structural abnormality.

Discussion

This is, to our knowledge, the first study to appraise the quality of research related to autonomic functioning and sudden death in patients with RTT. We identified 3 key themes from a review of the articles relating to autonomic dysregulation: (A) brainstem modulation of breathing, (B) electrical instability of the cardiovascular system and (C) neurochemical changes contributing to autonomic decline. Following a review of the articles relating to sudden death, we identified reduced heart rate variability, epilepsy and deleterious changes in cardiac repolarization (QT prolongation and T-wave changes) as potential risk factors for sudden death. These predisposing cardiac risk factors can lead to malignant ventricular arrhythmias and subsequent cardiorespiratory arrest. We will discuss the implications of these findings below.

Implications of themes

Theme A: Brainstem modulation of breathing

Based on the evidence related to this theme, impairments in vagally mediated changes to heart rate and oscillations in blood pressure can merge, and combined with hypoxia as a result of breathing dysregulation in RTT, cause hyperactivation of brainstem regions such as the nucleus of the solitary tract. This can lead to brainstem paroxysms. It is quite possible that in patients with RTT who present with a characteristic respiratory phenotype, repeated breath-holding and gasping may elicit increased firing of cardiorespiratory afferents in the brainstem that would, in turn, lead to brainstem hyperactivation and the brainstem shutting down, albeit transiently. This disturbance in cardiorespiratory homeostasis might decouple the cardiac and respiratory system and lead to severe consequences, such as sudden death.

Theme B: Electrical instability of the cardiovascular system

The evidence related to this theme strongly suggested that the underlying autonomic dysfunction causes electrical instability of the cardiovascular system. Although the findings for electrical instability agreed with other studies in patients with RTT with respect to abnormalities in the cardiac ECG[82] and cardiac repolarization (prolonged QT and T-wave changes),[49,77] a recent study involving a much larger sample (n = 100) found that the average QT interval was within the normal range for the RTT population examined.[43] However, a limitation of that study was the lack of follow-up data. It is therefore possible that abnormalities in cardiac repolarization could have occurred but were not captured by the study.

Looking at the broader clinical picture, it is probable that in specific scenarios, electrical instability might shift toward a more parasympathetic or sympathetic overdrive. Even in the absence of QTc prolongation,[43] it is plausible that cardiac rhythms might be particularly vulnerable, especially when a sympathetic–parasympathetic imbalance is dominant. This is because during basal conditions, the parasympathetic tone (rather than the sympathetic tone) seems to have a greater influence on QT and heart rate.[85] Although no clear relationship has been found between clinical features and autonomic dysfunction, certain clinical phenotypes, such as the forceful breathing phenotype[20] or those with the p.Arg294* mutation,[13] might be particularly susceptible to these shifts. Previous studies have also suggested that certain variants, such as the preserved speech variant, might even be resistant to these shifts compared to the classic RTT phenotype.[50]

Theme C: Neurochemical changes contributing to autonomic decline

The evidence related to this theme suggests that reduced levels of ghrelin and substance P are somehow associated with the correct functioning of the autonomic nervous system in RTT. The sympathoactivation driven by leptin in patients with RTT may be due in part to its suppression of ghrelin, and coinciding with the reduction of substance P expression in critical areas of the brainstem such as the nucleus of the solitary tract, suggest that dysregulation of these peptides is intimately associated with the autonomic decline in RTT.

Failure of some aspects related to the serotonergic neurotransmitter system in RTT appears to be important for propagating the deleterious phenotypes observed clinically. This system innervates into cardiorespiratory nexuses in the brainstem. Hence, any abnormality in this system can manifest as catastrophic circuit dysfunction, which has been suggested as a significant driver of mortality in sudden infant death.[86] This is further supported by the finding in patients with RTT that there is a failure to attenuate serotonin transporter binding, especially in critical regions that govern parasympathetic outflow, such as the DMX. All of these neurochemical changes probably destabilize the autonomic nervous system and contribute to autonomic decline.

Autonomic metrics in RTT

Although our review revealed that some form of desynchronization of the autonomic nervous system is present in patients with RTT, an important factor that needs to be considered is whether the LF/HF metric is a true reflection of this desynchronization. Eight studies[37,48–52,57,75] had used the LF/HF metric to quantify autonomic imbalance and showed that this metric was elevated in patients with RTT compared with typically developing controls.[49,50,57] However, studies have challenged the notion that the LF/HF metric is an

accurate measure of the sympathovagal balance.[87,88] Some have argued that the sympathovagal balance cannot be quantified by the LF/HF metric, because this ratio depends largely on a straightforward linear relationship between the autonomic nervous system and low- and high-frequency components.[89] Ventilatory mechanisms are likely to have significant influence and cause uncertainty for the relative contributions of the parasympathetic and sympathetic arms to the LF and HF components. In RTT this is a confounding factor, given that 14 different types of breathing dysregulations have been recognized so far.[19] Furthermore, although autonomic dysfunction in RTT is present alongside the progression of the disorder, it is unlikely to follow a linear trajectory. Although caution is warranted when extrapolating findings from animal models to humans, some studies using animal models of RTT have suggested that breathing abnormalities develop after normal postnatal development.[90] This implies that at birth compensatory mechanisms could nullify the autonomic dysfunction, but as the disorder progresses, those mechanisms are overridden, resulting in fluid excitation of cardiorespiratory pathways that are no longer held back by autonomic restraint. Even when nonlinear metrics are used to assess autonomic function, they can also be affected by respiration rate, position and other cardiorespiratory factors probably unique to the RTT population.

Brainstem mechanisms of autonomic function in Rett syndrome

Although no single unified theme emerged from the studies that could explain the mechanism of autonomic dysregulation in RTT, evidence from the included studies can be pooled and lead to a possible mechanism under the rubric of autonomic dysfunction in RTT. From the evidence presented, one defining factor that emerged from the themes is that the autonomic dysregulation progresses with the disorder. This raises the question of why this occurs.

Significant development of the autonomic nervous system occurs during the third trimester,[91] and evidence points to lower parasympathetic activity in infants born preterm.[92] Therefore, we can hypothesize that the autonomic dysfunction in certain RTT phenotypes is comparable to that of a preterm infant and parallels the autonomic dysfunction seen in familial dysautonomia.[93] The emergence of serotonergic (5-HT) pathways during this prenatal period is critical for the maturation of respiratory networks. Brain-derived neurotrophic factor (BDNF), through activation of tyrosine kinase B receptors, plays a critical role in inducing the 5-HT neuronal phenotype[94] and is dysregulated in RTT.[95] Although we urge caution in making inferences with studies done in animals, these findings are supported by evidence from animal models. First, mice lacking neurotrophic factors such as BDNF have severe respiratory impairments.[96] Second, BDNF expression is reduced in the nucleus of the solitary tract in *MECP2* knockout mice.[97] Third, neurons of the Kölliker–Fuse nucleus become responsive to BDNF after birth.[98] Fourth, studies in brainstem slices of the solitary tract in rats demonstrate that BDNF signalling through its modulation of glutamatergic excitatory transmission tonically regulates cardiovascular function.[99] Fifth, neuronal excitability is increased in the nucleus of the solitary tract in *MECP2*-null mice.[100] Although human studies are scarce, in a study examining BDNF expression in postmortem brainstem samples from 45 fetuses and newborns that died suddenly, the nuclei of the Kölliker–Fuse showed significant levels of developmental immaturity, evidenced by dysregulated BDNF expression.[101] Taken together, these observations suggest that as BDNF becomes dysregulated, the shift between inhibitory and excitatory neuronal firing in the nuclei of the Kölliker–Fuse and solitary tract become unbalanced, resulting in the abnormal firing of cardiorespiratory networks and altered autonomic tone.

From the evidence presented, we hypothesize that in people who are typically developing and people with RTT, neurotrophic maturation of serotonergic networks extends beyond the fetal epoch. Although the number of networks is reduced during childhood and adolescence in people who are typically developing, it remains unchanged in people with RTT, and the imbalances in the inhibition and excitation of cardiorespiratory networks continue to persist across the stages of the disorder. The emergence of breathing abnormalities in RTT does not occur from birth. Recent time-to-onset data have indicated that by the age of 5 years, breath-holding is reported in 63.8% of people with RTT and hyperventilation in 50.8%.[13] It is therefore possible that compensatory mechanisms stabilize neurotrophic-induced maturation of serotonergic networks during early life. This idea correlates with the finding that impaired brainstem serotonergic deficiency is a precursor for sudden infant death syndrome.[86] As the disorder progresses, compensatory mechanisms become weakened, because far fewer serotonergic networks are pruned back. In this scenario, autonomic dysfunction might "re-emerge" or "catch up" and drive the different autonomic phenotypes seen in RTT across the age ranges.[20] Different "emergence patterns" might also be associated with age and drive clinical symptoms; for example, abdominal bloating appears to be more frequent in patients older than 20 years of age,[13] and T-wave abnormalities appear to be more frequent in stages III and IV of the disorder compared with stage I.[77] Moreover, there is a pattern of increasing QTc with age.[43] A comparable mechanism of catch-up autonomic dysfunction has been suggested for young adults who were born prematurely.[102] The state of autonomic dysfunction in RTT is likely to be highly complex, and given the pervasive nature of the autonomic nervous system, a range of control or compensatory mechanisms might be in operation, which can become imbalanced in different situations.

Clinical impact of the themes and management of patients with Rett syndrome

Brainstem modulation of breathing, electrical instability of the cardiovascular system and neurochemical changes that contribute to autonomic decline emerged as the dominant themes in this review, raising the question of what factors arising from these themes could increase brainstem vulnerability in patients with RTT and trigger events that cause acute

cardiorespiratory distress. Figure 3 presents an overview of the factors that need to be considered. The themes have significant clinical implications, which are discussed below.

Sudden death in epilepsy

The incidence of sudden death in epilepsy (SUDEP) in RTT is unknown; however, given the sporadic nature of epileptic seizures in patients with RTT, it is possible that seizures are another risk factor for sudden death in RTT. This possibility is supported by evidence showing that the root mean square score is reduced in people with RTT,[68] and that lower root mean square scores are associated with increased SUDEP-7 Risk Inventory scores.[103] Over the lifespan of a person with RTT, the risk of developing epilepsy is about 90%; however, seizure frequency and remission are highly variable, with a clinical picture of epilepsy reflecting a dynamic flux of intermittent remission and relapsing over the lifespan.[104] It is important to achieve optimal seizure control to prevent the risk

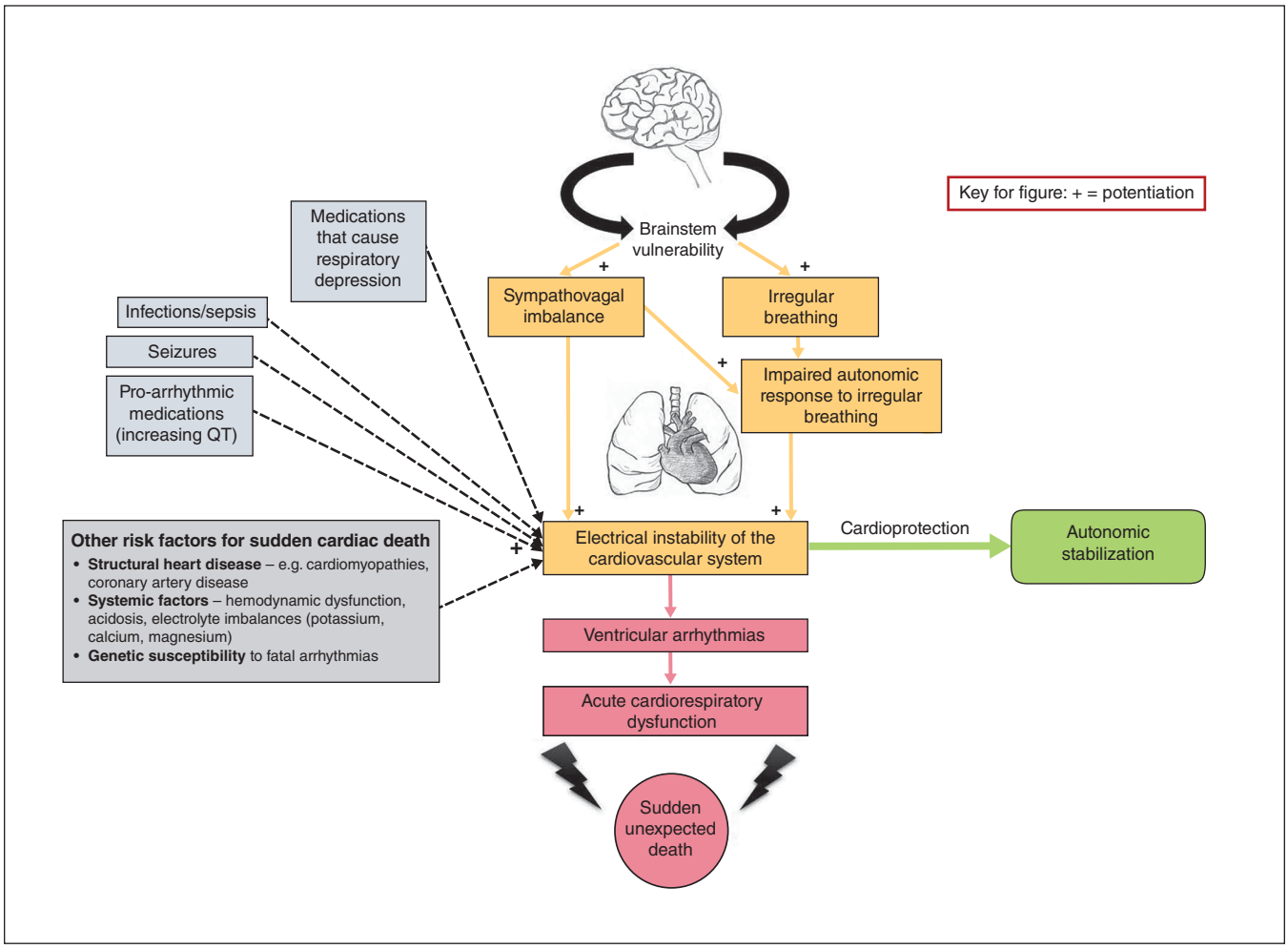

Fig. 3: Potentiation of brainstem vulnerability in RTT. Brainstem vulnerability in RTT leads to impaired cardiorespiratory autonomic control that presents with irregular breathing and modulations in the sympathovagal imbalance. Both hyperventilation and breath-holding shift the equilibrium, which is not sufficiently compensated for by an appropriate increase in vagal tone. These abnormalities result in heightened cardiac electrical instability and increased susceptibility to arrhythmias, represented by reduced heart rate variability and ECG abnormalities (prolonged QTc, T-wave abnormalities). In patients with Rett syndrome, intervening events such as epileptic seizures and infections/sepsis might enhance this underlying autonomic impairment and precipitate an acute cardiorespiratory dysfunction that can occasionally result in sudden death. For the same reasons, pro-arrhythmic medications and those that induce central respiratory depression (e.g., benzodiazepines) must be used with extreme caution in these patients, because they could worsen the underlying autonomic instability. Patients who present with a combination of potentiating factors such as those with significant breathing abnormalities (hyperventilation/breath-holding), uncontrolled generalized epileptic seizures and QT prolongation might be at increased risk for sudden death. A formal cardiac assessment would be warranted in people with suspected QT prolongation to evaluate the use of β-adrenergic blockers, even though their efficacy in reducing cardiac mortality in people without definite long QT syndrome has not yet been demonstrated. Other factors that can increase the risk of sudden cardiac death include structural and functional heart defects, transient systemic factors such as metabolic or hemodynamic abnormalities, and genetic predisposition to fatal arrhythmias. These factors may act individually or in combination. ECG = electrocardiogram; QT = Q and T waves on electrocardiogram; QTc = corrected QT; RTT = Rett syndrome.

of SUDEP, especially in those with significant breathing irregularities, who are more prone to develop a central hypoventilation syndrome. In terms of seizure control, it should also be noted that the regular use of benzodiazepines or barbiturates to achieve seizure control, or the use of benzodiazepines to manage increased muscular tone, increases the risk of respiratory arrest by direct suppression of the breathing centres in the brainstem. It is therefore preferable to use non-benzodiazepine medications as regular treatments for patients with RTT, and to save benzodiazepines for rescue medications in cases of prolonged seizures or status epilepticus. In such life-threatening settings, where benzodiazepines represent first-line treatment,[105] it is important to administer an adequate dose per kilogram of weight, because suboptimal doses can lead to scenarios in which multiple administrations are used, leading to an increased risk of medication-induced respiratory arrest.[106]

Formal cardiac assessment

It is imperative to identify patients with an increased predisposition for QT prolongation, because of the increased risk of torsadogenic events, especially if concomitant risk factors such as recurrent convulsive seizures and severe breathing irregularities are present. Patients who have suspected long QT might benefit from formal cardiac assessment. Prophylactic treatment with β-blockers could be an option.[107] Given the unique symptom profile of this population, the risk:benefit ratio of treatments must be considered carefully. In patients with RTT considered to be at very high risk (QT > 550 ms) despite β-blocker or patients who have marked T-wave abnormalities,[77] an implantable cardioverter-defibrillator could be another option. A new scoring system for selecting appropriate patients for cardioverter-defibrillator placement might help to facilitate this process if this treatment option were considered.[108]

Medications that can prolong QT

Medications that can cause prolongation of the QT (for example, antibiotics or psychiatric medications) must be avoided when possible. If a compound with a known pro-arrhythmic risk needs to be used, a β-blocker can be considered as a prophylactic option before starting the "at-risk" medication. Moreover, patients must be monitored closely during titration, which can be performed according to the principle of the effective dose with minimal side effects model, in which the lowest dose to achieve sufficient management of symptoms with the least side effects is used.[109]

Other considerations for managing EBAD

Even if there is evidence[110] that cardioselective β-blockers are more effective in preventing ventricular arrhythmias in people with long QT, nonselective β-blockers such as propranolol might also be considered for their efficacy in managing anxiety symptoms and challenging behaviours in patients with autonomic dysregulation.[111] Buspirone can also be an option for managing the symptoms of EBAD. Previously using a biometric-guided strategy, we have used buspirone to stabilize heart rate variability and improve the symptoms of EBAD in a patient with RTT.[15] The use of wearable sensor technology offers a noninvasive approach for monitoring physiologic signals and tracking the symptoms of EBAD in patients with RTT who are unable to communicate.

Limitations

Carrying out this review was complex, because no standardized method has been established for monitoring autonomic function in RTT, so it was difficult to make definitive inferences across studies that used different measures. Nevertheless, the studies using autonomic metrics to quantify autonomic dysfunction are highly important, and alongside observational studies, were valuable in terms of furthering our understanding of impairments in the autonomic nervous system and causes of sudden death in RTT. We developed the search terms to capture the majority of research studies done into autonomic dysfunction and sudden death in patients with RTT, but the search terms might not have captured all of the relevant information. For example, our search terms were unable to pick up a study relating to cardiac abnormalities in RTT.[82] Nevertheless, our broad search terms were able to capture 39 studies in RTT in which autonomic function had been explored. A wide range of assessment tools exist for assessing the quality of the literature. However, given a lack of tools for assessing the quality of literature in the rare disease population, we decided to choose a tool that had previously been used in RTT[32] to minimize the ambiguity of using quality-assessment tools in the RTT literature. The overlapping nature of the themes also highlighted the double-counting aspects of some subjects that were a part of each theme. The frequency of themes revealed some overlap, so the number of participants specified for each theme (as presented in Fig. 2) may not be an accurate representation. For example, given the overlap between breathing irregularities (theme 1) and heart rate variability metrics (theme 3), the same 151 patients described by Pini and colleagues[20] appeared for both. This overlap was evident for other complementary themes, such QTc changes (theme 4) and vagal imbalance (theme 5), where studies overlapped.[48–50,52,57] Whenever it was possible to state whether subjects were double-counted, we have clearly stated this in the text. Given the limited patient population, it is not surprising that multiple publications reported on patients recruited from recognized databases, which have cumulatively improved our understanding of autonomic dysfunction in RTT.

One of the limitations of a grounded-theory approach to thematic analysis is the unconscious application of researcher bias. Pre-existing knowledge and conceptions of the literature might influence the generation of themes rather than letting themes emerge. We agree that a true inductive analysis might not always be possible because of our prior knowledge of the subject area, but we were able to use a balanced approach to the thematic analysis. To manage the notion that pre-existing knowledge might over-influence themes, the authors reviewed the thematic analysis, and the 7 themes that emerged and were grouped into 3 final themes were based on consensus agreement.

Conclusion

The themes identified in this study contribute to our understanding of autonomic dysfunction and sudden death in patients with RTT, and presented options for clinical management. The themes support a role for autonomic dysfunction in increasing the probability of sudden death in RTT, but our findings were not definitive and should be interpreted with caution. Based on our synthesis of available evidence, other co-occurring factors may be related to sudden death in RTT, such as secondary dysautonomia arising from epileptic seizures; cardiorespiratory failure; infection (sepsis/aspiration pneumonia); systemic factors; or acute gastric dilation.[112,113]

Our findings also strongly suggest that autonomic dysfunction is an important feature of EBAD and is likely the fulcrum for the emotional and behavioural dysregulation observed in these patients. Because autonomic restraint is diminished in RTT, gastrointestinal, sleep and cardiorespiratory impairments are likely to have an adverse effect on internalizing and externalizing behaviours in people with RTT. Given that recent data from the natural history study has shown that internalizing behaviours such as anxiety are phenomenologically similar to those described in the psychiatric population,[114] it opens an important area for future research.

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