

Autonomic Nervous System Dysregulation: Breathing and Heart Rate Perturbation During Wakefulness in Young Girls with Rett Syndrome

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ABSTRACT: This study characterizes cardiorespiratory dysregulation in young girls with *MECP2* mutation–confirmed Rett syndrome (RS). Respiratory inductance plethysmography of chest/abdomen and ECG was obtained during daytime wakefulness in 47 girls with *MECP2* mutation–confirmed RS and 47 age-, gender-, and ethnicity-matched controls (ages 2–7 y). An in-home breath-to-breath and beat-to-beat characterization was conducted and revealed that breathing was more irregular, with an increased breathing frequency, mean airflow, and heart rate in RS *versus* controls. There was a decreased correlation between normal breathing and heart rate variability, and an exaggerated increase in heart rate response to breathholds in RS *versus* controls. We conclude that girls with RS have cardiorespiratory dysregulation during breathholds as well as during “normal” breaths and during breaths before and subsequent to breathholds. This dysregulation may offer insight into the mechanisms that render girls with RS more vulnerable to sudden death. (*Pediatr Res* 60: 443–449, 2006)

RS is one of a growing number of disorders characterized by autonomic nervous system dysfunction/dysregulation (1). Diagnosis of RS is based on clinical criteria (2–4), with more than 85% of identified girls having mutations in *MECP2* on the X chromosome (5; B. Roa, personal communication). The RS phenotype includes normal development until 6–18 mo of age, then regression with slowing of head circumference growth, loss of language, development of stereotypical hand movements, gait and truncal apraxia, EEG abnormalities, seizures, spasticity, and scoliosis (2).

Breathing irregularities consistent with autonomic dysregulation in RS include characteristic patterns variably described as hyperventilation, Valsalva maneuvers, apnea, apneusis, breathholding, and rapid shallow breathing (6–17). These irregularities are reported to occur near-exclusively during

wakefulness (8,17). Age may play a role, as more breathholds and forceful breathing are reported in 5–10-y-old patients compared with more of a Valsalva pattern in young adults (13). Conclusions regarding imbalance of sympathovagal input have been made primarily from heart rate and blood pressure monitoring during spontaneously occurring breathholds, coupled with analytical measures derived from these signals (4,11,13). Julu and others proposed a disturbance in cardiovascular and respiratory system integration, describing their findings as central autonomic dysfunction (4,11,13). Further support for autonomic dysregulation comes from observations of decreased heart rate variability, longer corrected QT intervals, sinus bradycardia, gaseous abdominal distension, sweating, cool extremities (3,7,18–23), flushing and temperature dysregulation (M. Coenraads, personal communication).

Despite survival into adulthood, Kerr and colleagues (24,25) reported that 20–26% of RS deaths are sudden and unexpected, and that the deaths occur primarily during wakefulness. QT prolongation and nonspecific ST changes in RS suggest cardiac causes for sudden death, though sudden death has been reported with normal QT intervals (26). Here we further characterize the autonomic phenotype of cardiorespiratory dysregulation in young girls with *MECP2* mutation–confirmed RS, studied in their home environment. The intent was to characterize breath-to-breath irregularities and cardiorespiratory changes associated with breathholds, rather than a catalogue of breathing abnormalities (4,13).

METHODS

Rett patients. Girls aged 2–7 y with clinical RS and a *MECP2* mutation were recruited.

Control subjects. Healthy girls matched for age/ethnicity to girls with RS were recruited. Exclusion criteria included obstructive sleep apnea and any family history for three generations of RS, sudden infant death syndrome, congenital central hypoventilation syndrome, Hirschsprung disease, neural

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Abbreviations: AMP, amplitude of respiratory inductance plethysmography sum; CV, coefficient of variation; LF, low-frequency heart beat components; *MECP2*, gene encoding methyl-CpG-binding protein 2 on the X chromosome; RMS, root-mean-square amplitude of correlation; RS, Rett syndrome; T_E, expiratory time; T_I, inspiratory time; T_I/T_{TOT}, duty cycle; T_{TOT}, cycle length (T_I + T_E)

crest tumors, familial dysautonomia, and apparent life-threatening events in infancy.

Consent process. The study was approved by the Rush University Medical Center Institutional Review Board. Consent and HIPAA forms were reviewed and signed by parents of study subjects.

Recording technology. Data were recorded in each subject's home using noninvasive ambulatory LifeShirt technology (VivoMetrics, Inc., Ventura, CA) to record breathing using a fabric zipper-front vest with two intrinsic inductance plethysmography bands at chest and abdomen, heart rate using three surface chest electrodes, and Hb saturation using a pulse oximeter. Participant data were recorded on a removable memory flashcard and coded to ensure participant anonymity and objectivity in data analysis.

Data collection protocol. Girls with RS and matched controls were instructed to wear the LifeShirt monitor continuously, for two consecutive hours on two consecutive mornings, and to watch singing videos while in the seated position. This instruction was included because a breathhold is typically demonstrated just before making voice to sing, and because girls with RS are typically seated at rest. Since one of the intents of the study was to compare the physiologic changes that accompany breathholds it was essential to assure such events occurred with regularity among control subjects.

Wave form computerized scoring. Inductance plethysmography waveforms (chest, abdomen, sum), ECG, Hb saturation, and pulse wave form were exported, then loaded into Igor Pro (WaveMetrics, Lake Oswego, OR) for analysis using custom-developed software. End-inspiration (shown as +) and end-expiration (shown as ×) were detected through automated routines (Fig. 1) and data points were manually repositioned to insure proper breath detection. Individual breaths were classified as nonbreathhold breaths, breathholds, or contamination by artifact; breathholds were identified by wave form configuration (Fig. 2A). R waves were detected on ECG trace and verified; their timing was used to compute heart rate (HR), R-R interval and R-R irregularity. Movement artifact on the pulse oximetry channel was a consistent problem among RS, precluding analysis.

Data sampling algorithm. Study duration was 127 ± 18 and 118 ± 13 min (mean \pm SD) in RS and controls, respectively.

Cardiorespiratory variables included in analysis. Respiratory variables included: inspiratory time (T_I), expiratory time (T_E), cycle length ($T_{TOT} = T_I + T_E$), duty cycle (T_I/T_{TOT}), T_{TOT} irregularity, breathhold T_{TOT} (start of one breathhold to start of next breathhold), breathhold T_{TOT} irregularity, inductance plethysmography amplitude sum (AMP) (Fig. 1), peak and mean inspiratory flow, and change in end-expiratory and end-inspiratory volumes. Heart beat variables included heart rate and R-R irregularity. Irregularity was defined for both respiratory and heartbeat variables as fractional change in T_{TOT} (or R-R interval) from one cycle to the next: $Irreg(n) = [T_{TOT}(n) - T_{TOT}(n-1)]/T_{TOT}(n-1)$. Irregularity measures included CV to indicate overall variability and/or an irregularity score to indicate variability comparing consecutive cycles.

Wave form analysis specific to breathholds. Breaths were classified as being five or fewer breaths before or following a breathhold, or at least five breaths from a breathhold in either direction ("normal breaths") (Fig. 3A). R-R intervals were similarly grouped.

Cross-correlation analysis of breathing and heart rate. The relationship between breathing and heart rate was examined using cross-correlation applied to a 30-min section of stable waveforms. There were two distinct

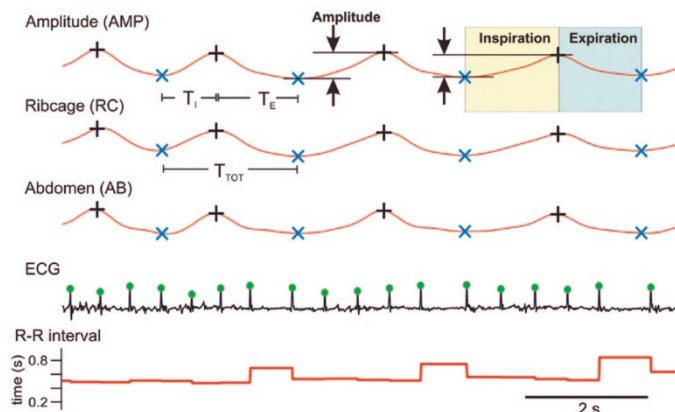


Figure 1. Inductance plethysmography waveforms [amplitude (AMP), ribcage (RC), and abdomen (AB)] and ECG (R-R interval) from a 7-y-old girl with RS indicating end-inspiration and end-expiration, T_I and T_E , as well as R wave peak.

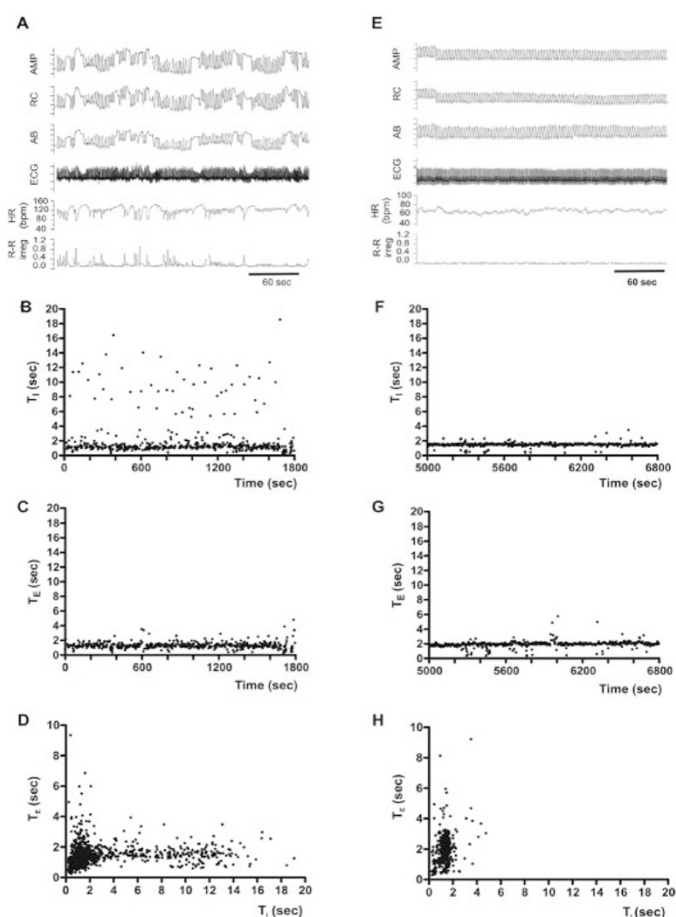


Figure 2. Breaths and breathholds in one 7-y-old girl with RS (A–D) and her age-matched control (E–H). Inductance plethysmography waveforms (AMP, RC, AB), ECG, heart rate (HR), and R-R irregularity in RS (A) and a control (E). B, F: Sequential histograms of consecutive T_I (ordinates, B, F) and T_E values (ordinates, C, G) are plotted over time (abscissa). D, H: Scatter plot of T_E vs T_I .

components: the first corresponding to a series of peaks at intervals of T_{TOT} , with the largest peak at approximately zero lag, and the second corresponding to a broad peak with a maximum at 4–6 s lag. Because of overlap, we alternately filtered out one component and measured the strength of the other by applying perfect frequency-domain filters to the Fourier-transformed correlation, then transforming back into the time domain. To isolate the respiratory component, a bandpass filter was applied from $0.7 \times f$ to $2.0 \times f$, where f is respiratory frequency, measured from autocorrelation performed on AMP for the same 30 min section of recording. To isolate the broad peak (heart rate component), a bandpass filter was applied from 0.03 Hz (1/30 s) to $0.7 \times f$. The cutoff frequency of $0.7 \times f$ was chosen to provide optimal separation while preserving the broad peak configuration. Similarly, the 0.03 Hz cutoff was chosen to preserve the shape of the broad peak while eliminating baseline shifts and other very low frequency (LF) components. After filtering, root-mean-square (RMS) amplitude of the correlation over the 20-s window centered on zero-lag (for both the f and LF heart beat components), and location and amplitude of the peak in the LF component were measured.

Statistical analysis. Data are presented as mean \pm SD or \pm SE. t Test was used to compare mean differences between RS cases versus controls. Significance was applied to $p < 0.05$.

RESULTS

Study subjects. Forty-seven girls with *MECP2* mutation-confirmed RS and 47 matched controls were studied: 44 Caucasian, two biracial, and one multiracial child in each group. Mean ages (SD) were 4.7 y (1.3) and 4.8 y (1.3), respectively.

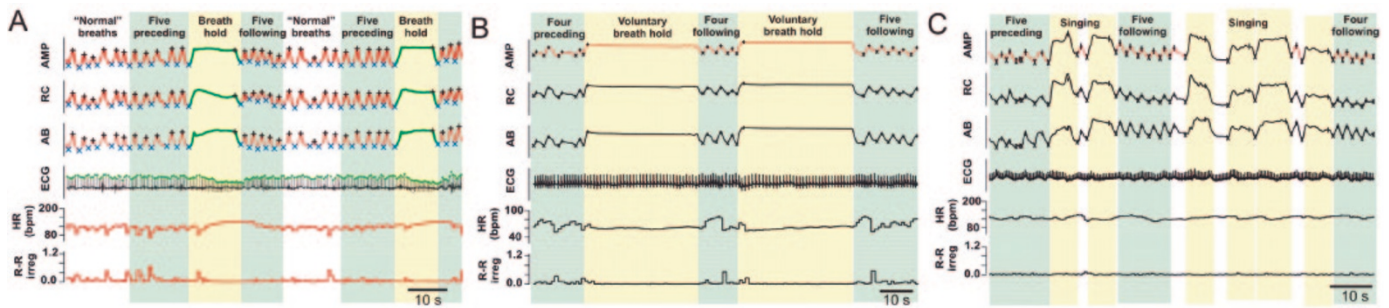


Figure 3. Characterization of spontaneous breathholds in one 7-y-old girl with RS (A) and voluntary (B) and pre-singing breathholds (C) in two different 7-y-old controls. Inductance plethysmography waveforms (AMP, RC, AB), ECG, HR, and R-R irregularity are shown for A, B, and C. Data categories: “normal” breaths, breathholds, five breaths preceding and following breathholds.

Qualitative data analysis: breathholds. RS patients exhibit spontaneous and frequent occurrence of breathholds (Fig. 2A), with rib cage, abdomen, and sum waveforms maintained in inspiratory phase with variable breathhold amplitude. Maintenance of inspiration at an amplitude similar to or exceeding the amplitude of preceding or following breaths suggests that this activity pattern is neither Valsalva nor central apnea. Rib cage and abdomen deflections in the same direction indicate that this activity pattern is not paradoxical breathing. However, study technology does not allow unambiguous discrimination between apneusis and breathholding (surface EMG would be required) and Valsalva maneuver (intrathoracic pressure measurements would be required). Thus, we simply use the term “breathholds.” Plotting T_I of consecutive individual breaths over time reveals frequent occurrence of prolonged T_I values (>4 s) that reflect spontaneous breathholds (Fig. 2B). Prolonged T_E values compatible with central apnea were absent in the sequential T_E histogram (Fig. 2C). Likewise, plots of T_I against T_E reveal a large spread of T_I , (<20 s), but not T_E (Fig. 2D).

The representative control exhibits regular breathing without spontaneous breathholds (Fig. 2E). Prolonged inspiratory (Fig. 2F) and expiratory durations (Fig. 2G) are absent in sequential T_I (Fig. 2F) and T_E histograms (Fig. 2G). Plotting T_I against T_E results in a larger spread of T_E compared with T_I (Fig. 2H), which is distinctly different from the child with RS.

Breathholds occurred frequently, but not regularly in RS. Inter-breathhold intervals could be short (Fig. 4A) or prolonged (Fig. 4B). The plot of 100 consecutive inter-breathhold intervals (IBI, Fig. 4C) illustrates the absence of obvious regularity in breathhold occurrence, but time between two consecutive breathholds tended to be longer (Fig. 4C) if the duration of the first breathhold was long (Fig. 4D) ($r^2 = 0.381$; slope $p < 0.0001$) (Fig. 4, A, and B). Intervals between consecutive brief breathholds were short (Fig. 4A), whereas a long IBI followed a longer breathhold (Fig. 4B).

Heart rate response in RS. Figures 2A, 3A, and 4A and B demonstrate a pronounced increase in heart rate with each breathhold ($16.1 \pm 9.7\%$ in the representative girl in Figure 4, $p < 0.0001$). Although the percentage heart rate increase per breathhold was variable (Fig. 4E), heart rate measured at exhalation onset tended to be higher after long duration

breathholds when compared with shorter duration breathholds (Fig. 4F).

Comparison between breathholds in RS and voluntary and pre-singing breathholds in controls. We qualitatively compared breathholds in a child with RS (Fig. 3A) with breathholds voluntarily produced in a control (Fig. 3B). Voluntary breathholds (Fig. 3B) were longer than spontaneous RS breathholds (Fig. 3A), but respiration tracings were otherwise not different. However, it was neither practical nor safe to instruct 2–7 y olds to breathhold repeatedly in the home without clinical supervision. A practical alternative to voluntary breathholds was singing along to a familiar videotape (Fig. 3C). During a pre-singing breathhold, diaphragmatic activity was maintained in a phase commonly referred to as postinspiration (27). Like breathholds in RS (Fig. 3A), respiratory activity for the pre-singing control breathhold was maintained in inspiration in the sum, rib cage, and abdomen channels (Fig. 3C) and the amplitude of these breathholds could exceed the amplitude of preceding nonbreathhold breaths. Despite similarities in the respiratory traces, available technology does not allow us to determine whether voluntary breathholds or the breathhold before singing in controls have the same neuronal basis as breathholds observed in RS, *i.e.* apneusis *versus* breathholds.

The heart rate response to the RS breathhold was qualitatively very different from the response to a voluntary control breathhold (Fig. 3). In RS, the heart rate increased during the breathhold (Fig. 3A) but remained unchanged in the control (Fig. 3B). Heart rate change was not significant in the control voluntary breathhold ($6.2 \pm 12.85\%$, $p = 0.58$), or the pre-singing breathhold ($-4.4 \pm 26.6\%$) (Fig. 3C); though the latter sometimes produced brief IBI (Fig. 5A), they did not occur regularly (Fig. 5B). In contrast to RS (Fig. 4D), the IBI in the control did not depend on breathhold duration (Fig. 5C), heart rate was not altered in a regular manner by consecutive breathholds (Fig. 5D), and there was no dependency between breathhold duration and heart rate (Fig. 5E).

Quantitative data analysis: cardiorespiratory timing parameters. To quantify differences in breathing behavior, data were separated into four categories: 1) “normal” breaths (Table 1, supplemental material online); 2) breathholds (Table 2, supplemental material online); 3) 5 breaths preceding a breathhold (Table 3, supplemental material online); and

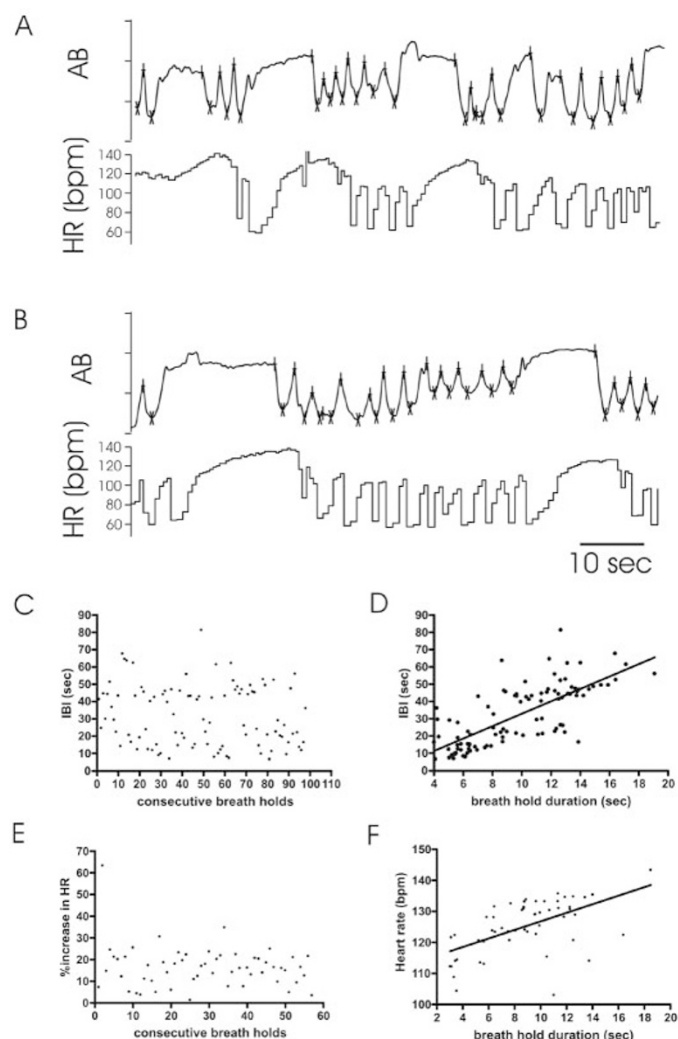


Figure 4. Breathholds in RS. (A, B) Inductance plethysmography waveforms and HR are shown. (C) Sequential histogram plots IBI (ordinate) between 100 consecutive breathholds (abscissa). (D) Histogram plots breathhold duration (abscissa) against interval to subsequent breathhold (IBI, ordinate) ($p < 0.00001$, $r^2 = 0.65$). (E) Sequential histogram plots percent HR increases (ordinate) for 60 consecutive breathholds (abscissa). (F) Histogram plots heart rate responses to breathholds (ordinate) against duration (abscissa) ($p < 0.0001$, $r^2 = 0.32$).

4) 5 breaths following a breathhold (Table 4, supplemental material online). Data expressed as percent change comparing RS *versus* controls are provided in Results; raw data are provided in Tables 1–4 (supplemental material online).

“Normal” breaths. T_{TOT} was significantly shorter in RS when compared with matched controls (-18.89% ; $p < 0.00001$) and cycles were more irregular (CV: 62.54% , $p < 0.0001$; irregularity score: 68.93% , $p < 0.00001$), demonstrating consistency with Figure 2, B and F. The T_{TOT} decrease was due to decreased T_I (-10.19% ; $p < 0.007$) and T_E (-25.16% ; $p < 0.00001$), consistent with Figure 2, C and D [CV: 55.4% (T_I), 56.13% (T_E), both $p < 0.00001$].

Time to peak AMP was not different, but mean and peak AMP/ T_I were enhanced in RS (17.94% , 17.51% , both $p < 0.00001$), as was associated irregularity (CV: 35.27% , 29.4% , $p < 0.00001$).

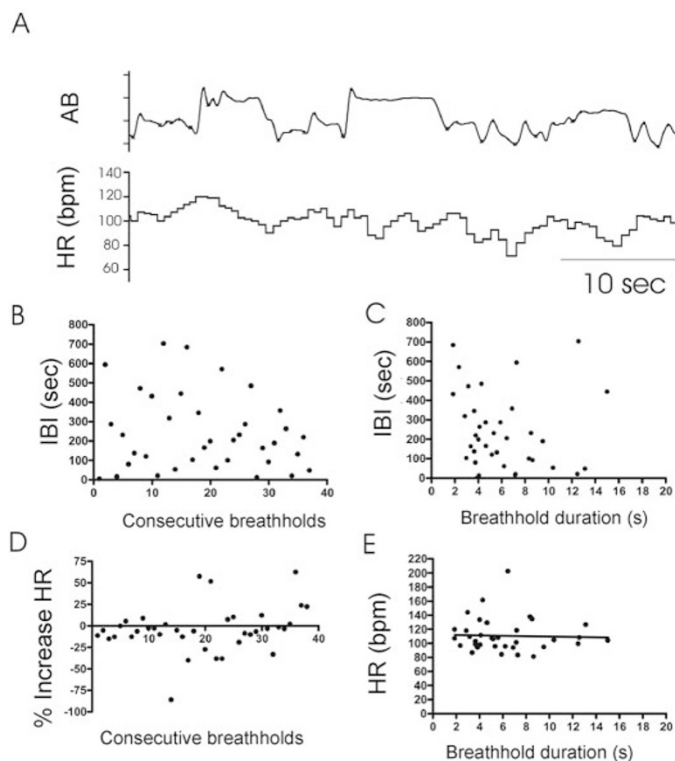


Figure 5. “Breathholds” in controls. (A) Inductance plethysmography waveform (AB), and HR are shown. (B) Sequential histogram plots IBI (ordinate) between 39 consecutive breathholds (abscissa) in a 7-y-old control. (C) Histogram plots breathhold duration (abscissa) against intervals to subsequent breathholds (IBI, ordinate). (D) Sequential histogram plots percent HR changes (ordinate) for 39 consecutive breathholds (abscissa). (E) Histogram plots heart rate responses to breathholds (ordinate) against duration (abscissa).

Heart rate was increased in RS (mean R-R interval: -9.41% , $p < 0.0001$) and was more regular (irregularity score: -29.17% , $p < 0.04$).

Breathholds. Inter-breathhold intervals and T_{TOT} were not significantly different between RS and controls, allowing us to match on average frequency and duration of breathholds. However, timing of breathholds differed: T_I : 21.93% ($p < 0.002$), T_E : -14.85% ($p < 0.018$). T_E was more irregular (CV: 15.25% , $p < 0.0025$) in RS *versus* controls.

Time to peak AMP of breathholds was shorter ($-8.56 \pm 13.84\%$, $p < 0.001$), peak AMP/ T_I (17.54% , $p < 0.01$), and associated variability were decreased (CV: -10.98% , $p < 0.01$).

During breathholds, mean R-R interval decreased in RS (-4.28% , $p < 0.04$), but not irregularity score or CV.

Breaths preceding and following breathholds. T_{TOT} for the five breaths before and after breathholds was shorter in RS *versus* controls (-12.16% , $p < 0.009$; -12.7% , $p < 0.006$, respectively) and more irregular (CV before: 32.79% , $p < 0.0001$; after: 32.25% , $p < 0.00001$; irregularity score before: 44.12% , $p < 0.00006$; after: 38.54% , $p < 0.00005$). The five breaths before and after breathholds demonstrated decreased T_E (before: -19.14% , $p < 0.00005$; after: -20.46% , $p < 0.00002$). Both T_I and T_E were more irregular (CV before: T_I : 27.67% , $p < 0.001$; after: 25.10% , $p <$

0.0002; before: T_E : 26.22%, $p < 0.0004$; after: 27.25%, $p < 0.00001$).

Time to peak AMP did not differ, but mean and peak AMP/ T_I of the five breaths before a breathhold increased (19.3%, $p < 0.007$ in RS; 18.04%, $p < 0.013$ in controls).

Heart rate was increased among RS (mean R-R interval: -7.32%, $p < 0.0004$ for five breaths before a breathhold, -7.22%, $p < 0.0005$ for five breaths after a breathhold).

Cross-correlation analyses. Cross-correlation methods evaluated differences in cardiorespiratory interactions. In a representative girl with RS each breathhold (Fig. 6A, green) was associated with an initial decrease (Fig. 6A, “zero lag decrease,” light green arrow) and a subsequent increase in heart rate (Fig. 6A, “delayed increase,” dark green). During each “normal” breath there was a zero-lag increase in heart rate (Fig. 6A, “zero lag increase,” red arrows). Two different responses to breathholds and “normal” breaths were reflected in cross-correlation analyses between AMP and heart rate waveforms (Fig. 6B). The upper graph showing the unfiltered correlation between heart rate and respiratory activity confirms a peak at zero lag indicating an instantaneous heart rate increase during each respiratory cycle and a second peak

reflecting the delayed heart rate increase (described above). Comparison with a matched control (Fig. 6C) suggests that peak correlation at zero lag in RS (Fig. 6B, upper graph) was attenuated, which becomes more evident in the respiratory domain after wave form filtering (Fig. 6, B and C, middle graphs). The delayed heart rate response was enhanced in RS (Fig. 6C, upper graph), and is consistent with heart rate traces in Figures 2A, 3A, and 4A and B). The LF band (Fig. 6B, lower graph) illustrates the heart rate response to breathholds: an initial decrease in correlation between heart rate and respiration during zero lag and a broad increased correlation over a period of 3–8 s in RS (Fig. 6B, lower graph), but not in the control (Fig. 6C, lower graph).

Cross-correlation analyses performed for all girls with RS and their matched controls confirmed these observations. Correlation analysis between respiration and heart rate was measured at zero lag for all breaths and related heart rates and provided as RMS values (Fig. 7, A and B). RMS amplitude for RS was significantly decreased at zero lag (Fig. 7A). The heart rate low frequency band to characterize the response to respiratory activity peaked at approximately 4 s (Fig. 7B), and did not differ in RS versus controls. Peak amplitude of heart rate correlation of at least one second lag revealed significantly increased amplitude in the heart rate LF band correlation in RS (Fig. 7C).

DISCUSSION

Previous studies aimed at categorizing various forms of breathing disturbances in RS (such as hyperventilation Valsalva maneuver, apneas, apneusis, and breathholds). In the present study, every consecutive breath and heart beat of a large cohort of 47 young girls with *MECP2* mutation-confirmed RS and 47 age-, gender-, and ethnicity-matched controls were evaluated in a cycle-by-cycle manner in their home environment during daytime wakefulness. This approach revealed three previously unreported observations. Even when breathing is apparently normal in RS, it was 1) more irregular than normal breathing in controls, 2) mean inspiratory flow was increased, and 3) breathing frequency was significantly higher, primarily due to a decrease in expiratory duration. With respect to heart beat regulation, we observed: 1) R-R interval was shorter in RS, 2) normal breathing and heart rate variability had a decreased correlation, and 3) the heart rate response to breathholds was increased in RS versus controls. While controls showed a decreased heart rate during breathholding, the heart rate was increased in RS. It must be emphasized that, for obvious reasons, it is not possible to identify a “perfect” control behavior to match the “pathologic” breathholds commonly observed in RS. We thus opted to provoke repeated “breathholds” in controls by encouraging them to sing, which may have different mechanisms for inducing heart rate changes. The heart rate response of RS has been previously reported by Julu *et al.* (4,11,13) and was attributed to be comparable to the response to a Valsalva maneuver. However, without further measurements, including intrathoracic pressure, EMG, and blood pressure, which were not assessed in the present study,

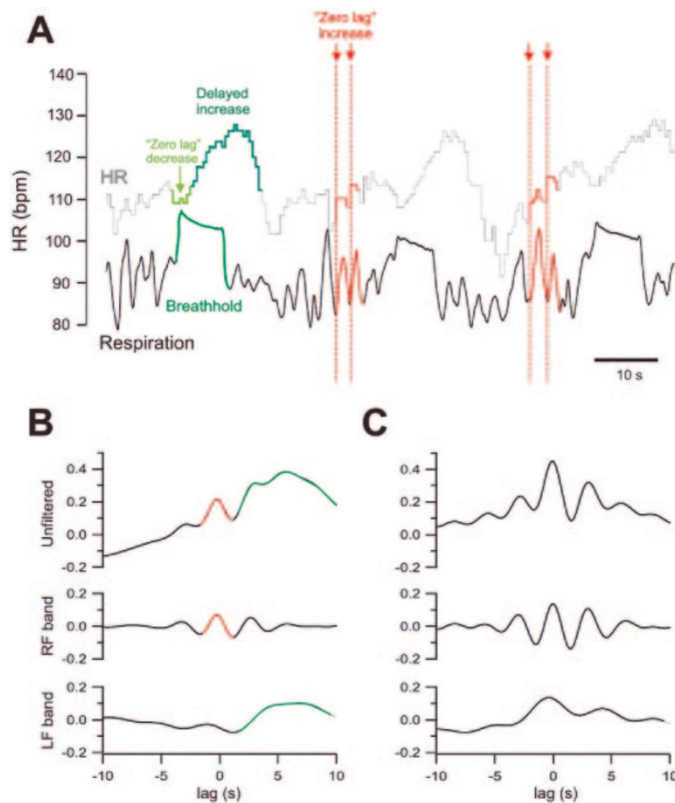


Figure 6. Cross-correlation analysis in one 7-y-old with RS and her matched control. (A) HR (upper trace, light gray) and respiratory inductance channel are shown (AMP = unitless, lower trace, black). One breathhold (green) and four representative “normal” breaths (red) are marked to highlight differential heart rate responses. During breathholds an initial decrease (“zero-lag decrease”) is followed by a delayed heart rate increase (“delayed increase”). During “normal” breaths HR increases immediately (“zero-lag increase”). (B) Correlation and filtering for the example shown in A (RS). Top panel = unfiltered; middle panel = respiratory frequency (RF) band; bottom panel = low-frequency (LF) heart rate band. (C) Example from a matched control with peaks at about 5 s and zero lag in LF component.

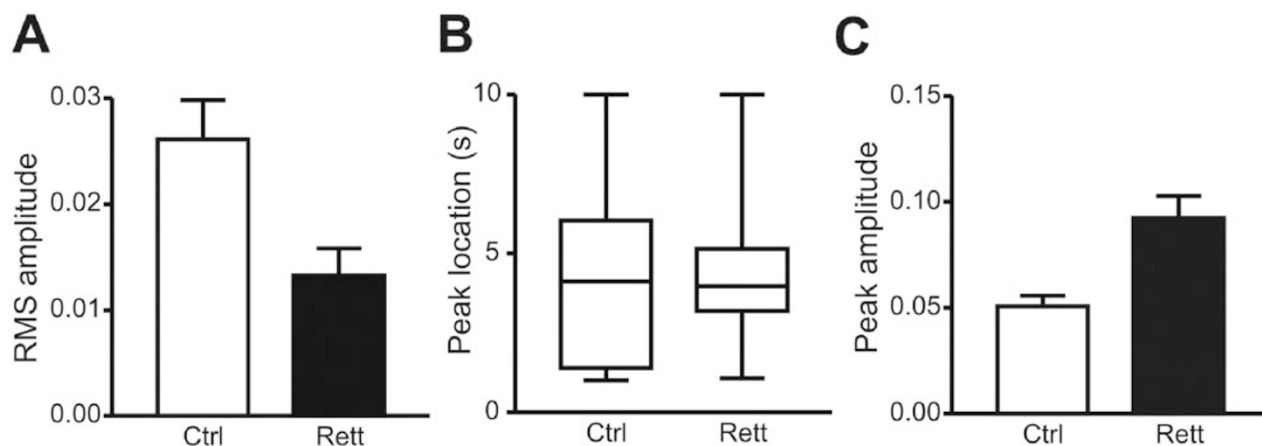


Figure 7. Cross-correlation analysis of cardiorespiratory interactions in RS and matched controls. (A) Amplitude of respiratory frequency component, measured as root-mean-square (RMS) amplitude for RS vs controls: $p < 0.005$. (B) Location of first peak in LF component at more than one second of lag in response to respiratory activity. Panel B = data for RS vs controls: $p = 0.6$. (C) Amplitude of first peak in LF heart rate component at more than one second of lag during breathholds. Panel C = data for RS vs controls: $p < 0.006$.

an unambiguous identification of these breathholds is not possible. Our interpretation of the waveforms is that girls with RS demonstrate the breathhold during inspiration (in contrast to the Valsalva expiratory behavior).

Prior publications have relied on conventional in-hospital physiologic recordings using traditional scoring methods for measuring apnea, bradycardia, and Hb desaturation. These studies focused on disparate patterns of breathing, typically referring to breathholds as apnea or Valsalva maneuvers, and mostly with focus on what induces the breathhold pattern (6–17). Published studies report a varying number of subjects (2–47, median = 18), a broad age range at study (1–32 y), typically unmatched and not necessarily healthy controls, and varying terms to describe the waveforms recorded. In contrast, our study includes a large number of girls, all with *MECP2* mutation-confirmed RS ($n = 47$), in a finite age range (ages 2–7 y), with matched controls having no symptoms/family history of autonomic disease. Moreover, no existing study used nonlinear analysis.

Our cross-correlation analysis revealed distinct differences in heart rate responses to breathholds among RS versus controls. During “normal” breaths, the heart rate response was instantaneous in RS and controls, but during breathholds, girls with RS had an initial subtle decrement in heart rate followed by a delayed, exaggerated heart rate response. Considering the frequent reoccurrence of breathholds, with associated overshoot and lag in heart rate response, one could hypothesize that cardiorespiratory homeostasis would be threatened in the most severely affected girls thereby increasing their risk of sudden death (25). The known decreased heart rate variability and prolonged QT_c (19,20,23), together with these repeated breathholds, could potentially explain the incidence of sudden death during wakefulness in RS. Some otherwise healthy individuals with long QT syndrome manifest symptoms when swimming (28,29), an activity associated with repetitive breathholds, though the specific arrhythmogenic influence of swimming is unknown. It is reasonable to consider that RS is a model for repeated breathholds that, like swimming, creates a challenge eliciting aberrant dynamics resulting in sudden

deaths in non-Rett children with long QT syndrome. Even in the absence of long QT syndrome or other channelopathies (30), one might hypothesize that the cardiac rhythm channel milieu may be compromised in RS with repeated breathholding and cardiorespiratory disassociation.

As already mentioned, our results are associated with several caveats: these studies were (by design) completed in the home environment and not supervised by medical personnel. The technique we used to create “breathholds” among controls is clearly not identical physiologically to the breathholds spontaneously occurring in RS. Finally, our data collected in young girls cannot be extrapolated to older girls with RS.

The disturbed heart rate response and irregular breathing are presumably the result of an aberrant medullary neuronal network. Studies in a mouse model for RS indicate that the irregular respiratory rhythm is explained by disturbed medullary levels of norepinephrine and serotonin (31). These animal studies are consistent with disturbances in serotonin transporter as described in human patients with RS (32). Further elucidation of the mechanisms underlying cardiorespiratory dysregulation and their neurochemical basis will ideally lead to intervention strategies to modify the cardiorespiratory phenotype in RS.

In summary, our study indicates that girls with RS demonstrate faster, deeper, and more erratic breathing as well as heart rate dysregulation during wakefulness when compared with matched controls. These results also reveal a profound dysregulation of the heart rate response to breathholds, and breaths preceding breathholds in young girls with RS. Taken together, these findings may offer insight into potential intervention strategies and into understanding mechanisms responsible for sudden death in Rett Syndrome.

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Table 1. Raw data from 47 Rett cases and 47 matched controls for “normal” breaths

Variable	Measures	Rett cases	Controls
T_{TOT} (s)	Mean	2.043 ± 0.41	2.52 ± 0.44
	CV	0.37 ± 0.09	0.23 ± 0.06
	Irregularity	0.35 ± 0.13	0.21 ± 0.08
T_I (s)	Mean	0.948 ± 0.166	1.055 ± 0.16
	CV	0.480 ± 0.127	0.309 ± 0.09
T_E (s)	Mean	1.095 ± 0.283	1.463 ± 0.29
	CV	0.47 ± 0.12	0.30 ± 0.08
$T_{I/TOT}$	Mean	0.47 ± 0.04	0.43 ± 0.02
	SD	0.12 ± 0.03	0.09 ± 0.02
Amplitude*	Mean	= 1	= 1
	CV	0.08 ± 0.03	0.07 ± 0.03
Mean AMP/ T_I	Mean	1.22 ± 0.21	1.03 ± 0.14
	CV	0.58 ± 0.13	0.43 ± 0.13
Peak AMP/ T_I	Mean	2.05 ± 0.28	1.74 ± 0.26
	CV	0.56 ± 0.12	0.43 ± 0.13
R-R interval (s)	Mean	0.52 ± 0.06	0.58 ± 0.06
	CV	0.10 ± 0.04	0.10 ± 0.03
	Irregularity	0.03 ± 0.03	0.05 ± 0.02

Values expressed as ± SD.

* Amplitude (AMP) = respiratory inductance plethysmography waveform sum signal; values normalized to 1, equivalent to 100% of baseline.

Table 3. Raw data from 47 Rett cases and 47 matched controls for the last five breaths before a breathhold

Variable	Measures	Rett cases	Controls
T_{TOT} (s)	Mean	2.19 ± 0.48	2.49 ± 0.43
	CV	0.43 ± 0.13	0.32 ± 0.08
	Irregularity	0.44 ± 0.15	0.30 ± 0.09
T_I (s)	Mean	1.05 ± 0.27	1.09 ± 0.23
	CV	0.58 ± 0.20	0.45 ± 0.12
T_E (s)	Mean	1.14 ± 0.27	1.40 ± 0.24
	CV	0.54 ± 0.12	0.43 ± 0.08
$T_{I/TOT}$	Mean	0.48 ± 0.04	0.44 ± 0.03
	SD	0.14 ± 0.02	0.12 ± 0.02
Amplitude*	Mean	1.29 ± 0.63	1.15 ± 0.14
	CV	0.10 ± 0.05	0.09 ± 0.04
Mean AMP/ T_I	Mean	1.44 ± 0.49	1.21 ± 0.19
	CV	0.61 ± 0.12	0.58 ± 0.13
Peak AMP/ T_I	Mean	2.50 ± 0.90	2.12 ± 0.35
	CV	0.58 ± 0.12	0.59 ± 0.15
R-R interval (s)	Mean	0.52 ± 0.05	0.56 ± 0.05
	CV	0.11 ± 0.04	0.11 ± 0.03
	Irregularity	0.04 ± 0.03	0.04 ± 0.02

Values expressed as ± SD.

* Amplitude (AMP)* = respiratory inductance plethysmography waveform sum signal.

Table 2. Raw data from 47 Rett cases and 47 matched controls for breathholds

Variable	Measures	Rett cases	Controls
T_{TOT} for breathholds (s)	Mean	33.5 ± 26.2	45.7 ± 35.9
	CV	1.34 ± 0.74	1.37 ± 0.33
	Irregularity	2.03 ± 1.72	2.53 ± 1.27
T_{TOT} (s)	Mean	5.94 ± 1.62	5.65 ± 0.88
	CV	0.42 ± 0.11	0.38 ± 0.09
T_I (s)	Mean	3.77 ± 1.33	3.09 ± 0.61
	CV	0.65 ± 0.13	0.63 ± 0.11
T_E (s)	Mean	2.18 ± 0.67	2.56 ± 0.61
	CV	0.72 ± 0.13	0.63 ± 0.12
$T_{I/TOT}$	Mean	0.61 ± 0.08	0.54 ± 0.06
	SD	0.22 ± 0.03	0.22 ± 0.03
Amplitude*	Mean	2.33 ± 1.01	2.86 ± 0.71
	CV	0.38 ± 0.10	0.31 ± 0.10
Time to peak AMP/ T_I	Mean	0.76 ± 0.10	0.83 ± 0.09
	SD	0.28 ± 0.04	0.25 ± 0.05
Mean AMP/ T_I	Mean	1.37 ± 0.62	1.61 ± 0.43
	CV	0.70 ± 0.14	0.72 ± 0.10
Peak AMP/ T_I	Mean	3.35 ± 1.32	4.07 ± 1.04
	CV	0.51 ± 0.12	0.57 ± 0.12
R-R interval (s)	Mean	0.52 ± 0.05	0.54 ± 0.05
	CV	0.12 ± 0.04	0.11 ± 0.03
	Irregularity	0.04 ± 0.03	0.04 ± 0.01

Values expressed as ± SD.

* Amplitude (AMP) = respiratory inductance plethysmography waveform sum signal.

Table 4. Raw data from 47 Rett cases and 47 matched controls for the first five breaths after a breathhold

Variable	Measures	Rett cases	Controls
T_{TOT} (s)	Mean	2.18 ± 0.48	2.49 ± 0.42
	CV	0.42 ± 0.09	0.32 ± 0.07
	Irregularity	0.42 ± 0.12	0.31 ± 0.09
T_I (s)	Mean	1.06 ± 0.26	1.08 ± 0.22
	CV	0.58 ± 0.14	0.47 ± 0.12
T_E (s)	Mean	1.13 ± 0.28	1.41 ± 0.24
	CV	0.54 ± 0.10	0.43 ± 0.08
$T_{I/TOT}$	Mean	0.48 ± 0.04	0.44 ± 0.02
	SD	0.14 ± 0.02	0.13 ± 0.02
Amplitude*	Mean	1.26 ± 0.59	1.16 ± 0.13
	CV	0.10 ± 0.04	0.10 ± 0.04
Mean AMP/ T_I	Mean	1.40 ± 0.47	1.23 ± 0.20
	CV	0.62 ± 0.12	0.58 ± 0.11
Peak AMP/ T_I	Mean	2.45 ± 0.85	2.16 ± 0.35
	CV	0.58 ± 0.12	0.58 ± 0.12
R-R interval (s)	Mean	0.50 ± 0.05	0.54 ± 0.05
	CV	0.10 ± 0.04	0.11 ± 0.03
	Irregularity	0.03 ± 0.02	0.04 ± 0.02

Values expressed as ± SD.

* Amplitude (AMP) = respiratory inductance plethysmography waveform sum signal.