Relation between circulating levels of GH, IGF-1, ghrelin and somatic growth in Rett syndrome

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Abstract

Background: Most cases of Rett syndrome (RTT) are caused by mutations in methyl CpG binding protein 2 (MECP2), and individuals with RTT have somatic growth failure, growth arrest of brain, epilepsy, and intellectual disability (ID). Ghrelin is a peptide hormone which stimulates growth hormone (GH) secretion from the pituitary gland. Ghrelin and GH regulate insulin-like growth factor-1 (IGF-1) synthesis, and this GH/IGF-1 axis is an endocrine axis involved in energy and sleep homeostasis and plays crucial roles in somatic and brain growth. This study aimed to determine whether circulating ghrelin, GH and IGF-1 reflect somatic and brain growth in RTT patients.

Methods: We examined anthropometric data and circulating ghrelin, GH, and IGF-1 in 22 female RTT patients with epilepsy and ID (RTT-Ep/ID) and 14 age-matched females with epilepsy and ID (non-RTT-Ep/ID).

Results: Body mass index (BMI) and height/length were significantly lower in RTT-Ep/ID than in non-RTT-Ep/ID in patients less than 20 years old. Plasma ghrelin in RTT-Ep/ID patients showed a significant inverse correlation with weight but had no significant correlations with BMI or height. Head circumference in both groups showed a significant positive correlation with circulating ghrelin and a significant negative correlation with circulating IGF-1. The ratio of octanoyl-ghrelin to total-ghrelin (O/T-ratio) is used as an indicator to estimate the biological activity of ghrelin. Among pre-adolescents, O/T-ratios were significantly higher in the RTT-Ep/ID group than in the non-RTT-Ep/ID group ($P < 0.05$). Conclusions: Timing of growth spurts differed between the RTT-Ep/ID and non-RTT-Ep/ID groups, possibly due to a common (but yet unknown) mechanism of growth failure. Ghrelin/GH/IGF-1 axis function was aberrant in both the RTT-Ep/ID and non-RTT-Ep/ID groups. The initial clinical course of Rett syndrome affects the development of the sleep–wake cycle and locomotion in early infancy, both of which may be based on the dysfunction of the aminergic neurons modulated by ghrelin/GH/IGF-1 axis. Further study with a larger sample size should help clarify the precise mechanisms controlling the somatic growth and hormonal features in Rett syndrome.

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Keywords: Rett syndrome; MECP2; Intellectual disability; Growth; Ghrelin; GH; IGF-1

1. Introduction

Rett syndrome (RTT; MIM 312750) is an X-linked neurodevelopmental disorder caused by mutations in
methyl CpG binding protein 2 (MECP2) [1]. RTT is characterized by somatic growth failure following the deceleration of head growth, intellectual disability, erratic and purposeless rhythmic movement and sleep disruption [2,3]. Somatic growth failure is a major aspect of the developmental arrest. In a population-based cohort, the mean weight, height, and body mass index Z scores in subjects with RTT were below those of their age group in the general population and decreased steadily with age. Moreover, growth failure occurs less frequently in girls and women with better development and less morbidity typically associated with RTT, and in those with late truncation mutations or C terminal mutations of the MECP2 gene [4–6]. The growth hormone (GH)/insulin-like growth factor-1 (IGF-1) axis has essential roles in somatic growth. Ghrelin is a peptide hormone involved in the GH/IGF-1 axis. Ghrelin secreted during fasting promotes the secretion of GH through the GH secretagogue receptor (GHS-R) and this in turn promotes the synthesis and secretion of IGF-1 [7,8]. The Ghrelin/GH/IGF-1 axis is an endocrine axis involved in energy and sleep homeostasis [9]. Plasma concentration of ghrelin is negatively regulated by circulating IGF-1 [8]. GH regulates somatic growth and development directly through the activation of GH receptors and indirectly through IGF-1 [10,11]. IGF-1 mediates tissue formation and remodeling, bone growth, postnatal growth and muscle metabolism [11,12]. IGF-1 is widely expressed in the central nervous system (CNS) [13], where it regulates neuronal and glial cell proliferation, and strongly promotes neuronal cell survival and synaptic maturation [13,14]. In genetically modified mice, postnatal overexpression of IGF-1 contributed to brain overgrowth characterized by an increase in the number of neurons and oligodendrocytes [13]. In contrast, ablation of IGF-1 and IGF-1 receptor (IGF-1R) expression resulted in growth retardation not only of body but also of brain [14]. In the CNS, ghrelin is synthesized mainly at the hypothalamus [15], whereas its receptor, GHS-R type 1a, is broadly distributed within the CNS [11]. Ghrelin promotes cell proliferation in both the embryonic and adult nervous systems [11] and stimulates the proliferation of neuronal precursor cells through GHS-R [16]. Moreover, ghrelin modifies the sleep–wake (S–W) rhythm by increasing wakefulness and decreasing the duration of REM sleep periods via GHS-R in the hypothalamus and pituitary gland [17]. S–W rhythm is related to GH ultradian rhythmicity in humans [18]. Maximal GH release occurred within minutes of the sleep onset of stage 3 or 4 sleep [17]. Ghrelin secretion is pulsatile and displays an ultradian rhythmicity. The number of peaks and the interval between peaks of ghrelin are similar to those observed for GH secretion, whereas peak amplitudes are much more important for GH [17]. Consequently, ghrelin and the GH/IGF-1 axis play crucial roles not only in somatic growth and but also in CNS development. In our previous work, plasma ghrelin levels were high during infancy in RTT patients, then decreased whereas plasma ghrelin levels increased at puberty in healthy controls [19]; however, we did not examine the relationship between somatic growth disturbances and circulating levels of GH and IGF-1, in RTT. Moreover, we did not compare plasma ghrelin levels between patients with RTT and patients with epilepsy and intellectual disability (Ep/ID), although there is a high incidence of Ep/ID in RTT patients [19]. Therefore, in the present study we compared the circulating ghrelin, GH and IGF-1 concentrations and anthropometric data, i.e., weight, height, body mass index (BMI), and occipito-frontal head circumference (OFC), in RTT and non-RTT patients with Ep/ID.

2. Methods

Clinical diagnosis of RTT was confirmed in 22 female patients according to the recently proposed RTT Diagnostic Criteria [2]. The age of our RTT-Ep/ID patients ranged from 4.0 to 37.5 years old. RTT patients manifested sleep disruptions (18/22) and periodic breathing (14/22). Plasma concentrations of ghrelin, GH and IGF-1 were measured in the RTT-Ep/ID patients and in 14 age-matched female patients with epilepsy and intellectual disability (Ep/ID; age range 3.3–23.9 years old). MECP2 mutations were confirmed in all 22 RTT-Ep/ID patients by MECP2 gene analysis. All had a developmental quotient (DQ) or intelligence quotient (IQ) below 20. Of the 14 patients with non-RTT-Ep/ID, seven had profound retardation (IQ < 20), one had severe ID (IQ = 20–34), two had moderate ID (IQ = 35–49), three had mild ID (IQ = 50–69), and one had an IQ below 70 (precise score unknown). None of the participants received autonomic nerve regulators or had undergone gastrostomy. We also collected the participants’ clinical data (including age for developmental comparisons): 0–10 yr-olds [RTT-Ep/ID, n = 7; non-RTT-Ep/ID, n = 6], 10–20 yr-olds [RTT-Ep/ID, n = 10; non-RTT-Ep/ID, n = 6], and over-20-year-olds [RTT-Ep/ID, n = 5; non-RTT-Ep/ID, n = 2], weight, height, BMI and occipito-frontal head circumference (OFC). These data were converted into standard deviation (Z score) values based on the U.S. National Center for Health Statistics/World Health Organization references [20]. Written informed consent was obtained from a parent for each patient. The study protocol was approved by the Ethics Committee of the Kurume University School of Medicine.

3. Measurement of plasma ghrelin levels

The extraction of plasma ghrelin from blood was performed by a method described previously [21,22]. The separated plasma samples were stored at −80 °C within
5 min to prevent degradation of rapidly regulated proteins. The plasma samples were semi-purified with a Sep-Pak C18 cartridge before the ghrelin radioimmunoassay (RIA). Two ghrelin-specific RIAs were used; one, named N-RIA, recognizes the N-terminal portion of octanoyl-modified active ghrelin, and the other, named C-RIA, recognizes the C-terminal portion of ghrelin irrespective of its octanoyl modification. The plasma level of octanoyl-ghrelin, which is post-transnationally octanoylated at Ser3, was measured by N-RIA [21,23]. The plasma level of total ghrelin, i.e. the sum of the non-octanoyl and octanoyl ghrelin levels, was measured by C-RIA.

3.1. Measurement of serum growth hormone (GH) and insulin-like growth factor-1 (IGF-1) levels

Serum concentrations of GH and IGF-1 were measured in duplicate by immunoradiometric assays according to the manufacturer’s protocol (Active Growth Hormone IRMA DSL-1900 and Active Non-Extraction IGF-1 IRMA DSL-2800, respectively, Diagnostics System Laboratories, Webster, TX) or a radioimmunoassay kit (SRL, Tokyo). Each assay was calibrated with manufacturer-supplied standards.

3.2. Statistical analysis

The concentrations of plasma total- and octanoyl-ghrelin and serum GH and IGF-1 were compared between the two subject groups by t-tests, and Pearson’s correlation coefficients were used to measure monotonic associations between variables. The data are summarized as mean ± standard deviations (s.d.). P-values ≤0.05 were considered significant.

4. Results

The mean values of BMI-for-age and height/length-for-age Z scores in RTT-Ep/ID patients were significantly lower than those of non-RTT-Ep/ID patients (Table 1). Conversely, the octanoyl-/total-ghrelin ratios in RTT-Ep/ID patients were significantly higher than those of non-RTT-Ep/ID patients. The developmental data (Table 2) show that the serum GH concentrations in RTT-Ep/ID patients were significantly lower than those of non-RTT-Ep/ID patients between the ages of 0 and 10 years. The means of the height/length-for-age Z score of RTT-Ep/ID patients between the ages of 0 and 20 years were significantly lower than those of non-RTT-Ep/ID patients within the same age range. Over 20 years old, the mean of the height/length-for-age Z score of RTT-Ep/ID patients was similar to that of non-RTT-Ep/ID patients. On the other hand, the octanoyl-/total-ghrelin ratios of RTT-Ep/ID patients between the ages of 0 and 20 years were significantly higher than those of non-RTT-Ep/ID patients within the same age range. There were no significant differences in plasma concentrations of total- and octanoyl-ghrelin or serum concentrations of GH and IGF-1 between the two groups. Plasma total- and octanoyl-ghrelin concentrations, and the serum GH and IGF-1 concentrations showed no significant correlation with height/length-for-age Z score in either group. As shown in

Table 1
Characteristics of the RTT-Ep/ID and non-RTT-Ep/ID patients.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>RTT-Ep/ID (n = 22)</th>
<th>Non-RTT-Ep/ID (n = 14)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± s.d.</td>
<td>Range</td>
<td>Mean ± s.d.</td>
</tr>
<tr>
<td>Age (years)</td>
<td>16.44 ± 8.56</td>
<td>4.00–37.50</td>
<td>11.77 ± 6.23</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>28.90 ± 12.44</td>
<td>11.60–54.00</td>
<td>31.53 ± 13.82</td>
</tr>
<tr>
<td>Weight-for-age (Z score)</td>
<td>−0.86 ± 2.17</td>
<td>−4.35–2.52</td>
<td>0.35 ± 1.56</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>15.57 ± 3.64</td>
<td>9.70–22.80</td>
<td>17.41 ± 3.69</td>
</tr>
<tr>
<td>BMI-for-age (Z score)</td>
<td>−2.18 ± 2.17</td>
<td>−7.91–0.50</td>
<td>−0.47 ± 1.73</td>
</tr>
<tr>
<td>Height/length (cm)</td>
<td>133.01 ± 19.59</td>
<td>88.10–156.5</td>
<td>131.41 ± 23.46</td>
</tr>
<tr>
<td>Height/length-for-age (Z score)</td>
<td>−2.68 ± 0.85</td>
<td>−3.99–1.02</td>
<td>−1.30 ± 1.01</td>
</tr>
<tr>
<td>OFC (cm)</td>
<td>50.64 ± 2.48</td>
<td>46.50–54.30</td>
<td>50.77 ± 2.46</td>
</tr>
<tr>
<td>OFC-for-age (Z score)</td>
<td>0.52 ± 1.73</td>
<td>−2.41–3.08</td>
<td>0.70 ± 1.57</td>
</tr>
<tr>
<td>Total ghrelin (fmol/ml)</td>
<td>127.80 ± 87.62</td>
<td>39.72–442.72</td>
<td>164.77 ± 113.27</td>
</tr>
<tr>
<td>Octanoyl ghrelin (fmol/ml)</td>
<td>17.76 ± 8.80</td>
<td>2.75–32.13</td>
<td>12.56 ± 9.47</td>
</tr>
<tr>
<td>Octanoyl-/total ghrelin ratio</td>
<td>16.26 ± 6.64</td>
<td>5.91–29.31</td>
<td>7.68 ± 3.78</td>
</tr>
<tr>
<td>GH (ng/ml)</td>
<td>1.62 ± 2.60</td>
<td>0.05–11.50</td>
<td>2.10 ± 1.91</td>
</tr>
<tr>
<td>IGF-1 (ng/ml)</td>
<td>168.25 ± 96.12</td>
<td>60.31–375.00</td>
<td>201.57 ± 92.69</td>
</tr>
<tr>
<td>IGF-1/GH ratio</td>
<td>618.13 ± 1194.27</td>
<td>30.43–5540.00</td>
<td>367.79 ± 601.71</td>
</tr>
</tbody>
</table>

The data are means ± s.d. Ep: epilepsy; ID: intellectual disability; RTT: Rett syndrome; OFC: occipito-frontal head circumference. The means of BMI-for-age Z score, height/length-for-age Z score, and octanoyl-/total ghrelin ratio in the RTT-Ep/ID group were significantly different compared to those of the non-RTT-Ep/ID group.

* p < 0.05 (t-test).
** p < 0.01 (t-test).

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Table 2: Developmental characteristics of the RTT-Ep/ID and non-RTT-Ep/ID patients.

Table 3, plasma concentrations of total-ghrelin showed significantly negative correlations with age, weight, and OFC-for-age Z score in both RTT-Ep/ID and non-RTT-Ep/ID patients, whereas the serum IGF-1 concentrations showed significantly positive correlations with weight, BMI-for-age and OFC-for-age Z score in RTT-Ep/ID patients. The octanoyl-total-ghrelin ratio showed a significantly positive correlation with OFC-for-age Z score only in RTT-Ep/ID patients. No statistical analysis to present definite relationships between genotype and phenotype is possible because of the small sample size, as shown in Supplementary Table 1.

5. Discussion

It is well known that patients with RTT exhibit short statures compared to healthy individuals with normal somatic growth [2]. The mean growth of length, weight and head circumference in classic RTT fell below growth chart levels for the normative population and growth failure occurs less frequently in girls with RTT, who frequently RTT-Ep/ID patients achieved growth in height equivalent to that of non-RTT-Ep/ID patients. Previously, we and others have reported that the values for occipito-frontal head circumference (OFC) in RTT-Ep/ID patients were significantly smaller than those in healthy controls [2,19]. Although eating difficulties may be caused by inadequate dietary intake, growth problems in Rett syndrome are also known to be related to the specific genotypes. Eating difficulties and growth failure in RTT patients with low levels of plasma ghrelin are also presumed to be caused by MECP2 mutations. However, we did not identify any statistically significant overall correlations between the Z score and genetic profile because of small sample size.

In the present study, the time points for growth spurts in RTT-Ep/ID children were delayed compared to those in non-RTT-Ep/ID children, whereas subsequently RTT-Ep/ID patients achieved growth in height equivalent to that of non-RTT-Ep/ID patients. Previously, we and others have reported that the values for occipito-frontal head circumference (OFC) in RTT-Ep/ID patients were significantly smaller than those in healthy controls [2,19]. However, in this study there was no significant difference in OFC values between the RTT-Ep/ID and non-RTT-Ep/ID groups in most statistically significant differences between any genotype groups. Isaacs et al. previously found that microcephaly was associated with lower weight-for-age Z scores [24]. We previously reported that the mean values of weight, BMI, height/length and OFC-for-age Z scores in RTT patients were lower than those of healthy controls, and that eating difficulties in RTT patients were significantly correlated with the plasma levels of total and octanoyl-ghrelin [19]. Although eating difficulties may be caused by inadequate dietary intake, growth problems in Rett syndrome are also known to be related to the specific genotypes. Eating difficulties and growth failure in RTT patients with low levels of plasma ghrelin are also presumed to be caused by MECP2 mutations. However, we did not identify any statistically significant overall correlations between the Z score and genetic profile because of small sample size.

In the present study, the time points for growth spurts in RTT-Ep/ID children were delayed compared to those in non-RTT-Ep/ID children, whereas subsequently RTT-Ep/ID patients achieved growth in height equivalent to that of non-RTT-Ep/ID patients. Previously, we and others have reported that the values for occipito-frontal head circumference (OFC) in RTT-Ep/ID patients were significantly smaller than those in healthy controls [2,19]. However, in this study there was no significant difference in OFC values between the RTT-Ep/ID and non-RTT-Ep/ID groups. In most
children with postnatal-onset microcephaly, developmental outcome and somatic growth were markedly retarded [25]. In children with epilepsy, it was reported that onset of epileptic symptoms was preceded by a reduction in brain volume [26]. In disorders associated with ID, reductions in dendritic branch complexity and dendritic length, both of which bring about a reduction of brain volume, have been reported to be common pathological features [27]. These data supports the suggestion that the short stature and microcephaly of both groups may have been affected by epilepsy and intellectual disability during early infancy. However, the median age of onset of epilepsy in RTT is around 4 years [3]. This does not coincide with the timing of the deceleration of head growth. The deceleration of head growth and the characters of neuronal architecture may be partly determined by the genotype. On the other hand, the neurons and neuronal systems involved in the development of S-W rhythm and locomotion are affected in early infancy of RTT [28]. Segawa reported that this pathophysiology was based on the dysfunction of the aminergic neurons of the brainstem in early infancy. This causes autistic tendency and failure in synaptogenesis of the cortex and consequently causes microcephaly. Furthermore, this causes failure in restriction of atonia into REM stage. This induces dysfunction of the pedunculopontine nuclei (PPN) and consequently dysfunction of the dopamine neurons. This causes dysfunction of the supplementary motor area through the ascending pathway of the basal ganglia to the thalamus, consequently causes loss of purposeful hand use and induces the characteristic stereotyped hand movements. Ghrelin depolarizes PPN postsynaptically and dose-dependently via GHS-Rs [29]. The metabolic rate of girls with RTT was lower while sleeping, but not while awake, than in healthy controls [30]. Short stature, microcephaly and disorder of the circadian S-W cycle of RTT in early infancy may reflect the dysfunction of aminergic neurons modulated by the ghrelin/GH/IGF-1 axis.

In the present study, circulating levels of GH, IGF-1 and ghrelin in RTT-Ep/ID patients did not differ significantly from those in non-RTT-Ep/ID patients. Furthermore, the levels of circulating GH, IGF-1 or ghrelin were not significantly correlated with height in either group. On the other hand, our present study revealed a significant positive correlation between body weight and serum IGF-1 levels in RTT-Ep/ID patients. Within the RTT-Ep/ID group, we also found a significant inverse correlation between plasma octanoyl-ghrelin (active ghrelin) level and body weight. These findings are in line with those of previous reports demonstrating a positive correlation between serum IGF-1 level and body weight in a group of healthy children with normal growth [31]. Our findings are also supported by previous reports showing that the secretion of total ghrelin is negatively regulated by circulating IGF-1 through a negative-feedback loop [32]. IGF-1 ameliorates the RTT-like symptoms in a mouse model of the disease [33]. An Italian pilot study of RTT revealed that there are no risks associated with IGF1 administration [34].

In general, bone mineral deficits and bone-related disorders including fractures and scoliosis were common in RTT and deficits in bone mineral density were identified across a broad range of MECP2 mutations [35].

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Table 3
Correlation among anthropometric data and circulating ghrelin, GH and IGF-1 between the RTT-Ep/ID and non-RTT-Ep/ID patients.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total ghrelin</th>
<th>Octanoyl ghrelin</th>
<th>Octanoyl/Total ghrelin ratio</th>
<th>IGF-1</th>
<th>GH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Non-RTT-Ep/ID (n = 14)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>-0.62*</td>
<td>-0.49</td>
<td>0.05</td>
<td>0.36</td>
<td>-0.43</td>
</tr>
<tr>
<td>Weight-for-age (Z score)</td>
<td>-0.55**</td>
<td>-0.41</td>
<td>0.07</td>
<td>0.25</td>
<td>-0.40</td>
</tr>
<tr>
<td>BMI-for-age (Z score)</td>
<td>-0.08</td>
<td>-0.07</td>
<td>-0.00</td>
<td>0.04</td>
<td>-0.24</td>
</tr>
<tr>
<td>Height/Length-for-age (Z score)</td>
<td>0.06</td>
<td>0.21</td>
<td>0.47</td>
<td>0.18</td>
<td>0.10</td>
</tr>
<tr>
<td>OFC-for-age (Z score)</td>
<td>-0.60*</td>
<td>-0.50</td>
<td>0.05</td>
<td>0.52</td>
<td>-0.67*</td>
</tr>
<tr>
<td><strong>RTT-Ep/ID (n = 22)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>-0.44*</td>
<td>-0.37</td>
<td>0.21</td>
<td>0.25</td>
<td>0.01</td>
</tr>
<tr>
<td>Weight-for-age (Z score)</td>
<td>-0.63**</td>
<td>-0.52*</td>
<td>0.37</td>
<td>0.62**</td>
<td>0.25</td>
</tr>
<tr>
<td>BMI-for-age (Z score)</td>
<td>-0.10</td>
<td>0.09</td>
<td>0.32</td>
<td>0.65**</td>
<td>0.24</td>
</tr>
<tr>
<td>Height/Length-for-age (Z score)</td>
<td>-0.20</td>
<td>0.05</td>
<td>0.23</td>
<td>-0.04</td>
<td>-0.20</td>
</tr>
<tr>
<td>OFC-for-age (Z score)</td>
<td>-0.72**</td>
<td>-0.55**</td>
<td>0.47</td>
<td>0.58**</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Pearson’s correlation coefficients were used to measure monotonic associations in the RTT-Ep/ID and non-RTT-Ep/ID groups. The plasma total-ghrelin concentrations showed a significantly negative correlation with age, weight-for-age Z score and OFC-for-age Z score in both the RTT and non-RTT-Ep/ID patients. The plasma octanoyl-ghrelin concentrations showed a significantly negative correlation with weight and OFC-for-age Z score only in the RTT-Ep/ID patients. The serum IGF-1 concentrations showed a significantly positive correlation with weight-for-age Z score, BMI-for-age Z score and OFC-for-age Z score only in the RTT-Ep/ID patients. Octanoyl/total-ghrelin ratio showed a significantly positive correlation with OFC-for-age Z score only in the RTT-Ep/ID patients. The serum GH concentrations showed a significantly negative correlation with OFC-for-age Z score only in non-RTT-Ep/ID patients. Abbreviations are explained in Table 1.

* p < 0.05
** p < 0.01
Australian Rett syndrome cohort study, the p.R168X and p.T158 M mutations predicted the low value of the areal bone mineral density and bone mineral content for all bone outcomes [36]. The activated ghrelin/GH/IGF-1 axis stimulates longitudinal bone growth and increases the body weights of growing children [37,38]. However, a study by Caffarelli et al., reported that plasma levels of ghrelin did not reflect longitudinal bone growth in female RTT patients within a growing period and both age and height were independent predictors of total body bone mineral density [39]. Similarly, the short stature of our RTT-Ep/ID patients (a consequence of insufficient longitudinal bone growth), could not be predicted by their circulating levels of ghrelin, GH or IGF-1. These findings in RTT may imply that ghrelin stimulation is insufficient to induce the required peak amplitudes of GH secretion [40], and this may be caused by the dysfunction of aminergic neurons from early infancy.

Octanoyl ghrelin is a major active form of ghrelin which is post-translationally modified with an octanoyl-group at its Ser3 residue [7]. In fact, the ratio of octanoyl-ghrelin to total-ghrelin (O/T-ratio) is used as an indicator to estimate the biological activity of ghrelin [41]. In our study, the O/T-ratio of patients less than 20 years old was significantly higher in the RTT-Ep/ID group than in the non-RTT-Ep/ID group. In addition, this O/T-ratio exhibited a significantly positive correlation with OFC-for-age Z score only in RTT-Ep/ID patients. In comparison to non-RTT-Ep/ID patients, RTT-Ep/ID patients below the age of 20 had shorter height, smaller OFC, and a higher O/T-ratio. This unexpected finding may reflect alterations in respect of endocrine control by the ghrelin/GH/IGF-1 axis. On the other hand, these results coincide temporally with early infancy. These phenomena appear to occur independently and concurrently, as the result of epigenetic processes that temporally and spatially control gene activity during ontogenesis. Organ patterning and size are based on the spatiotemporal formation of morphogen gradients [42,43]. The MECP2 gene determines cell fate, morphology and proliferation through posttranslational modifications [44]. In RTT, epigenetic regulation of gene expression involved in the morphogens linked to the growth of bone and brain and the enzymes mediating the modification of ghrelin may be improperly and irreversibly influenced by MECP2 mutation in early infancy.

This study has two major limitations. One is that we obtained results from single-time-point assays, and the other is the relatively small sample size of the groups (22 RTT-Ep/ID patients, 14 non-RTT-Ep/ID patients). The use of provocation tests (i.e. GHRH-loading test for GH) or measurement of the circadian profiles of ghrelin and other somatotropic hormones in a larger number of RTT-Ep/ID and non-RTT-Ep/ID patients, would allow us to evaluate the various functions of the ghrelin/GH/IGF-1 axis in more detail.

In conclusion, we found in this study a difference in the timing of growth-spurts between RTT-Ep/ID and non-RTT-Ep/ID groups, which might be due to a common (but yet unknown) mechanism of microcephaly. We also found that the regulatory functions of the ghrelin/GH/IGF-1 axis were aberrant in both the RTT-Ep/ID and non-RTT-Ep/ID groups. Further study with a larger sample size should reveal the precise mechanisms controlling the anthropometric and hormonal features in Rett syndrome.

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**Appendix A. Supplementary data**

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.braindev.2013.11.007.

**References**


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