Ghrelin improves dystonia and tremor in patients with Rett syndrome: a pilot study

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Abstract

Background: Dystonia occurs in approximately 60% of patients with Rett syndrome (RTT) and severely impairs their quality of life. However, an effective standard therapy has not been established. In a previous study, ghrelin levels were significantly decreased in patients with RTT, in particular, among patients over 10 years old. This prompted speculation that ghrelin may play an important role in RTT. **Objectives:** Four patients, including two adults, with severe dystonia and tremor, were recruited. **Methods:** Ghrelin was intravenously administered at a dose of 3 μg/kg, once-daily for 3 days, followed by once every 3 weeks. Objective evaluation was performed, including scoring for different clinical features (SDCF), the Burke-Fahn-Marsden Dystonia Rating Scale (BFMDRS) and the Visual Analog Scale (VAS). **Results:** The SDCF, BFMDRS, autonomic dysfunction and VAS scores were markedly improved in two patients with severe dystonia and head tremor. **Conclusion:** Ghrelin may improve extrapyramidal symptoms in patients with RTT.

Abbreviations: Rett syndrome; RTT, SDCF; scoring for different clinical features, BFMDRS; Burke-Fahn-Marsden Dystonia Rating Scale

Introduction

Rett syndrome (RTT), a neurodevelopmental disorder that mainly affects females, is caused by mutations in the *MECP2* gene. It is characterized by normal early development, followed by the loss of psychomotor skills and acquired purposeful hand skills, and the onset of stereotypic hand movements and gait disturbances [1-5]. Extrapyramidal involvement manifests as stereotypic movements, dystonia, Parkinsonian tremor, rigidity, and hypokinesia. Dystonia occurs in 59.0% to 63.3% of patients with RTT and more severely affects patients older than 8 years [6-8]. Although both generalized and focal dystonia reduce the patients' quality of life and motor functioning, apparent life-threatening episodes are also observed in RTT [9]. Few reports have shown improvements in dystonia and autonomic dysfunction in patients with RTT [9]. Various candidate drugs have been studied, including the muscarinic cholinergic antagonist trihexyphenidyl and dopamine agonists; however, these agents failed to ameliorate dystonia, tremor and autonomic dysfunction.

Ghrelin, a 28-amino acid peptide isolated from the stomach as an endogenous ligand for growth hormone secretagogue receptor 1a (GHS-R), is expressed in both the stomach and hypothalamus [10]. It has multiple physiological actions, including stimulating somatic growth, increasing appetite [11], enhancing gut motility, and regulating autonomic function [12], notably, the activity of the sympathetic nervous system and the stress response [12]. According to some reports, GHS-R is expressed in the central nervous system and regulates

dopamine signalling [13].

As shown by previous research, the biological activity of ghrelin and its localization are related to clinical phenotypes of RTT. Plasma levels of ghrelin were decreased, particularly, in patients older than 10 years [14, 15]. Thus, it was speculated that ghrelin may play an important role in the pathophysiology of RTT. The present study enrolled four RTT patients with confirmed *MECP2* mutations, who were intravenously administered ghrelin.

2. Patients and Methods

2.1 Patients

The study enrolled four Japanese females with RTT, aged between 12 and 32 years (mean 21.75±8.18 years). Each patient had an *MECP2* mutation and was diagnosed as having RTT. Patient information, including activity levels in daily life, clinical symptoms and signs, distribution of dystonia, drug treatments, including antiepileptic drugs, dopamine, antimuscarinic cholinergic agents, is shown in Table 1. The study protocol was approved by the Institutional Review Board of Kurume University. All patients provided written informed consent to participate in this study.

2.2 Study design

Patients were hospitalized for 7 days in Kurume University Hospital (Kurume city, Japan). The pre-treatment period was defined as 2 days before ghrelin administration. Ghrelin was intravenously administered at a dose of 3 μg/kg for 5 min, once-daily for 3 days. Thereafter, for patients 1 and 2, who exhibited dystonia, received the same dose of intravenous ghrelin was administered over 2 days every 3 weeks. These patients were clinically evaluated on multiple occasions (see Fig. 1).

2.3 Ghrelin

Synthetic human ghrelin (active form) was obtained from Peptide Institute Inc (Osaka, Japan) and prepared as described in a previous report [16-22]. Examination by Japan Food

Research Laboratories (Tokyo, Japan) did not find any traces of endotoxin in the ghrelin solution. In brief, recently applied clinical regimens of ghrelin administration were modified. Serum growth hormone (GH) and IGF-1 levels were measured before and after ghrelin administration.

2.4 Biochemistry and endocrinology parameters

Blood samples for measurement of biochemical and endocrine parameters were taken in the morning on day 1 of ghrelin treatment, after an overnight fast of at least 10 h. For ghrelin assays, blood samples were collected in tubes containing 1-mg/ml EDTA-2Na and 500-U/mL aprotinin. After immediate centrifugation at 4ºC, the plasma samples were acidified by 1 normal HCL and stored at -80ºC. A fluorescent enzyme immunoassay was utilized to measure GH levels. An immmunoradiometric assay (SRL Co Ltd, Tokyo, Japan) was utilized to measure serum IGF-1 levels. Plasma levels of intact and des-octanoyl ghrelin were measured with an Active Ghrelin and Desacyl-Ghrelin ELISA kit (LSI Medience Corporation, Tokyo, Japan).

2.5 Clinical evaluation of ghrelin treatment

Objective evaluations were performed, including scoring for different clinical features (SDCF), which consists of comprehensive clinical and neurological findings [1]. The score ranges from 0 to 40, with 40 points being the worst score. The Burke-Fahn-Marsden Dystonia Rating Scale (BFMDRS) score (range of 0 to 120) was assessed by two paediatric

neurologists (KY, TM), with lower scores corresponding to less dystonia [23, 24]. The Visual Analog Scale (VAS) was administered by caregivers and physical therapists [25], and scored between 1 and 10, with lower scores corresponding to more severe dysfunction.

3. Results

3.1 At the cut-off date for this report, patients 1 and 2 had continued to receive intravenous ghrelin injections. Patient 1 had been followed for 2 years and patient 2 for 10 months. Patients 3 and 4 received ghrelin for only the first 3 days. This was because their parents had other children, and consent was only given for the first 7 days of hospital admission and follow-up at 1 month.

3.2 Changes in biochemistry and endocrinology parameters

During this study, complete blood count, and liver and kidney function were not significantly changed. Plasma levels of active and des-acyl ghrelin, before and after ghrelin administration, are shown in Table 2. After ghrelin administration, plasma ghrelin levels displayed an increase after 15 min, followed by a gradual decline until 90 min. Plasma levels of active ghrelin in the four patients were 25.1, 34.7, 20.6, and 60.6 before ghrelin administration, with an immediate rise to 3384.0, 9749.6, 10562.0, and 11361.9, respectively, within 15 min after ghrelin administration. In the four patients, GH levels changed from 0.2, 3.0, 2.9, and 0.6 ng/ml, respectively, before ghrelin administration, to peak levels of 35.0, 24.2, 89.9, and 18.1 ng/ml, respectively, after ghrelin administration. Changes in IGF-1 levels were from 214 to

168 ng/ml, 175 to 193 ng/ml, and 285 to 244 ng/ml, in Patients 1, 2 and 3, respectively. Data were not obtained from Patient 4. Neither glucose levels nor serum IGF-1 levels were significantly changed.

3.3 Changes in clinical observations

Patient 1 developed severe dystonia at 13 years of age, which was dominant on the left side, including neck-twisting, and trunk and shoulder rotation; thus, the head tightly contacted the shoulder (Fig. 2A). She had severe scoliosis and exhibited a Cobb angle of 86º. She displayed a resting tremor with 4 to 5 Hz rhythmic involuntary movements of the head and both hands. Treatment with trihexyphenidyl and pramipexole was ineffective. The patient lost 4 kg in body weight from a starting weight of 28 kg, although she underwent gastrostomy at 18 years of age due to diarrhoea and difficulty in swallowing food. Nutrition was maintained by both gastric tube feeding and oral feeding by her mother. No other stereotypic hand movements were observed. At 1 week after intravenous ghrelin administration, dystonia of the neck, and shoulder and trunk rotation were markedly improved (Fig. 2B). SDCF scores improved by a total of 2 points, with gains being made with involuntary movement and oromotor feeding scores. Peripheral circulation of extremities was also improved, but the score was not affected because the patient already had atrophic changes. The BFMDRS score changed from 108.5 to 100.0 because of improvements in shoulder, neck and trunk dystonia. VAS scores for dystonia, tremor and sympathetic vasomotor reflexes (VMR) were improved

(Table 3). In addition, the patient's stool condition and nutrition was improved. Finally, her weight returned to 28 kg without tube feeding.

Patient 2 presented with dystonia, most prominently in the right lower extremity (Fig. 2C), and the right side of the face, chest and trunk. After ghrelin administration, dystonia improved markedly (Fig 2D). The continuous dystonic toe positioning (video segment B) was markedly improved after ghrelin administration (video segment D), as was her gait disturbance (video segments A and C). She showed occasional stereotypic hand movements, a kyphotic posture and mild scoliosis. She exhibited a Cobb angle of -20º. After ghrelin administration, her gait disturbance and dystonia were markedly improved, based on her BFMDRS score. An improvement of her BFMDRS score from 67 to 54.5 was attributed to the lower extremity and gait scores both reducing from 4 to 2, as well as mild improvements in the trunk and mouth. The SDCF score was reduced from 20 to 18 because of improvements in the scales for dystonia and mood disturbance of prominent agitation. The VAS, including right dystonic foot posture (striatal toe), tremor, right face and neck, were also markedly improved. Constipation was ameliorated after ghrelin administration (Table 3).

Patient 3 was bedridden and exhibited quadriplegia, with severe breathing abnormalities and sleep disturbance. She developed slight rigidity and scoliosis. Her refractory constipation was improved. Patient 4 required wheelchair assistance and had severe breathing abnormalities. She developed slight rigidity, but not scoliosis. Her refractory constipation was

improved.

In all four patients, improvement of constipation and other symptoms was observed at 1 week after ghrelin treatment and continued for 3 weeks.

3.4 Adverse effects

Ghrelin administration was well-tolerated. There were no adverse effects, except for transient flushing of the face in one patient.

4. Discussion

Extrapyramidal signs, including stereotypic hand movements, gait disturbance, bruxism, bradykinesia, hypomimia, scoliosis, rigidity and dystonia, are observed in almost all patients with RTT, and they affect the daily lives of patients [6-8]. These findings suggest that the extrapyramidal system, mainly the striatum, is affected in patients with RTT, and neuronal dysfunction in the basal ganglia may be responsible for movement disorders such as stereotypies and Parkinsonism [6, 7]. Therefore, the management of extrapyramidal signs is important. Previous studies demonstrate that between 59% [6, 7] and 63% [8] of RTT patients have dystonia (one of the extrapyramidal signs), with the frequency of dystonia increasing with age.

Apparent life-threatening episodes have also been related to dystonia [9]. However, only a few reports have focused on these movement disorders in patients with RTT [6-9]. Candidate

therapies for extrapyramidal signs include dopamine agonists, muscarinic cholinergic antagonists and botulinum toxin. However, only one study showed that trihexyphenidyl improved dystonic movement and apparent life-threatening episodes [9]. In our previous studies, the dopamine agonist pramipexole, and the muscarinic cholinergic antagonists trihexyphenidyl and biperiden, were not clinically effective. Previous neuropathological studies have reported a decrease in melanin content in the zona compacta of the substantia nigra and a reduction in the number of basal forebrain cholinergic neurons [26]. Patients with RTT were reported to have Parkinsonian symptoms [6-8], and Mecp2-null mice ($Mecp2^{null/y}$) showed dysfunction of the dopaminergic system [27]. Ghrelin is involved in the GH/IGF-1 axis and regulates somatic growth. Ghrelin also has multiple physiological functions, including increasing appetite, enhancing the motility of the gut, and regulating autonomic functions [10-12]. As shown in some studies, the ghrelin receptor is expressed in the central nervous system and regulates dopamine signalling [13]. Ghrelin protected dopaminergic neurons in the substantia nigra from MPTP-induced neurotoxicity in a model of Parkinson's disease [28], an effect mediated by its antioxidant properties [29]. Peripherally administered ghrelin activates GHS-Rs in the ventral tegmental area (VTA) and induces bimodal effects on mesolimbic dopaminergic neurotransmission during food consumption [30]. Systemic ghrelin administration induced an increase in dopamine levels in the nucleus accumbens via activation of μ-opioid receptors in the VTA [31].

Recent clinical trials assessing the effects of ghrelin on various diseases, including chronic heart failure [17], functional dyspepsia [18], anorexia nervosa [19], anorexia after gastrostomy [20], esophagectomy [21] and chronic obstructive pulmonary disease, have been reported [22]. Garin et al. reviewed clinical trials examining the dosage, route of administration, clinical efficacy and safety of ghrelin [32]. A total of 124 studies were reviewed, comprising 1,850 participants with various disorders such as gastrectomy and anorexia, as well as healthy controls. The major outcomes included an increase in energy intake and an increase in body weight and/or body mass. Ghrelin was found to have an excellent short-term safety profile with few transient adverse effects. Nagaya et al. reported that ghrelin improved left ventricular fraction, exercise capacity, and muscle wasting in 10 patients with chronic heart failure compared with a healthy control group [17]. In six patients with functional dyspepsia, hunger sensation was significantly enhanced compared with controls. No severe adverse effects were observed [18]. A pilot study in five patients with restricting-type anorexia nervosa demonstrated that ghrelin decreased gastrointestinal symptoms, and increased hunger sensation and daily energy intake, without serious adverse events [19]. In a prospective, randomized, placebo-controlled phase II study, short-term ghrelin administration was safe, and it was successful in lessening postoperative body weight loss, and improving appetite and food intake after total gastrectomy in 11 patients compared with a placebo group [20]. Ghrelin was administered to 10 patients after esophagectomy,

leading to increased oral food intake and attenuated weight loss. No side effects were observed [21]. Miki et al. described the effects of ghrelin treatment in cachectic patients with chronic obstructive pulmonary disease. Although ghrelin improved symptoms and respiratory strength, without adverse events, there was no significant difference from placebo in the 6-min walk test [22].

The effective dose level and delivery route include 3-μg/kg administered intravenously, once or twice a day for 10 days to 3 weeks, in particular, following gastrointestinal tract surgery. In the present pilot study, once-daily doses of 3-μg/kg ghrelin were administered for 3 days, and the clinical efficacy was evaluated. The short time-period of ghrelin treatment was due to the burden on the caregivers and siblings of patients with RTT, and limitations on hospital admission of patients with RTT. Two patients with severe dystonia and tremor showed marked improvements. Sympathetic VMR were markedly improved in one patient. We thought that a limited timeframe of ghrelin administration may have less efficacy in Patients 3 and 4 due to the lack of change in SDCF scores. Intravenous ghrelin does not appear to be beneficial when used acutely. However, subacute efficacy may be considered, as recurrent administrations may improve extrapyramidal symptoms. The parents of Patients 3 and 4 only provided written informed consent for the first 7 days of admission. Constipation was improved in all patients with RTT. VMR, constipation, and cold feet and hands are important supportive clinical findings of autonomic dysfunction in patients with RTT. Autonomic

dysfunction and cardiorespiratory impairment have been described in patients with RTT [33]. Future therapeutic trials of potential compounds, including BDNF, BDNF mimetics, IGF-1, IGF-1-related agents, NMDA receptor inhibitors, read-through compounds, aminoglycoside-like compounds, small molecules, desipramine, GABA uptake inhibitors and other drugs, should be performed [34-36]. None of these candidate drugs have been tested on older patients with movement disorders. The effect of ghrelin on movement disorders is still unknown. The efficacy of ghrelin may be explained by one or more of the following hypotheses: 1) ghrelin protects dopaminergic neurons of the substantia nigra against neurotoxicity via its antioxidative properties and by activation of adenosine 5'-monophosphate activated protein kinase to provide neuroprotection in Parkinsonian-like conditions [28,29,37]; 2) ghrelin induces dopamine synthesis in the mesolimbic and nigrostriatal dopamine system via activation of μ-opioid receptors [30,31]; and, 3) ghrelin stimulates the proliferation of neural progenitors, neural survival, neurite growth, and synapse formation via IGF-1 [35, 36]. Hypotheses 1 and 2 may account for our results. Ghrelin also plays an important role in regulating the imbalance of sympathetic and parasympathetic neurons by activating the parasympathetic nervous system and reducing the hypertonic state of the sympathetic nervous system [12]. Hypothesis 3 is difficult to evaluate because only serum IGF-1 levels were measured, and measurement of IGF-1 levels in cerebrospinal fluid was not performed owing to the difficulty of lumbar puncture. The physiological effects of

ghrelin, including regulation of the sympathetic and parasympathetic nervous systems, may account for the improvements in clinical symptoms, including appetite, stool condition, body weight, and VMR (the latter, only in Patient 1).

There are some limitations to this pilot study that may influence the interpretation of the results. The principal limitation is the small sample size. The second limitation is a lack of randomized controls.

In conclusion, based on these preliminary data, intravenous ghrelin administration is safe and improves severe dystonia, tremor and VMR in patients with RTT. These effects continued for 2 years in Patient 1 and for 10 months in Patient 2 when followed by maintenance therapy every 3 weeks. A double-blind, randomized, placebo-controlled study is indispensable for developing ghrelin as an effective therapy for extrapyramidal symptoms in patients with RTT. The present findings will contribute toward future investigations of the therapeutic potential of ghrelin in RTT patients.

Author Contributions

KY, MH, SN, YY and KO performed the follow-ups and evaluated the patients. RO, YN, HO measured ghrelin level. MK adjusted the ghrelin samples. TM and KY designed the study protocol and prepared the manuscript. All the authors contributed to this paper and approved its submission.

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Conflict of interest

None of the authors have conflicts of interest to declare.

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Legends:

Figure 1.

Flow chart of study protocol. Patients were hospitalized for 7 days. The pre-treatment period was defined as 2 days before ghrelin administration. Ghrelin was intravenously administered at a dose of 3 μg/kg for 5 min, once-daily for 3 days. Thereafter, patients 1 and 2, who exhibited dystonia, received the same dose of intravenous ghrelin was administered over 2 days every 3 weeks. These patients were clinically evaluated on separate occasions by two paediatric neurologists. Arrows indicate the day of ghrelin administration. *Neurological evaluations were performed at two years after ghrelin administration in patient 1, and 10 months in patient 2.

Figure 2.

Photographs of Patient 1. A: Before ghrelin administration. The patient showed severe dystonia, which was dominant on the left side, and included neck twisting, and trunk and shoulder rotation, causing the head to contact the shoulder. B: At 1 week after ghrelin administration, dystonia was markedly improved, and the patient could hold her head and neck in the middle of her trunk. Photographs of Patient 2. C: Before ghrelin administration, the patient exhibited marked dystonic posture (striatal toe) of the right lower extremity. D: At 1 week after ghrelin administration, the right striatal toe was improved.

Video:

Video recording of Patient 2 before and 9 months after ghrelin administration.

Segment A: Before ghrelin administration, her gait was impaired because of dystonic posture.

Segment B: The patient also showed marked dystonia in her right foot (striatal toe). Segment C: Her gait was markedly improved in a steady and smooth manner. Segment D: After ghrelin administration, the right dystonic foot (striatal toe) was improved.

Highlights

1. Ghrelin improved dystonia and tremor in two Rett syndrome patients with severe dystonia.
2. Vasomotor reflexes were ameliorated in one Rett syndrome patient.
3. No severe adverse effects were observed.

Figure 1. The timeline of the present study

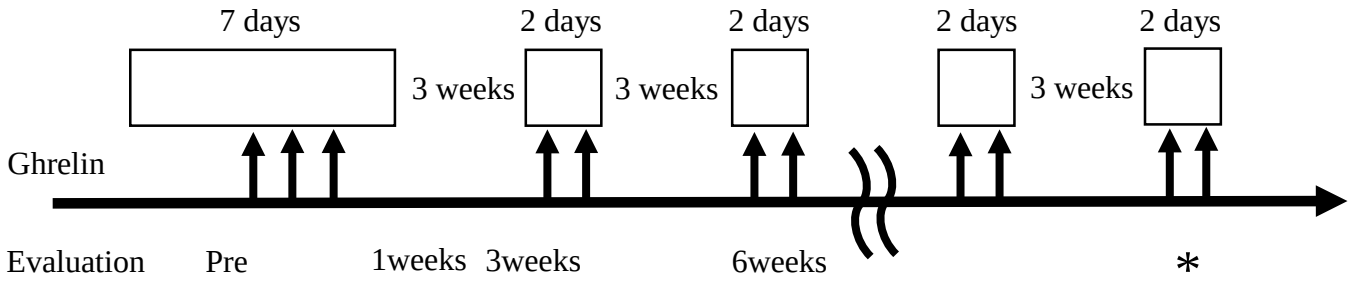

Figure2. The Change of dystonia for ghrelin

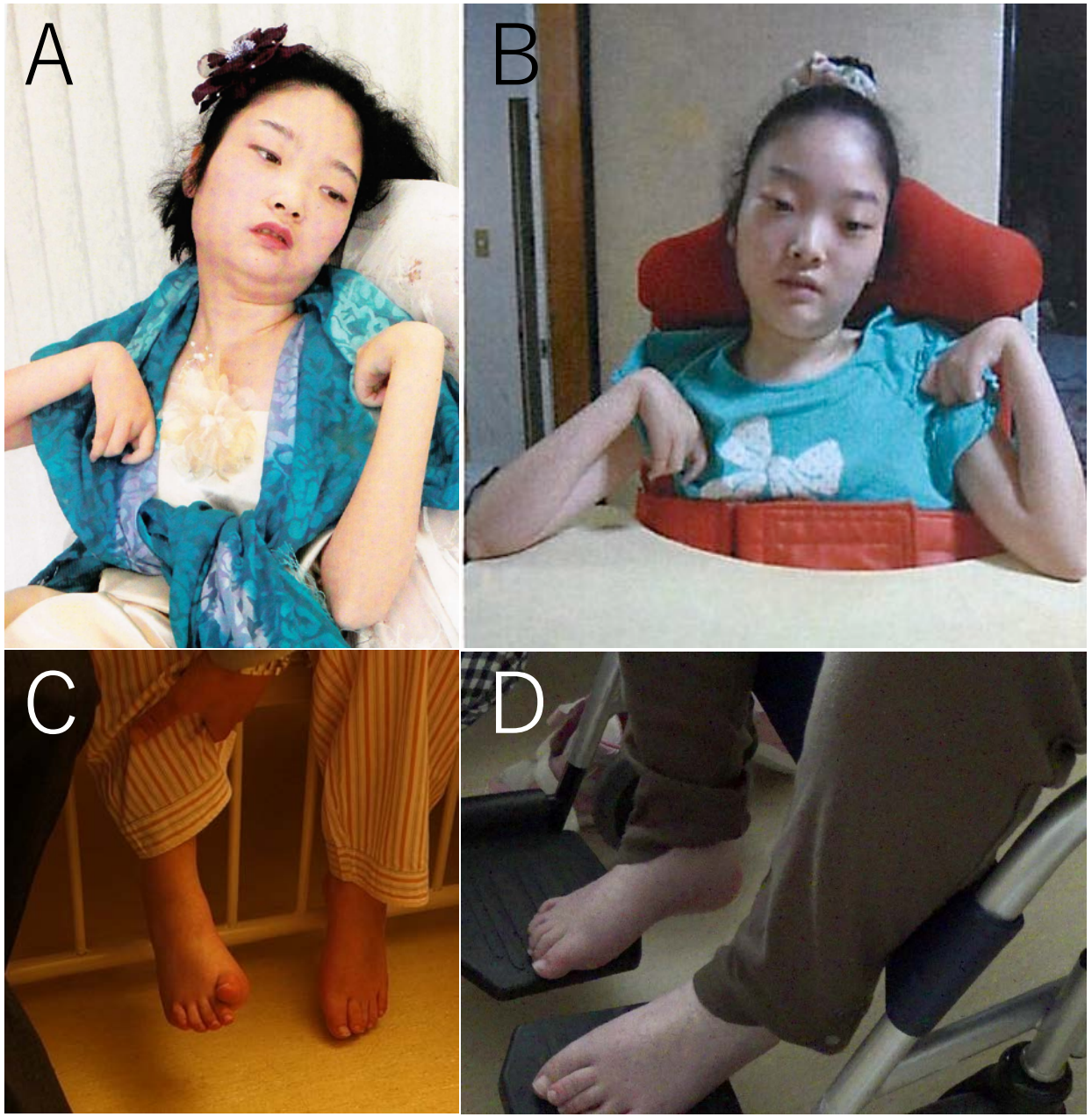

Table 1. Clinical profile of four patients with Rett syndrome

Patient No	1	2	3	4
Age on entry (yrs)	21	32	22	12
MECP2 mutation	p.S134C	p.P399QfsX4	p.R270X	p.G232A
Diagnosis	Typical RTT	Atypical RTT	Typical RTT	Typical RTT
Height (cm)	150	147	133	122
Weight (kg)	29	55	32	19
BMI (kg/m^2)	12.9	25.5	18.1	12.8
ADL	Bedridden Oral feeding and gastric stoma feeding	Standing up Walking and Eating with mother's assistance	Bedridden Oral feeding by mother	Can roll over but often bedridden Oral feeding by mother
Clinical symptoms	Scoliosis Difficulties in opening mouth Tremor of the head and hands VMR Constipation	Agitation Tremor of the head and hands Constipation	Breathing abnormalities seizure Constipation	Breathing abnormalities Constipation
Dystonia	neck, shoulder, and trunk rotation	Right lower extremities, right side of the face, chest, trunk		
AEDs	CBZ, VPA, CLB, LEV	none	CBZ, VPA, CLB	CBZ, VPA, CLB
Dopamine agonist	pramipexol	L-DOPA	none	none
Muscarinic cholinergic antagonist	trihexyphenidyl	none	none	none

RTT: Rett syndrome, ADL: active daily life, SDCF: scoring for different clinical features, a score of 0 represents no signs and the worst score was 40, BFMDRS: Burke-Fahn-Marsden Dystonia Rating Scale, 0 represents no dystonia with higher scores representing more severe dystonia, VMR: vasomotor reflex, AEDs: antiepileptic drugs, CBZ: carbamazepine, VPA: valproic acid, CLB: clobazam, LEV: levetiracetam, yrs: years.

Table 2. Changes in ghrelin, GH, IGF-1 and BG after administration of ghrelin

Case No		**1**	**2**	**3**	**4**
ghrelin; fmol/ mL					
Before	AG	25.1	34.7	20.6	60.6
	DG	83.2	101.3	119.1	343.5
Immediate (within 15 min)	AG	3384	9749.6	10562.0	11361.9
	DG	5333.1	6867.2	6455.1	6720.2
30 min	AG	914.8	351.3	804.4	1486
	DG	765.4	2852.6	1261.1	2472.2
60 min	AG	112.1	100.7	80.1	257.2
	DG	274.4	226.4	445.4	1320.8
90 min	AG	no data	64.5	36.4	141.2
	DG		94.1	237.7	547.5
GH; ng/ mL					
pre		0.2	3.01	2.9	0.6
immediate		17.61	2.71	8.16	1.13
30 min		35.02	24.22	89.9	18.07
60 min		13.04	7.03	23.57	7.37
90 min		no data	2.8	7.03	2.46
IGF-1; ng/ mL					
pre		214	175	285	no data
post		168	193	244	no data
BG; mg/ dL					
pre		86	86	77	86
immediate		89	84	77	92
30 min		91	88	80	88
60 min		94	88	79	89
90 min		no data	90	82	93

GH: growth hormone, IGF-1: insulin-like growth factor-1, BG: blood glucose, AG: active ghrelin, DG: des-acyl ghrelin.

Table 3. Summary of the clinical effects of ghrelin treatment

Patient No		Before	1 wk after	3 wks after	*
1	SDCF	31		31	29
	BFMDRS	108.5		108.5	100
	VAS	Dystonia; 5	8 (CG)	5 (CG)	8 (CG), 7 (PT)
		Tremor; 5	8 (CG)	5 (CG)	8 (CG), 8 (PT)
		VMR; 5	8 (CG)	6 (CG)	8 (CG)
		Constipation; 5	8 (CG)	7 (CG)	8 (CG)
2	SDCF	20		20	18
	BFMDRS	67		67	54.5
	VAS	Dystonia; 5	7 (CG)	5 (CG)	8 (CG), 8 (PT)
		Tremor; 5	10 (CG)	5 (CG)	10 (CG), 10 (PT)
		Striatal toe; 5	10 (CG)	6 (CG)	10 (CG), 10 (PT)
		Constipation; 5	9 (CG)	7 (CG)	9 (CG)
3	SDCF	31		31	
	VAS	Constipation; 5	7 (CG)	6 (CG)	
4	SDCF	26		26	
	VAS	Constipation; 5	8 (CG)	5 (CG)	

Wks: weeks; mos: months; VAS: visual analogue scale, 5 represents the baseline before ghrelin administration, 10 = markedly improved, 1 = markedly worsened; SDCF: scoring for different clinical features, a score of 0 represents no signs and the worst score was 40; BFMDRS: Burke-Fahn-Marsden Dystonia Rating Scale, 0 represents no dystonia with higher scores representing more severe dystonia; VMR: vasomotor reflex; CG: caregivers, PT: physical therapist. *At two years after ghrelin administration for patient 1 and 10 months for patient 2.