機能障害が神経細胞の機能に重大な影響を及ぼしていることが想定されている。

4. レット症候群患者の検査と治療：全人的な取組みの必要性

レット症候群の症状は神経系を中心とした発達障害に端を発するが、心機能や呼吸運動の障害、側彎などの骨格の障害など全身性で多彩な症状を呈するため、専門多科にわたった全人的取り組みが必要である。具体的には、多領域にわたるチームによる定期的な検査、加療が求められる。特に留意すべき合併症は、成長障害、栄養摂取、神経系、消化管機能、運動機能、コミュニケーション機能、整形外科、歯科の合併症である。

1) 必要な検査、評価

①発達評価
②栄養摂取 / 摂食、消化機能(便秘や胃食道逆流現象など)、身体測定
③睡眠、呼吸異常の評価
④てんかんおよび抗てんかん薬の評価
⑤心電図・ホルター心電図での QT 延長の有無の検査
⑥自律神経障害の評価(脳幹機能、サーモスタットによる末梢循環評価)
⑦側彎検査
⑧歯科的評価(齲歯治療、咬合などの歯科衛生管理など)

2) 具体的な対症治療

治療は、患者ごとに異なる。治療・医学的管理は対症的であるが、各患者の症状に応じて栄養学、理学療法、作業療法、言語療法などの専門家との多領域のアプローチを行う。家族に対する精神的、社会的支援は重要であるが、具現化しているものは少ない。

①患者・家族間の交流は精神的な支援を得るだけでなく、情報交換としても重要である。わが国には三団体があり、サマーキャンプなどの活動をしている〔NPO 法人レット症候群支援機構(大阪府)、日本レット症候群協会(石川県)、さくらんぼ会(福岡県)〕。
②てんかんの治療は、小児神経科専門医により治療と管理を行うのが望ましい。てんかんには一般的な治療と管理を要する。トピラマート(topiramate)は、てんかん発作に有効なだけでなく、呼吸異常の改善も報告されている。
③睡眠障害には、メラトニン(melatonin)が有効なことがある。
④高栄養液や高繊維食は便秘の予防に有効である。食事療法の効果がない場合には、マグネシウムなどの便軟化剤を用いる。また、食事は逆流防止剤や小さく刻んでとろみをつけたり、胃食道逆流の予防のための体位変換や骨密度減少を予防するためのカルシウム摂取などが勧められる。
⑤側彎や痙縮、拘縮の予防のために、早期から理学療法を行うことは移動能を維持する上で重要である。
⑥ QT 延長がある患者には、β遮断薬やペースメーカーが有効であることがある。

3) 注意すべき薬物

レット症候群の患者は、QT 延長などの循環器機能不全が高い危険率で発生するため、薬剤の選択は重要である。以下の薬剤には十分な注意が必要である。

・プロテアーゼ阻害薬：インジナビル、リトナビルなど
・抗精神病薬：チオリダジンなど
・三環系抗うつ薬：イミプラミンなど
・抗不整脈薬：キニジン、ソタコール、アミオダロンなど
・麻酔薬：チオペンタール、サクシニルコリンなど
・抗生物質：エリスロマイシンなどのマクロライド系抗菌薬、ケトコナゾールなどの抗真菌薬など

5. レット症候群の研究最前線―治療に向けて

2013 年 5 月現在、「レット症候群」をキーワードに PubMed 検索をかけると、2385 件の研究論文がみつかる。そのうち、1999 年の責任遺伝子発見以降では 1647 件を数え、2001 年のモデルマウスの発表以降では 1557 件に上る。最近 10 年間、世界中で急速に研究が進められているが、未だ有効な治療法はない。ここでは、近年のわが国におけるレット症候群の研究と治療法開発の最前線を概説する。

1) わが国のレット症候群の研究

1980 年代より臨床生理学的研究がなされ、近年では臨床遺伝学的、あるいは分子生物学的研究を中心に精力的に進められている。我々は、2009 年から厚生労働省科学研究費補助金事業としていくつかの研究に取り組ん

できた。以下、簡単に紹介する。

■ 基礎研究から

2001年に、*Mecp2*欠損マウスがレット症候群の症状を模していることが報告されると、これまで数多くの種類の*Mecp2*遺伝子改変マウスが作られた。その詳細は省くが、病態解析や治療法開発の研究に大いに役立っている。我々は、このマウスとレット症候群患者の脳からMECP2の標的遺伝子として*IGFBP-3*をみつけた[23]。このIGFBP-3は生体内でインスリン様成長因子Ⅰ(IGF-Ⅰ)の細胞間輸送や組織内濃度調節を行っている。IGF-Ⅰはニューロリギン(NLG)を活性化し、GABA受容体やグルタミン酸受容体を形成する働きがある[24,25]。そのため、MECP2の機能障害がIGFBP-3の量的異常を引き起こし、さらに細胞内IGF-Ⅰの異常をもたらし、シナプス形成や機能維持に重大な欠陥をもたらすものと考えられている(図3)。

他に、*Mecp2*欠損マウスによる呼吸障害の病態解明や*Cdkl5*欠損マウスによる多角的分子機序の解明が行われている。前者では、セロトニン/ノルアドレナリン再取り込み阻害薬による症状の改善がみられ、これは脳幹の呼吸中枢核の機能や形態学的異常の改善と相関していることを明らかにした。また、国内のいくつかの施設では、レット症候群患者からiPS細胞を作製し、病態研究や治療法開発に取り組んでいる。アンジェルマン症候群の責任遺伝子を含む15番染色体q11-13領域はインプリンティング遺伝子や非翻訳RNAが多くみつかっている。この領域の遺伝子発現機構とMECP2との分子間相互関係の解明も行われている。アンジェルマン症候群の一部に*MECP2*遺伝子変異がみつかっていることと、症状の類似性があることから、レット症候群との分子遺伝子学的関連性の解明は、自閉性障害の病態理解には重要である。

図3 想定される、*MECP2*とIGFBP-3が関与する神経細胞の分子病態

■ 臨床研究から

わが国では、少数の限られた施設にレット症候群の患者が集まる傾向にあるため、大規模な臨床研究は少なかった。

最近の研究から、レット症候群の生物マーカーに血中グレリン濃度が利用できる可能性が出てきた。既に、*Mecp2*欠損マウスの研究から、血中グレリン濃度の低下をみつけている。これを基に、レット症候群患者と対照例の空腹時血中グレリン濃度、成長ホルモンやIGF-Ⅰの血中濃度を測定した結果、レット症候群患者の体重と血中グレリン濃度で負の相関があることがわかった。特に前思春期では、レット症候群患者の活性型グレリン濃度が対照例に比して有意に高値であった[7]。この血中グレリン濃度は、幼小児期の身体発育の生物マーカーになるものと考えられている。

2) 現在試みられている治療法

*Mecp2*欠損マウスの研究で、*Mecp2*発現を回復すると症状が改善されることが報告されている。このことは、完全な症状の回復は難しいまでも、積極的な介入によって治療の可能性が期待できることを意味している。これまでの臨床治験では有効性が支持されているものはないが、欧米で進められている治験を紹介する。米国にあるInternational Rett Syndrome Foundation(IRSF)を通じて、臨床研究や治験についての情報公開と治験参加者の募集を行っている(http://www.rettsyndrome.org/research-programs/clinical-trials-and-databases)。

■ IGF-Ⅰ

*Mecp2*欠損マウスの研究から、IGF-Ⅰの投与によって活動量の上昇、異常呼吸の改善、心拍数の安定化、生存率の改善といった有効性が報告される[26]と、IGF-Ⅰの治験が始まった。そこでは、6例のレット症候群患者に6か月間の投与を行った結果、5例で呼吸運動の改善と3例で運動機能の改善が得られた[27]。現在、米国で第Ⅱ相治験が進んでいるところである。

■ デシプラミン

デシプラミンは三環系抗うつ薬で、セロトニン/ノルアドレナリン再取込み阻害作用を有する薬剤である。2007年に*Mecp2*欠損マウスで呼吸運動と生存率の改善が報告された[28]。その後、フランスの6施設共同で治験が行われ

ている。

■ デキストロメトルファン

デキストロメトルファンは鎮咳薬として日常診療で使われているが、非選択的セロトニン再取込み阻害作用とNMDA型グルタミン酸受容体阻害作用がある。米国で35例のレット症候群患者で治験を行い、副作用はほとんどなく、脳波上のてんかん発作波を有意に減らしたと報告されている。現在、第Ⅱ相治験が進んでいるところである。

■ その他の治療の試み

*Mecp2*欠損マウスを用いた実験段階であるが、いくつかの興味深い試みがなされている。

骨髄移植

2012年、正常ミクログリアを骨髄移植することにより、体重増加、無呼吸の減少などの呼吸運動の改善、運動機能の改善、生存率の改善が得られたことが報告された[29]。しかし、神経細胞は*Mecp2*遺伝子変異を有したままであり、長期にわたる経過が不明であることなど臨床応用には多くの問題がある。

フィンゴリモド

フィンゴリモドは多発性硬化症の治療薬として使われているが、スフィンゴシン1受容体を介してBDNFの発現を上昇する。2012年に、これを*Mecp2*欠損マウスに投与して、協調運動と生存率の改善が報告されている[30]。

これら以外にも様々な試みがなされてきたが、副作用などの問題から中止に終わったものも少なくない。今後も様々な取り組みがなされ、その中から有効な治療法がみつかることを切に願う。

6. おわりに

レット症候群に限らず、幼小児期に起こる進行性の病気はその家族と周囲に大きな影響を及ぼす。欧米では、早くから患者家族が医療者や研究者とチームを組んで治療法の開発を進めている。わが国では、2009年に初めて厚生労働省の研究事業が始まり、疫学調査が行われた。既に約25年の遅れがある。ようやく、臨床研究や治験に向けた取り組みができつつある。また、わが国の基礎研究も本稿で紹介した以外に、ユニークな取り組みがなされている。これらの広く多彩な研究が統合されて、1日も早く治療法をみつけることが求められている。

現在、レット症候群患者データベース登録を行っている。これは近い将来の臨床研究、治験を推進することを目的とし、患者・患者家族・医療関係者が一体となったプロジェクトである。欧米豪では、既に20年以上の歴史があり、これを基に治験が進められている。わが国では始まったばかりであるが、レット症候群患者にとって有意義なものとなることが期待されている。その詳細はホームページ〔NPO法人レット症候群支援機構(http://www.npo-rett.jp)、久留米大学医学部小児科(http://www.ped-kurume.com)〕に譲るが、多くのレット症候群患者の登録が必要である。また、ここに引用した厚生労働省科学研究費補助金による研究事業の報告書は、NPO法人レット症候群支援機構のホームページで閲覧可能である。

レット症候群は国から難病指定を受け、小児慢性特定疾患の対象疾患である。したがって、所定の手続きをすれば、障害の内容と程度により公的給付や医療助成を受けることができる。こうしたことは、レット症候群に限らず、診療を行う上で知っておくべきことである。

本稿を終えるにあたり、全国アンケート調査にご協力いただいた医療機関の先生方に感謝申し上げます。また、本稿をご高閲していただいた厚生労働省障害者対策総合研究事業(神経・筋疾患分野)「レット症候群の早期診断と治療をめざした統合的研究」研究班班員の先生方と、NPO法人レット症候群支援機構の方々に感謝申し上げます。

レット症候群と関連した疾患の情報については、下記へお問い合わせください。

伊藤雅之(国立精神・神経医療研究センター神経研究所疾病研究第二部) itoh@ncnp.go.jp

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Brain & Development xxx (2013) xxx–xxx

BRAIN & DEVELOPMENT
Official Journal of the Japanese Society of Child Neurology

www.elsevier.com/locate/braindev

Original article

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

Munetsugu Hara [a], Yoshihiro Nishi [b], Yushiro Yamashita [c], Rumiko Hirata [c], Satoru Takahashi [d], Shin-ichiro Nagamitsu [c], Hiroshi Hosoda [e], Kenji Kangawa [e], Masayasu Kojima [f], Toyojiro Matsuishi [c,*]

[a] *Department of Neonatology, Medical Center for Maternal and Child Health, St. Mary's Hospital, Kurume, Fukuoka 830-8543, Japan*
[b] *Department of Physiology, Kurume University School of Medicine, 67 Asahi-machi, Kurume, Fukuoka 830-0011, Japan*
[c] *Department of Pediatrics and Child Health, Kurume University School of Medicine, 67 Asahi-machi, Kurume, Fukuoka 830-0011, Japan*
[d] *Department of Pediatrics, Asahikawa Medical University, Asahikawa, Hokkaido 078-8510, Japan*
[e] *Department of Biochemistry, National Cardiovascular Center Research Institute, Suita, Osaka 565-8565, Japan*
[f] *Institute of Life Science, Kurume University, Hyakunenkohen, Kurume, Fukuoka 839-0864, Japan*

Received 18 January 2013; received in revised form 6 November 2013; accepted 18 November 2013

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.

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

* Corresponding author. Tel.: +81 942 31 7565; fax: +81 942 38 1792.
E-mail address: tmatsu@med.kurume-u.ac.jp (T. Matsuishi).

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http://dx.doi.org/10.1016/j.braindev.2013.11.007

Please cite this article in press as: Hara M et al. Relation between circulating levels of GH, IGF-1, ghrelin and somatic growth in Rett syndrome.. Brain Dev (2013), http://dx.doi.org/10.1016/j.braindev.2013.11.007

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

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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 $\leqslant 0.05$ 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.

Characteristics	RTT-Ep/ID ($n = 22$)		Non-RTT-Ep/ID ($n = 14$)		*p*
	Mean ± s.d	Range	Mean ± s.d	Range	
Age (years)	16.44 ± 8.56	4.00–37.50	11.77 ± 6.23	3.25–23.92	0.09
Weight (kg)	28.90 ± 12.44	11.60–54.00	31.53 ± 13.82	11.40–61.00	0.56
Weight-for-age (*Z* score)	−0.86 ± 2.17	−4.35–2.52	0.35 ± 1.56	−2.22–3.12	0.06
BMI (kg/m^2)	15.57 ± 3.64	9.70–22.80	17.41 ± 3.69	12.41–25.65	0.15
BMI-for-age (*Z* score)	−2.18 ± 2.17	−7.91–0.50	−0.47 ± 1.73	−3.02–3.16	0.02*
Height/length (cm)	133.01 ± 19.59	88.10–156.5	131.41 ± 23.46	91.30–169.30	0.83
Height/length-for-age (*Z* score)	−2.68 ± 0.85	−3.99–−1.02	−1.30 ± 1.01	−3.47–0.94	0.00**
OFC (cm)	50.64 ± 2.48	46.50–54.30	50.77 ± 2.46	46.80–54.30	0.88
OFC-for-age (*Z* score)	0.52 ± 1.73	−2.41–3.08	0.70 ± 1.57	−2.19–3.08	0.76
Total ghrelin (fmol/ml)	127.80 ± 87.62	39.72–442.72	164.77 ± 113.27	21.92–454.75	0.28
Octanoyl ghrelin (fmol/ml)	17.76 ± 8.80	2.75–32.13	12.56 ± 9.47	2.00–30.84	0.10
Octanoyl-/total-ghrelin ratio	16.26 ± 6.64	5.91–29.31	7.68 ± 3.78	3.45–18.14	0.00**
GH (ng/ml)	1.62 ± 2.60	0.05–11.50	2.10 ± 1.91	0.15–5.75	0.56
IGF-1 (ng/ml)	168.25 ± 96.12	60.31–375.00	201.57 ± 92.69	47.00–350.00	0.31
IGF-1/GH ratio	618.13 ± 1194.27	30.43–5540.00	367.79 ± 601.71	15.06–2333.33	0.47

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.

Characteristics	0–10 (years)		p	10–20 (years)		p	>20 (years)		p
	RTT-Ep/ID ($n = 7$) Mean ± s.d.	Non-RTT-Ep/ID ($n = 6$) Mean ± s.d.		RTT-Ep/ID ($n = 10$) Mean ± s.d	Non-RTT-Ep/ID ($n = 6$) Mean ± s.d		RTT-Ep/ID ($n = 5$) Mean ± s.d.	Non-RTT-Ep/ID ($n = 2$) Mean ± s.d.	
Weight-for-age (Z score)	−3.14 ± 0.78	−0.28 ± 1.53	0.00**	−0.42 ± 1.78	0.16 ± 1.02	0.48	1.44 ± 0.78	2.78 ± 0.48	0.64
BMI-for-age (Z score)	−1.74 ± 1.11	0.28 ± 2.17	0.05	−3.02 ± 2.87	−1.39 ± 1.13	0.21	−1.09 ± 1.11	0.05 ± 0.00	0.15
Height/length-for-age (Z score)	−2.84 ± 0.77	−1.24 ± 0.78	0.00**	−2.96 ± 0.65	−1.73 ± 0.89	0.01*	−1.88 ± 0.77	−0.17 ± 1.57	0.13
OFC-for-age (Z score)	−1.20 ± 0.98	−0.43 ± 1.34	0.26	1.06 ± 1.55	1.26 ± 1.16	0.79	1.87 ± 0.98	2.41 ± 0.94	0.50
Total ghrelin (fmol/ml)	208.34 ± 107.84	226.22 ± 157.49	0.81	91.56 ± 45.68	123.50 ± 18.27	0.13	87.51 ± 107.84	104.25 ± 30.25	0.61
Octanoyl ghrelin (fmol/ml)	26.85 ± 4.28	17.16 ± 11.33	0.09	12.15 ± 6.32	7.87 ± 3.62	0.16	16.27 ± 4.28	12.83 ± 14.09	0.68
Octanoyl-/total-ghrelin ratio	14.91 ± 5.63	7.98 ± 1.54	0.01*	15.84 ± 7.80	6.36 ± 2.67	0.01*	19.00 ± 5.63	10.80 ± 10.39	0.22
GH (ng/ml)	0.93 ± 0.96	3.05 ± 1.90	0.03*	2.32 ± 3.65	1.62 ± 1.91	0.67	1.16 ± 0.96	0.68 ± 0.52	0.65
IGF-1 (ng/ml)	127.11 ± 43.34	154.00 ± 103.39	0.55	183.38 ± 119.92	250.00 ± 76.60	0.25	195.60 ± 43.34	199.00 ± 35.36	0.96
IGF-1/GH ratio	302.77 ± 232.00	418.65 ± 938.17	0.76	480.89 ± 644.60	310.07 ± 204.78	0.54	1334.12 ± 2368.34	388.34 ± 244.59	0.62

The data are means ± s.d. The RTT-Ep/ID and non-RTT-Ep/ID groups were divided into the following age groups: 0–10 years old, 10–20 years old, and over 20 years old. The means of the weight-for-age Z score, height/length-for-age Z score, octanoyl-/total ghrelin ratio and the serum GH concentrations in the 0–10-years-old group with RTT were significantly different compared to those of the non-RTT-Ep/ID group in the same age range. The means of height/length-for-age Z score and octanoyl-/total-ghrelin ratio in the 10–20-years-old group with RTT were significantly different compared to those of the non-RTT-Ep/ID group in the same age range. Abbreviations are explained in Table 1.
* $p < 0.05$ (t-test).
** $p < 0.01$ (t-test).

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 show better development, less morbidity typically associated with RTT, and late truncation mutations [5]. RTT patients with C-terminal deletions had the highest Z scores for weight and BMI. Their BMI Z scores were significantly higher when compared with all other mutations [4]. BMI, weight, and height Z scores of RTT patients without enteral support did not identify 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

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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.

Characteristics	Total ghrelin	Octanoyl ghrelin	Octanoyl/total ghrelin ratio	IGF-1	GH
Non-RTT-Ep/ID (n = 14)					
Age (years)	−0.62*	−0.49	0.05	0.36	−0.43
Weight-for-age (*Z* score)	−0.53*	−0.41	0.07	0.25	−0.40
BMI-for-age (*Z* score)	−0.08	−0.07	−0.00	0.04	−0.24
Height/Length-for-age (*Z* score)	0.06	0.21	0.47	0.18	0.10
OFC-for-age (*Z* score)	−0.60*	−0.50	0.05	0.52	−0.67**
RTT-Ep/ID (n = 22)					
Age (years)	−0.44*	−0.37	0.21	0.25	0.01
Weight-for-age (*Z* score)	−0.63*	−0.52*	0.37	0.62**	0.25
BMI-for-age (*Z* score)	−0.10	0.09	0.32	0.65**	0.24
Height/Length-for-age (*Z* score)	−0.20	0.05	0.23	−0.04	−0.20
OFC-for-age (*Z* score)	−0.72**	−0.55**	0.47*	0.58**	0.22

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.
* $p < 0.05$.
** $p < 0.01$.

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]. In an

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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 development. 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 irrelevantly 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.

Acknowledgments

We are grateful to Dr. Masayuki Itoh, National Institute of Neuroscience and Dr. Eiichiro Tanaka, Kurume University School of Medicine for their valuable advice. This work was partly supported by a Grant-in-Aid for Scientific Research (No. 30389283 to M.H., Nos. 22591145 to Y.N. and 21591338 to T.M.) from the Japan Society for the Promotion of Science and research grant from the Ministry of Health, Labor and Welfare of Japan (Intramural Research Grant [21B-5] for Neurological and Psychiatric Disorders of NCNP, and Research on Intractable Diseases 21-110 and 22-133 to T.M.).

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.

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Please cite this article in press as: Hara M et al. Relation between circulating levels of GH, IGF-1, ghrelin and somatic growth in Rett syndrome.. Brain Dev (2013), http://dx.doi.org/10.1016/j.braindev.2013.11.007

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Brain & Development xxx (2013) xxx–xxx

BRAIN & DEVELOPMENT
Official Journal of the Japanese Society of Child Neurology

www.elsevier.com/locate/braindev

Case report

A haploinsufficiency of *FOXG1* identified in a boy with congenital variant of Rett syndrome

Akira Kumakura [a,*], Satoru Takahashi [b], Kazuki Okajima [b], Daisuke Hata [a]

[a] *Department of Pediatrics, Kitano Hospital, The Tazuke Kofukai Medical Institute, Osaka, Japan*
[b] *Department of Pediatrics, Asahikawa Medical University, Asahikawa, Japan*

Received 10 November 2012; received in revised form 1 September 2013; accepted 13 September 2013

Abstract

Background: Forkhead box G1 gene (*FOXG1*) mutations and deletions are associated with a congenital variant of Rett syndrome (RTT). Nucleotide alterations of the coding region of *FOXG1* have never caused dysmorphic features. *Patient:* An 8-year-old boy with the congenital variant of RTT who showed severe psychomotor deterioration, epilepsy, acquired microcephaly, and involuntary movements including jerky movements of the upper limbs and tongue protrusion. He showed dysmorphic features including round face, anteverted nostrils, and tented upper lips. Brain magnetic resonance imaging showed hypoplasia of the frontal lobes and the rostral part of the corpus callosum. The molecular cytogenetic analysis confirmed a *de novo* deletion of 14q12 including *FOXG1* in this patient. *Conclusion:* We identified the smallest deletion of 14q12 involving *FOXG1* among those previously reported. Dysmorphic facial features are a characteristic for the patients with chromosomal deletion including *FOXG1*. In our patient, *C14orf23* is the only transcript other than *FOXG1*. Therefore, *C14orf23* might be responsible for facial dysmorphism.

Keywords: Rett syndrome; Congenital variant; *FOXG1*; *C14orf23*; Dismorphic facial features

1. Introduction

Rett syndrome (RTT), a severe neurodevelopmental disorder with characteristic clinical features including psychomotor deterioration, acquired microcephaly, seizures, and loss of purposeful hand movements, has incidence of 1:10,000 female births. It is the second most common cause of severe mental retardation in females. About 90% of typical RTT cases are attributable to mutations in the methyl-CpG-binding protein 2 gene (*MECP2*) located on the X chromosome. Therefore, the affected patients have been exclusively females [1]. Mutational analyses conducted for RTT patients without *MECP2* abnormalities have revealed mutations in the cyclin-dependent kinase-like 5 gene (*CDKL5*) on Xp22 or mutations in the forkhead box G1 gene (*FOXG1*) on 14q12. *CDKL5* mutations are associated with the early onset seizures variant of RTT in both females and males [2,3]. Both loss of function mutations and microdeletions of *FOXG1* have been identified in patients with the congenital variant of RTT, accounting for 0.6% in patients with RTT [4–9]. The congenital variant of RTT is characterized by brain malformation that is specific to the forebrain, severe psychomotor deterioration, and involuntary movements including tongue protrusion and stereotyped jerky movements of the upper limbs. *FOXG1* is a brain-specific transcriptional factor that is necessary for fetal neurogenesis. Lack of *FOXG1* function suppresses neural stem cell self-renewal and promotes premature cortical neural expansion, engendering

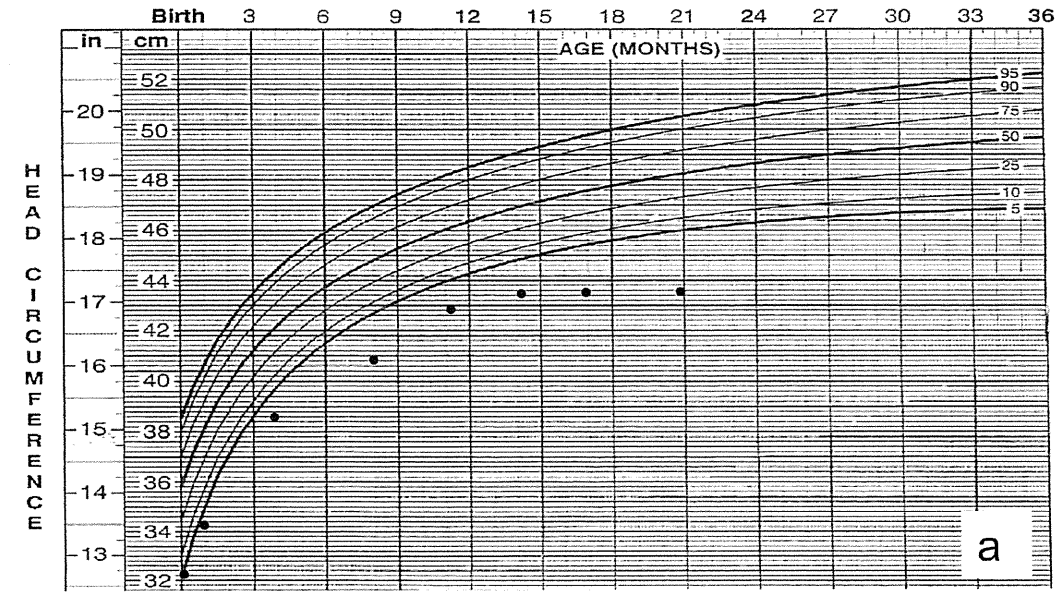
* Corresponding author. Address: Department of Pediatrics, Kitano Hospital, The Tazuke Kofukai Medical Institute, 2-4-20 Ohgimachi, Kita-Ku, Osaka 530-8480, Japan. Tel.: +81 6 6312 8824; fax: +81 6 6312 8867.
E-mail address: a-kumakura@kitano-hp.or.jp (A. Kumakura).

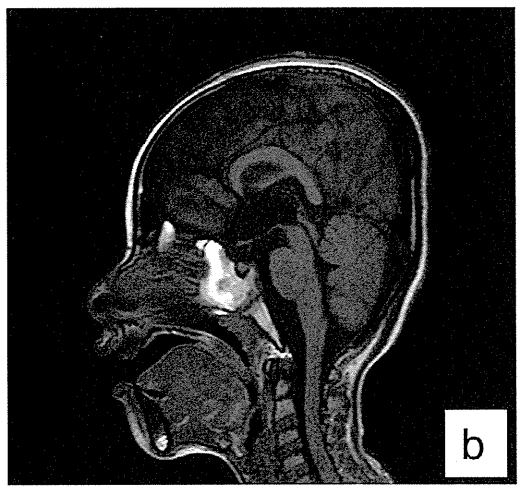
0387-7604/$ - see front matter © 2013 Published by Elsevier B.V. on behalf of The Japanese Society of Child Neurology.
http://dx.doi.org/10.1016/j.braindev.2013.09.006

an insufficient quantity of telencephalic neurons [10–12]. This report describes a Japanese boy who showed postnatal developmental deterioration and arrested head growth after 10 months of age. Moreover, he showed irregular jerky movements of the upper limbs. We initially diagnosed him as having dyskinetic or athetotic cerebral palsy. However, according to the diagnostic criteria for classical and variant RTT [13], this patient was regarded as having a congenital variant of RTT. Therefore, we conducted *FOXG1* mutational analysis, which revealed a *de novo* deletion of *FOXG1* at 14q12.

2. Case report

The patient, an 8-year-old boy, was born to non-consanguineous, healthy Japanese parents at 38 weeks gestation after an uneventful pregnancy. His birth weight and length were, respectively, 2680 g (−0.78 SD) and 49.0 cm (−0.17 SD). He had normal occipito–frontal circumference (OFC) of 32.0 cm (−0.86 SD) with no auxological abnormality. He showed no asphyxia or jaundice. He had no siblings and no family history of neuromuscular diseases, metabolic disorders, dysmorphic syndrome, or other developmental disorders. He had developed with no complications during the neonatal period. However, he showed developmental delay and deterioration after 3 months of age. His motor skills had progressed to rolling over. Subsequently, his head control deteriorated and he became less able to roll over, being bedridden. He showed severe mental retardation with no explosive language, but with deficient social reciprocal communication including eye contact and eye





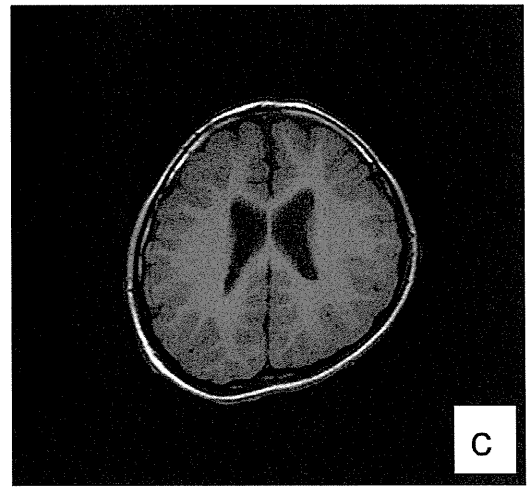


Fig. 1. (a) Growth curve of occipito-frontal circumference shows postnatal microcephaly became more evident between 4 and 8 months of age. (b and c) Brain magnetic resonance imaging (MRI) shows hypoplasia of the rostral part of the corpus callosum ((b) TR/TE = 529.283/13.000) and frontal lobes ((c) TR/TE = 505.224/13.000).

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gaze. His sleep pattern did not acquire circadian rhythm. He needed no enteral tube feeding. Postnatal microcephaly became more evident at 4–8 months of age (Fig. 1a). In addition to acquired microcephaly, he had dysmorphisms including a round face, anteverted nostrils, and tented upper lips. Physical examination revealed severe truncal hypotonia. He demonstrated dyskinesic involuntary movements: peculiar jerky movements of the upper limbs pushed in different directions and tongue protrusion. He showed no stereotypic hand washing or hand mouthing, as patients with RTT typically do. Ophthalmological and audiological examinations yielded normal results. Chromosomal analysis revealed normal karyotype, 46, XY. Extensive metabolic investigations including serum amino-acid quantification, serum acylcarnitine profile quantification, and urine organic acid quantification revealed no abnormality.

At three years of age, he experienced unprovoked seizures: nocturnal tonic seizures and sometimes hypermotor seizures. Interictal electroencephalography (EEG) revealed sharp waves over the bilateral frontopolar areas. Therefore, we diagnosed him as having symptomatic focal epilepsy. Brain magnetic resonance imaging (MRI) revealed hypoplasia of the frontal lobes and the rostral part of the corpus callosum (Fig. 1b and c). Antiepileptic drugs including zonisamide, phenytoin, and phenobarbital controlled his epileptic seizures well. From six years of age, atypical absence and tonic seizures appeared. Interictal EEG showed high-amplitude slow activity and diffuse slow spike and wave complex predominantly over the frontal areas. These seizures were treated with valproate, topiramate, and lamotrigine, which produced some improvement in seizure frequency. These clinical and radiological features are compatible with those of the congenital variant of RTT.

3. Genetic analysis

After obtaining written informed consent from his parents, genomic DNA was extracted from the peripheral blood leukocytes of the patient and his parents and was used for mutation screening. The compatible primers for polymerase chain reaction (PCR) were used to obtain DNA fragments spanning the entire *FOXG1* coding region [4]. Mutation screenings were performed by direct sequencing of the exon1-derived PCR products. Direct sequencing of the entire *FOXG1* coding region yielded a normal result. Screening of the patient's

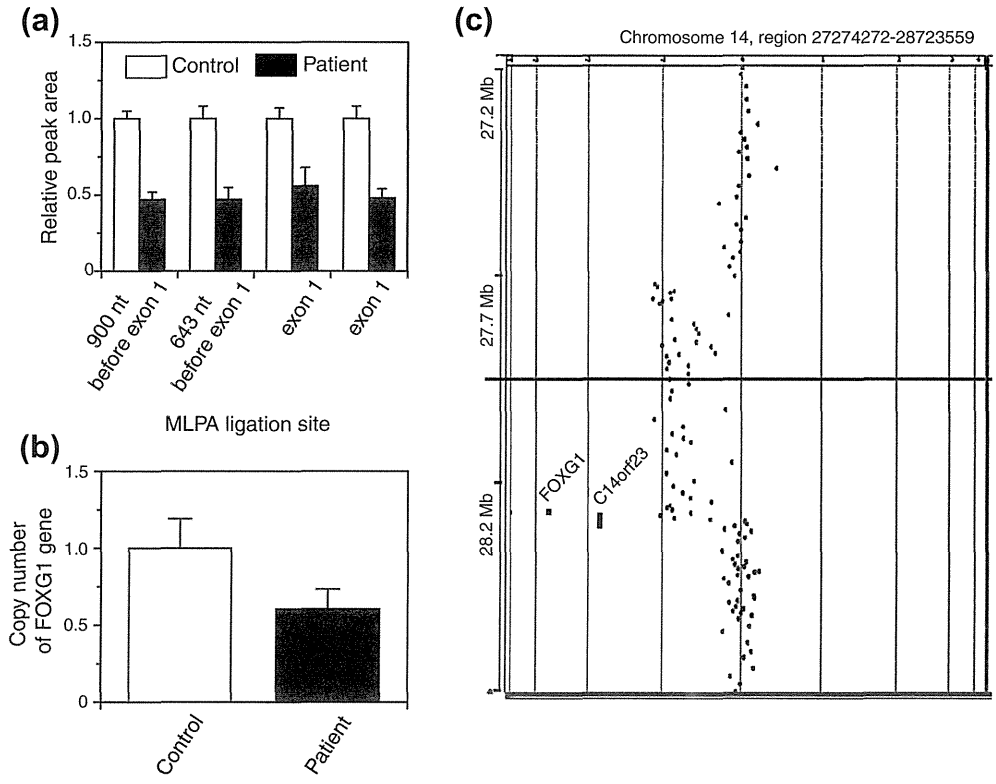

Fig. 2. Heterozygous deletion of the *FOXG1* in the patient. (a) MLPA analysis performed on DNA from the patient revealed deletion of exon 1 in *FOXG1* and a region upstream of exon 1. Results indicate the relative peak area of a probe target sequence with normalization against normal male samples and are shown as means ± SD ($n = 4$). (b) The number of *FOXG1* copies was ascertained using quantitative real-time PCR assay based on the relative amplification of the target sequence (*FOXG1*) and the internal standard *RNaseP*. Results show the ratio of *FOXG1* versus *RNaseP* gene copies, shown as means ± SD ($n = 4$). (c) The array-CGH result shows the log2 intensity ratios of the patient versus reference DNA. A 0.54-Mb deletion was detected at 14q12. This region includes only two genes: *FOXG1* and *C14orf23*.

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DNA using an MLPA kit (MLPA-P075-A1; MRC-Holland, Amsterdam, The Netherlands) revealed a deletion in 14q12 including exon 1 of *FOXG1* and a region upstream of exon 1 (Fig. 2a). Gene dosage analysis was performed using quantitative real-time PCR [4], which confirmed the deletion of *FOXG1* in the patient (Fig. 2b). Testing of the patient's parents confirmed that the deletion of *FOXG1* was *de novo*. To define the boundary of the deleted region, array-based comparative genomic hybridization (aCGH) analysis was performed using a high-resolution 400 K array (Agilent Technologies Inc., Santa Clara, CA, USA) according to the manufacturer's instructions. As a consequence, 540 Kb deletion was confirmed at 14q12 from 27.78 to 28.32 Mb (Fig. 2c; according to UCSC Human Genome Browser, on March 2006 Assembly). Only two genes are included in the region: *FOXG1* and a putative gene, *C14orf23*, with unknown function.

4. Discussion

This report described a Japanese boy with a *de novo* heterozygous deletion of *FOXG1*. *FOXG1*-related disorders consist of 14q12 microdeletion syndrome, loss of function mutation in *FOXG1* and 14q12 microduplication syndrome [4–9,14,15]. *FOXG1* is located on the autosomal chromosome. However, *FOXG1* abnormalities have been found more frequently in females than in males, probably because of the predominance of females in the diagnosis of RTT. This case report confirmed that *FOXG1* haploinsufficiency causes the congenital variant of RTT in males as well as in females.

His neurological symptoms and brain MRI findings were consistent with a diagnosis of congenital variant of RTT. Patients with 14q12 microdeletion or *FOXG1* point mutation show cardinal clinical features including severe psychomotor deterioration after 3–6 months, acquired microcephaly, truncal hypotonia, epilepsy, and involuntary movements such as tongue protrusion and stereotyped jerky movements of the upper limbs. Brain MRI findings of patients with 14q12 microdeletion or *FOXG1* point mutation are indicate hypogenesis of the rostral part of the corpus callosum and delayed myelination that is specific to frontal lobe. In 2006, Bisgaard et al. reported the first case of microdeletion in chromosome band 14q12, resulting in haploinsufficiency for *FOXG1* [4,5]. Since that first case, more than 10 such cases have been reported [6–8]. In 2008, Ariani et al. reported the first two cases with point mutations of *FOXG1* [4]. In 2009, Yeung et al. reported a case of microduplication in chromosome band 14q12 including *FOXG1* [14]. A considerable phenotypic overlap exists between patients with 14q12 microdeletion, loss of function of mutation in *FOXG1*, 14q12 microduplication, and our patient (presented in

Table 1
Summary of clinical findings of this case, 14q12 microdeletion, *FOXG1* point mutation, and 14q12 microduplication.

	This study	14q12 microdeletion [4,5]	*FOXG1* point mutation [3]	14q12 microduplication [9,10]
Psychomotor deterioration	After 3 months	After 3–6 months	After 3 months	Sometimes after 3 months
Developmental delay	Postnatal onset	Postnatal onset	Postnatal onset	From birth
Hypotonia	+	+	+	Sometimes
Microcephaly	Postnatal onset	Postnatal onset	Postnatal onset or congenital	Sometimes postnatal
Epilepsy	Refractory	Treatable	Treatable	Sometimes refractory infantile spasms
Involuntary movements				
Jerky movements	+	+	+	–
Tongue protrusion	+	+	+	–
Hand stereotypies	–	+	+	–
Sleep disturbance	+	Sometimes	Sometimes	–
Feeding problems	–	+	Sometimes	–
Brain MRI				
Corpus callosum	Hypogenesis	Sometimes agenesis	Hypogenesis	Hypogenesis
White matter	Delayed myelination (frontal lobe)	Delayed myelination	Delayed myelination	Reduction of white matter volume
Cortex	No abnormality	Not reported	Gyral simplification (frontal lobe)	Not reported
Dysmorphisms	Round face Anteverted nostril Tented upper lips	Epicanthic folds Bulbous nasal tip Depressed nasal bridge Tented upper lips	Not significant	Mid face hypoplasia Flat nasal bridge Small palpebral fissures

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the Table 1), suggesting a dosage-sensitive role for *FOXG1* in brain development [14]. FOXG1 plays an important role in forebrain development [10–12]. These developmental abnormalities, which were specific to the forebrain, appear to be a key feature associated with *FOXG1* haploinsufficiency, although patients with 14q12 microduplication showed no specific abnormalities of the brain MRI [14,15].

Facial dysmorphisms including epicanthic folds, bulbous nasal tip, depressed nasal bridge, and tented upper lips have often been demonstrated in patients with 14q12 microdeletions. By contrast, these features are not seen in patients with *FOXG1* point mutations. It seems likely that the facial dysmorphisms are caused by a contiguous deletion of other genes at 14q12. However, our patient and a previously reported patient [16] who have deletions of only two genes, *FOXG1* and a putative gene *C23orf14C14orf23*, also show distinctive facial features similar to patients with 14q12 microdeletions. Because the identified deletion was the smallest among those previously reported, this deletion narrowed the critical region for facial dismorphism. Consequently, *C14orf23* might be responsible for facial dysmorphism. Further investigations must be conducted to elucidate the function of *C14orf23* for facial dysmorphism.

Acknowledgment

We thank the members of the patient's family, whose help and participation made this work possible.

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Please cite this article in press as: Kumakura A et al. A haploinsufficiency of *FOXG1* identified in a boy with congenital variant of Rett syndrome. Brain Dev (2013), http://dx.doi.org/10.1016/j.braindev.2013.09.006