genome-wide UPD samples in unbiased methylation screens such as bisulphite genome sequencing will aid the identification of additional imprinted loci, which will facilitate study of genetic diseases associated with aberrant imprinting. The general trend until now has been that, while imprinted genes play an important role in fetal development and behavior, evolutionary forces dictated by the genetic conflict have allowed for a lack of conserved imprinting between mouse and humans (54). However, our screen has identified new human-specific imprinted transcripts, all of which have conserved gene orthologues in many taxa. These genes have selected imprinting as a mechanism of transcriptional regulation in humans despite the risk of being functional hemizygous.

MATERIALS AND METHODS

The human reciprocal genome-wide UPD samples

Genomic DNA isolated from three previously described Beckwith–Weidemann syndrome-like cases (16–18) and one Silver–Russell syndrome-like patient (19) was used in this study. Each of these cases had undergone extensive molecular characterization to confirm genome-wide UPD status and level of mosaicism. We used DNA isolated from leukocytes as these samples had minimal mosaicism of a biparental cell line. The genome-wide BWS samples had 9, 11 and 15% biparental contribution, whereas the genome-wide SRS sample had 16%.

Human tissues

Two independent tissue collections were used in this study. All tissues were collected after obtaining informed consent. The Spanish collection was from the Hospital St Joan De Deu tissue cohort (Barcelona, Spain). Normal peripheral blood was collected from adult volunteers aged between 19 and 60 years old. A selection of normal brain samples was obtained from BrainNet Europe/Barcelona Brain Bank. The Japanese tissues were collected at the National Center for Child Health and Development (Tokyo, Japan) and at the Saga University Hospital.

DNA was extracted using either the standard phenol/chloroform extraction method or the QIAamp DNA Blood Midi Kit (Qiagen). RNA was extracted using either Trizol (Invitrogen) or Sepasol®-RNA I Super G (Nacalai Tesque) and cDNA synthesis was carried out as previously described (54). Ethical approval for this study was granted by the Institutional Review Boards at the National Center for Child Health and Development and Saga University and Hospital St Joan De Deu Ethics Committee (Study number 35/07) and IDIBELL (PR006/08).

Cell lines and mouse crosses

Wild-type mouse embryos and placentas were produced by crossing C57BL/6 with *Mus musculus molosinus* (JF1) mice. C57BL/6 (B6) mice were purchased from Sankyo Labo Service Corporation, Inc. (Tokyo, Japan) and JF1/Ms (JF1) mice were obtained from the Genetics Strains Research Center at the National Institute of Genetics, Japan. All animal husbandry and breeding was approved and licensed by the National Research Institute for Child Health and Development, Japan (Approved number A2010–002).

Illumina Infinium methylation27 BeadChip microarray analysis

Approximately 1 μg DNA from the reciprocal genome-wide UPDs, placenta, leukocytes, brain, muscle, fat, buccal cells was subjected to sodium bisulphite treatment and purified using the EZ GOLD methylation kit (ZYMO, Orange, CA, USA). This DNA was then hybridized to the Illumna Infinium Human Methylation27 BeadChip microarray either at the Centro Nacional de Investigaciones Oncologicas (Madrid, Spain) or Genome Science Division, Research Center for Advanced Science and Technology (University of Tokyo, Japan) using Illumina-supplied reagents and protocols. The loci included on this array and the technologies behind the platform have been described previously (55). Before analyzing the methylation data, we excluded possible sources of technical biases that could alter the results. We discarded 109 probes because they had a false-positive rate >0.1. We also excluded 261 probes because of the lack of signal in one of the 11 DNA samples analyzed. Lastly, prior to screening for novel imprinted DMRs, we excluded all X chromosome CpG sites. Therefore, in total we analyzed 26 152 probes in all DNA samples. All hierarchical clustering and β-value evaluation was performed using the Cluster Analysis tool of the BeadStudio software (version 3).

Allelic methylation analysis

A panel of placenta-, leukocyte-, brain- and kidney-derived DNAs were genotyped to identify heterozygous samples. These DNA were converted using the EZ GOLD methylation kit. Approximately 100 ng of converted DNA was used for each bisulphite PCR. Bisulphite-specific primers (Supplementary Material, Table S1) which incorporate the SNPs were used with Hotstar Taq polymerase (Qiagen, West Sussex, UK). Amplifications were performed using either 45 cycles or a nested PCR using 35 cycles for each round. The subsequent PCR products were cloned into pGEM-T Easy vector (Promega) for subsequent sequencing.

Allelic expression analysis

Genotypes on DNA were obtained for exonic SNPs identified in the UCSC browser (NCBI36/hg18, Assembly 2006) by PCR. Sequences were interrogated using Sequencher v4.6 (Gene Codes Corporation, MI) to distinguish informative heterozygote samples. Informative samples were analyzed by RT–PCR. All primers, with the exception of those targeting *FAM50B*, are intron-crossing and incorporated the heterozygous SNP in the resulting amplicon (Supplementary Material, Table S1). RT–PCRs were performed using cycle numbers determined to be within the exponential phase of the PCR, which varied for each gene, but was between 32 and 40 cycles.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

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Conflict of Interest statement. None declared.

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ORIGINAL ARTICLE

Radiological evaluation of dysmorphic thorax of paternal uniparental disomy 14

Osamu Miyazaki · Gen Nishimura · Masayo Kagami · Tsutomu Ogata

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Abstract
Background The "coat-hanger" sign of the ribs with a bell-shaped thorax has been known as a radiological hallmark of the paternal uniparental disomy 14 (upd(14)pat).
Objective To quantitatively determine the differences in thoracic deformity between upd(14)pat and other bone diseases with thoracic hypoplasia and to establish the age-dependent evolution.
Materials and methods The subjects comprised 11 children with upd(14)pat. The angle between the 6th posterior rib and the horizontal axis was measured (coat hanger angle; CHA). The ratio of the mid- to widest thorax diameter (M/W ratio) was calculated for the bell-shaped thorax.
Results CHA ranged from +28.5 to 45° (mean; 35.1°±5.2) in upd(14)pat, and from −19.8 to 21° (−3.3±13°) in bone dysplasias ($p<0.01$). The M/W ratio ranged from 58% to 93% (75.4±10) in upd(14)pat, and from 80% to 92% (86.8±3.3) in bone dysplasias ($p<0.05$). Serial radiographs revealed that CHA remained constant during early childhood, while the M/W ratio gradually increased with age.
Conclusion The "coat-hanger" sign of upd(14)pat provides a distinctive radiological gestalt that makes it possible to differentiate the disorder from other skeletal dysplasias. By contrast, the bell-shaped thorax is significant only in the neonatal period.

Keywords UPD14 · Plain radiograph · Coat-hanger sign · Bell-shaped thorax

O. Miyazaki (✉)
Department of Radiology,
National Center for Child Health and Development,
2-10-1 Okura,
Seatagaya-ku, Tokyo 157-8535, Japan
e-mail: osamu-m@rc4.so-net.ne.jp

G. Nishimura
Department of Radiology,
Tokyo Metropolitan Children's Medical Center,
2-8-29 Musashidai,
Fuchu-shi, Tokyo 183-8561, Japan

M. Kagami · T. Ogata
Division of Clinical Genetics and Molecular Medicine,
National Center for Child Health and Development,
2-10-1 Okura,
Seatagaya-ku, Tokyo 157-8535, Japan

Introduction

Uniparental disomy (UPD) refers to the inheritance of a pair of chromosomes from only one parent. UPD is a relatively common phenomenon. The inheritance of both, or parts of both, maternal chromosomes (heterodisomic maternal UPD) has been found to become more prevalent as parental age becomes more advanced [1]. It is well established that UPD for chromosomes 6, 7, 11, 14 and 15 is associated with recognized syndromes, including Prader-Willi syndrome (maternal UPD 15), Angelman syndrome (paternal UPD 15), and Beckwith-Wiedemann syndrome (paternal UPD 11) [2].

The paternal UPD 14 phenotype (upd(14)pat) is a recently recognized genetic condition that is caused by an aberration of the imprinting center in chromosome 14. The clinical hallmarks of upd(14)pat are thoracic hypoplasia and abdominal wall defect. Mild facial dysmorphism and developmental delay are also noted. In addition, upd(14)pat presents with a distinctive radiological finding: the "coat-hanger" appearance of the ribs and a bell-shaped thorax [3]. In the past, upd(14)pat was often misdiagnosed as bone dysplasias with thoracic hypoplasia, as in Jeune syndrome [4], because attention was not paid to the morphological differences of the thorax between upd(14)pat and other genetic bone diseases. Previous reports on

upd(14)pat have been based on a single case or a limited number of cases. To date, there has been no radiological report involving a large series of upd(14)pat cases. Although a previous report suggested that the dysmorphic thorax in upd(14)pat ameliorated in the mid-childhood period [5], it remains to be determined how the thoracic deformity in upd (14)pat evolves with age. The purpose of this study was to quantitatively determine the differences in the thoracic deformity between upd(14)pat and other genetic bone diseases, and to establish the age-dependent radiological evolution of the thoracic hypoplasia in upd(14)pat.

Materials and methods

The subjects comprised 11 children (6 girls and 5 boys) with upd(14)pat phenotypes proven on molecular grounds [5, 6]. Three of the 11 children had been managed in our hospital, and 8 were referred to our institution for molecular diagnosis. The molecular diagnoses included seven cases of paternal uniparental disomy, two of microdeletion and two of epimutation. The initial radiographs available for the analysis were obtained in the neonatal period (n=8), and at 7, 24 and 32 months of age (n=1). Sequential radiological evaluation was feasible in 4 of 11 children up to 5 years of age. The study was approved by the institutional review board at the National Center for Child Health and Development.

To assess for the "coat-hanger" sign, the angle between the 6th posterior rib and the horizontal axis was measured (coat hanger angle, CHA; an upward angle was defined as +, and a downward angle as −). The ratio of the mid- to widest thorax diameter (M/W ratio) was calculated for the bell-shaped thorax (Figs. 1, 2). For comparison, both indexes were evaluated in nine cases with bone dysplasia with thoracic hypoplasia, including thanatophoric dysplasia (n=6), Ellis-van Creveld syndrome (n=2) and asphyxiating thoracic dysplasia (n=1). These cases were selected from our radiology database. The children's ages ranged from 21 weeks of gestation to 6 years of age (mean: 11 months of age). Both indexes were also evaluated in five children with respiratory distress syndrome (RDS) and without skeletal abnormalities that could be assessed to determine the evolution of the normal thoracic morphology. In the RDS group, serial follow-up radiographs were available from the neonatal period up to 2 years to 6 years of age (mean 4.2). The measurement of CHA and M/W ratio was performed using an accessory digital tool from a PACS

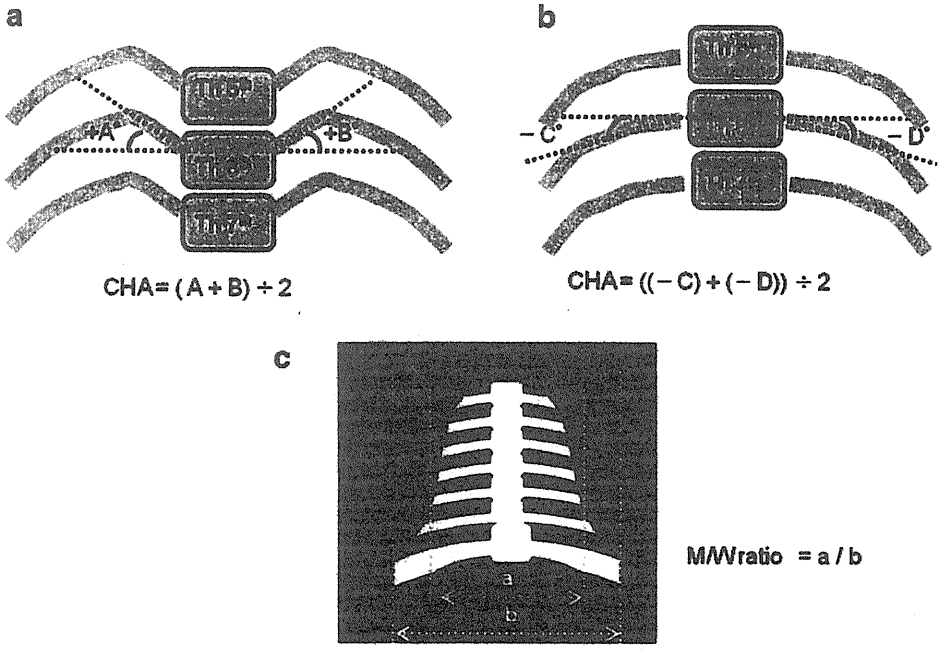

Fig. 1 **a**, **b** Diagram of coat-hanger angle (CHA) and mid/widest ratio. CHA refers to the average of the angles between the peak point of both 6th posterior ribs and the horizontal axis. If there is no peak point of the 6th posterior ribs, the center of the ribs is utilized instead. The horizontal axis is defined as a line passing through two points of both 6th cost-vertebral junctions. An upward angle is defined as +, and a downward angle as −. CHA is thought to be a quantitative index of the coat-hanger sign. **c** The ratio of mid- to widest thorax (M/W ratio) refers to the ratio of the narrowest diameter of the mid-thorax to the widest diameter of the basal thorax. In most cases with upd(14)pat, the thorax showed medial concavity with the top of approximately the 6th rib (the narrowest mid-thorax) and downward sloping toward the 9th to 11th ribs (the widest basal thorax). M/W ratio is thought to be a quantitative index of dysmorphic bell-shaped thorax

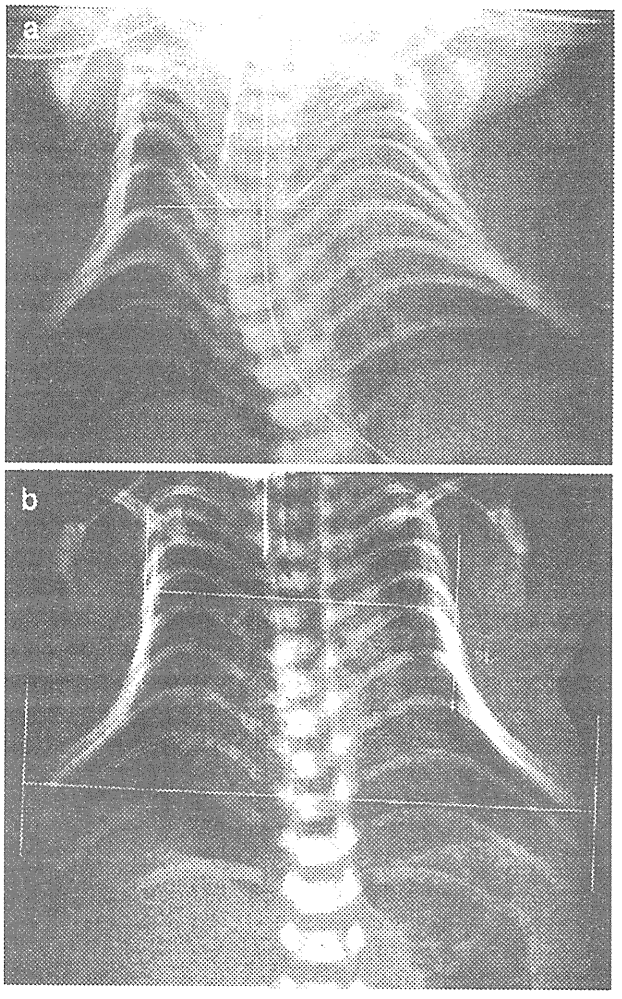

Fig. 2 Examples of CHA and M/W ratio. **a** The 6th posterior ribs show upward bowing that provides the coat-hanger sign. The CHA of this case (patient #7 in Table 1) was 45° (the measurement was 48° for the right and 42° for the left). **b** The M/W ratio was 58% in this case (patient #5 in Table 1). This is an example of severe bell-shaped thorax in upd(14)pat

system (Centricity ™ RA 1000 Ver.3.0, GE Healthcare, Milwaukee, WI) on the PACS monitor, or using area and protractor commercial software (Lenara Ver2.21, Vector, Tokyo) on a personal computer monitor. An unpaired two-tailed t-test was used for statistical evaluation.

Results

Clinical and measurement data are summarized in Table 1 and Fig. 3. All 11 children with upd(14)pat showed a severe upward sweep of the posterior rib or increased CHA, ranging from +28.5 to 45° (mean ± SD; 35.1°±5.2) (Figs. 2, 3). Children with bone dysplasias presented with variable manifestations of the posterior rib, and CHA ranged from −19.8 to 21° (mean ± SD; −3.3±13°) (Figs. 3, 4). The difference in CHA was statistically significant between the upd(14)pat and bone dysplasia groups ($P<0.01$). According to this result, approximately +25° was the estimated cut-off line of CHA to differentiate upd(14)pat from skeletal dysplasias (Fig. 3). The M/W ratio ranged from 58% to 93% (mean±SD; 75.4±10) in the upd(14)pat group, while it ranged between 80% and 92% (mean±SD; 86.8±3.3) in the skeletal dysplasia group (Fig. 3). The difference an unpaired two-tailed t-test in the M/W ratio was, though statistically significant, less conspicuous than that in CHA ($P<0.05$). There was considerable overlap in the range of the M/W ratio between the upd(14)pat and skeletal dysplasia groups.

The age-dependent evolution of CHA and M/W ratio in the upd(14)pat and RDS groups is shown in Fig. 5. In the four children with upd(14)pat, CHA remained unaltered regardless of age, ranging from 25° to 45°. In the RDS group ($n=5$), CHA was constant regardless of age, ranging from −6.4 to 10° (mean −0.6) at birth and from −8 to 7.3° thereafter (Fig. 5). The M/W ratio of the upd(14)pat group was smaller than that of the RDS group in the neonatal period. However, it increased gradually with age and finally caught up with that observed in the RDS group (Figs. 6, 7).

Discussion

The clinical manifestations of upd(14)pat have been well established to date. The hallmarks of this condition include a small thorax, laryngomalacia, hypoplastic abdominal wall, short limbs with joint contractures, craniofacial dysmorphism, and mental retardation [2]. In addition, several reports on the prenatal diagnosis of upd(14)pat suggested the common occurrence of polyhydramnios and preterm delivery in upd(14)pat [2, 7]. A few reports on upd (14)pat have detailed the radiological manifestations, such as disproportionately short limbs, spurring of lower femoral and upper tibial metaphyses, absent glenoid fossa, shortened iliac wing with flaring, thin and elongated clavicle, hypoplastic scapular neck, kyphoscoliosis, hypoplasia of the maxilla and mandible, a broad nasal bridge, wide sutures and multiple wormian skull bones, contractures of the wrists with ulnar deviation, and stippled calcification [3, 8–10]. However, these findings are so mild that alone they do not determine the diagnosis. Instead, the distinctive thoracic deformity in upd(14)pat, termed the coat-hanger sign as introduced by Offiah et al. [3], enables a definitive diagnosis to be made. Sutton et al. [8] described the thoracic deformity of upd(14)pat as "anterior ribs bowed caudally (downward), and posterior portions of the ribs bowed cranially (upward)," and these configurations are combined in the characteristic coat-hanger sign of the ribs

Table 1 Summary of clinical details, measurement of rib angle, coat-hanger angle (CHA), and ratio of mid- to widest thorax (M/W ratio). *GW* gestational week, *TD* thanatophoric dysplasia, *ATD* asphyxiating thoracic dysplasia, *EvC* Ellis-van Creveld syndrome, *RDS* respiratory distress syndrome

Case	Gender	Age (months)[a]	Molecular or clinical diagnosis	Right rib angle (°)	Left rib angle (°)	CHA (°)	M/W ratio (%)
upd(14)pat patients							
1	f	0	upd	36	31	33.5	80
2	m	0	upd	43	41	42	66
3	m	0	upd	27	46	36.5	80
4	m	7	upd	32	38	35	80
5	m	0	deletion	27	30	28.5	58
6	f	0	Epimutation	35	23	29	77
7	f	0	Epimutation	48	42	45	65
8	f (45,XX)	0	upd	30	34	32	69
9	f	0	upd	46	32	39	74
10	m	24	upd	28	38	33	87
11	f	32	decision	32	33	32.5	93
mean		5.7		35	35.82	35.1	75.4
TD group patients							
1	m	21GW	TD	−9.9	−13.7	−11.8	80
2	f	6	TD	−3.7	12	1	85.6
3	m	21GW	TD	−11.7	−13.9	−12.8	86
4	Unknown	20GW	TD	−19.6	−20	−19.8	86
5	m	0	TD	7	−12	−2.5	87
6	m	21GW	TD	−15	−21	−18	87
7	m	84	ATD	4	2	3	88
8	f	11	EvC	9.6	10.3	9.95	90
9	m	24	EvC	14	28	21	92
mean		11		−2.8	−3.1	−3.3	86.8
RDS patients							
1	m	0	RDS	1.8	4	2.9	90
2	m	0	RDS	1.2	−14	−6.4	81.7
3	m	0	RDS	−6.9	−4.1	−5.2	84
4	m	0	RDS	−6	−2	−4	91
5	f	0	RDS	11.3	8.7	10	85
mean		0		0.28	−1.48	−0.54	86.3

[a] Age at which time the initial radiograph was available

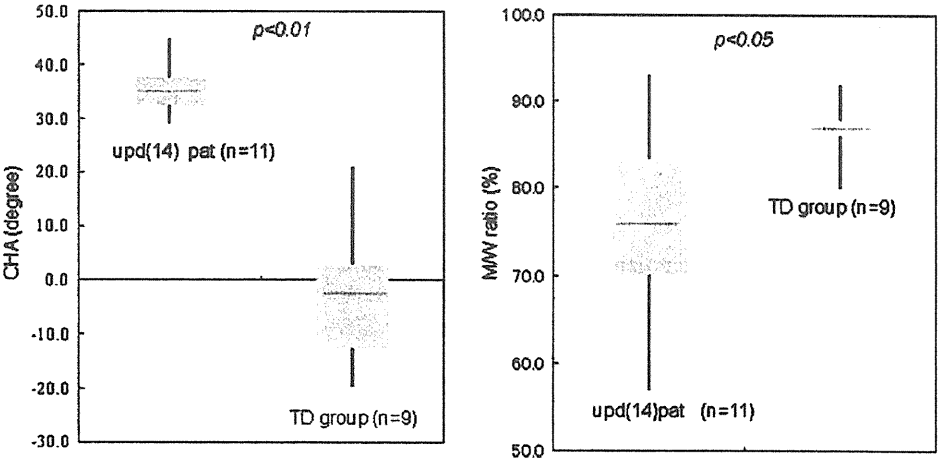

Fig. 3 Box plot of CHA and M/W ratio with the median, interquartile interval and range

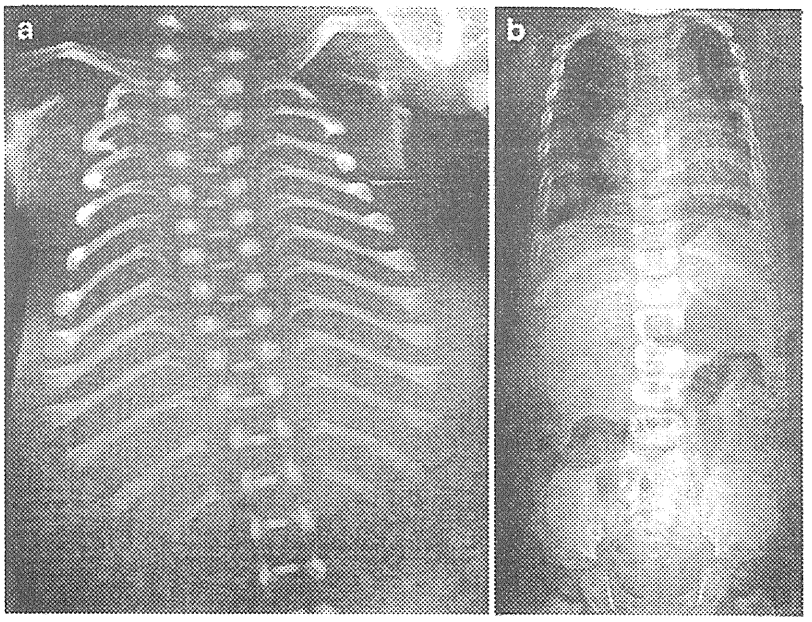

Fig. 4 Examples of the thoracic appearance and measurement of bone dysplasias with thoracic hypoplasia. **a** Thanatophoric dysplasia (TD) type 1 (stillbirth at 21 weeks of gestation). Note a narrow thorax with cupped anterior ends as well as short long bones with metaphyseal cupping. The posterior ribs show downward sloping. The CHA was −18°, and the M/W ratio was 87%. Despite the presence of severe thoracic hypoplasia in TD, its morphology is different from that seen in upd(14)pat (Fig. 2). **b** Ellis-van Creveld (EvC) syndrome (2 years of age). The thorax appears narrow, and a trident appearance of the acetabula is seen. Posterior ribs show upward sloping. The CHA was 21°, and the M/W ratio was 92%. The morphological pattern of the thorax differs from that of upd(14)pat

on the chest radiograph. Sutton et al. concluded that the skeletal phenotype in upd(14)pat involves primarily the axial skeleton, with little to no effect on the long bones. Very small changes of the long bones in upd(14)pat correspond with those of the mouse model (UPD of the distal segment of mouse chromosome 12) [11]. Consequently, it is assumed that imprinted genes on human chromosome 14 and mouse chromosome 12 play a role in axial skeletal formation and ossification [8, 11].

In the subsequent articles on upd(14)pat, all 11 affected children presented unexceptionally with the coat-hanger sign [5, 6, 12]. It was thought that the upward posterior rib bowing and downward anterior rib bowing (the coat-hanger appearance) in upd(14)pat contrast with the horizontally oriented ribs generally seen in disorders with thoracic hypoplasia. Based on the radiological sign, along with other radiological findings, it is not difficult to differentiate upd(14)pat from other genetic disorders involv-

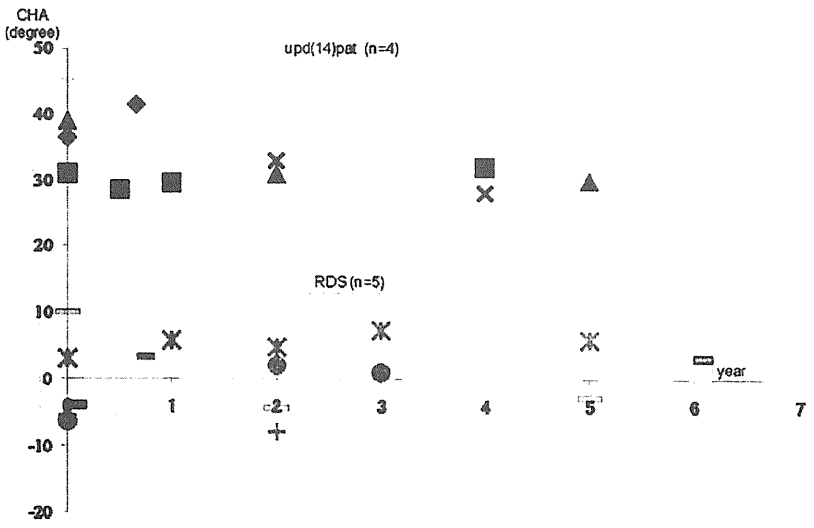

Fig. 5 Comparative observation of age-dependent transition of CHA between the upd(14)pat and respiratory distress syndrome (RDS) groups. Individual shapes represent individual patients

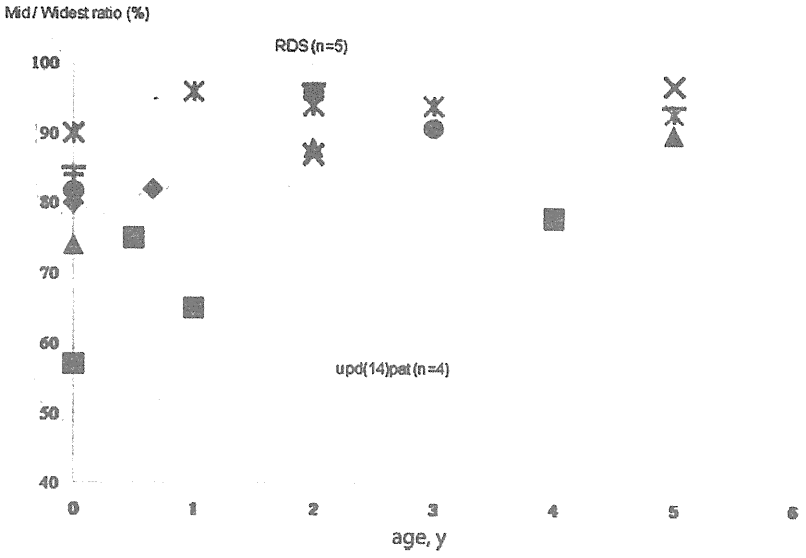

Fig. 6 Comparative observation of age-dependent transition of M/W ratio between the upd(14) pat and RDS groups. Individual shapes represent individual patients

ing thoracic hypoplasia, such as thanatophoric dysplasia, asphyxiating thoracic dysplasia and metatropic dysplasia [13]. However, there are several disorders wherein thoracic hypoplasia is the sole radiological hallmark, including Barnes syndrome, Shwachman-Diamond syndrome and the mildest cases of asphyxiating thoracic hypoplasia. Thus, we thought that quantitative analyses of the coat-hanger sign could elucidate how different the thoracic hypoplasia

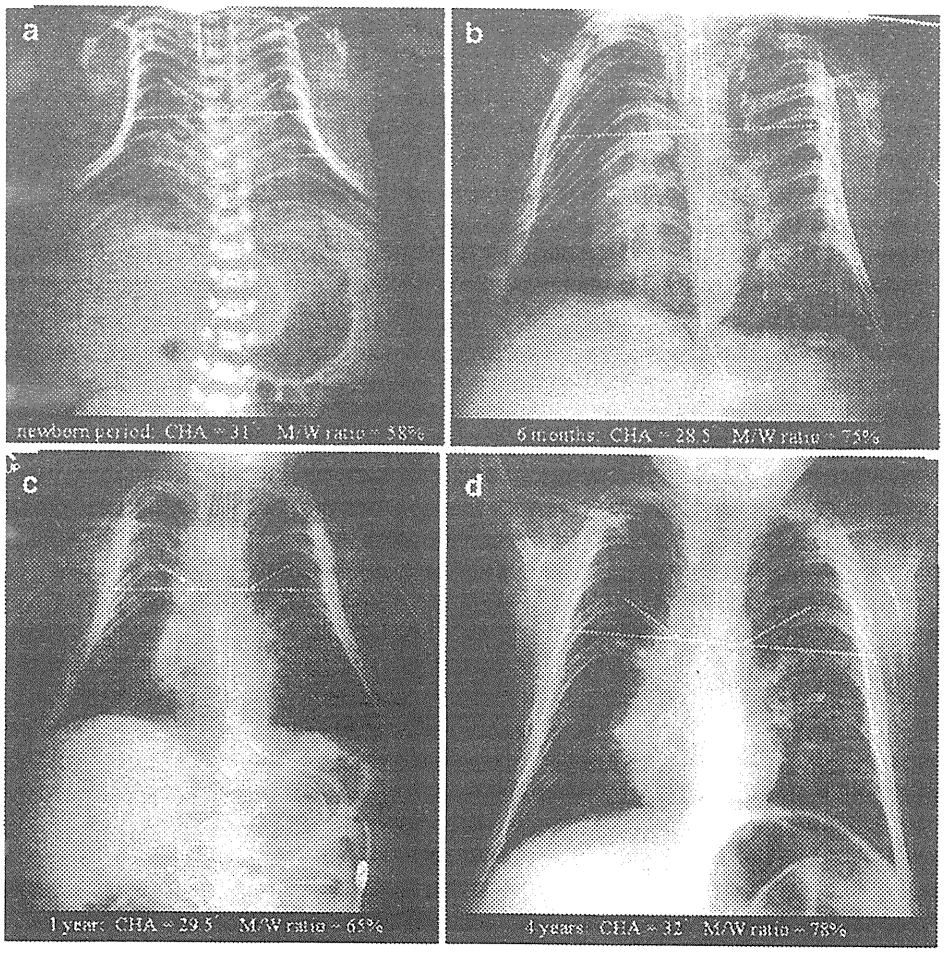

Fig. 7 Serial images of the thorax deformity in upd(14)pat. In this case, four images taken at different ages were available: (**a**) neonatal period, (**b**) 6 months, (**c**) 1 year and (**d**) 4 years. The CHA was almost consistent regardless of age, while the M/W ratio increased with advancing age. The coat-hanger sign and bell-shaped thorax are readily identifiable in the neonatal period. The diagnosis is not straightforward in childhood, yet close observation combined with CHA measurement points to the coat-hanger sign

in upd(14)pat is from the thoracic hypoplasia in other genetic disorders, and presumed that the measurement of CHA (mean 35.1°) and M/W ratio (mean 75.4%) might be helpful when the diagnosis of upd(14)pat is in question. As comparison groups, we included not only cases of severe bone dysplasias but also RDS. Neonates with RDS may present with a small chest [14], and it is not uncommon for them to undergo repeated examinations of chest radiographs because of the association with chronic lung disease.

Kagami et al. [5] reported the age-dependent evolution of the thoracic deformity of upd(14)pat in two children, which was said to ameliorate in mid-childhood. Their observation corresponded with the improvement of the M/W ratio with age described here. By contrast, however, CHA persisted consistently until mid-childhood. This finding indicates that the coat-hanger sign is still discernable during mid-childhood. Radiological findings are presumed to be the only clue to the presence of upd(14)pat after mid-childhood. Serial radiographs (newborn, 2 years and 9 years), as illustrated by Cotter et al. [15] also warrant our observation.

A drawback of this study is that it includes a limited number of cases and available radiographs with uneven quality, such as chest radiographs with some obliquity and radiographs taken in the supine position in the neonatal period vs. the upright position in childhood. Even taking into account these technical problems, however, we believe that our quantitative analyses, particularly the measurement of the CHA, are a valid way to characterize the distinctive thoracic deformity in upd(14)pat.

Conclusion

The coat-hanger sign of upd(14)pat was quantitatively represented by CHA, and was found to be more severe than that seen in other genetic bone diseases and to persist into early childhood; thus, the findings will help in the diagnosis of upd(14)pat even after infancy. By contrast, the bell-shaped thorax represented by M/W ratio was significant only in the neonatal period, and its diagnostic value declined with age.

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CASE REPORT

Growth hormone supplement treatment reduces the surgical risk for Prader–Willi Syndrome patients

Yutaka Nakamura · Toshiro Nagai · Takahiro Iida · Satoru Ozeki · Yutaka Nohara

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Abstract
Introduction Many complications have been reported to occur with surgery for scoliosis in Prader—Willi Syndrome (PWS). However, growth hormone (GH) treatment has contributed to improvements in height, body composition, bone density and breathing functions in PWS patients. The purpose of this study was to investigate patients who underwent surgery for scoliosis in PWS.
Materials There were 136 PWS patients being followed-up by the Pediatrics Department of our hospital. Among these, we investigated nine patients who had undergone surgery. Their mean age was 11 years. The mean follow-up period was 6 years 10 months.
Results The mean body mass index was 22.5 kg/m^2. GH therapy was administered to eight patients. Brace treatment was performed in two patients. Spinal correction and fusion were performed in six patients, and the growing rod method was performed in three patients. Necessary re-operations were performed in two patients. For the total 11 surgeries in the nine patients, the mean blood loss was 397 ml and the mean operation time was 4 h and 20 min. The mean Cobb angles were 76.0 degrees preoperatively and 35.8 degrees at follow-up. Regarding complications, one patient experienced early dislodgment of the hook and one patient experienced a superior wound infection.
Conclusion There were no severe complications such as deep infections or neurovascular damage. A few obese patients underwent surgery, but there were no dangerous complications. Overall, we consider that GH treatment before surgery may reduce postoperative complications. The growing rod method was effective for PWS patients who resisted brace treatment owing to mental retardation.

Keywords Prader–Willi syndrome · Scoliosis · Surgery · Growth hormone · Growing rod

Y. Nakamura (✉) · T. Iida · S. Ozeki
Department of Orthopedic Surgery, Dokkyo Medical University Koshigaya Hospital, 2-1-50 Minami-Koshigaya, Koshigaya, Saitama 343-8555, Japan
e-mail: nakayuta@dokkyomed.ac.jp

T. Nagai
Department of Pediatrics, Dokkyo Medical University Koshigaya Hospital, 2-1-50 Minami-Koshigaya, Koshigaya, Saitama 343-8555, Japan

Y. Nohara
Department of Orthopedic Surgery, Dokkyo Medical University Hospital, 880 Kitakobayashi, Mibu-machi, Shimotsuga-gun, Tochigi 321-0293, Japan

Background

Prader–Willi Syndrome (PWS) is caused by abnormalities of chromosome 15 and is characterized by weakness of muscle tension, imperfect function of the hypothalamus and pituitary gland, hypogonadism, overeating and obesity. Its orthopedic characteristics include scoliosis, hip dysplasia and lower limb alignment abnormalities. In addition, bone fractures caused by osteoporosis are problematic. Although the prevalence of scoliosis in PWS is 15–86% [1], the prevalence of PWS itself is rare, with only one person per 10,000–20,000 people affected.

Many complications have been reported to occur in surgery for severe scoliosis in PWS [2]. The problems associated with surgery for the treatment of scoliosis in PWS are severe obesity, short height, osteoporosis, sleep apnea, breathing restriction by an obstacle, mental retardation, character action abnormalities and diabetes.

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Growth hormone (GH) supplement treatment has recently become commonly used worldwide. GH treatment contributes to improvements in height, body composition, bone density and breathing functions in PWS [3]. Recently, we consider that there has been a decrease in severely obese patients with PWS.

In this study, we investigated patients who had undergone surgery for scoliosis (Cobb angle of >45 degrees) in PWS, in comparison with conventional surgical reports.

Materials and methods

There were 136 PWS patients being followed-up from November 2002 to August 2009 by the Department of Pediatrics at Dokkyo Medical University Koshigaya Hospital. All the patients were diagnosed using fluorescence in situ hybridization or methylation tests. Scoliosis was identified in 39 patients (31%), of whom 13 patients had Cobb angles of >45 degrees. We investigated nine of these patients (four males and five females) who had undergone surgery. Their mean age was 11 years (range 4–20 years). Six patients had an inherited deletion of chromosome 15q11–13 and three patients did not have a deletion. We focused on the following two aspects: (1) the body mass using the body mass index (BMI), presence of mental retardation, presence and periods of GH supplement treatment, presence of brace treatment and type of scoliosis curve; and (2) the surgical methods, blood loss, operation time, presence of autologous blood transfusion, presence of allogeneic blood transfusion, complications and changes in the Cobb angle and kyphosis angle (before surgery, after surgery and at final follow-up).

Results

The mean follow-up period was 6 years 10 months (range 9–153 months). Regarding the clinical characteristics, the average BMI was 22.5 kg/m^2, and the individual BMIs reflected 4 thin patients (BMI: <18.5 kg/m^2), 2 average-sized patients (BMI: 18.5–25.0 kg/m^2), 1 slightly obese patient (BMI: 25.0–30.0 kg/m^2) and 1 severely obese patient (BMI: >30.0 kg/m^2). Mental retardation was found in all patients. GH therapy was administered in eight of the nine patients, and the mean preoperative period of GH therapy was 5.0 years. Brace treatment was only performed in two patients. The types of scoliosis curves were classified by the methods of Lenke et al. [4] and are shown in Table 1.

Regarding the surgical characteristics, spinal correction and fusion were performed in six patients (anterior method in three patients; posterior method in three patients) at the initial surgery, and the growing rod method was performed in three patients. Necessary additional operations were performed in two patients for additional correction and fusion surgery owing to progressive scoliosis at the upper end of the fused level. In the total 11 surgeries performed in the 9 patients, the mean blood loss was 397 ml (range 150–900 ml) and the mean operation time was 4 h 20 min. Allogeneic blood transfusion was performed in one patient. Autologous blood transfusion was performed in five surgeries for four patients. Regarding complications, one patient experienced early dislodgment of the hook after the first surgery and one patient experienced a superior wound infection. However, there were no dangerous complications such as deep infections or neurovascular damage (Table 2). The mean Cobb angles were 76.0 degrees (range 45–85 degrees) before surgery, 34.6 degrees (range 13–55 degrees) after surgery and 35.8 degrees (range 15–55 degrees) at follow-up (Table 3).

Case 1

Case 1 was a 5-year-old girl. She was diagnosed with a hereditary form of PWS with deletion of chromosome 15q11–13 at 1 year of age. GH supplement treatment (1.8 mg/week) and a diet therapy were started when she was 2 years 6 months. Scoliosis was observed in this patient at 3 years of age, and she was subsequently introduced to our Orthopedics Department. The Cobb angle was 29 degrees at T10–L4 and the sagittal alignment was 19 degrees at T10–L4 at the first consultation. However, the scoliosis deteriorated to 80 degrees at T10–L4 and the sagittal alignment was 32 degrees of kyphosis at T1–T12 and 33 degrees of lordosis at L1–S1 at 5 years of age. We tried to use an under-arm brace treatment during the observation period, but she hated the brace because of her mental retardation and was unable to put it on. At the time of surgery, her height was 96.0 cm, her weight was 14.8 kg and her BMI was 16.1 kg/m^2. There were no abnormal data in blood analyses and no neurological deficits. There were also no abnormal findings by CT or by MRI including anomalies and deformities of the spinal cord and vertebrae. The GH supplement treatment had been performed for 3 years 3 months before surgery. Anterior correction and fusion were performed with the Mykres system of spinal instrumentation. After the surgery, the scoliosis was corrected to a Cobb angle of 22 degrees and the sagittal alignment was 16 degrees of lordosis at T10–L4. The quantity of bleeding was 229 ml and the operation time was 3 h 54 min. At 4 years after surgery, the Cobb angle was 35 degrees at T10–L4, with 23 degrees of kyphosis at T1–T12 and 34 degrees of kyphosis at L1–S1 (Fig. 1).

Table 1 Preoperative data

Age (years old)/gender		Presence of GH treatment (periods: years and months)	Type of scoliosis	BMI (kg/m^2)	Brace treatment	Follow-up period (years and months)
At surgery	At onset of scoliosis					
5/F	3	3 years 3 months	6C	16.1	Impossible	75
15/M	4	5 years 2 months	3C	17.0	Impossible	67
17/M	16	4 years 8 months	2C	17.0	Possible	113
11/F	4	5 years 2 months	3C	24.5	Impossible	21
15/M	14	8 years 11 months	5C	20.3	Impossible	60
8/F	1	–	2C	40.9	Impossible	153
11/M	7	5 years 6 months	3B	15.2	Impossible	77
17/M	15	8 years 10 months	5C	29.2	Impossible	120
5/F	1	2 years 3 months	1C	15.7	Possible	12

The types of scoliosis were classified by the Lenke classification [4]

GH growth hormone; *BMI* body mass index

Table 2 Surgical data

Age/ gender at surgery	Surgical methods	Instrumentation	Surgical level	Blood loss	Operation time (hours, minutes)	Autologous blood transfusion (ml)	Allogeneic blood transfusion (ml)	Complications and additional surgery
5/F	ACF	Mykres	T11–L3	229	3′54″	Impossible	None	None
15/M	1st: PCF	Mykres	T3–L3	860	5′11″	600	800	L3 screw pulled out
16/M	2nd: PCF	Mykres	T3–L4	210	3′30″	None	None	None
17/M	PCF	Mykres	T3–L3	900	4′40″	600	None	Superior infection
11/F	Growing rod	Mykres	T2–L3	250	3′00″	Impossible	None	Early hook dislodgment
15/M	ACF	Mykres	T12–L2	260	4′10″	Impossible	None	None
8/F	1st: ACF	Zeilke	T10–L2	300	4′32	600	None	Progress, upper fused level
19/F	2nd: PCF	Legacy	T1–L1	650	5′19	1,030	None	None
11/M	Growing rod	Mykres	T12–L1	120	2′20″	Impossible	None	None
17/M	PCF	Mykres	T3–L3	440	7′20″	1,200	None	None
5/F	Growing rod	Mykres	T2–L2	150	3′43″	Impossible	None	None

ACF anterior correction fusion, *PCF* posterior correction fusion, *1st* first surgery, *2nd* second surgery

Cases with distraction surgery using the growing rod method are excluded

Case 2

Case 2 was a 17-year-old boy. He was diagnosed with a hereditary form of PWS with uniparental disomy in the Pediatrics Department of our hospital at 3 years of age. GH supplement treatment (4.6 mg/week) and a diet therapy were started when he was 8 years 6 months. Scoliosis was observed in this patient at 15 years of age, and he was subsequently introduced to our Orthopedics Department. The Cobb angle was 35 degrees at T9–L4 at the first consultation. However, the scoliosis deteriorated to a double curve with Cobb angles of 45 degrees at T4–T9 and 52 degrees at T9–L3 and the sagittal alignment was 32 degrees at T1–T12 and 31 degrees at L1–S1 at 17 years of age. We tried to use an under-arm brace treatment during the observation period, but he hated the brace owing to his mental retardation and was unable to put it on. At the time of surgery, his height was 157.9 cm, his weight was 72.8 kg and his BMI was 29.2 kg/m^2. There were no abnormal data in blood analyses and no neurological deficits. There were also no abnormal findings by CT or by MRI including anomalies and deformities of the spinal cord and vertebrae. The GH supplement treatment had been performed for 8 years 10 months before surgery. Posterior correction was performed with the Mykres system of spinal instrumentation at T3–L3. After the surgery, the scoliosis was corrected to Cobb angles of 13 degrees at T4–T9 and 13 degrees at T9–L3, and the sagittal alignment was 35 degrees of kyphosis at T1–T12 and 9 degrees of lordosis at L1–S1. The quantity of bleeding was 440 ml and the

Table 3 Radiographic data

Age/gender at surgery	Level	Major curve (Cobb angle)			T1–T12 (Kyphosis)		L1–S1 (lordosis)	
		Presurgery	Postsurgery	Final F/U	Preoperative	Final F/U	Preoperative	Final F/U
5/F	T10–L4	80	22	35	32	23	33	34
15/M	T3–12	69	35	40	4.7	35	20	27
17/M	T8–L3	47	27	27	20	43	34	34
11/F	T10–L4	72	44	57	18	46	31	40
15/M	T6–T10	46	36	40	28	38	26	30
	T10–L3	59	39	39				
8/F	T2–T7	71	45	45	34	53	26	49
	T7–L2	71	55	55				
11/F	T4–L1	53	27	27	31	30	33	32
17/M	T4–T9	45	13	16	32	35	31	9
	T9–L3	52	13	15				
5/F	T6–L1	65	40	26	55	46	52	46

The values are in degrees

F/U follow-up

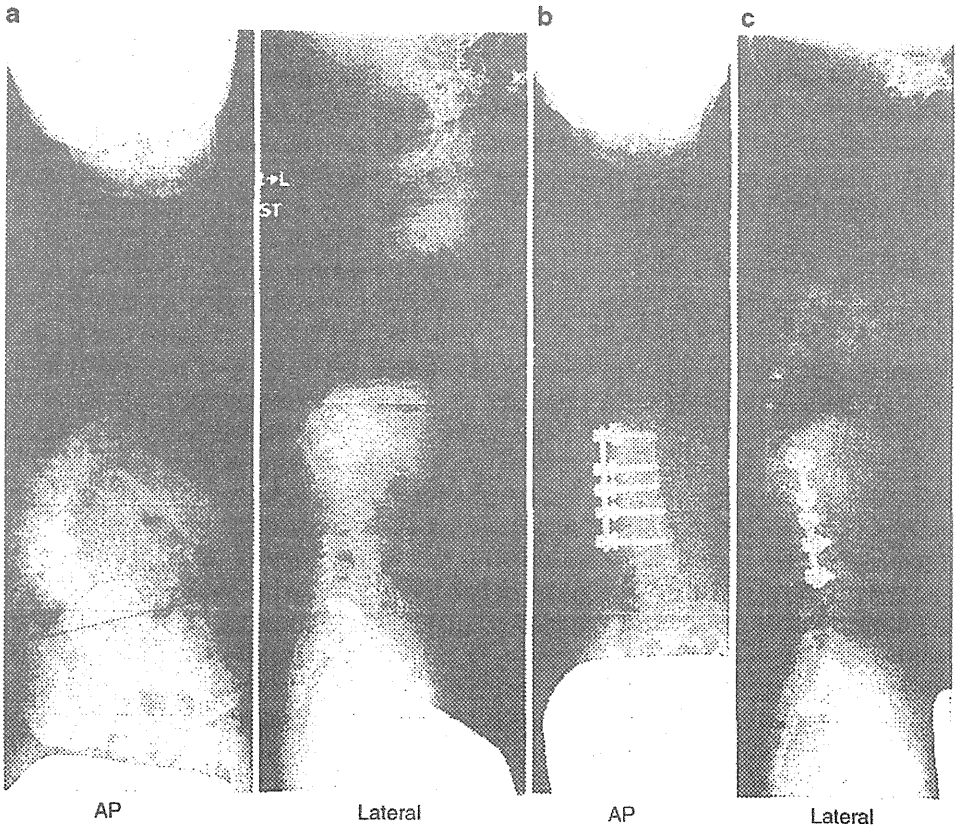

Fig. 1 Case with anterior correction and fusion. **a** Before surgery. **b** After surgery. **c** Final follow-up

operation time was 7 h 20 min. At 1 year after the surgery, the Cobb angles were 16 degrees at T4–T9 and 15 degrees at T9–L3. These results were good without any correction loss (Fig. 2).

Case 3

Case 3 was an 11-year-old boy. He was diagnosed with a hereditary form of PWS with deletion of chromosome

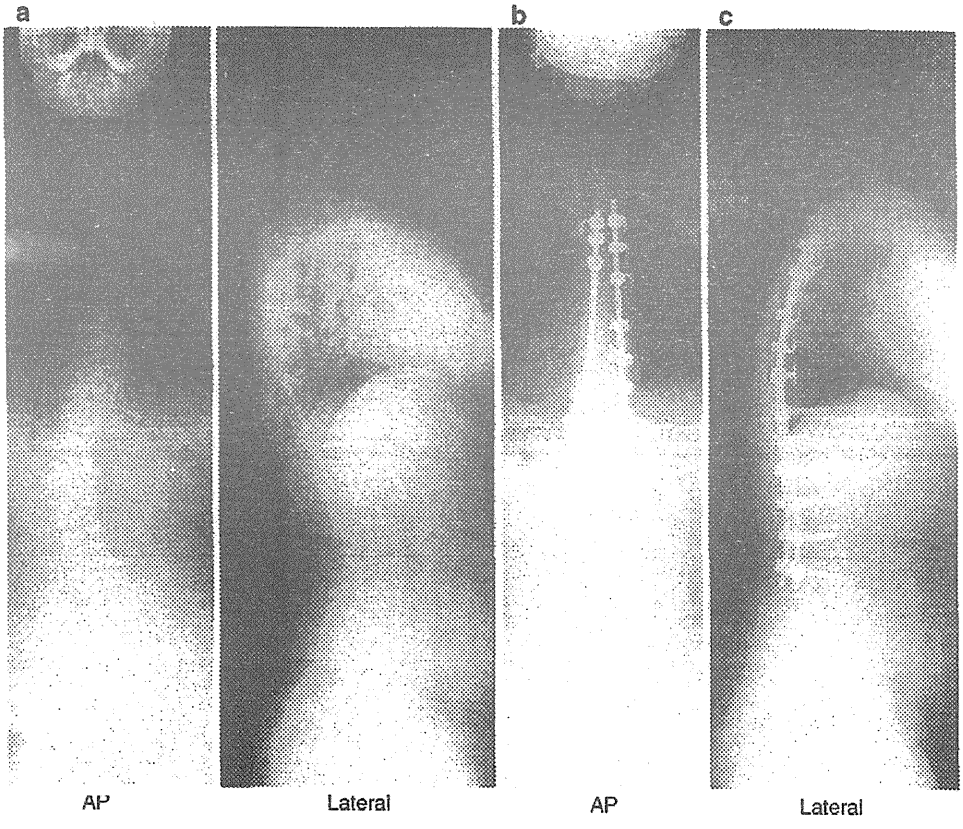

Fig. 2 Case with posterior correction. a Before surgery. b After surgery. c Final follow-up

15q11–13 soon after birth. GH supplement treatment (4 mg/week) and a diet therapy were started when he was 5 years 11 months. Scoliosis was observed in this patient at 7 years of age, and he was subsequently introduced to our Orthopedics Department. The Cobb angle was 37 degrees at T6–L1 at the first consultation. However, the scoliosis deteriorated to a Cobb angle of 55 degrees at T2–L3 and the sagittal alignment was 31 degrees of kyphosis at T1–T12 and 33 degrees of lordosis at L1–S1 at 11 years of age. We tried to use an under-arm brace treatment during the observation period, but he hated the brace owing to his mental retardation and was unable to put it on. At the time of surgery, his height was 148 cm, his weight was 72 kg, his BMI was 15.2 kg/m^2 and he had leptosomatic habitus. There were no abnormal data in blood analyses and no neurological deficits. There were also no abnormal findings by CT or by MRI including anomalies and deformities of the spinal cord and vertebrae. The GH supplement treatment had been performed for 5 years 6 months before surgery. In consideration of his bone fragility, we performed the growing rod method, which can maintain growth, and it was planned with two stages of surgery to produce a stronger anchor. In the first surgery, a hook was anchored in the upper edge region and a screw was attached at the lower edge, and autologous iliac bone was only transplanted on the edges. After 6 months, a strong anchor was confirmed with bone fusion. Next, posterior correction was performed with the Mykres system of spinal instrumentation at T2–L1. A straight tandem connector was used at the thoracolumbar area, and the hook and pedicle screw with the strong anchor through bone fusion were connected with a rod. After this surgery, the scoliosis was corrected to a Cobb angle of 27 degrees and the sagittal alignment was 30 degrees of kyphosis at T1–T12. The quantity of bleeding was 120 ml and the operation time was 2 h 20 min. A doctor from the Department of Pediatrics provided mental support to the patient both preoperatively and postoperatively. Presently, rod extension of 1.5 cm is still being continued every 6 months (Fig. 3).

Discussion

Holm and Laurnen [5] reported that the spinal curvatures in 15–20% of the PWS patients eventually require surgical management. In our study, the frequency of patients who needed surgery was 9.6% (13/136). Accadbled et al. [2] described 16 patients who underwent surgery from 1997 to 2004 for scoliosis in PWS. Regarding complications, there were nine major complications, including four cases of severe kyphosis above the fusion, two deep infections and one transient paraplegia. The four kyphosis cases required

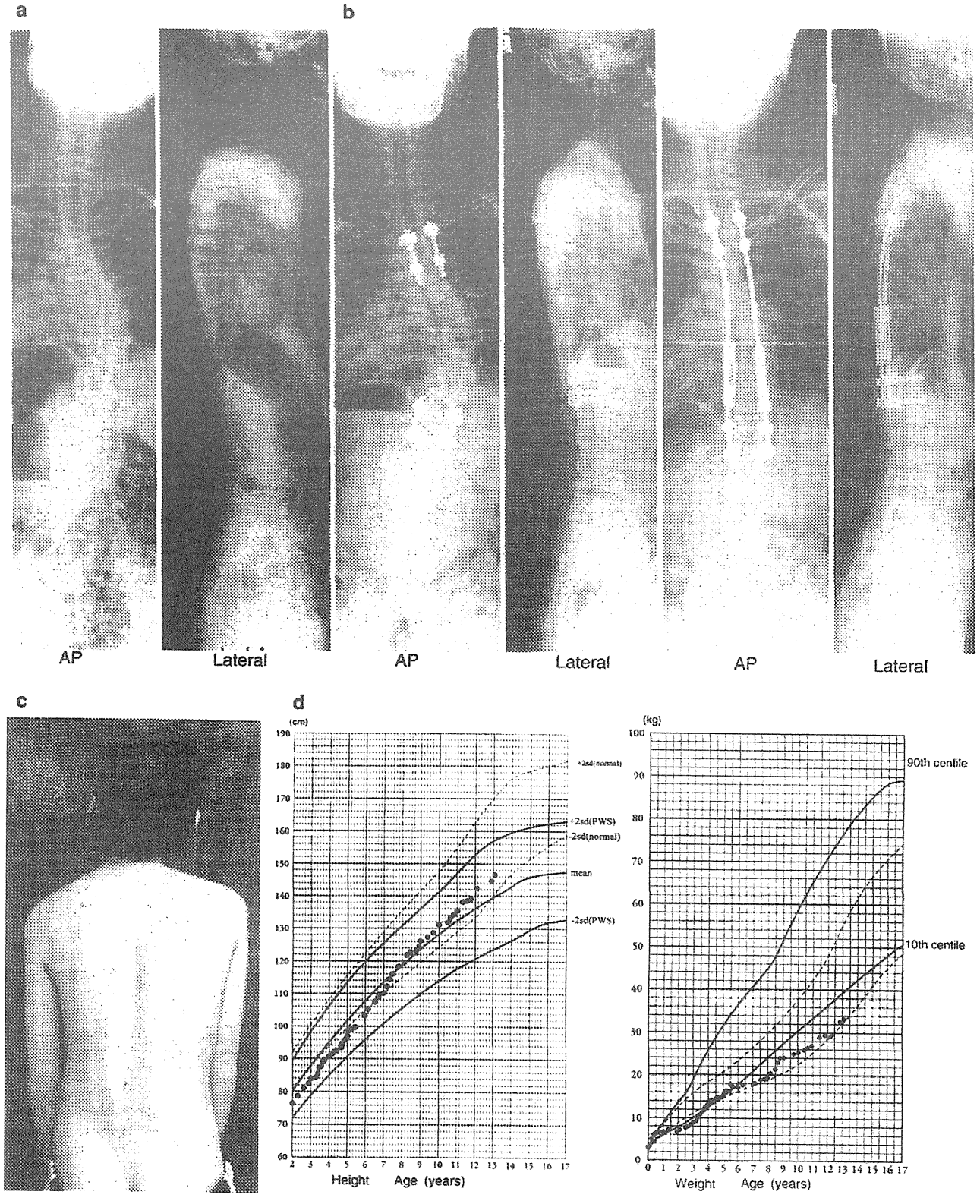

Fig. 3 Case with the growing rod procedure. **a** Preoperative photograph and X-ray. **b** After surgery. **c** Final follow-up. **d** Height in PWS male with respect to age. Weight in PWS male with respect to age

reoperations, three of which were for complications involving permanent spinal cord injury. In that study, GH supplement treatment was administered to 7 of the 16 patients, but most of the patients were obese. Furthermore, the surgeries involved the use of Harrington or Luque instrumentations, which are no longer used.

Weiss and Goodall [6] performed a systematic review in 2009, and concluded that the rate of complications after spinal fusion in patients with scoliosis in PWS was very high and that their death rates were higher than those of patients with adolescent idiopathic scoliosis. Furthermore, the long-term side-effects of the interventions were detrimental, such that the risk–benefit ratios favored conservative approaches over spinal fusion surgery.

As a cause of suffering after surgery for scoliosis in PWS, Rees et al. [1] reported that postoperative respiratory complications can become serious owing to restrictive respiratory disorders caused by the severe obesity peculiar to PWS, deterioration of the quantity of ventilation by the small height and imperfect function of the respiratory muscles with myopathy. Furthermore, they pointed out that preoperative breathing function training is difficult in PWS patients because of their mental retardation. They further stated that a bleeding tendency is brought on by weakness of the blood vessel walls owing to connective tissue abnormalities. In addition, we were concerned about the risk of surgery because there is higher risk of infection associated with the high frequency of diabetes in PWS.

Nohara et al. [7] reported a case of a 14-year-old patient who was severely obese (BMI: 34.4 kg/m^2) and had obstructive pulmonary disease and sleep apnea. After correction surgery, breathing control by a ventilator was required owing to atelectasis for 13 days. In addition, Kakutani et al. [8] described a case of a 17-year-old boy who was severely obese (BMI: 37.5 kg/m^2) and had a Cobb angle of 75 degrees at T10–L3 with a very rigid spinal deformity. In that report, the authors had hoped to operate by anterior release, posterior correction and fusion. However, anterior release was impossible because of a restrictive pulmonary disorder caused by the severe obesity. The authors described that the correction could not be achieved at a satisfactory level, because only posterior correction was able to be performed. However, Tokutomi et al. [9] reported a case of cardiopulmonary impairment caused by severe kyphoscoliosis in PWS. A breathing function imperfection can decrease the quality of life of PWS patients with severe kyphoscoliosis. We need to make a greater effort to improve the surgical outcomes.

On the other hand, Gurd and Thompson [10] described an operation for scoliosis in PWS in a 7-year-old girl. They reported that the girl underwent surgery although her laminas were too weak to hold a Harrington rod. In a recent report from Japan, Yamada et al. [11] reported that a patient with Cobb angles of 43 degrees at T1–T5, 60 degrees at T5–T11 and 52 degrees at T11–L4 was successfully treated surgically using modern instrumentation (XIS-SS system) via a posterior approach. Nohara et al. [7] reported good results in a 12-year-old girl with PWS who had a BMI of 21.4 kg/m^2 and a Cobb angle of the main curve of 106 degrees at T6–L1. A Smith-Petersen osteotomy at T2–L3, anterior release at T7–T12 and correction and fusion at T6–T10 were performed in this patient. After the surgery, the Cobb angle was corrected to 20 degrees and the correction rate was 82%. In addition, Kakutani et al. [8] reported that T12–L3 anterior correction fusion was performed in an 11-year-old girl. Her BMI was 23 kg/m^2 and the quantity of bleeding was 477 ml. Her Cobb angle was corrected to 23 degrees postoperatively from 69 degrees preoperatively without any complications.

There are reports about difficulties associated with management connected with the severe obesity that is peculiar to PWS, as well as difficulties associated with breathing management and mental retardation. Therefore, surgery remains controversial in PWS based on previous reports, considering that there are high rates of complications in patients with PWS undergoing surgery and the health-related benefits of such surgery in these patients are unclear. It is difficult to make clear decisions regarding the optimal approaches for surgical treatment or conservative treatment for PWS patients. Indications for surgery are necessary to determine individual cases.

Recently, the treatment of PWS has improved remarkably in the field of pediatrics. GH supplement treatment was started in Japan in January 2001, and many reports have described its utility and positive effects such as height acceleration, body mass reduction and muscular strength improvement worldwide. However, there are some concerns regarding the risks involved in GH treatment of PWS patients. First, there was a report of sudden death owing to the use of GH treatment [12]. Although the relationship between the sudden death and GH treatment was completely unclear, Nagai et al. [13] subsequently reported that the causes of sudden unexpected death did not differ between PWS patients with or without GH treatment. GH treatment has now become contraindicated for patients who are seriously obese or have a high breathing obstacle. Therefore, careful use of GH treatment is necessary in PWS patients.

Second, the possibility of deterioration in scoliosis was suggested, although we have particularly reported that there was no connection between the frequencies of scoliosis in GH treatment and non-treatment groups [14, 15]. Based on the backgrounds of these studies, GH treatment has recently been recommended at an early age, and it is assumed that its long-term use is not a problem. It is important that PWS patients are treated intensively from 3 years of age, because obesity begins at around this time.

GH supplement treatment is effective for height acquisition, body composition improvement, natural activity and muscular strength improvement, but the greatest purpose of GH use is body composition improvement. All of our patients except one were treated with long-term GH supplement treatment in the Department of Pediatrics from childhood.

Recently, a sex hormone supplement treatment has also been shown to be effective for body composition, mental and bone density improvements. Osteoporosis is caused by imperfect sex hormone secretion in PWS, and this symptom was reported to lead to a risk of more than 29% for a bone fracture once in the lifetime of PWS patients [16]. Preoperatively, improvement in the bone density is important.

Regarding complications, only early dislodgment of the hook and a superior infection were encountered. Fortunately, there were no dangerous complications such as neurological deficits, vascular damage or deep infections. However, an additional operation was performed in two patients. One patient was an 8-year-old girl whose surgery only involved anterior fusion around the apex of the scoliosis to restrain its progression. After 11 years, the kyphoscoliosis had deteriorated at the upper fused level. Accadbled et al. [2] also reported that 4 of 16 patients had progression of serious kyphosis at the upper fused level during their follow-up periods. In our case, the growing rod method was indicated at the present time. In the other case, a screw had been pulled out at the lowest level of the correction level. This patient underwent surgery using sublaminar wiring by a polyethylene tape. However, it was considered that the influence of osteopenia and uncontrolled behavior associated with the mental retardation contributed to the loose screw.

Fortunately, the operative outcomes in our patients were better than in those of some other reports [5, 6]. Some reasons are: first, the Pediatrics Department provided intense preoperative therapy with GH supplement treatment, sex hormone supplement treatment, diet and so on for a long term owing to the completely uncontrolled general conditions in all patients. As a result, the patients had a reduced BMI and improved bone density before surgery. Although breathing function evaluation was difficult because all PWS patients have mental retardation, our patients can be expected to show improvements in their breathing functions. In our patients, we operated using anterior correction fusion. If patients are not obese and do not have breathing function imperfections, surgeons will be able to perform anterior surgery. Therefore, the GH supplement treatment administered was a very effective therapy. In other words, the GH supplement treatment reduced the surgical risk in PWS patients. Consequently, spinal surgeons are able to choose greater ranges of operative methods for successful correction fusion for difficult cases.

Second, in our institution, the spinal surgeons were supported in the preoperative and postoperative therapies by pediatricians who had abundant experience of PWS patients. In addition, there was cooperation by the families of the patients who were well educated about the characteristics of PWS by their pediatricians. Sometimes PWS patients exhibited uncontrolled behavior for orthopedics preoperatively or postoperatively owing to their mental retardation. It is considered that these supports were necessary for smooth treatment. Therefore, the medical team care of orthopedic surgeons and pediatricians was indispensable for the treatment of this disease.

Recently, the dual growing rod technique [17] has been developed. Since all PWS patients have mental retardation, many of them hate brace treatment. In our patients, the brace-wearing rate was low (2/9). When these patients are infants and young children, corrective surgery is required. The dual growing rod technique was found to be an effective method. However, this method requires a lot of distraction surgery to maintain steady growth.

With the development of spinal instrumentation for pediatric surgery, instrumentation for the growing rod method was developed using the strong claw technique, thereby enabling a low profile with an increase in the correction power. In addition, a tandem connector for infants was developed, meaning that the distraction surgery became less invasive. Subsequently, we devised a better technique for the growing rod method of spinal correction, so that we could obtain a strong anchor production for bone fragility in infants or young children. The resulting spinal corrective surgery was separated into two operations. First, anchor production alone was performed with a hook at the upper end, a pedicle screw at the lower end and a bone graft from the autologous iliac bone. After the anchors became strong with complete bone fusion, correction fusion surgery was performed in a second operation.

It is considered that the device for anchor formation that leads to stronger bone fusion was effective for avoiding early dislodgment of the hooks in patients who had bone fragility as infants or young children.

Conclusions

In this study, the frequency of patients with PWS who required surgery in this study was 9.6% (13/136). GH therapy was administered in eight of the nine patients examined who underwent surgery. The mean BMI was 22.5 kg/m^2, and a few obese patients underwent surgery. Regarding complications, one patient experienced early dislodgment of the hook and one patient experienced a superior wound infection. There were no severe complications such as deep infections or neurovascular damage.

Therefore, we consider that GH treatment before surgery may reduce postoperative complications. Brace treatment was only performed in two of the nine patients. The growing rod method was effective for PWS patients who resisted brace treatment owing to mental retardation.

Conflict of interest None of the authors has any potential conflict of interest.

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ORIGINAL INVESTIGATION

Spread of X-chromosome inactivation into chromosome 15 is associated with Prader–Willi syndrome phenotype in a boy with a t(X;15)(p21.1;q11.2) translocation

Satoru Sakazume · Hirofumi Ohashi · Yuki Sasaki · Naoki Harada · Katsumi Nakanishi · Hidenori Sato · Mitsuru Emi · Kazushi Endoh · Ryoichi Sohma · Yasuhiro Kido · Toshiro Nagai · Takeo Kubota

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Abstract X-chromosome inactivation (XCI) is an essential mechanism in females that compensates for the genome imbalance between females and males. It is known that XCI can spread into an autosome of patients with X;autosome translocations. The subject was a 5-year-old boy with Prader–Willi syndrome (PWS)-like features including hypotonia, hypo-genitalism, hypo-pigmentation, and developmental delay. G-banding, fluorescent in situ hybridization, BrdU-incorporated replication, human androgen receptor gene locus assay, SNP microarrays, ChIP-on-chip assay, bisulfite sequencing, and real-time RT-PCR were performed. Cytogenetic analyses revealed that the karyotype was 46,XY,der(X)t(X;15)(p21.1;q11.2),−15. In the derivative chromosome, the X and half of the chromosome 15 segments showed late replication. The X segment was maternal, and the chromosome 15 region was paternal, indicating its post-zygotic origin. The two chromosome 15s had a biparental origin. The DNA methylation level was relatively high in the region proximal from the breakpoint, and the level decreased toward the middle of the chromosome 15 region; however, scattered areas of hypermethylation were found in the distal region. The promoter regions of the imprinted *SNRPN* and the non-imprinted *OCA2* genes were completely and half methylated, respectively. However, no methylation was found in the adjacent imprinted gene *UBE3A*, which contained a lower density of LINE1 repeats. Our findings suggest that XCI spread into the paternal chromosome 15 led to the aberrant hypermethylation of *SNRPN* and *OCA2* and their decreased expression, which contributes to the PWS-like features and hypo-pigmentation of the patient. To our knowledge, this is the first chromosome-wide methylation study in which the DNA methylation level is demonstrated in an autosome subject to XCI.

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S. Sakazume (✉) · R. Sohma · Y. Kido · T. Nagai
Division of Pediatrics, Dokkyo University Koshigaya Hospital, 2-1-50 Minami Koshigaya, Koshigaya, Saitama 343-8555, Japan
e-mail: saka343@dokkyomed.ac.jp

H. Ohashi
Division of Genetics, Saitama Children's Medical Center, Hasuda, Japan

Y. Sasaki · N. Harada
Department of Molecular Genetic Research and Analysis, Advanced Medical Science Research Center, Mitsubishi Chemical Medience Corporation, Tokyo, Japan

K. Nakanishi · H. Sato · M. Emi
DNA Chip Research Inc, Yokohama, Japan

H. Sato · M. Emi
Department of Neurology, Hematology, Metabolism, Endocrinology and Diabetology, Yamagata University School of Medicine, Yamagata, Japan

K. Endoh · T. Kubota
Department of Epigenetic Medicine, Faculty of Medicine, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Chuo, Japan

Introduction

X-chromosome inactivation (XCI) is a genetic mechanism in females in which one of the two X chromosomes are stochastically inactivated in the early stages of embryonic development. In each cell, the inactivated X-chromosome is randomly chosen; therefore, paternal and maternal X chromosomes have a 50% probability of being inactivated, and females are functional mosaic of two cell populations (Lyon 1961, 1962).