CLINICAL REPORT

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West Syndrome Associated With Mosaic Duplication of *FOXG1* in a Patient With Maternal Uniparental Disomy of Chromosome 14

Jun Tohyama,[1,2]* Toshiyuki Yamamoto,[3] Kana Hosoki,[4] Keisuke Nagasaki,[2] Noriyuki Akasaka,[1] Tsukasa Ohashi,[1] Yu Kobayashi,[1] and Shinji Saitoh[4]

[1]Department of Pediatrics, Epilepsy Center, Nishi-Niigata Chuo National Hospital, Niigata, Japan

[2]Department of Pediatrics, Niigata University Medical and Dental Hospital, Niigata, Japan

[3]Tokyo Women's Medical University Institute for Integrated Medical Sciences, Tokyo, Japan

[4]Department of Pediatrics, Hokkaido University Graduate School of Medicine, Sapporo, Japan

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FOXG1 on chromosome 14 has recently been suggested as a dosage-sensitive gene. Duplication of this gene could cause severe epilepsy and developmental delay, including infantile spasms. Here, we report on a female patient diagnosed with maternal uniparental disomy of chromosome 14 and West syndrome who carried a small supernumerary marker chromosome. A chromosomal analysis revealed mosaicism of 47,XX, + mar[8]/46,XX[18]. Spectral karyotyping multicolor fluorescence in situ hybridization analysis confirmed that the marker chromosome was derived from chromosome 14. A DNA methylation test at *MEG3* in 14q32.2 and microsatellite analysis using polymorphic markers on chromosome 14 confirmed that the patient had maternal uniparental disomy 14 as well as a mosaic small marker chromosome of paternal origin containing the proximal long arm of chromosome 14. Microarray-based comparative genomic hybridization analysis conclusively defined the region of the gain of genomic copy numbers at 14q11.2-q12, encompassing *FOXG1*. The results of the analyses of our patient provide further evidence that not only duplication but also a small increase in the dosage of *FOXG1* could cause infantile spasms.

Key words: West syndrome; maternal uniparental disomy; chromosome 14; supernumerary marker chromosome; *FOXG1*; mosaic duplication

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INTRODUCTION

Mutations in *FOXG1* on chromosome 14 are associated with the congenital variant of Rett syndrome [Shoichet et al., 2005; Jacob et al., 2009]. Recently, *FOXG1* was described as a dosage-sensitive gene. The duplication of this gene could cause severe epilepsy and developmental delay, including infantile spasms [Yeung et al., 2009; Brunetti-Pierri et al., 2011]. Maternal uniparental disomy 14 (upd(14)mat) is characterized by pre- and postnatal growth retardation, neonatal hypotonia, small hands and feet, feeding difficulty, precocious puberty, and truncal obesity [Kotzot and Utermann, 2005; Mitter et al., 2006]. Upd(14)mat syndrome demonstrates a Prader-Willi-like phenotype during infancy [Mitter et al., 2006; Hosoki et al., 2009] but complications of seizures are rarely observed. Upd(14)mat is reported in carriers of Robertsonian translocations involving chromosome 14 and is also found in patients with normal karyotypes and supernumerary marker chromosomes (SMCs) [Mitter et al., 2006]. The presence of SMCs has often increased chromosome dosage, which results in the increased expression of dosage-sensitive genes.

To add new insight regarding the genetic cause of West syndrome phenotype, we report on a female patient diagnosed with upd(14)mat and West syndrome who carried a small SMC derived from the chromosome 14q11.2 to 14q12 region encompassing *FOXG1*.

*Correspondence to:
Jun Tohyama, Department of Pediatrics, Epilepsy Center, Nishi-Niigata Chuo National Hospital, 1-14-1 Masago, Nishi-ku, Niigata 950-2085, Japan. E-mail: jtohyama@masa.go.jp
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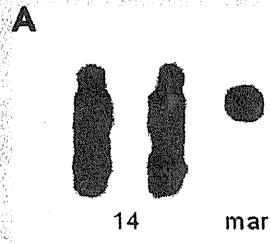
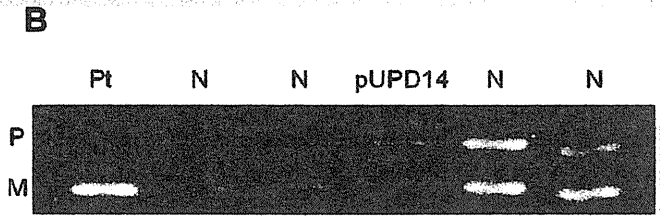
CLINICAL REPORT

The female patient was the first daughter born to healthy, nonconsanguineous Japanese parents with an unremarkable family history. Intrauterine growth retardation was noted during pregnancy. The child was delivered at 40 weeks and 5 days of gestation by cesarean because of cervical insufficiency. At birth, birth weight (BW) was 2,140 g (−2.4 SD), birth length (BL) was 48 cm (−0.4 SD), and occipitofrontal head circumference (OFC) was 28 cm (−3.9 SD). After delivery, the infant had episodic vomiting and was admitted to the neonatal intensive care unit. She received nasogastric tube feeding for 11 days due to feeding difficulty. During infancy, she had hypotonia. At 4 months, she developed epileptic seizures with upward eye deviation, and, at 5 months, infantile spasms. Her electroencephalogram (EEG) showed hypsarrhythmia. At 5 months, she was diagnosed as having West syndrome, and referred to our hospital. When she was admitted, her BW was 5.6 kg (−1.9 SD), BH was 62 cm (−1.1 SD), and OFC was 41 cm (+0.2 SD). She had mild dysmorphic features including a frontal bossing, small mouth, and small hands. A hemangioma on the left forehead was noted. A neurological examination revealed mild hypotonia without muscle weakness. A brain MRI and comprehensive metabolic screening were normal. Infantile spasms were not controlled despite an optimal dose of sodium valproate and zonisamide. Treatment with adrenocorticotropic hormone (ACTH) was started at age 6 months and successfully controlled her seizures. Subsequently, clobazam was added to improve her EEG, and she had no relapse of infantile spasms until she was 6 years old. Her EEG at 5 years 11 months was normal. At 3 years 11 months, her BH was 87.5 cm (−3.9 SD). Because of her short stature, growth hormone therapy was started at age four and was effective. The patient had mild psychomotor delay. At age six, she was able to speak a few words. Her intelligent quotient by a modified Binet method was 40 at age 5 years and 8 months.

Cytogenetic and Molecular Genetic Analysis

A chromosomal analysis was performed using the G-banding of cultured lymphocyte and spectral karyotyping (SKY) multicolor fluorescence in situ hybridization (FISH) method. Through the analysis of 26 metaphase cells, we found that the patient had a mosaic chromosome of 47,XX, + mar[8]/46,XX[18] (Fig. 1A). The origin of the SMC was not identified by conventional G banding.





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Locus	Region	Pt			Father		Mother	
D14S261	14q11.2	299	277	301	299	301	277	301
D14S283	14q11.2	139	139	139	139	151	139	149
D14S275	14q12	155	149	149	155	149	149	149
D14S70	14q13.1	–	106	106	106	104	106	104
D14S288	14q21.2	–	208	208	206	218	208	206
D14S276	14q22.3	–	241	245	241	243	241	245
D14S63	14q23.2	–	189	189	191	195	189	189
D14S258	14q24.2	–	197	203	197	197	197	203
D14S74	14q24.3	–	305	305	301	309	305	305
D14S68	14q31.3	–	328	328	326	332	328	328
D14S280	14q32.12	–	247	253	241	245	247	253
D14S65	14q32.2	–	138	138	138	138	138	138
D14S985	14q32.2	–	249	249	247	251	249	249
D14S292	14q32.33	–	89	89	91	91	89	89

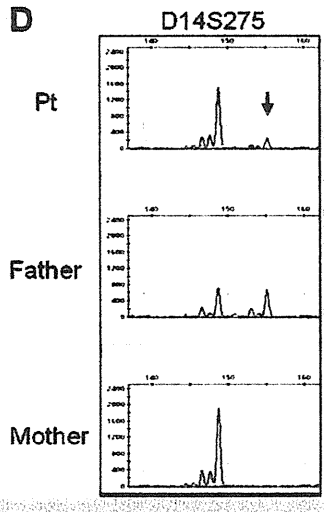

FIG. 1. Cytogenetic and molecular genetic examinations of chromosome 14. A: G-banding of chromosome 14 and the marker. B: *MEG3* methylation test. The *MEG3* methylation test demonstrated that the patient showed only a maternal unmethylated signal. P, paternal methylated signal; M, maternal unmethylated signal; Pt, patient; N, normal control; pUPD14, paternal uniparental disomy 14. C: Microsatellite analysis using polymorphic markers on chromosome 14. Putative haplotypes are indicated by color. The patient showed a combination of maternal uniparental heterodisomy and isodisomy of the entire chromosome 14, as well as additional paternal inheritance for only the proximal long arm of chromosome 14 (shown in blue). D: Fragment analysis at D14S275. Fragment analysis at D14S275 showed a small peak of paternal inheritance (indicated by arrow) showing the mosaic status of the marker of paternal origin. [Color figure can be seen in the online version of this article, available at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1552-4833].

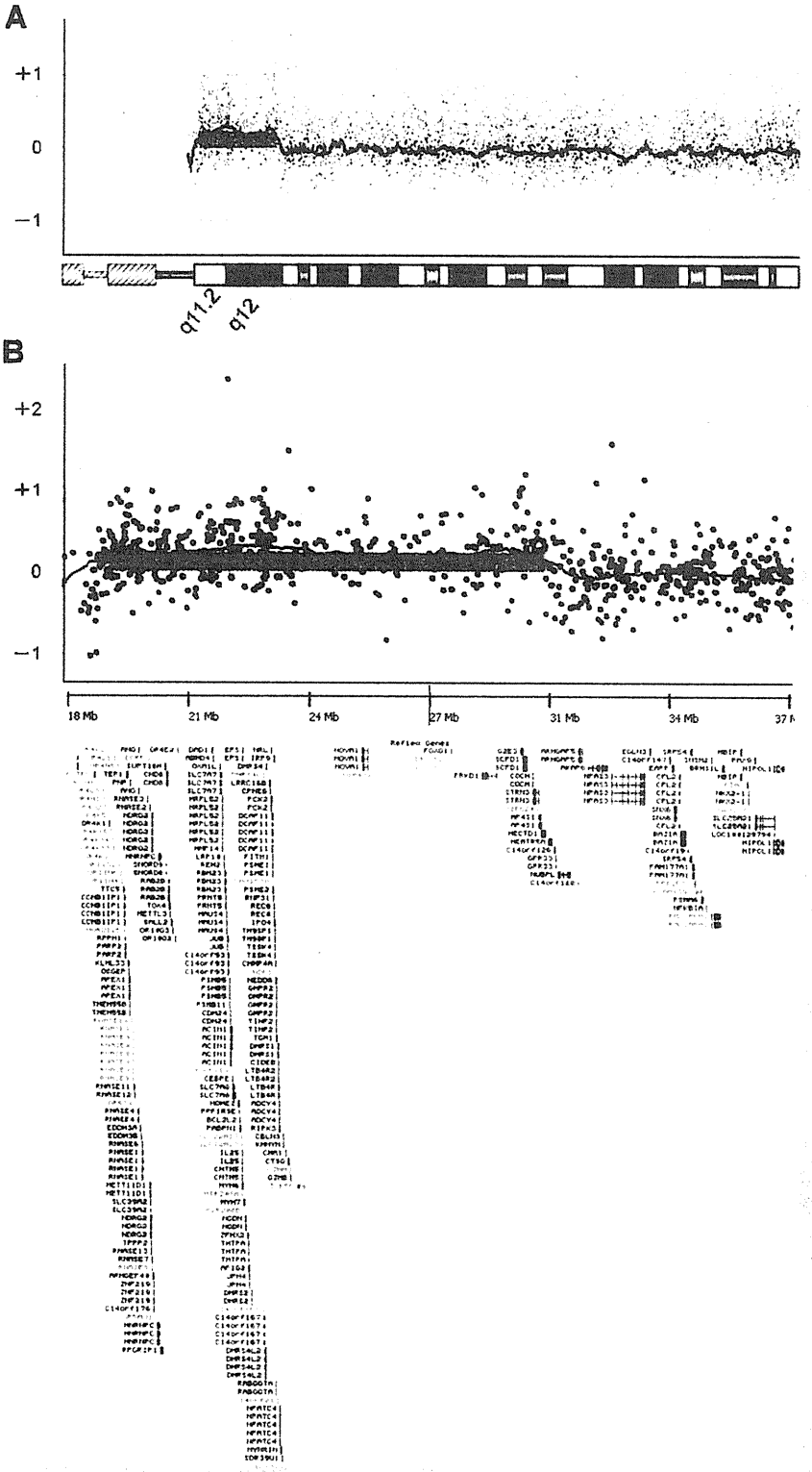

FIG. 2. Result of aCGH analysis for chromosome 14. A: Chromosome view indicating a genomic copy number gain of 14q11.2q12. The mean log2 ratio of this aberration region is 0.24, which indicates mosaicism of this marker chromosome. B: Aberration region expanded in gene view. The locations of the RefSeq Genes from the UCSC genome browser are shown under the gene view. [Color figure can be seen in the online version of this article, available at http://onlinelibrary.wiley.com/journal/10.1002/(ISSN)1552-4833].

SKY FISH analysis showed that the marker chromosome was derived from chromosome 14. Suspecting a relationship between the presence of marker chromosome 14 and upd(14)mat, we performed a DNA methylation test at *MEG3* in 14q32.2 [Hosoki et al., 2009], resulting in the abnormal hypomethylation of this gene (Fig. 1B). To confirm the origin of chromosome 14, microsatellite analysis using polymorphic markers on chromosome 14 was performed using ABI PRISM Linkage Mapping Set v2.5 (Applied Biosystems, Foster City, CA). Microsatellite polymorphism analysis indicated that both alleles of chromosome 14 were derived from the patient's mother and marker chromosome 14 was from her father (Fig. 1C). Fragment analysis at D14S275 showed a small peak of paternal inheritance, indicating the mosaic status of the marker of paternal origin (Fig. 1D). To further define the region of marker chromosome 14, microarray-based comparative genomic hybridization (aCGH) analysis performed using a 105K microarray kit (Agilent Technologies, Santa Clara, CA). The gain of genomic copy numbers was detected at 14q11.2-q12 indicating the molecular karyotype as arr14q11.2q12(19,761,035–30,941,609) × 1–1.5 (Fig. 2A,B). *FOXG1* was located in this region. Both parents had normal karyotypes.

DISCUSSION

The present patient showed intrauterine growth retardation, feeding difficulty during the neonatal period, mild hypotonia, and postnatal growth retardation. These findings fit well with those of upd(14)mat. Chromosomal analysis revealed mosaicism of 47,XX, + mar(14)/46,XX. From the association of the clinical findings of this patient and the presence of small SMC 14, we suspected that her clinical symptoms were related to upd(14)mat and performed a DNA methylation test at *MEG3* in 14q32.2 and microsatellite polymorphism analysis. We successfully confirmed that her condition was upd(14)mat. Upd(14)mat is manifested in clinical features overlapping the Prader-Willi phenotype, particularly during infancy. Therefore, this syndrome is considered to be underestimated. Hosoki et al. [2009] recommended performing the *MEG3* methylation test for all undiagnosed infants with hypotonia.

Infantile spasms or seizures are uncommon complications of upd(14)mat. We postulated that an increased dosage of some genes in extra SMC could be responsible for West syndrome. To identify the affecting gene, we performed aCGH analysis. The analysis showed an increased dosage of 14q11.2-q12. These regions contain 124 RefSeq genes including *FOXG1*. Recently, *FOXG1* on 14q12 was reported to be a dose-sensitive gene, and duplication of this gene could cause severe epilepsy and developmental retardation [Yeung et al., 2009; Brunetti-Pierri et al., 2011]. Several patients with *FOXG1* haploinsufficiency have been associated with a Rett-like syndrome and epilepsy [Shoichet et al., 2005; Jacob et al., 2009]. Deletion of this gene could cause seizures, but not infantile spasms. On the other hand, duplication of this gene is reported to cause infantile spasms or seizures during infancy. Yeung et al. [2009] first reported a patient with 4.45 Mb microduplication in 14q12. This patient showed infantile spasms at 6 months. In addition, Brunetti-Pierri et al. [2011] studied six patients with duplication of the 14q12 region. In their series, the size of the duplication varied between ~3 and 14.5 Mb with the patient carrying the largest duplication showing a 14.5 Mb duplication in 14q11.2-q13.1. The shortest region of overlap for the duplicated regions in the six patients contained only three genes, including *FOXG1*. Three of the six patients showed infantile spasms. The authors concluded that *FOXG1* represented the most interesting candidate for explaining the abnormal neurodevelopment phenotypes [Brunetti-Pierri et al., 2011]. The present patient also showed infantile spasms. However, her seizures are not refractory and are well controlled by anti-epileptic drugs and ACTH therapy. Her developmental delay is also not so severe. The 14q11.2-q12 region involved in our patient was almost equal in size to the largest duplication in Brunetti-Pierri's series. *FOXG1* is a dose-sensitive gene, and the results of our patient strongly suggested that an increased dosage of a small amount of this gene might lead to a milder West syndrome and milder intellectual disability.

The West syndrome has a heterogeneous etiology. Recent molecular biological approaches have identified several causative genes. To date, *ARX*, *CDKL5*, *STXBP1*, and *SPTAN1* have been reported as being associated with West syndrome [Kato et al., 2006; Otsuka et al., 2010; Saitsu et al., 2010]. These previous reports state that haploinsufficiency or small mutations of these genes are related to their phenotypes. In addition, duplication of *FOXG1* was recently reported to cause severe epilepsy and developmental delay, including infantile spasms. Epilepsies associated with increasing gene dosage are rare [Ramocki et al., 2010; Brunetti-Pierri et al., 2011]. The results of the study of our patient will provide further evidence that not only duplication but also a small increasing dose of *FOXG1* could cause infantile spasms or seizure during early infancy. Of course, in our patient, the contribution of other genes in 14q11.2-q12 could not be excluded.

The first patient with upd(14)mat had a Robertsonian translocation (13;14) [Temple et al., 1991]. This syndrome was also reported in carriers of Robertsonian translocation involving chromosome 14 and in patients with normal karyotypes [Mitter et al., 2006]. Other chromosomal rearrangements frequently associated with upd are small SMCs [Starke et al., 2003; Liehr et al., 2004]. Mitter et al. [2006] reported 10 patients with upd(14)mat, two of whom had SMC 14. In our patient, we were also able to determine that the marker chromosome was derived from chromosome 14 by SKY FISH, microsatellite polymorphism analysis, and aCGH analysis. The coexisting of small marker chromosome 14 and upd(14)mat is likely to be originated in functional trisomic rescue or gamete complementation in the formation of the chromosome aberration in our patient [Kotzot, 2002].

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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

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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

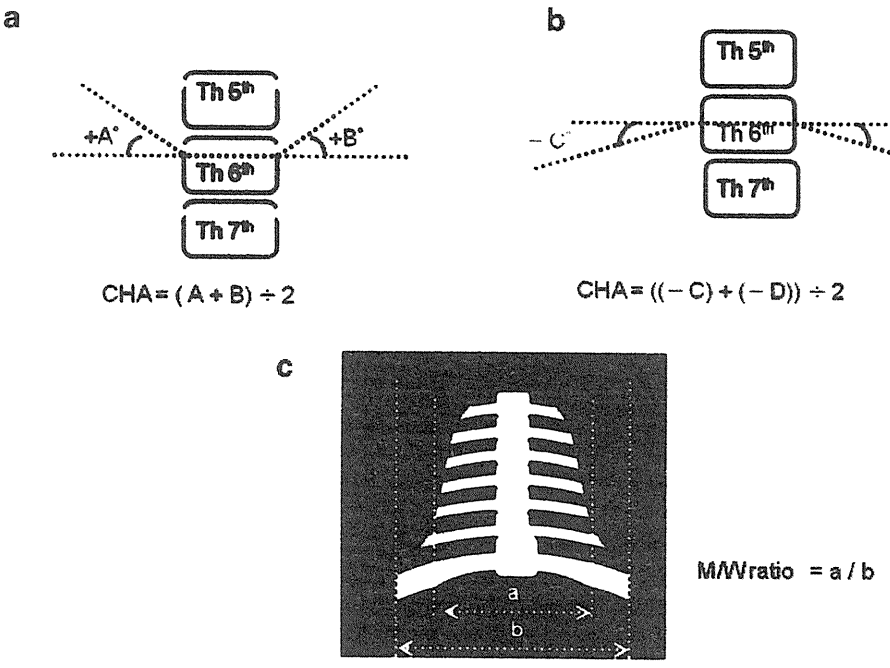

Fig. 1 **a**, **b** Diagram of coat-hanger angle (CHA) and mid/widest ratio. CHA refers to the average of the angles between the peak point of both 6th posterior ribs and the horizontal axis. If there is no peak point of the 6th posterior ribs, the center of the ribs is utilized instead. The horizontal 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

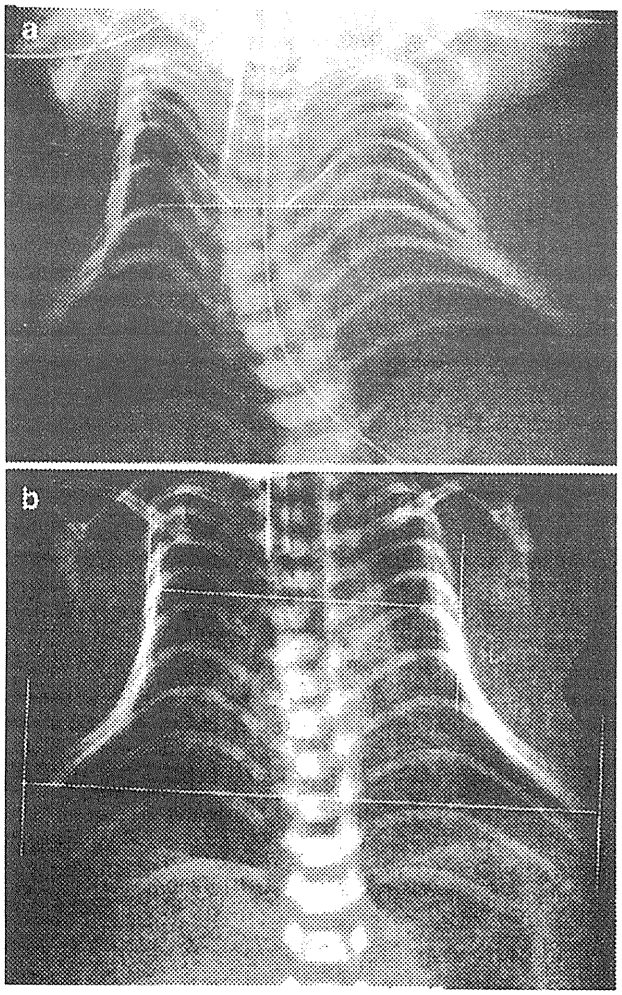

Fig. 2 Examples of CHA and M/W ratio. **a** The 6th posterior ribs show upward bowing that provides the coat-hanger sign. The CHA of this case (patient #7 in Table 1) was 45° (the measurement was 48° for the right and 42° for the left). **b** The M/W ratio was 58% in this case (patient #5 in Table 1).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

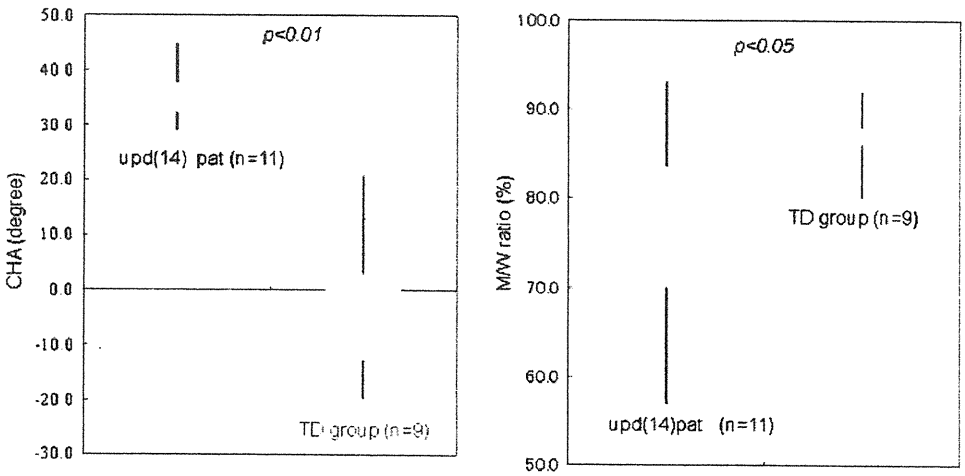

Fig. 3 Box plot of CHA and M/W ratio with the median, interquartile interval and range

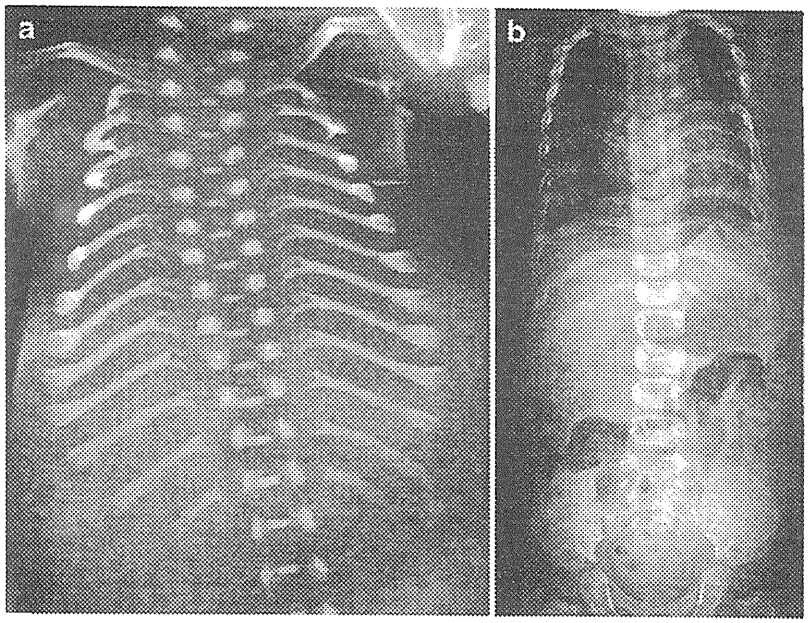

Fig. 4 Examples of the thoracic appearance and measurement of bone dysplasias with thoracic hypoplasia. **a** Thanatophoric dysplasia (TD) type 1 (stillbirth at 21 weeks of gestation). Note a narrow thorax with cupped anterior ends as well as short long bones with metaphyseal cupping. The posterior ribs 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-

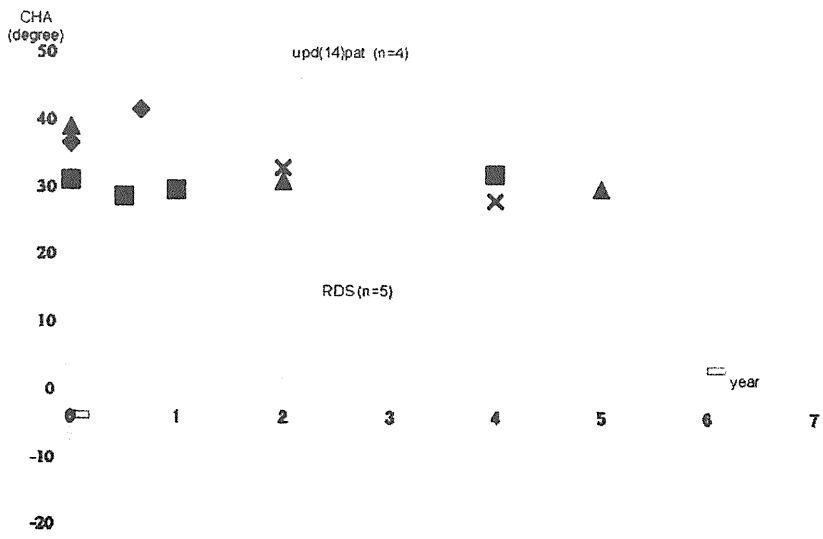

Fig. 5 Comparative observation of age-dependent transition of CHA between the upd(14)pat and respiratory distress syndrome (RDS) groups. Individual shapes represent individual patients

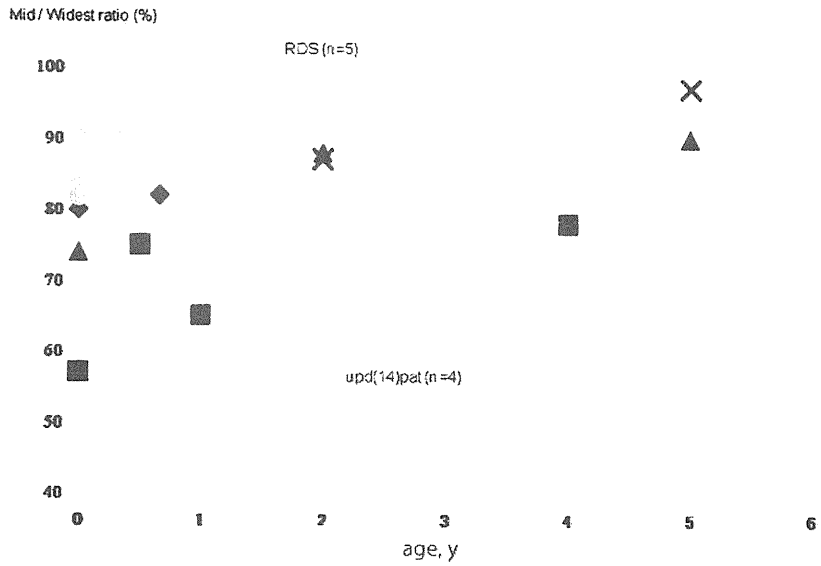

Fig. 6 Comparative observation of age-dependent transition of M/W ratio between the upd(14) pat and RDS groups. Individual shapes represent individual patients

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

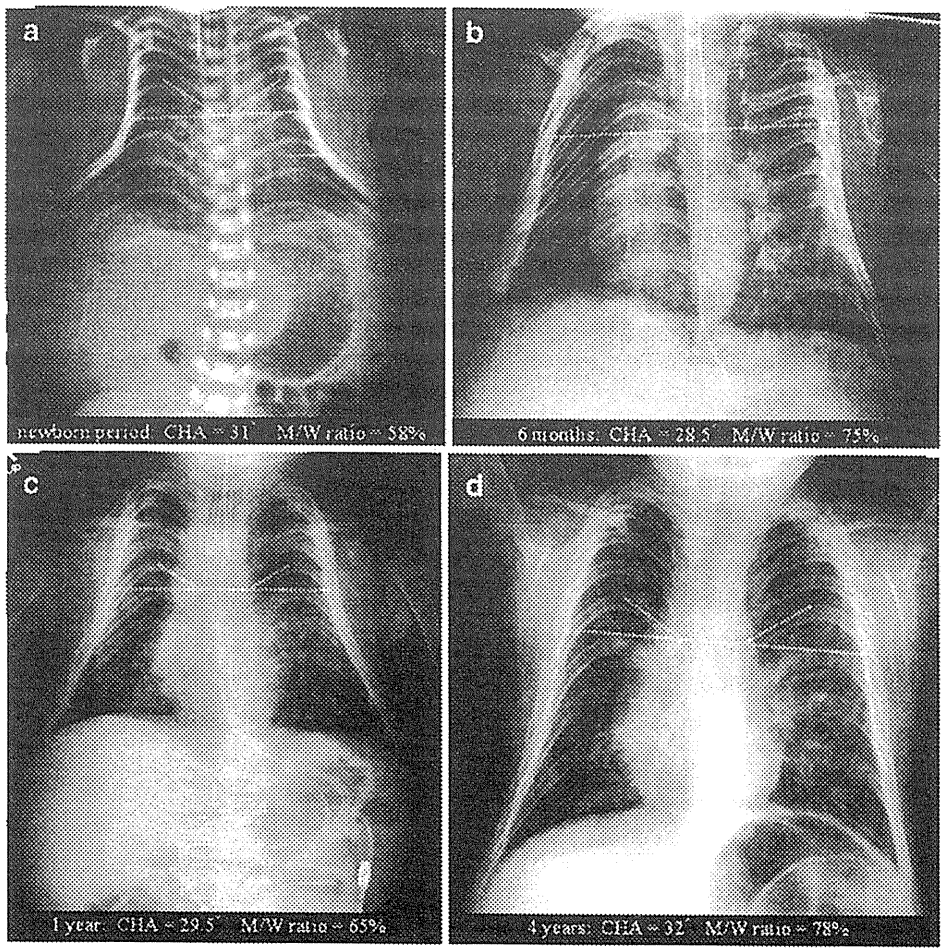

Fig. 7 Serial images of the thorax deformity in upd(14)pat. In this case, four images taken at different ages were available: (**a**) neonatal period, (**b**) 6 months, (**c**) 1 year and (**d**) 4 years. The CHA was almost consistent regardless of age, while the M/W ratio increased with advancing age. 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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Relative frequency of underlying genetic causes for the development of UPD(14)pat-like phenotype

Masayo Kagami[1], Fumiko Kato[1], Keiko Matsubara[1], Tomoko Sato[1], Gen Nishimura[2] and Tsutomu Ogata*[,1,3]

Paternal uniparental disomy 14 (UPD(14)pat) results in a unique constellation of clinical features, and a similar phenotypic constellation is also caused by microdeletions involving the *DLK1-MEG3* intergenic differentially methylated region (IG-DMR) and/or the *MEG3*-DMR and by epimutations (hypermethylations) affecting the DMRs. However, relative frequency of such underlying genetic causes remains to be clarified, as well as that of underlying mechanisms of UPD(14)pat, that is, trisomy rescue (TR), gamete complementation (GC), monosomy rescue (MR), and post-fertilization mitotic error (PE). To examine this matter, we sequentially performed methylation analysis, microsatellite analysis, fluorescence *in situ* hybridization, and array-based comparative genomic hybridization in 26 patients with UPD(14)pat-like phenotype. Consequently, we identified UPD(14)pat in 17 patients (65.4%), microdeletions of different patterns in 5 patients (19.2%), and epimutations in 4 patients (15.4%). Furthermore, UPD(14)pat was found to be generated through TR or GC in 5 patients (29.4%), MR or PE in 11 patients (64.7%), and PE in 1 patient (5.9%). Advanced maternal age at childbirth (≥35 years) was predominantly observed in the MR/PE subtype. The results imply that the relative frequency of underlying genetic causes for the development of UPD(14)pat-like phenotype is different from that of other imprinting disorders, and that advanced maternal age at childbirth as a predisposing factor for the generation of nullisomic oocytes through non-disjunction at meiosis 1 may be involved in the development of MR-mediated UPD(14)pat.
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Keywords: genetic cause; maternal age effect; monosomy rescue; UPD(14)pat subtype

INTRODUCTION

Human chromosome 14q32.2 carries a ~1.2 Mb imprinted region with the germline-derived primary *DLK1-MEG3* intergenic differentially methylated region (IG-DMR) and the post-fertilization-derived secondary *MEG3*-DMR, together with multiple imprinted genes.[1,2] Both DMRs are methylated after paternal transmission and unmethylated after maternal transmission in the body, whereas in the placenta the IG-DMR alone remains as a DMR and the *MEG3*-DMR is rather hypomethylated irrespective of the parental origin.[2,3] Furthermore, it has been shown that the unmethylated IG-DMR and *MEG3*-DMR of maternal origin function as the imprinting centers in the placenta and the body, respectively, and that the IG-DMR acts as an upstream regulator for the methylation pattern of the *MEG3*-DMR in the body but not in the placenta.[3]

As a result of the presence of the imprinted region, paternal uniparental disomy 14 (UPD(14)pat) (OMIM #608149) causes a unique constellation of body and placental phenotypes such as characteristic face, bell-shaped small thorax, abdominal wall defect, polyhydramnios, and placentomegaly.[2,4,5] Furthermore, consistent with the essential role of the DMRs in the imprinting regulation, microdeletions and epimutations affecting the IG-DMR or both DMRs of maternal origin result in UPD(14)pat-like phenotype in both the body and the placenta, whereas a microdeletion involving the maternally inherited *MEG3*-DMR alone leads to UPD(14)pat-like phenotype in the body, but not in the placenta.[2,3]

Of the three underlying genetic causes for UPD(14)pat-like phenotype (UPD(14)pat, microdeletions, and epimutations), UPD(14)pat is primarily generated by four mechanisms, that is, trisomy rescue (TR), gamete complementation (GC), monosomy rescue (MR), and post-fertilization mitotic error (PE).[6] TR refers to a condition in which chromosome 14 of maternal origin is lost from a zygote with trisomy 14 formed by fertilization between a disomic sperm and a normal oocyte. GC results from fertilization of a disomic sperm with a nullisomic oocyte. MR refers to a condition in which chromosome 14 of paternal origin is replicated in a zygote with monosomy 14 formed by fertilization between a normal sperm and a nullisomic oocyte. PE is an event after formation of a normal zygote. In this regard, a nullisomic oocyte specific to GC and MR is produced by non-disjunction at meiosis 1 (M1) or meiosis 2 (M2), and non-disjunction at M1 is known to increase with maternal age, probably because of a long-term (10–50 years) meiotic arrest at prophase 1.[7]

However, relative frequency of the genetic causes for UPD(14)pat-like phenotype remains to be determined, as well as that of underlying mechanisms for the generation of UPD(14)pat. Here, we report our data on this matter, and discuss the difference in the relative frequency

[1]Department of Molecular Endocrinology, National Research Institute for Child Health and Development, Tokyo, Japan; [2]Department of Radiology, Tokyo Metropolitan Children's Medical Center, Fuchu, Japan; [3]Department of Pediatrics, Hamamatsu University School of Medicine, Hamamatsu, Japan
*Correspondence: Professor T Ogata, Department of Pediatrics, Hamamatsu University School of Medicine, Hamamatsu 431-3192, Japan. Tel: +81 53 435 2310; Fax: +81 53 435 2312; E-mail: tomogata@hama-med.ac.jp
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among imprinted disorders and the possible maternal age effect on the relative frequency.

PATIENTS AND METHODS

Patients

This study comprised 26 patients with UPD(14)pat-like phenotype (9 male patients and 17 female patients) (Table 1). Of the 26 patients, 18 patients have been reported previously; they consisted of nine sporadic patients with full UPD(14)pat,[4,5] one sporadic patient with segmental UPD(14)pat,[4] the proband of sibling cases and four sporadic patients with different patterns of microdeletions involving the unmethylated DMRs of maternal origin,[2,3] and three patients with epimutations (hypermethylations) of the two normally unmethylated DMRs of maternal origin.[2] The remaining eight patients were new sporadic cases.

Phenotypic findings of the 26 patients are summarized in Supplementary Table 1; detailed clinical features of patients 6 and 16–25 are as described previously,[2–4] and those of the eight new patients 3, 5, 10–14, and 26 are shown in Supplementary Table 2, together with those of patients 1, 2, 4, 7–9, and 15 in whom detailed phenotypes were not described in the previous report.[5] All the 26 patients were identified shortly after birth because of the unique bell-shaped thorax with coat-hanger appearance of the ribs on roentgenograms obtained because of asphyxia. Subsequent clinical analysis revealed that 25 of the 26 patients exhibited both body and placental UPD(14)pat-like phenotype, whereas the remaining one previously reported patient (patient 22) manifested body, but not placental, UPD(14)pat-like phenotype.[3] The karyotype was found to be normal in 25 patients, although cytogenetic analysis was not performed in one previously reported patient who died of respiratory failure at 2 h of age (patient 6).[4] One patient (patient 15) was conceived by *in vitro* fertilization-embryo transfer.[5] This study was approved by the Institute Review Board Committee at the National Center for Child Health and Development, and performed after obtaining written informed consent.

Analysis of underlying genetic causes in patients with UPD(14)pat-like phenotype

We sequentially performed methylation analysis, microsatellite analysis, and fluorescence *in situ* hybridization (FISH), using leukocyte genomic DNA samples and lymphocyte metaphase spreads of all the 26 patients with UPD(14)pat-like phenotype. The detailed methods were as reported previously.[2,3] In brief, methylation analysis was performed for the IG-DMR (CG4 and CG6) and the *MEG3*-DMR (CG7 and the CTCF-biding sites C and D) by combined bisulfite restriction analysis and bisulfite sequencing. Microsatellite analysis was performed for multiple loci on chromosome 14, by determining the sizes of PCR products obtained with fluorescently labeled forward primers and unlabeled reverse primers. FISH analysis was carried out for the IG-DMR and the *MEG3*-DMR using 5104-bp and 5182-bp long PCR products, respectively, together with the RP11-566I2 probe for 14q12 utilized as an internal control.

In this study, furthermore, oligonucleotide array-based comparative genomic hybridization (CGH) was also performed for the imprinted region of non-UPD(14)pat patients, using a custom-build oligo-microarray containing 12 600 probes for 14q32.2–q32.3 encompassing the imprinted region and ~10 000 reference probes for other chromosomal region (4×180K format, Design ID 032112) (Agilent Technologies, Palo Alto, CA, USA). The procedure was as described in the manufacturer's instructions.

Analysis of subtypes in patients with UPD(14)pat

UPD(14)pat subtype was determined by microsatellite analysis.[8,9] In brief, heterodisomy for at least one locus was regarded as indicative of TR- or GC-mediated UPD(14)pat (TR/GC subtype), whereas isodisomy for all the informative microsatellite loci was interpreted as indicative of MR- or PE-mediated UPD(14)pat (MR/PE subtype) (for details, see Supplementary Figure S1). Here, while heterodisomy and isodisomy for a pericentromeric region in the TR/GC subtype imply a disomic sperm generation through M1

Table 1 Summary of patients examined in this study

Patient	*Genetic cause*	*UPD(14)pat subtype*	*Maternal age at childbirth (years)*	*Paternal age at childbirth (years)*	*Remark*	*Reference*
1	UPD(14)pat	TR/GC [M1]	31	35		5
2	UPD(14)pat	TR/GC [M1]	28	29		5
3	UPD(14)pat	TR/GC [M1]	29	38		This report
4	UPD(14)pat	TR/GC [M1]	36	41		5
5	UPD(14)pat	TR/GC [M2]	30	30		This report
6	UPD(14)pat	MR/PE	42	Unknown		4,5
7	UPD(14)pat	MR/PE	31	28		5
8	UPD(14)pat	MR/PE	32	33		5
9	UPD(14)pat	MR/PE	26	35		5
10	UPD(14)pat	MR/PE	38	38		This report
11	UPD(14)pat	MR/PE	26	32		This report
12	UPD(14)pat	MR/PE	41	36		This report
13	UPD(14)pat	MR/PE	30	28		This report
14	UPD(14)pat	MR/PE	39	34		This report
15	UPD(14)pat	MR/PE	42	37	Born after IVF-ET	5
16	UPD(14)pat	MR/PE	36	36		4,5
17	UPD(14)pat-seg.	PE	27	24	Segmental isodisomy	4,5
18	Microdeletion		31	34		2
19	Microdeletion		33	36		2
20	Microdeletion		28	27		2
21	Microdeletion		27	37	IG-DMR alone	3
22	Microdeletion		25	25	*MEG3*-DMR alone	3
23	Epimutation		35	36		2
24	Epimutation		28	26		2
25	Epimutation		27	30		2
26	Epimutation		33	33		This report

Abbreviation: IVF-ET, *in vivo* fertilization-embryo transfer using parental gametes.
The microdeletions in patients 18–22 are different in size.

and M2 non-disjunction respectively,[9] such discrimination between M1 and M2 non-disjunctions is impossible for the development of a nullisomic oocyte. Furthermore, it is usually impossible to discriminate between TR and GC, although the presence of trisomic cells is specific to TR. Similarly, it is also usually impossible to discriminate between MR and PE, although identification of segmental isodisomy or mosaicism is unique to PE (PE subtype).

Analysis of parental ages

We examined parental ages at childbirth in patients of different underlying causes and different UPD(14)pat subtypes. Statistical significance of the relative frequency was examined by the Fisher's exact probability test, and that of the median age by the Mann–Whitney's *U*-test. $P<0.05$ was considered significant.

RESULTS

Analysis of underlying causes in patients with UPD(14)pat-like phenotype

For the eight new sporadic patients, methylation analysis invariably revealed hypermethylation of both DMRs, and microsatellite analysis showed UPD(14)pat in seven patients and biparentally inherited homologs of chromosome 14 in the remaining one patient (patient 26). FISH analysis for patient 26 identified two signals for the two DMRs, and subsequently performed array CGH analysis showed no evidence for genomic rearrangements (Supplementary Figure S2). Thus, patient 26 was assessed to have an epimutation affecting the two DMRs. Furthermore, the results of array CGH analysis confirmed the presence of microdeletions in patients 18–21 and the absence of a discernible microdeletion in patients 23–25 (Supplementary Figure S2) (array CGH analysis was not performed in patient 22 with a 4303-bp microdeletion[3] because of the lack of DNA sample available). Thus, together with our previous data, all the 26 patients with UPD(14)pat-like phenotype had genetic alteration involving the imprinted region on chromosome14q32.2.

Consequently, the 26 patients with UPD(14)pat-like phenotype were classified as follows: (1) 16 sporadic patients with full UPD(14)pat and 1 sporadic patient with segmental UPD(14)pat (UPD(14)pat group); (2) the proband of the sibling cases and two sporadic patients with different patterns of microdeletions involving the two DMRs, one sporadic patient with a microdeletion involving the IG-DMR alone in whom the *MEG3*-DMR was epimutated, and one patient with a microdeletion involving the *MEG3*-DMR alone (deletion group); and (3) four patients with epimutations (hypermethylations) of both DMRs (epimutation group) (Figure 1 and Table 1).

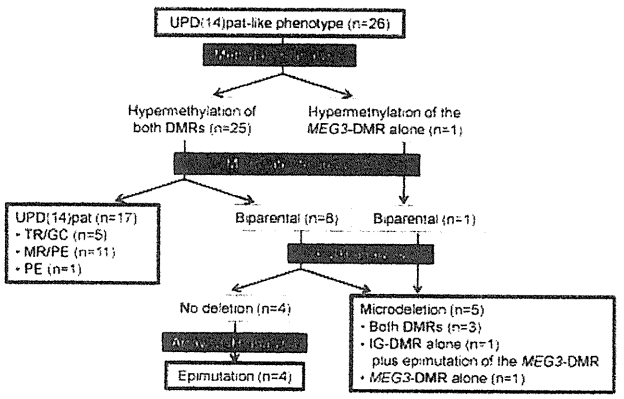

Figure 1 Classification of 26 patients with UPD(14)pat-like phenotype.

Analysis of subtypes in patients with UPD(14)pat

Heterozygosity for at least one locus indicative of TR/GC subtype was identified in five patients (patients 1–5), and the disomic pattern of pericentromeric region indicated M1 non-disjunction in patients 1–4 and M2 non-disjunction in patient 5. Full isodisomy consistent with MR/PE subtype was detected in 11 patients (patients 6–16), and segmental isodisomy unique to PE subtype was revealed in 1 patient (patient 17) (Table 1, Figure 1, and Supplementary Figure S3).

Analysis of parental ages

The distribution of parental ages at childbirth is shown in Figure 2. The advanced maternal age at childbirth (≥35 years) was predominantly observed in the MR/PE subtype of UPD(14)pat. Furthermore, while the relative frequency of aged mothers (≥35 years) did not show a significant difference between the MR/PE subtype of UPD(14)pat (6/11) and (i) other subtypes of UPD(14)pat (1/6) ($P=0.159$), (ii) deletion group (0/5) ($P=0.057$), and (iii) epimutation group (1/4) ($P=0.338$), it was significantly different between the MR/PE subtype and the sum of other subtypes of UPD(14)pat, deletion group, and epimutation group (2/15) ($P=0.034$). Similarly, while the median maternal age did not show a significant difference between the MR/PE subtype of UPD(14)pat (36 years) *vs* (i) other subtypes of UPD(14)pat (29.5 years) ($P=0.118$), (ii) deletion type (28 years) ($P=0.088$), and (iii) epimutation type (30.5 years) ($P=0.295$), it was significantly different between the MR/PE subtype of UPD(14)pat and the sum of other subtypes of UPD(14)pat, deletion group, and epimutation group (29 years) ($P=0.045$).

The paternal ages were similar irrespective of the genetic causes and the UPD(14)pat subtypes. In addition, the median paternal age was comparable between the TR/GC subtype of UPD(14)pat that postulates the production of a disomic sperm (35.0 years) and the sum of other subtypes of UPD(14)pat, deletion group, and epimutation group that assumes the production of a normal sperm (33.5 years) ($P=0.322$).

DISCUSSION

This study revealed that the UPD(14)pat-like phenotype was caused by UPD(14)pat in 65.4% of patients, by microdeletions in 19.2% of patients, and by epimutations in 15.4% of patients. Although the relative frequency of underlying genetic factors for the development of UPD(14)pat-like phenotype has been reported previously,[10] most data are derived from our previous publications. Thus, the present results are regarded as the updated and extended data on the relative frequency. For the relative frequency, it is notable that 25 of the 26 patients were confirmed to have normal karyotype, although chromosome analysis was not performed in patient 6. Thus, while Robertsonian translocations involving chromosome 14 is known to be a

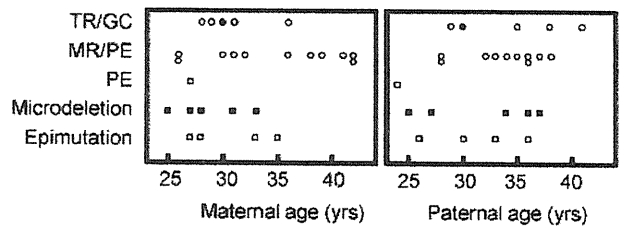

Figure 2 The distribution of parental ages at childbirth according to the underlying genetic causes for the development of UPD(14)pat-like phenotype and UPD(14)pat subtypes. Of the five plots for the TR/GC subtype, open and black circles indicate the TR/GC subtype due to non-disjunction at paternal M1 and M2, respectively.

predisposing factor for the occurrence of UPD(14)pat,[11–16] such a possible chromosomal effect has been excluded in nearly all patients examined in this study.

The relative frequency of underlying causes has also been reported in other imprinting disorders.[8,17–19] The data are summarized in Table 2 (a similar summary has also been reported recently by Hoffmann *et al*).[10] In particular, the results in patients with normal karyotype are available in Prader–Willi syndrome (PWS).[8] Furthermore, PWS is also known to be caused by UPD, microdeletions, and epimutations affecting a single imprinting region,[8,19] although Silver–Russell syndrome and Beckwith–Wiedemann syndrome (BWS) can result from perturbation of at least two imprinted regions,[17,18] and BWS and Angelman syndrome can occur as a single gene disorder.[17,19] Thus, it is notable that the relative frequency of underlying causes is quite different between patients with UPD(14)pat-like phenotype and those with PWS.[8,19] This would primarily be due to the presence of low copy repeats flanking the imprinted region on chromosome 15, because chromosomal deletions are prone to occur in regions harboring such repeat sequences.[20] Indeed, two types of microdeletions mediated by such low copy repeats account for a vast majority of microdeletions in patients with PWS,[21] whereas the microdeletions identified in patients with UPD(14)pat-like phenotype are different to each other. This would explain why microdeletions are less frequent and UPD and epimutations are more frequent in patients with UPD(14)pat-like phenotype than in those with PWS.

Advanced maternal age at childbirth was predominantly observed in the MR/PE subtype. This may imply the relevance of advanced maternal age to the development of MR-mediated UPD(14)pat, because the generation of nullisomic oocytes through M1 non-disjunction is a maternal age-dependent phenomenon.[22] Although no paternal age effect was observed, this is consistent with the previous data indicating no association of advanced paternal age with a meiotic error.[23] For the maternal age effect, however, several matters should be pointed out: (1) the number of analyzed patients is small, although it is very difficult to collect a large number of patients in this extremely rare disorder; (2) of the MR/PE subtype, the advanced maternal age is a risk factor for the generation of MR-mediated UPD(14)pat, but not for the development of PE-mediated UPD(14)pat; (3) it is impossible to discriminate between maternal age-dependent M1 non-disjunction and maternal age-independent M2 non-disjunction in the MR and GC subtypes (however, GC must be extremely rare, because it requires the concomitant occurrence of a nullisomic oocyte and a disomic sperm); (4) of the TR/GC subtype, the advanced maternal age is a risk factor for the generation of GC-mediated UPD(14)pat, but not for the development of TR-mediated UPD(14)pat; and (5) if a cryptic recombination(s) might remain undetected in some patients with apparently full isodisomy, this argues that such patients actually have TR- or GC-mediated UPD(14)pat rather than MR- or PE-mediated UPD(14)pat. Thus, further studies are required to examine the maternal age effect on the generation of MR-mediated UPD(14)pat. In addition, while a relationship is unlikely to exist between advanced maternal age and microdeletions and epimutations, this notion would also await further investigations.

Such a maternal age effect is also expected in the TR/GC subtype maternal UPDs after M1 non-disjunction, because the generation of disomic oocytes through M1 non-disjunction is also a maternal age-dependent phenomenon.[7] Indeed, such a maternal age effect has been shown for PWS patients with normal karyotype; the maternal age at childbirth was significantly higher in patients with heterodisomy for a very pericentromeric region indicative of TR/GC subtype UPD(15)mat after M1 non-disjunction than in those with other genetic causes.[8,9] For various chromosomes other than chromosome 15, furthermore, since maternal age at childbirth is higher in patients with maternal heterodisomy than in those with maternal isodisomy,[24] this would also argue for maternal age effect on the development of maternal UPDs. However, in the previous studies on maternal UPDs other than UPD(15)mat, the available data are quite insufficient to assess the maternal age effect. For example, although a relatively large number of patients with UPD(14)mat phenotype have been reported in the literature (reviewed in reference Hoffmann *et al*),[10] we could identify only six UPD(14)mat patients with normal karyotype in whom maternal age at childbirth was documented and microsatellite analysis was performed.[25–30] Furthermore, the microsatellite data are insufficient to identify the subtype of UPD(14)mat and to distinguish between M1 and M2 non-disjunction in the TR/GC subtype. Thus, while the maternal age at childbirth may be advanced in five patients with apparently TR/GC-mediated UPD(14)mat (27, 35, 37, 41, and 44 years)[25–27,29,30] (the maternal age at childbirth in the remaining one

Table 2 Relative frequency of genetic mechanisms in imprinting disorders

	UPD(14)pat-like phenotype	*BWS*	*SRS*	*AS*	*PWS*
Uniparental disomy	65.4%	16%	10%	3–5%	25% (25%)
	UPD(14)pat	UPD(11)pat (mosaic)	UPD(7)mat	UPD(15)pat	UPD(15)mat
Cryptic deletion	19.2%	Rare	—	70%	70% (72%)
Cryptic duplication	—	—	Rare	—	—
Epimutation					
Hypermethylation	15.4%	9%	—	—	2–5% (2%)
Affected DMR	IG-DMR/*MEG3*-DMR	*H19*-DMR	—	—	*SNRPN*-DMR
Hypomethylation	—	44%	>38%	2–5%	—
Affected DMR		KvDMR1	*H19*-DMR	*SNRPN*-DMR	
Gene mutation	—	5%	—	10-15%	—
Mutated gene		*CDKN1C*		*UBE3A*	
Unknown		25%	>40%	10%	
Reference	This study	17	18	19	8, 19

Abbreviations: AS, Angelman syndrome; BWS, Beckwith–Wiedemann syndrome; PWS, Prader–Willi syndrome; SRS, Silver–Russell syndrome.
Patients with abnormal karyotypes are included in BWS and AS, and not included in SRS. In PWS, the data including patients with abnormal karyotypes are shown, and those from patients with normal karyotype alone are depicted in parentheses.

patient with apparently MR/PE-mediated UPD(14)mat is 40 years),[28] the notion of a maternal age effect awaits further investigations for UPD(14)mat.

Finally, it appears to be worth pointing out that methylation analysis invariably revealed hypermethylated DMR(s) in all the 26 patients who were initially ascertained because of bell-shaped thorax with coat-hanger appearance of the ribs. This indicates that methylation analysis of the DMRs can be utilized for a screening of this condition, and that the constellation of clinical features in the UPD(14)pat-like phenotype, especially the bell-shaped thorax with coat-hanger appearance of the ribs, is highly unique to patients with UPD(14)pat-like phenotype.

In summary, this study confirms the relative frequency of underlying genetic causes for the UPD(14)pat phenotype and reveals the relative frequency of UPD(14)pat subtypes. Furthermore, the results emphasize the difference in the relative frequency of underlying genetic causes among imprinted disorders, and may support a possible maternal age effect on the generation of the nullisomic oocyte mediated UPD(14)pat. Further studies will permit a more precise assessment on these matters.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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特　集　小児内分泌学の進歩2009

14番染色体母性片親性ダイソミーはPrader-Willi症候群の鑑別疾患である

鏡　雅代[*1]　細木　華奈[*2]　田中　藤樹[*3]
斎藤　伸治[*2]　緒方　勤[*1]

はじめに

遺伝子は通常親由来にかかわらず両方のアレルから発現するが，父親由来アレルからのみ発現する父性発現遺伝子（Paternal Expressed Genes：PEGs）と母親由来アレルからのみ発現する母性発現遺伝子（Maternal Expressed Genes：MEGs）が存在する[1]．これらの親由来により発現が異なる遺伝子をインプリンティング遺伝子という[1]．インプリンティング遺伝子は，クラスターとなって存在しドメインを形成することが多く，胎盤において強く発現している[2]．インプリンティング遺伝子は胎盤を形成する動物においてのみ同定されており，胎盤，胎児の発育に大きな役割を果たしていることが明らかとなっている[2]．

インプリンティング遺伝子の発現には，ゲノムに父親由来もしくは母親由来がマーキングされるゲノムインプリンティングというメカニズムがかかわっている．インプリントは，遺伝子のプロモーター領域にしばしば存在するCpG配列が繰り返されるCpG islandsのシトシンにメチル化修飾を入れることであり，遺伝子の発現はインプリントをうけることにより抑制される．親由来によってメチル化修飾が異なる領域をメチル化可変領域（Differentially methylated region：DMR）といい，いくつかのインプリンティングドメインにおいてはDMRがインプリンティングセンターとして作用する．DMRのメチル化修飾は，配偶子形成過程で完全に消去され，配偶子の性に一致して再度樹立される[1]．

14番染色体長腕の32.2領域（14q32.2）には，インプリンティング遺伝子がクラスターとなって存在しており，PEGとしてはDLK1，RTL1，MEGとしてはMEG3（GTL2），RTL1as（RTL1 antisense），MEG8，microRNAやsnoRNAがある[3,4]（**図1**）．DLK1，RTL1はタンパクをコードし，MEG3，MEG8，microRNA，snoRNAはタンパクをコードしないRNA遺伝子である．これらの遺伝子群の発現には，生殖細胞でDMRが確立しているDLK1-MEG3 intergenic DMR（IG-DMR）と受精後にDMRが確立するMEG3-DMRのメチル化状態が関与しており，ともに父親由来染色体でメチル化修飾をうけ，母親由来染色体ではメチル化修飾をうけない[3,4]．

[*1] 国立成育医療センター研究所小児思春期発育研究部
[*2] 北海道大学医学部小児科
[*3] 国立成育医療センター遺伝科
Masayo Kagami [*1], Kana Hosoki [*2], Toju Tanaka [*3], Shinji Saitoh [*2], Tsutomu Ogata [*1] : Maternal uniparental disomy 14 is an important differential diagnosis of Prader-Willi Syndrome.
[*1] Department of Endocrinology and Metabolism, National Research Institute for Child Health and Development, [*2] Department of Pediatrics, Hokkaido University Graduate School of Medicine, [*3] Division of Clinical Genetics and Molecular Medicine, National Center for Child Health and Development.

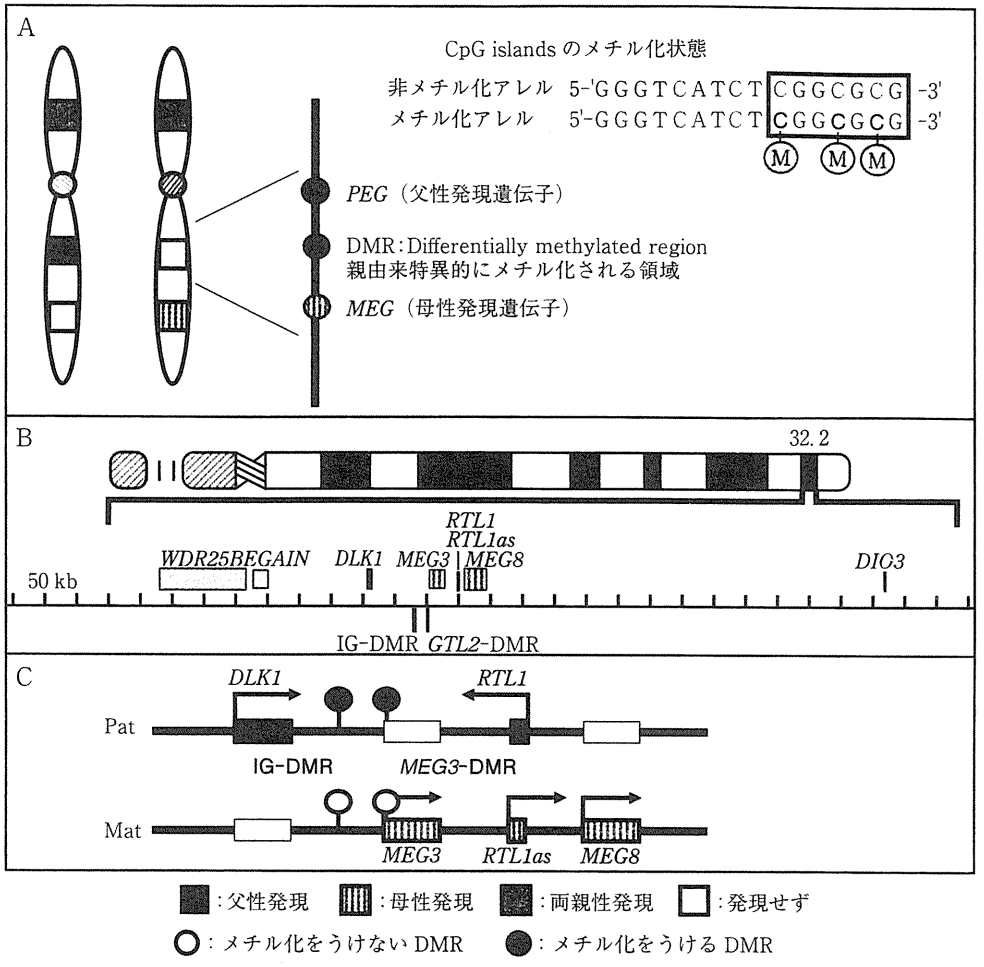

図1　14q32.2インプリンティング領域と制御機構
A. インプリンティング遺伝子のモデル　B. 14q32.2インプリンティング領域
C. DLK1-DIO3ドメインの調節機構

インプリンティング遺伝子群の存在を裏付けるように，14番染色体がともに母親に由来する14番染色体母親性ダイソミー(upd (14) mat)と，ともに父親に由来する14番染色体父親性ダイソミー(upd (14) pat) はその臨床像が異なる．upd (14) matは胎児期・出生後の成長障害，新生児期の筋緊張低下，小さな手，哺乳障害，思春期早発傾向，などの症状を示す[4,5]．一方，upd (14) patは，羊水過多，胎盤過形成，ベル型・コートハンガー型と形容される胸郭低形成，臍帯ヘルニアや腹直筋離開といった腹壁異常，特徴的顔貌を示す[4,6]．これらの臨床症状はMEGsとPEGsの発現異常により生じることが明らかとなっている[3,4]．

近年，Mitterらは Prader-Willi 症候群（PWS）を疑われた患者33例中4例で upd (14) mat であったと報告している[7]．我々はPWS症状陽性で，15q11-q13の欠失およびSNURF-SNRPNのDNAメチル化異常が否定された29症例において14染色体インプリンティングドメインの解析を行い，メチル化異常を認めた症例についてその臨床像について報告する．

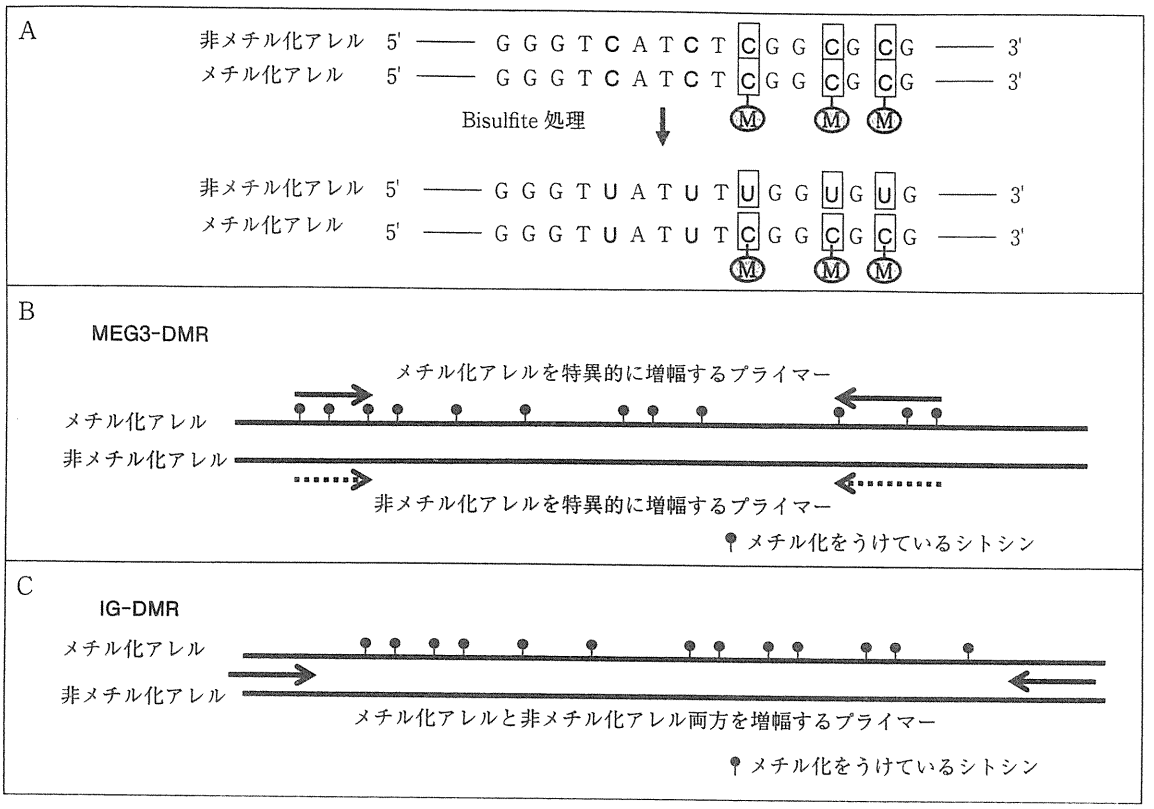

図2 メチル化解析の方法
A. Bisulfite 法 B. MEG3-DMR のメチル化テスト
C. IG-DMR の Bisulfite Sequence 法

1 対　象

PWS 表現型陽性で，15q11-q13 の欠失および SNURF-SNRPN の DNA メチル化異常が否定された 29 症例．

2 方　法

患者末梢血白血球よりゲノム DNA を抽出したのち，EZ DNA Methylation Kit (Zymo Research) を用いて Bisulfite 処理を行った．Bisulfite 処理を行うとシトシンはウラシルに変換され，最終的にはチミンに変換されるが，CpG island のなかに存在するメチル化をうけているシトシンは変換されない．この違いを用いて MEG3-DMR 中の父親由来のメチル化アレルを特異的に増幅するプライマーセットと母親由来のメチル化をうけないアレルを特異的に増幅するプライマーセットを作成し，メチル化テストを行った[8]．メチル化テストにおいて異常メチル化パターンを示した症例については，IG-DMR 中にメチル化アレルも非メチル化アレルも両方とも増幅するメチル化をうけたシトシンを含まない領域にプライマーセットを設計し，その PCR 産物を TOPO TA-cloning Kit (Invitrogen) を用いてクローニングしたのち直接シークエンス法でメチル化状態を解析した[4]（**図2**）．さらにダイソミーの有無を確認するため，両親のゲノム DNA と患者ゲノム DNA を用いて 14 番染色体上の 14 か所のマイクロサテライトマーカーでの genotyping を行いその親由来を解析した．マイクロサテライトマーカー解析でダイソミーが否定された症例は，微小欠失の有無を同定するために，FISH 解析を行った．FISH