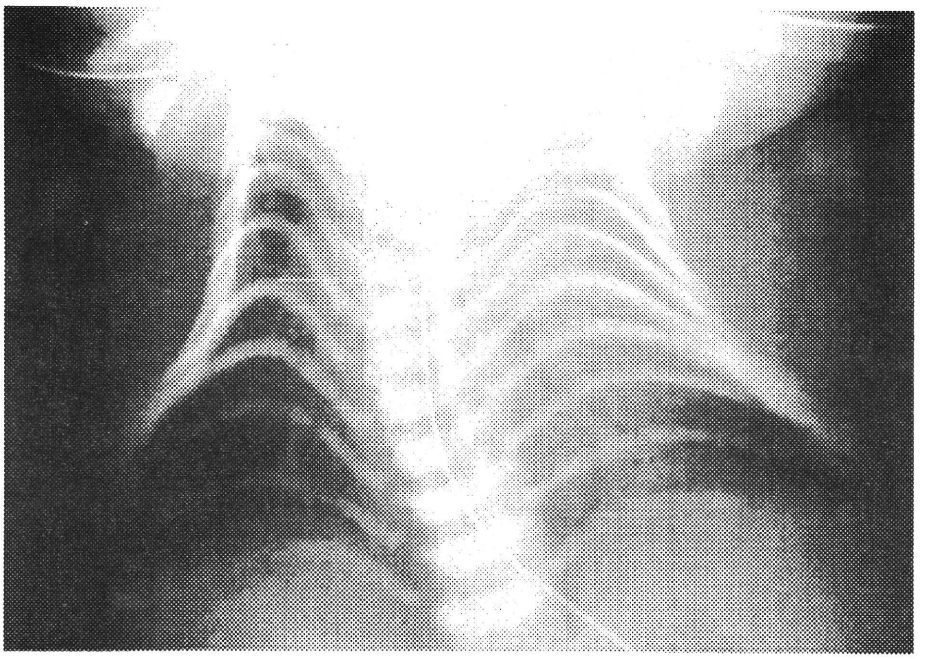
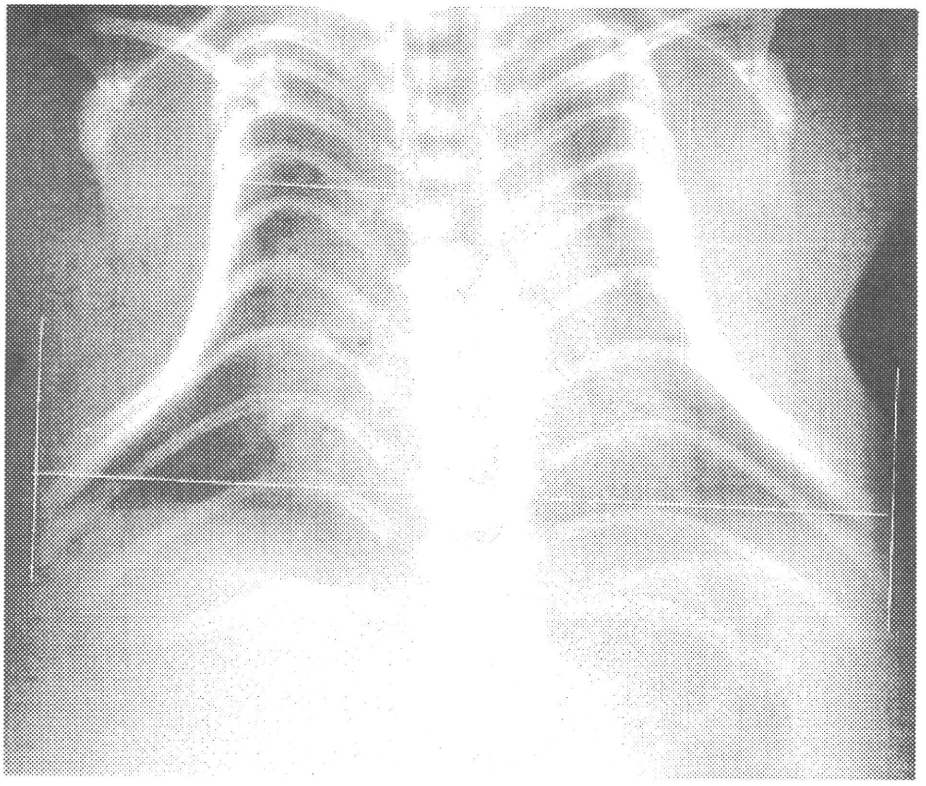


Fig. 2 Examples of the CHA and M/W ratio
a) The 6th posterior ribs show upward bowing that provides the "coat-hanger" sign. The CHA of this case (patient #7 in Table 1) was 45° (the measurement was 48° for the right and 42° for the left).

127x88mm (300 x 300 DPI)



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.

100x84mm (300 x 300 DPI)

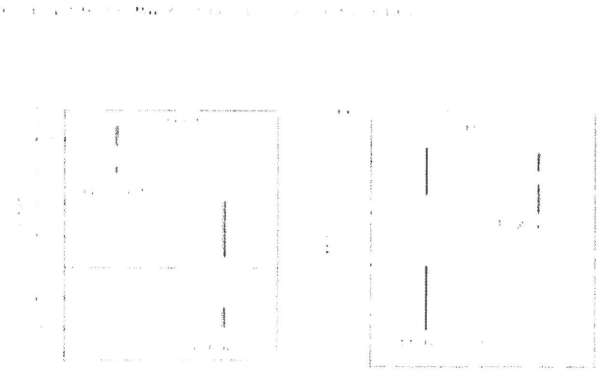


Fig. 3 Box plot of CHA and M/W ratio with the median, interquartile interval and range.
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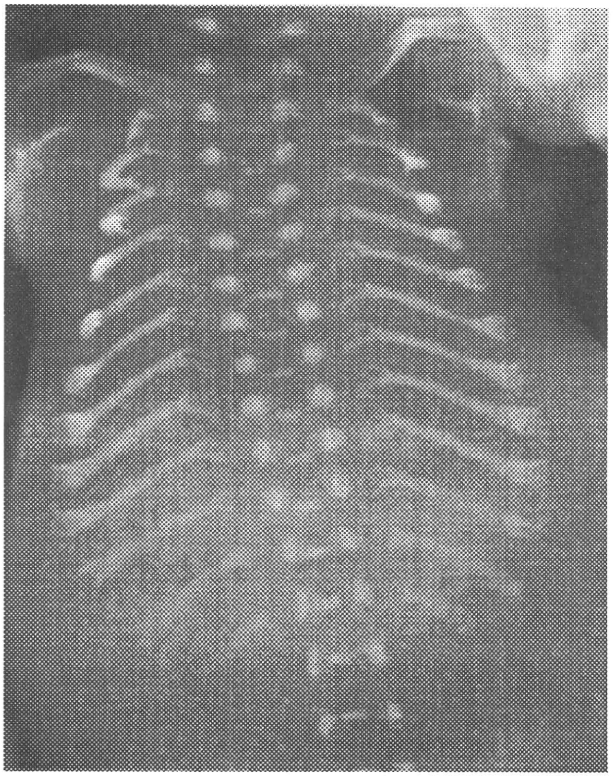
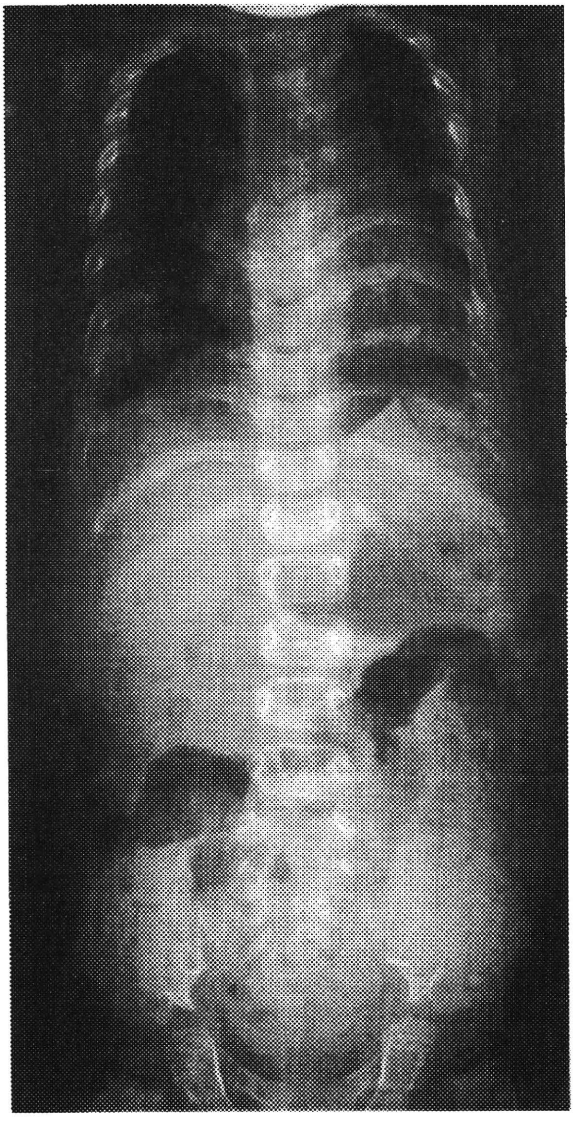


Fig. 4 Examples of the thoracic appearance and measurement of bone dysplasias with thoracic hypoplasia
a) Thanatophoric dysplasia type 1 (stillbirth at 21 weeks of gestation). Note a narrow thorax with cupped anterior ends as well as short long bones with metaphyseal cupping. The posterior ribs show downward sloping. The CHA was -18°, and the M/W ratio was 87%. Despite the presence of severe thoracic hypoplasia in thanatophoric dysplasia, its morphology is different from that seen in upd(14)pat (Fig. 2).

28x35mm (300 x 300 DPI)



b) Ellis-van Creveld syndrome (2 years of age). The thorax appears narrow, and 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.

123x243mm (300 x 300 DPI)

Fig. 5 Comparative observation of age-dependent transition of CHA between the upd(14)pat and RDS groups. Individual shapes represent individual patients.
15x11mm (600 x 600 DPI)

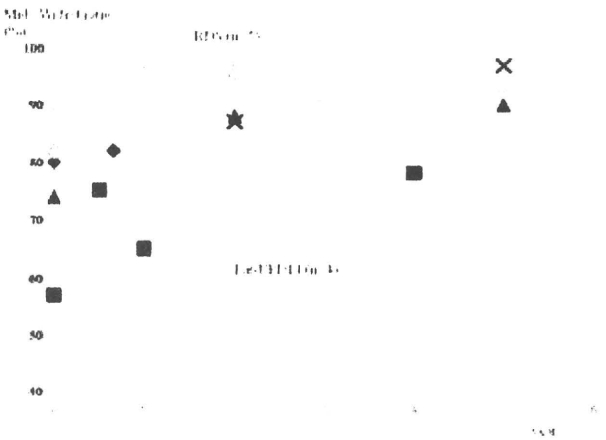


Fig. 6 Comparative observation of age-dependent transition of M/W ratio between the pat-UPD and RDS groups. Individual shapes represent individual patients.
15x11mm (600 x 600 DPI)

Fig. 7. Example of unchangeable coat hanger angle (CHA) and changeable M/W ratio in follow up case (case #5)

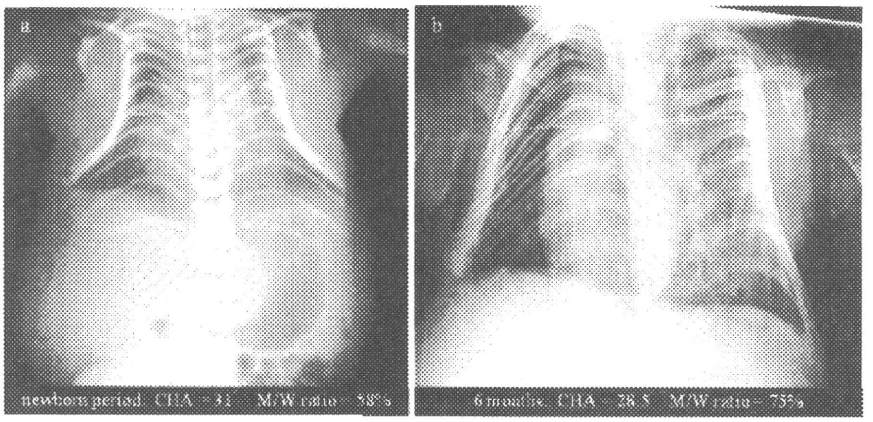
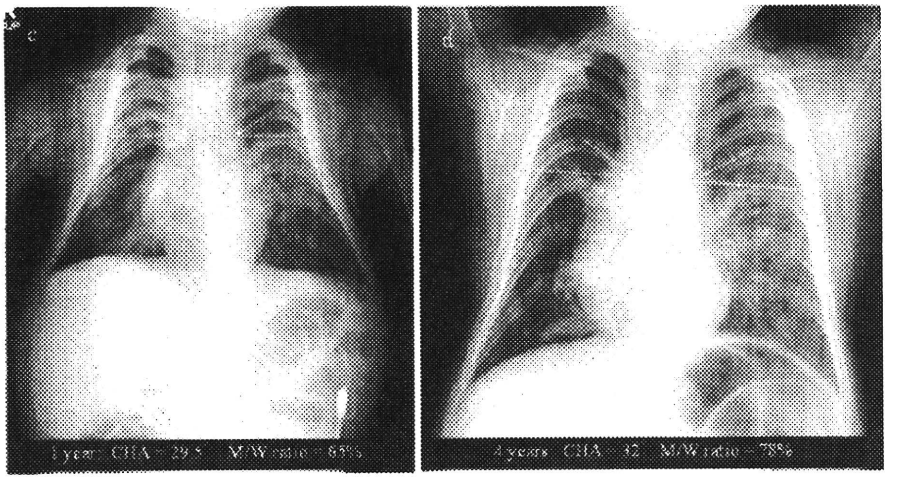


Fig 7 Serial images of the thorax deformity in upd(14)pat
In this case, four images taken at different ages were available: (a) neonatal period, (b) 6 months, (c) 1 year, and (d) 4 years. The CHA was almost consistent regardless of age, while the M/W ratio increased with advancing age. The "coat-hanger"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

81x60mm (300 x 300 DPI)

Fig. 7 Example of unchangeable coat hanger angle (CHA) and changeable M/W ratio in follow up case (case #5)



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81x60mm (300 x 300 DPI)

RESEARCH LETTER

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Prenatal Findings of Paternal Uniparental Disomy 14: Delineation of Further Patient

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TO THE EDITOR:

Human chromosome 14q32.2 carries a cluster of imprinted genes including paternally expressed genes such as *DLK1* and *RTL1* and maternally expressed genes such as *MEG3* (alias *GTL2*) and *RTL1as* (*RTL1* antisense), together with the germline-derived intergenic differentially methylated region (IG-DMR) and the postfertilization-derived *MEG3*-DMR [da Rocha et al., 2008; Kagami et al., 2008a]. Consistent with this, paternal uniparental disomy 14 (upd(14)pat) results in a unique phenotype characterized by facial abnormality, small bell-shaped thorax with coat-hanger appearance of the ribs, abdominal wall defects, placentomegaly, and polyhydramnios [Kagami et al., 2008a,b], and maternal uniparental disomy 14 (upd(14)mat) leads to less-characteristic but clinically discernible features including growth failure [Kotzot, 2004; Kagami et al., 2008a].

For upd(14)pat, this condition has primarily been identified by the pathognomonic chest roentgenographic findings that are obtained immediately after birth because of severe respiratory dysfunction [Kagami et al., 2008a]. However, upd(14)pat has also been suspected prenatally by fetal radiological findings suggestive of small thorax and other characteristic findings [Curtis et al., 2006; Yamanaka et al., 2010]. Here, we report on prenatal findings in a hitherto unreported upd(14)pat patient. The results will serve to the prenatal identification of similarly affected patients and appropriate neonatal care including respiratory management.

A 41-year-old gravida 1, para 0 Japanese woman was referred to Nagoya City University Hospital because of polyhydramnios at 24 weeks of gestation. The polyhydramnios was severe and required repeated amnioreduction (1,600 ml at 26 weeks, 1,800 ml at 29 weeks, 2,000 ml at 32 weeks, and 2,100 ml at 35 weeks). The fetal urine volume was normal (5–12 ml per hr). At 28 weeks of gestation, 3D ultrasound studies were performed, delineating dysmorphic face, anteverted nares, micrognathia and small thorax characteristic of upd(14)pat (Fig. 1), although the differential diagnosis included Beckwith–Wiedemann syndrome and several types of skeletal dysplasia. Thereafter, ultrasound studies were weekly carried out, indicating almost normal fetal growth and normal umbilical artery Doppler.

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At 37 weeks of gestation, a 2,778 g male infant was delivered by cesarean because of fetal distress. The placenta was 1,384 g (gestational age-matched reference, 510 ± 98 g) [Kagami et al., 2008b]. The patient had severe asphyxia, and immediately received appropriate management including mechanical ventilation for 6 days and nasal directional positive airway pressure at the neonatal intensive care unit. At birth, physical examination revealed hairy forehead, blepharophimosis, depressed nasal bridge, anteverted nares, small ears, protruding philtrum, puckered lips, micrognathia, short webbed neck, joint contractures, and diastasis recti, and roentgenograms showed typical bell-shaped thorax with coat-hanger appearance of the ribs (Fig. 2). Coax valga or kyphoscoliosis was uncertain. Discharge from hospital was 35 days after birth. On the last examination at 8 months of age, the patient

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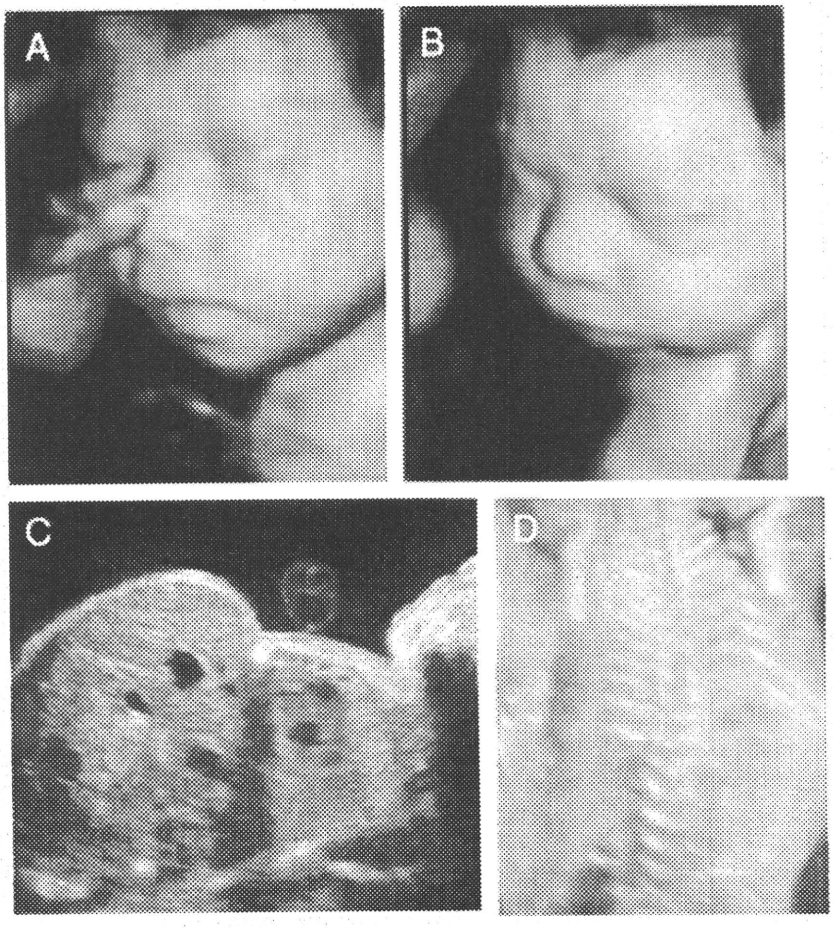

FIG. 1. Prenatal 3D findings at 28 weeks of gestation. A,B: Face appearance with blepharophimosis, depressed nasal bridge, anteverted nares, and micrognathia. C: Small thorax and polyhydramnios. D: Coat-hanger like appearance of the ribs.

required regular oropharyngeal suction and nasogastric tube feeding due to a poor swallowing reflex, and showed developmental delay. At the time of the last evaluation there was no seizure disorder.

To confirm the findings, cytogenetic and molecular studies were performed for the cord blood of the patient by the previously described methods [Kagami et al., 2008a]. This study was approved by the Institutional Review Board Committees at National Center for Child Health and Development and Nagoya City University, and performed after obtaining written informed consent. The karyotype was normal, and metaphase fluorescence in situ hybridization (FISH) analysis with a 202 kb BAC probe containing *DLK1* (RP11-566J3) and a 165 kb BAC probe containing *MG3* and *RTL1/RTL1as* (RP11-123M6) (http://bacpac.-chori.org/) delineated two signals with a similar intensity, respectively. Methylation analysis for bisulfite-treated genomic DNA indicated the presence of paternally derived hypermethylated IG-DMR (CG4 and CG6) and *MEG3*-DMR (CG7) and the absence of maternally derived hypomethylated DMRs. Furthermore, microsatellite analysis was performed using leukocyte genomic DNA of patient and parents, revealing uniparental paternal isodisomy for chromosome 14 (Table I, Fig. 3).

In this patient with molecularly confirmed upd(14)pat, ultrasound studies unequivocally showed typical upd(14)pat phenotypes such as thoracic abnormality and facial dysmorphic features. While this is the first report documenting the facial appearance of the affected fetus, small thorax has been suspected prenatally in five patients with upd(14)pat or epimutations of the IG-DMR and the *MEG3*-DMR, with coat-hanger appearance of the ribs being delineated in one patient [Curtis et al., 2006; Yamanaka et al., 2010]. In this regard, it is notable that polyhydramnios has invariably been identified in upd(14)pat by the second trimester [Kagami et al., 2008a]. It is recommended, therefore, to perform radiological studies for pregnant women with polyhydramnios, to suspect upd(14)pat-compatible clinical features of the fetus. This will permit appropriate counseling and delivery planning at a tertiary

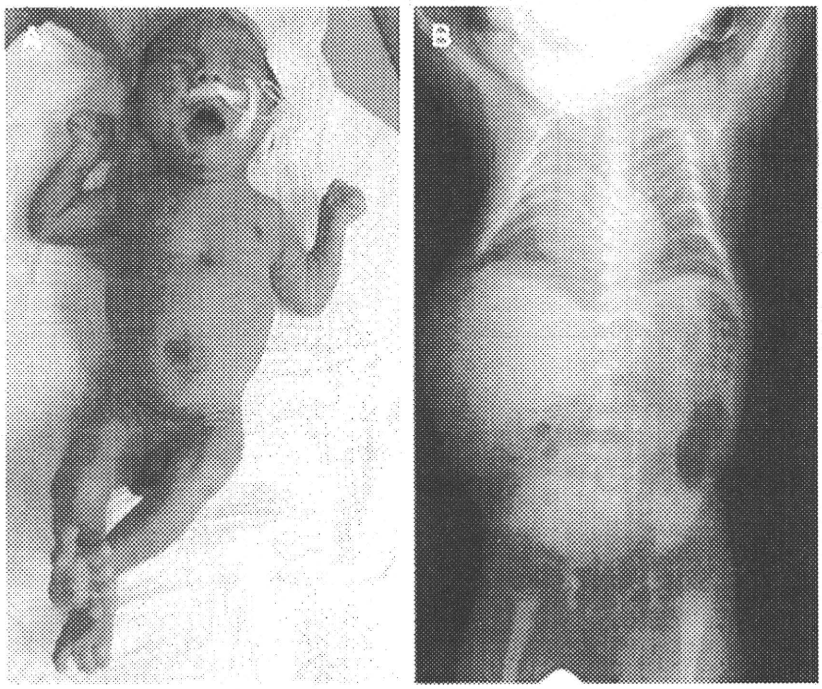

FIG. 2. Postnatal findings at 1 month of age. A: Front view. B: Chest roentgenogram showing bell-shaped thorax with coat-hanger appearance of the ribs.

center with neonatal intensive care as well as pertinent molecular studies using cord blood.

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We thank Dr. Saori Kaneko for her assistance in coordinating this research. We also acknowledge the cooperation of the patient's family in allowing us to publish their information.

TABLE 1. The Results of Microsatellite Analysis

Locus	Location	Mother	Patient	Father	Assessment
D14S80	14q12	98	98	98	N.I.
D14S608	14q12	200	194	194/210	Isodisomy
D14S588	14q23–24.1	114/126	114	114/122	N.I.
D14S617	14q32.12	139/169	143	143/165	Isodisomy
D14S250	14q32.2	159	159	159/167	N.I.
D14S1006	14q32.2	127/139	127	127/139	N.I.
D14S985	14q32.2	135/137	131	131/133	Isodisomy
D14S1010	14q32.33	134/142	142	142/144	N.I.
D14S1007	14q32.33	119	119	119	N.I.

N.I., not informative.
The Arabic numbers indicate the PCR product sizes in bp.
The imprinted region resides at 14q32.2.
D14S985 is located in the intron of *MEG3*.

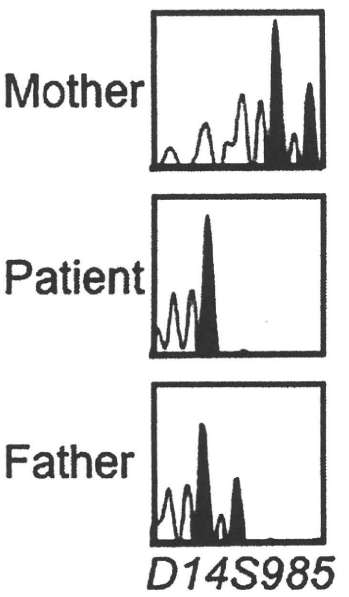

FIG. 3. Microsatellite analysis for *D14S985* residing in the intron of *MEG3*. One of the two peaks in the father is transmitted to the patient, and both of the two peaks in the mother are not inherited by the patient. The PCR fragment size: 135 and 137 bp in the mother, 131 bp in the patient, and 131 and 133 bp in the father. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com]

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Yamanaka M, Ishikawa H, Saito K, Maruyama Y, Ozawa K, Shibasaki J, Nishimura G, Kurosawa K. 2010. Prenatal findings of paternal uniparental disomy 14: Report of four patients. Am J Med Genet Part A 152A:789–791.

RESEARCH LETTER

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Prenatal Findings of Paternal Uniparental Disomy 14: Report of Four Patients

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TO THE EDITOR:

A common finding in neonates with paternal uniparental disomy for chromosome 14 (upd(14)pat) is the abnormal curvature of the ribs that is referred to as a "coat-hanger" appearance. The radiographs of the ribs together with other clinical findings usually lead to correct diagnosis. In the fetus, however, it is difficult to detect this deformity by ultrasonography or other clinical findings associated with upd(14)pat.

We encountered four patients with upd(14)pat at our hospital and followed them during the prenatal and postnatal periods. In one case, it was possible to visualize the typical deformation of the ribs prenatally by X-ray photos, which led to a suspicion of upd(14)pat and allowed us to prepare for postnatal management. Here we present the prenatal findings in our series of upd(14)pat.

All four cases were referred to our hospital, the Kanagawa Children's Medical Center, a tertiary care referral center, from 1999 to 2007. Clinical information is summarized in Table I. Cases 1 and 4 have been partly reported [Kurosawa et al., 2002; Ozawa et al., 2009]. In all four cases polyhydramnios was present prenatally, and all required serial amnioreductions. A small thorax was recognized in all. A fetal omphalocele was detected in two (Cases 1 and 4); however the shapes of these omphaloceles were not typical. Case 1 had a large omphalocele that included massive Wharton's jelly (Fig. 1a). Case 4 showed an omphalocele with a wide base of the hernia which led us to suspect diastasis recti. In contrast to a typical omphalocele, no constriction at the transverse view was observed at the base of the herniated part (Fig. 1b). As upd(14)pat was suspected in Case 3, we attempted to visualize the fetal thorax at 27 weeks of gestation using X-ray. However, due to the fetal position the results were inconclusive. Deformity of the extremities was detected by ultrasonography, but an MRI examination at 32 weeks of gestation did not show any additional findings. In Case 4, an X-ray photo taken at 33 weeks of gestation revealed the "coat-hanger appearance" of the fetal ribs which suggested upd(14)pat (Fig. 2a). This finding led us to inform the parents of the suspected diagnosis and prepare for postnatal care. In this case, an MRI performed at 31 weeks of gestation did not show the distinctive deformity of the thorax probably because it was used to evaluate the atypical shape of the omphalocele. This MRI could not clearly demonstrate the margin of the omphalocele arising from the abdominal wall in the sagital view of the fetal trunk. Because of polyhydramnios, an enlarged abdominal circumference with an atypical omphalocele and the suspicion of a narrow thorax, we suspected upd(14)pat and tried to visualize the deformity of the fetal ribs using three-dimensional ultrasonography (which was inconclusive) and X-ray, which showed the deformity.

Postnatally all infants were born preterm (32–36 weeks of gestation) and required mechanical ventilation at birth (Table I). The birth weights were larger than average for the gestational age. The placental weights were also larger than average for the birth weight and above +2 SD of the mean in three cases. All cases showed a "coat-hanger appearance" of the ribs, and Figure 2 shows the

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TABLE I Clinical Findings of Four Cases

	Case 1[a]	Case 2	Case 3	Case 4[a]
Maternal age	25	27	31	28
Paternal age	26	30	35	29
Fetal findings				
Ultrasonographic findings	Polyhydramnios, small thorax, omphalocele	Polyhydramnios, bell-shaped small thorax, skin edema of the head and neck, Small stomach[b], hepatomegaly, enlarged kidneys, slightly short femur	Polyhydramnios, small thorax, large abdomen, small stomach[b], radial hypoplasia, deformity of the foot	Polyhydramnios, small thorax, omphalocele, slightly short limbs, slightly enlarged lateral ventricles
Required amnioreduction	5 times after 25 weeks	6 times after 29 weeks	4 times after 26 weeks	3 times after 24 weeks
Amniotic fluid karyotyping	46, XX	46, XX	Not performed	46, XX
Mode of delivery	CS	VD	VD	CS
	Rupture of the membrane, Breech presentation (foot)			Arrest of labor
Neonatal outcome				
Gestational week at birth	32 weeks and 3 days	35 weeks and 3 days	34 weeks and 2 days	36 weeks and 3 days
Sex	Female	Female	Male	Female
Birth weight	2,213 g	2,930 g	2,508 g	3,372 g
Mechanical ventilation	Yes (for 1 year and 4 months)	Yes (for 3 years and 9 months and continuing)	Yes	Yes (for 5 days)
Placental weight (g)	470	710	830	970
DNA analysis	upd(14)pat	Epimutation	upd(14)pat	upd(14)pat
Survival	>8 years old	>3 years old	Died at 117 days	>1 year old
Developmental delay	+	+		+

CS, cesarean delivery; VD, vaginal delivery.

[a]Partly reported in Kurosawa et al. [2002] and Ozawa et al. [2009].

[b]It is hard to visualize the stomach pouch.

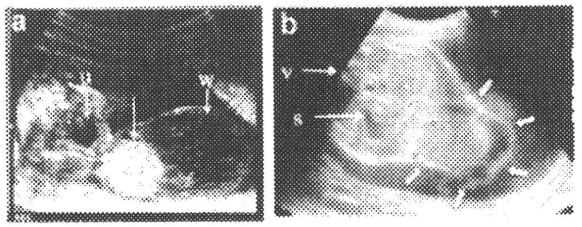

FIG. 1. Transverse view of the fetal abdomen by ultrasonography. a: At 19 weeks of gestation in Case 1. u, Urinary bladder; i, intestine; w, Wharton's jelly. The omphalocele containing the small intestine and massive Wharton's jelly. b: At 33 weeks of gestation in Case 4. v, Vertebra; s, stomach. Short arrows are showing the wide based omphalocele.

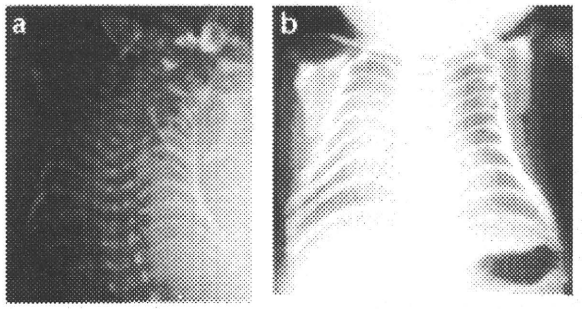

FIG. 2. Prenatal and postnatal chest X-ray photo of Case 4. a: 33weeks of gestation. b: After birth.

comparison of prenatal and postnatal X-rays in Case 4. Surgical repair of the omphalocele was successfully performed on day 1 in Cases 1 and 4 with both having herniation containing only the small intestine. All cases survived the neonatal period. Case 3 died at 177 days old due to hepatic failure. Case 1, a female, had turned 8 years old, and her physical health was good. Mental retardation and developmental delay were recognized in all living cases with varying degrees of severity.

The DNA analyses (kindly performed by Dr. Tsutomu Ogata and Dr. Masayo Kagami, Department of Endocrinology and Metabolism, National Research Institute for Child Health and Development, Tokyo.) demonstrated isodisomy of chromosome 14 was present in Cases 1, 3, and 4, and an epimutation of the 14q32.2 region in Case 2.

While neonates with upd(14)pat have some distinctive features, these are difficult to detect prenatally. Mattes et al. [2007] reviewed 19 cases of upd(14)pat including one overlapping case with this report (Case 1) [Kurosawa et al., 2002]. Together with our other three cases, a total of 22 cases have been reported. Polyhydramnios was mentioned in 20 cases. Therefore, when presented with polyhydramnios that requires serial amnioreductions, we recommend evaluation of the shape of both the thorax and abdomen of the fetus using medical imaging techniques in addition to ultrasonography. This is especially important when atypical omphalocele is present. The X-ray, which can be performed with less radiation compare to a three-dimensional or helical CT, is a simple method as long as the fetal position is suitable to visualize the distinctive shape of the thorax and once fetal ossification becomes detectable.

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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]．

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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.
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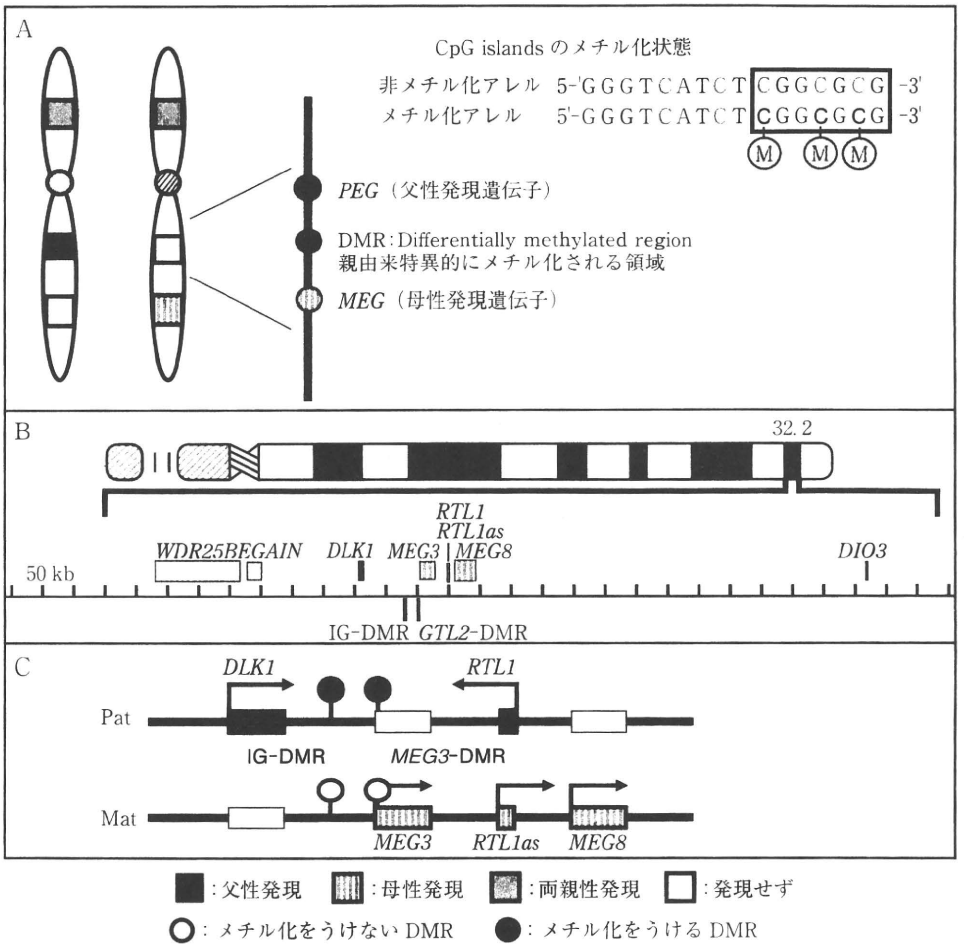

図1　14q32.2 インプリンティング領域と制御機構
A. インプリンティング遺伝子のモデル　B. 14q32.2 インプリンティング領域
C. DLK1-DIO3 ドメインの調節機構

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．

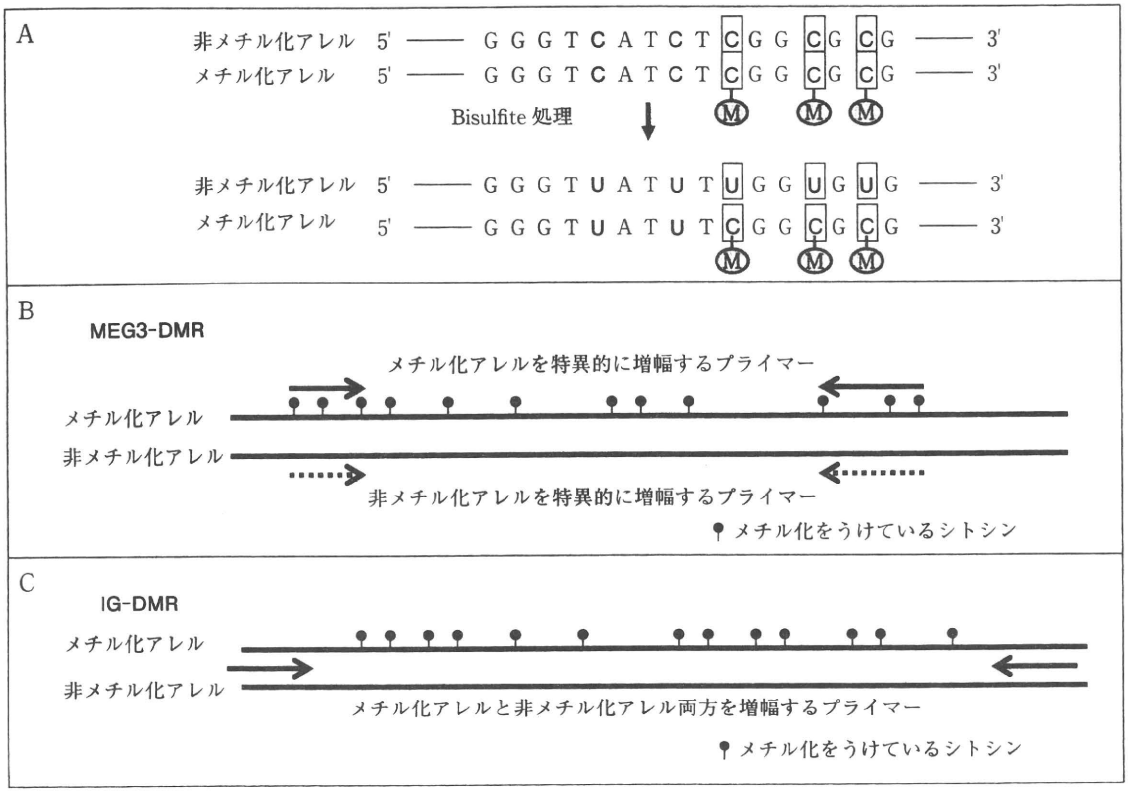

図2 メチル化解析の方法
A. Bisulfite 法 B. MEG3-DMR のメチル化テスト
C. IG-DMR の Bisulfite Sequence 法

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

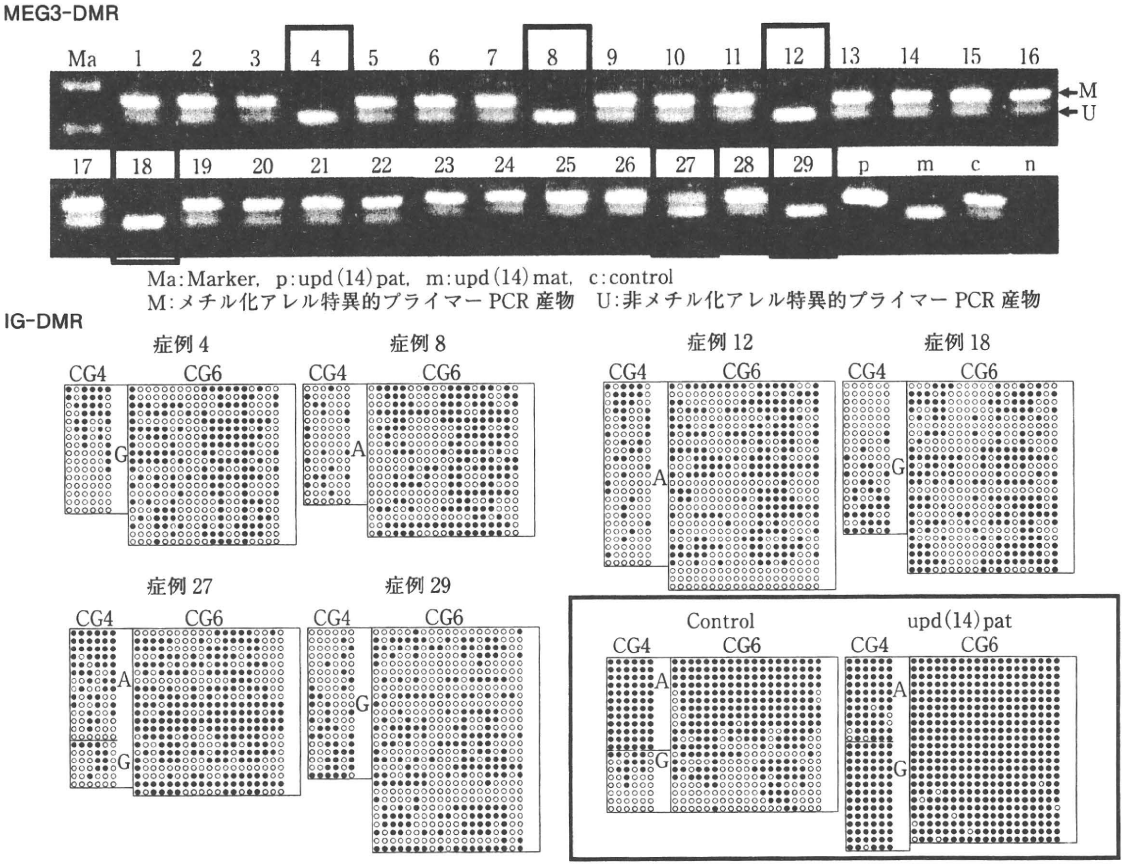

図 3　メチル化解析結果
上段：MEG3-DMR メチル化テスト結果　下段：Bisulfite Sequence 結果

プローブ 1，FISH プローブ 2 は，IG-DMR 領域を包含する 5,104 bp の領域と MEG3-DMR を包含する 5,182 bp の領域を LA-PCR で増幅した PCR-産物をラベルして作成した[4]．

3 結　果

＜メチル化解析＞

MEG3-DMR のメチル化テストにおいて，29 症例中 5 症例で upd(14) mat のメチル化パターンに一致する低メチル化を示した．症例 27 はメチル化アレル特異的プライマーでもバンドを得られたが，正常に比較してバンドの強度は低下していた．この 6 症例につき，IG-DMR のメチル化状態を解析したところコントロールに比較し低メチル化を示した．症例 27 は低メチル化傾向を示したが，モザイクにメチル化クローンが認められた（**図 3**）．

＜マイクロサテライトマーカー解析＞

症例 4，8，18，29 は母親性ダイソミーであることが証明された．症例 27 は母親性ダイソミーと正常のモザイクであった．症例 12 はダイソミーが否定された（**図 4 a，b，c**）．

＜FISH 解析＞

症例 12 はダイソミーが否定されたため，FISH 解析を行った．シグナルは FISH プローブ 1，2 ともに 2 コピー認められ，微小欠失は否定された（**図 4 d**）．