RESEARCH LETTER

AMERICAN JOURNAL OF medical genetics

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

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Received 5 March 2010; Accepted 2 August 2010

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.

How to Cite this Article:
Suzumori N, Ogata T, Mizutani E, Hattori Y, Matsubara K, Kagami M, Sugiura-Ogasawara M. 2010. Prenatal Findings of Paternal Uniparental Disomy 14: Delineation of Further Patient.
Am J Med Genet Part A 152A:3189–3192.

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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Published online 24 November 2010 in Wiley Online Library (wileyonlinelibrary.com)
DOI 10.1002/ajmg.a.33719

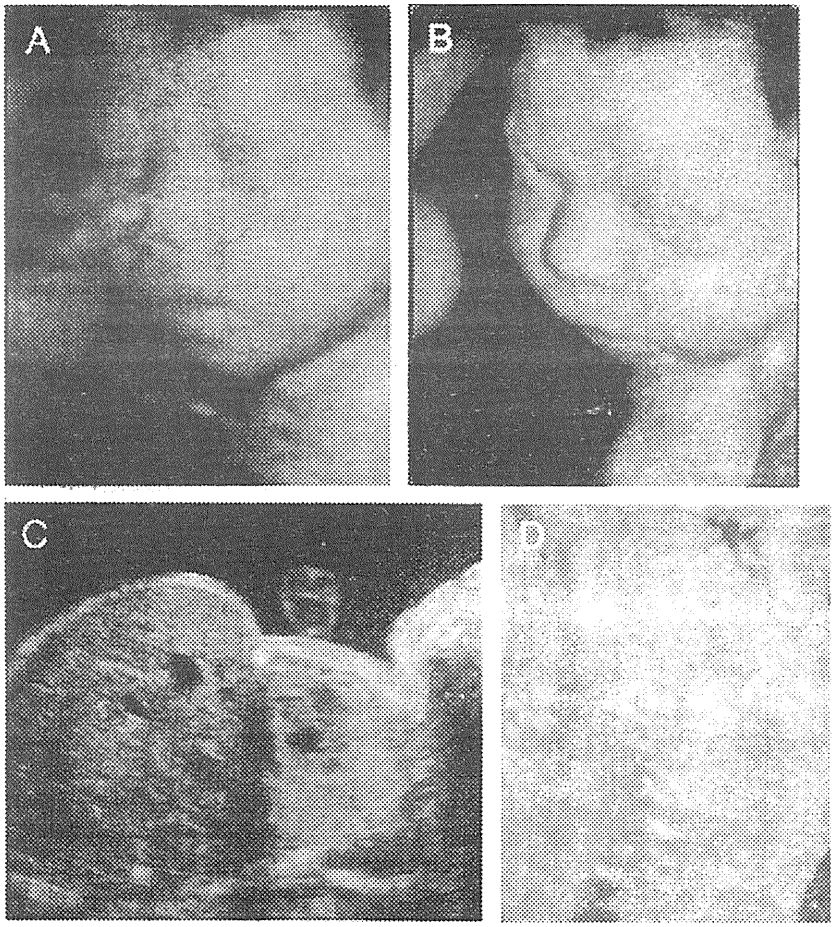

FIG. 1. Prenatal 3D findings at 28 weeks of gestation. A,B: Face appearance with blepharophimosis, depressed nasal bridge, anteverted nares, and micrognathia. C: Small thorax and polyhydramnios. D: Coat-hanger like appearance of the ribs.

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

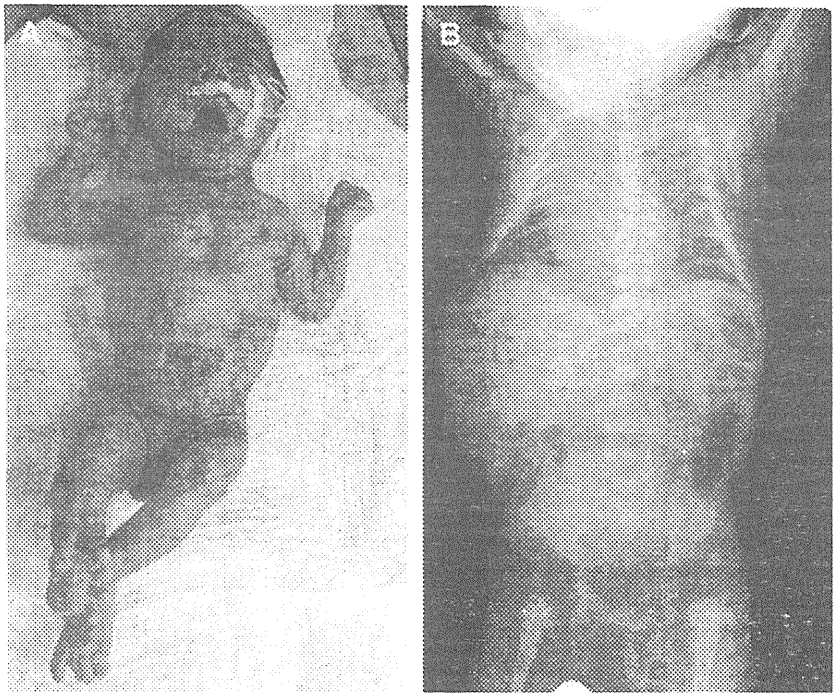

FIG. 2. Postnatal findings at 1 month of age. A: Front view. B: Chest roentgenogram showing bell-shaped thorax with coat-hanger appearance of the ribs.

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

ACKNOWLEDGMENTS

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 I. 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.

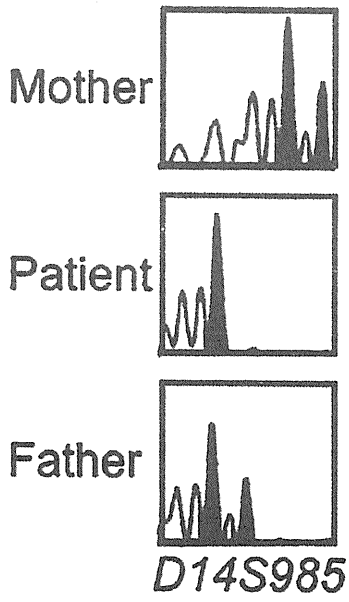

FIG. 3. Microsatellite analysis for *D14S985* residing in the intron of *MEG3*. One of the two peaks in the father is transmitted to the patient, and both of the two peaks in the mother are not inherited by the patient. The PCR fragment size: 135 and 137 bp in the mother, 131 bp in the patient, and 131 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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Journal of Human Genetics (2011) 56, 566–571
 1434-5161/11 $32.00
www.nature.com/jhg

ORIGINAL ARTICLE

Maternal age effect on the development of Prader–Willi syndrome resulting from upd(15)mat through meiosis 1 errors

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Prader–Willi syndrome (PWS) is primarily caused by deletions involving the paternally derived imprinted region at chromosome 15q11.2–q13 and maternal uniparental disomy 15 (upd(15)mat). The underlying mechanisms for upd(15)mat include trisomy rescue (TR), gamete complementation (GC), monosomy rescue and post-fertilization mitotic error, and TR/GC is mediated by non-disjunction at maternal meiosis 1 (M1) or meiosis 2 (M2). Of these factors involved in the development of upd(15)mat, M1 non-disjunction is a maternal age-dependent phenomenon. We studied 117 Japanese patients with PWS and identified deletions in 84 patients (Deletion group) and TR/GC type upd(15)mat through M1 non-disjunction in 15 patients (TR/GC (M1) group), together with other types of abnormalities. Maternal age was significantly higher in TR/GC (M1) group than in Deletion group (median (range), 37 (35–45) versus 30 (19–42); $P=1.0\times10^{-7}$). Furthermore, delayed childbearing age became obvious since the year 2003 in Japan, and relative frequency of TR/GC (M1) group was significantly larger in patients born since the year 2003 than in those born until the year 2002. The results imply that the advanced maternal age at childbirth is a predisposing factor for the development of upd(15)mat because of increased M1 errors.
Journal of Human Genetics (2011) **56**, 566–571; doi:10.1038/jhg.2011.59; published online 2 June 2011

Keywords: maternal age effect; meiosis 1; non-disjunction; Prader-Willi syndrome; upd(15)mat

INTRODUCTION

Prader–Willi syndrome (PWS) is a developmental disorder associated with various dysmorphic, neurologic, cognitive, endocrine and behavioral/psychiatric features.[1] It is caused by absent expression of paternally derived genes on the imprinted region at chromosome 15q11.2–q13, and previous studies have indicated that deletions of the paternally derived imprinted region and maternal uniparental disomy 15 (upd(15)mat) account for ~70 and ~25% of PWS patients, respectively.[1] The remaining PWS patients have rare abnormalities such as epimutations (hypermethylation) of the PWS imprinting center (IC), at the differentially methylated region encompassing exon 1 of *SNRPN* and microdeletions involving the PWS-IC or HBII-85 small nucleolar RNAs distal to the PWS-IC.[2–4]

Upd(15)mat are primarily caused by four mechanisms; that is, trisomy rescue (TR), gamete complementation (GC), monosomy rescue (MR) and post-fertilization mitotic error (PE).[5] TR refers to a condition in which chromosome 15 of paternal origin is lost from a zygote with trisomy 15, formed by fertilization between a disomic oocyte and a normal sperm. GC results from fertilization of a disomic oocyte with a nullisomic sperm. MR refers to a condition in which chromosome 15 of maternal origin is replicated in a zygote with monosomy 15, formed by fertilization between a normal oocyte and a nullisomic sperm. PE is an event after formation of a normal zygote. In this regard, a disomic oocyte specific to TR and GC 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.[6]

It is predicted, therefore, that the relative frequency of TR/GC-type upd(15)mat through M1 non-disjunction is high in PWS patients born to aged mothers and is increasing in countries where childbearing age is rising. In this context, previous studies have revealed a significantly higher maternal age in PWS patients with upd(15)mat than in those with deletions,[7,8] a significantly higher relative frequency of upd(15)mat in patients born to mothers aged $\geqslant$35 years than in those born to mothers aged $<$35 years[9] and a significantly increased relative frequency of upd(15)mat in PWS patients $<$5 years of age in United Kingdom where childbearing age is increasing.[10] In these

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Received 3 March 2011; revised 29 April 2011; accepted 8 May 2011; published online 2 June 2011

studies, however, as underlying mechanisms for upd(15)mat have not been examined, it remains to be clarified whether such maternal age effect on the occurrence of upd(15)mat is primarily mediated by M1 non-disjunction. Furthermore, after studying underlying mechanisms for upd(15)mat by microsatellite analysis, Robinson *et al.*[11] have mentioned that maternal age effect is similar between M1 and M2 errors. Thus, it remains to be clarified whether advanced maternal age is relevant to the occurrence of TR/GC type upd(15)mat through M1 errors.

Here, we report that the advanced maternal age at childbirth constitutes a risk factor for TR/GC type upd(15)mat through M1 non-disjunction.

MATERIALS AND METHODS

This study was approved by the Institute Review Board Committees at the National Center for Child Health and Development and Dokkyo University Koshigaya Hospital, and performed after obtaining informed consent.

PWS patients

This study consisted of 117 Japanese PWS patients (72 male patients and 45 female patients) who satisfied the following selection criteria: (1) normal karyotype in all the 50 lymphocytes examined, (2) hypermethylated PWS-IC that was confirmed by methylation analysis for bisulfite-treated leukocyte genomic DNA, using methylated and unmethylated allele-specific PCR primers (Supplementary Figure 1),[12] and (3) positive data on the maternal age at childbirth (parental age was not found in two aged patients who had left our follow-up and whose hospital records had been discarded and in one patient who was born after artificial insemination by donor).

Molecular studies

We performed fluorescence *in situ* hybridization analysis, microsatellite analysis and multiplex ligation-dependent probe amplification (MLPA) analysis. For fluorescence *in situ* hybridization analysis, an ~125-kb probe identifying a region encompassing *SNRPN* was hybridized to lymphocyte metaphase spreads, together with a CEP 15 probe for *D15Z1* and a probe for *PML* on 15q22 utilized as internal controls. The probe for the *SNRPN* region was labeled with digoxigenin and detected by rhodamine anti-digoxigenin, and the control probes were detected according to the manufacturer's protocol (Abbott, Chicago, IL, USA). For microsatellite genotyping, PCR amplification was performed for 13 microsatellite loci on chromosome 15, using fluorescently labeled forward primers and unlabeled reverse primers. Subsequently, the PCR products were determined for size on a CEQ8000 autosequencer (Beckman Coulter, Fullerton, CA, USA). For MLPA analysis, we utilized a commercially available MLPA probe mix (ME028-B1) for multiple segments on the chromosome 15 imprinted region, including the PWS-IC and three portions within the HBII-85 small nucleolar RNAs (MRC-Holland, Amsterdam, The Netherlands). The procedure was as described in the manufacturer's instructions. The primers utilized in this study are summarized in Supplementary Table 1.

Classification of PWS patients

The PWS patients were classified into several groups, according to the underlying (epi) genetic causes (Figure 1). In particular, upd(15)mat was divided into three groups by the previously reported methods[13] (Supplementary Figure 2): (1) heterodisomy for at least one of the three adjacent pericentromeric (<4 Mb from the centromere) microsatellite loci (*D15S541*, *D15S542* and *D15S1035*) was regarded as indicative of TR/GC type upd(15)mat through M1 non-disjunction (TR/GC (M1) group), (2) the combination of isodisomy for the pericentromeric microsatellite loci and heterodisomy for at least one middle to distal microsatellite loci was interpreted as indicative of TR/GC type upd(15)mat through M2 non-disjunction (TR/GC (M2) group) and (3) isodisomy for all the informative microsatellite loci was regarded as indicative of MR/PE type upd(15)mat (MR/PE group). However, it is usually impossible to distinguish between TR and GC, and between MR and PE on the basis of microsatellite data, although identification of segmental isodisomy or mosaicism with a normal cell lineage is unique to PE.[14,15]

Analysis of parental ages

We compared parental ages between different groups and between two different time periods (until the year 2002 and since the year 2003), and relative frequency of each group between the two time periods. The setting of the two time periods was based on the Annual Vital Statistics Data from the Japanese Ministry of Health, Labor and Welfare (http://www.mhlw.go.jp/toukei/list/81-1.html). The maternal age producing the largest number of live births changed from 25–29 years to 30–34 years, and that producing the third largest number of live births changed from 20–24 years to 35–39 years, between the two time periods (Supplementary Figure 3).

Statistical significance of the median age was examined by the Mann–Whitneys *U*-test, that of the correlation between parental ages by Spearman's rank correlation test, and that of relative frequency by the Fisher's exact probability test. $P<0.05$ was considered significant.

RESULTS

Classification of PWS patients

The results are shown in Figure 1. Fluorescence *in situ* hybridization analysis revealed heterozygous deletions in 84 of the 117 patients (Supplementary Figure 4;Deletion group). Then, microsatellite genotyping was carried out in 27 of the 33 patients without deletions, classifying 15 patients as TR/GC (M1) group, seven patients as TR/GC (M2) group and three patients as MR/PE group (Figure 2;in the remaining six patients, further studies were refused by the parents). There was no finding indicative of segmental isodisomy or mosaicism. Finally, MLPA was performed in the remaining two non-upd(15)mat patients, identifying no microdeletion affecting the PWS-IC. Thus, the two patients were classified as Epimutation group.

Analysis of parental ages

Distribution of parental ages in each group is shown in Figure 3a, and parental age data are summarized in Table 1. Maternal ages were invariably ⩾35 in TR/GC (M1) group. Furthermore, comparison of maternal ages in Deletion, TR/GC (M1) and TR/GC (M2) groups with >5 patients revealed significant difference between Deletion and TR/GC (M1) groups ($P=1.0\times10^{-7}$), but not between Deletion and TR/GC (M2) groups ($P=0.19$), and between TR/GC (M1) and TR/GC (M2) groups ($P=0.085$). Paternal ages showed similar tendency, with

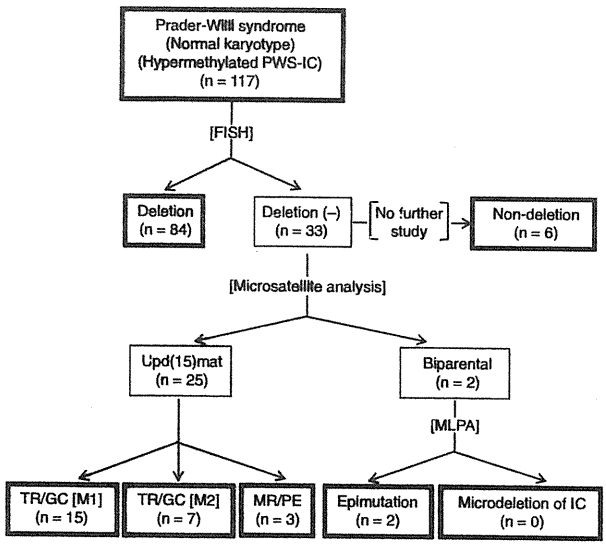

Figure 1 Classification of 117 Japanese patients with Prader–Willi syndrome phenotype.

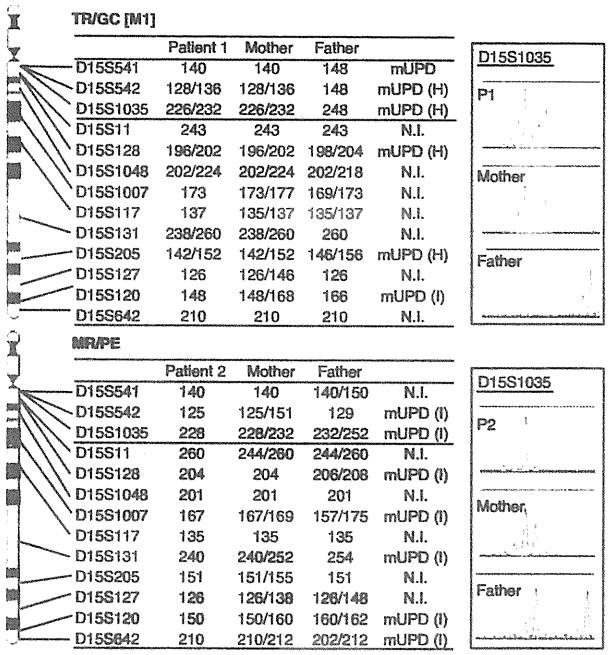

Figure 2 Chromosomal locations of the examined microsatellite loci and representative results. MUPD, maternal uniparental disomy (unknown for heterodisomy or isodisomy); mUPD (H), maternal uniparental heterodisomy; mUPD (I), maternal uniparental isodisomy; N.I., not informative. Pericentromeric loci are present in a heterodisomic status in patient 1, and this is consistent with trisomy rescue/gamete complementation (meiosis 1) (TR/GC (M1)) type maternal uniparental disomy 15 (upd(15)mat). For D15S1035, for example, both of the heterozygous maternal alleles are inherited by patient 1, whereas the homozygous paternal alleles are not transmitted to patient 1; this demonstrates mUPD (H) for this locus. In patient 2, all informative loci are present in an isodisomic condition, and this is compatible with monosomy rescue/post-fertilization mitotic error (MR/PE) type upd(15)mat. For D15S1035, for example, one of the two heterozygous maternal alleles is transmitted to patient 2, whereas both of the heterozygous paternal alleles are not inherited by patient 2; this demonstrates mUPD (I) for this locus.

significant difference between Deletion and TR/GC (M1) groups ($P=8.8\times10^{-5}$), but not between Deletion and TR/GC (M2) groups ($P=0.39$), and between TR/GC (M1) and TR/GC (M2) groups ($P=0.39$). However, whereas a significant correlation was observed between maternal and paternal ages in Deletion and TR/GC (M2) groups, there was no significant correlation between maternal and paternal ages in TR/GC (M1) group because of relatively advanced maternal ages in this group (Figure 3b). In addition, whereas maternal ages at childbirth were grossly similar between Deletion and TR/GC (M2) groups and the Japanese general population (the mean parental ages at childbirth in Japan were based on the data registered in the Ministry of Health, Labor and Welfare; http://www.mhlw.go.jp/toukei/list/81-1.html), they were obviously higher in TR/GC (M1) group than in the Japanese general population. Paternal ages at childbirth were grossly similar between Deletion group and the Japanese general population and tended to be higher in TR/GC (M1) and TR/GC (M2) groups than in the Japanese general population.

Relative frequency of each group markedly differed between 75 patients born until 2002 and 42 patients born since 2003 (Figure 3c). Here, TR/GC (M1) was indicated in three of the 75 patients born until the year 2002, and six non-deletion type patients were invariably born until the year 2002. Thus, TR/GC (M1) group accounted for at least three and up to nine of the 75 patients born until the year 2002, and 12 of the 42 patients born since the year 2003. Thus, the relative frequency of TR/GC (M1) was assessed to be significantly different, with the *P*-values being 1.8×10^{-7} for 3/75 versus 12/42, and 0.025 for 9/75 versus 12/42. In addition, there was no significant change in the parental ages of each group between the two time periods, although the maternal ages at birth of all the patients significantly differed between the two time periods.

DISCUSSION

The present study revealed deletions in 84 patients, upd(15)mat in 25 patients and epimutations in 2 patients. In addition, whereas microsatellite and MLPA analyses were not performed in six patients with non-deletion, the present and the previous studies argue that most of them have upd(15)mat, especially TR/GC (M1) type upd(15)mat.[1,13] Thus, the relative frequency of deletions, upd(15)mat and other rare causes appears to be similar between Japanese patient and previously reported Caucasian patients.[1]

Notably, the present study implies that advanced maternal age at childbirth constitutes a risk factor for the development of TR/GC (M1) type upd(15)mat. Indeed, maternal ages were significantly higher in TR/GC (M1) group than in Deletion group, which is free from maternal age effect. Although a significant difference was not found between maternal age-dependent TR/GC (M1) group and maternal age-independent TR/GC (M2) group, this would primarily be due to the small number of TR/GC (M2) group. Furthermore, the relative frequency of TR/GC (M1) group significantly increased since the year 2003 when delayed childbearing age became obvious, and the advanced maternal ages at birth since the year 2003 were primarily associated with the high frequency of TR/GC (M1) group rather than the advanced maternal ages in each group. Although it was impossible to distinguish between TR and GC, and between MR and PE,[16] this would not pose a major problem. The patients with M1 non-disjunction are included only in TR/GC (M1) group.

Paternal and environmental factors should also be considered for the present results. For a paternal factor, the frequencies of microdeletions and nullisomic sperms might increase with age.[17] However, paternal ages at childbirth in each group were similar between the two time periods, and the relative frequency of Deletion group actually decreased since the year 2003. Furthermore, whereas nullisomic sperms can be a background of the development of GC, concomitant occurrence of a nullisomic sperm and a disomic oocyte must be extremely rare. Rather, nullisomic sperms would primarily constitute an underlying factor for the development of maternal age-independent MR. For an environmental factor, it is predicted that chemical materials are increasing with time and that aged parents are exposed to such materials for a long time. In this regard, it has been reported that exposure to environmental chemicals may exaggerate the occurrence of aneuploidies in females.[18] Thus, the environmental factor might be relevant to the recent increase of TR/GC (M1) group, although it is unlikely that this factor constitutes the major cause of the increased TR/GC (M1) type upd(15)mat. In males, whereas it has been reported that exposure to chemical materials might facilitate the occurrence of PWS, the relative frequency of genetic causes remained unchanged in PWS patients born to such males.[19–21] Collectively, the effects of such non-maternal age factors would remain small, if any, although further careful examinations are required for the precise evaluation of the maternal age effect on the occurrence of TR/GC (M1).

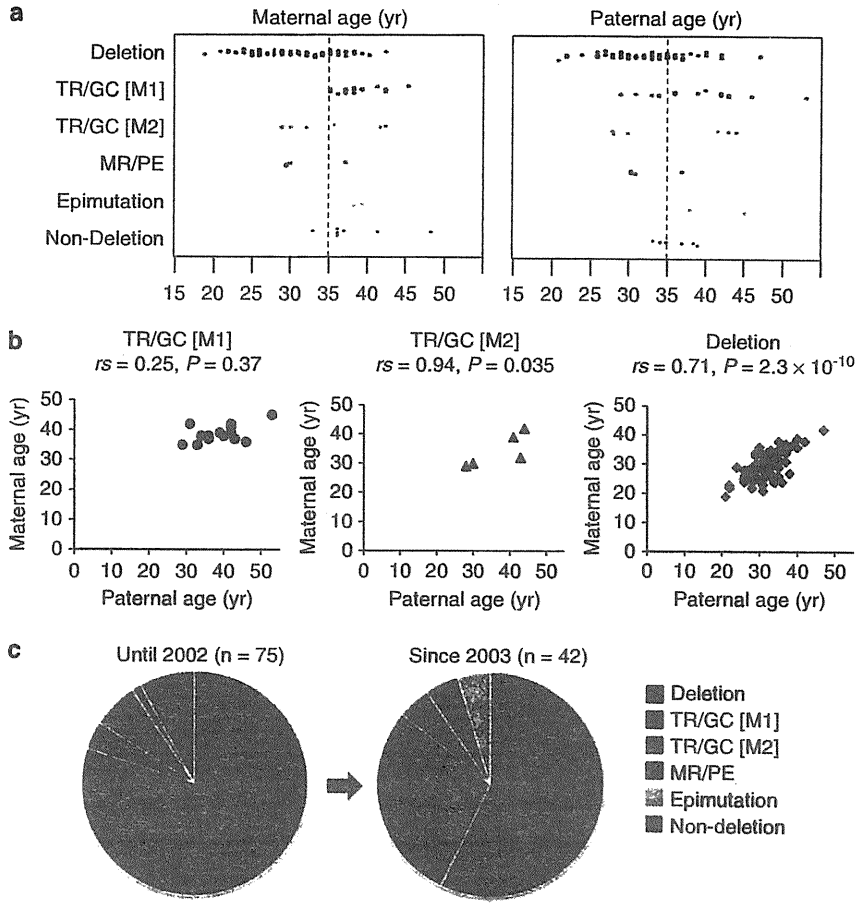

Figure 3 Analysis of parental ages at childbirth. (**a**) The distribution of parental ages in each group. The light pink and blue vertical bars represent the mean maternal and paternal ages at childbirth from the year 1970 to the year 2008. (**b**) Correlation between maternal and paternal ages at childbirth. Significant correlation is observed in trisomy rescue/gamete complementation (meiosis 2) (TR/GC (M2)) and Deletion groups, but not in trisomy rescue/gamete complementation (meiosis 1) (TR/GC (M1)) group because of relatively advanced maternal age. (**c**) Relative frequency of each group in 75 patients born until the year 2002 (n=60, 3, 5, 1, 0 and 6 for Deletion, TR/GC (M1), TR/GC (M2), monosomy rescue/post-fertilization mitotic error (MR/PE), epimutation and non-deletions groups, respectively) and in 42 patients born since the year 2003 (n=24, 12, 2, 2, 2 and 0 for Deletion, TR/GC (M1), TR/GC (M2), MR/PE, Epimutation and Non-deletions groups, respectively).

Several points should be made with regard to the present study. First, we classified upd(15)mat primarily on the basis of the results of three pericentromeric microsatellite loci, with the assumption of no recombination between the centromere and the three loci, as have been employed in the previous study.[13] The methods would be basically acceptable, because the three loci reside within a 4 Mb region from the centromere and a recombination is relatively rare in the centromeric regions.[22] However, it remains possible that a cryptic recombination(s) might have occurred in the pericentromeric region.

Second, upd(15)mat may also be caused by maternal age-dependent meiotic sister chromatid pre-division that can lead to aneuploid oocytes, including disomic oocytes specific to TR/GC.[23] In this regard, as such disomic oocytes can have various patterns of isodisomic and heterodisomic regions, it is impossible to discriminate between upd(15)mat through sister chromatid pre-division and that through conventional meiotic non-disjunction by microsatellite analysis. Thus, the patients classified as TR/GC (M1) group may have upd(15)mat due to maternal age-dependent conventional non-disjunction at M1 and maternal age-dependent sister chromatid pre-division, whereas those classified as TR/GC (M2) group may have upd(15)mat due to maternal age-independent conventional non-disjunction at M2 and maternal age-dependent sister chromatid pre-division. However, even if not all the patients classified as TR/GC (M1) group have upd(15)mat due to conventional non-disjunction at M1, it can be concluded that maternal age-dependent factors still have a critical role in the occurrence of upd(15)mat in patients classified as TR/GC (M1) group. In addition, possible mixture of maternal age-dependent and -independent factors in patients classified as TR/GC (M2) group may be relevant to the lack of significant difference in the maternal age between TR/GC (M2) and Deletion groups, and between TR/GC (M2) and TR/GC (M1) groups.

Lastly, whereas fluorescence *in situ* hybridization analysis has been routinely performed at commercial laboratories since the year 1993 in Japan, detailed molecular studies including microsatellite analysis are usually available only in institutional laboratories. Thus, a substantial fraction of patients without deletions may have remained undiagnosed or misdiagnosed, without receiving further studies including micro-

Table 1 Parental ages (year) at childbirth

	Deletion	*TR/GC (M1)*	*TR/GC (M2)*	*MR/PE*	*Epimutation*	*Non-deletion*	*All patients*	*General population*
Maternal age								
Total								
Median	30	37	31	30	38.5	36	32	27.5–30.9
Range	19–42	35–45	29–42	29–37	38–39	30–48	19–48	
Number	84	15	7	3	2	6	117	
Until 2002								
Median	29	37	32	29	—	36	30[a]	
Range	19–42	35–37	29–42	—	—	30–48	19–48	
Number	60	3	5	1	0	6	75	
Since 2003								
Median	32.5	38.5	35.5	33.5	38.5	—	35[a]	
Range	23–39	35–45	30–41	30–37	38–39	—	23–45	
Number	24	15	2	2	2	0	42	
Paternal age								
Total								
Median	32.5	40	35.5	31	41.5	36	33	30.6–33.0
Range	21–47	29–53	28–44	28–37	38–45	33–39	21–53	
Number	82[b]	15	6[c]	3	2	6	114[b,c]	
Until 2002								
Median	32.5	43	35.5	28	—	36	33	
Range	21–47	33–43	28–44	—	—	33–39	21–47	
Number	58[b]	3	4[c]	1	0	6	72[b,c]	
Since 2003								
Median	32.5	39.5	35.5	34	41.5	—	34.5	
Range	22–40	29–53	30–41	31–37	38–45	—	22–53	
Number	24	12	2	2	2	0	42	

Abbreviations: GC, gamete complementation; M1, meiosis 1; M2, meiosis 2; MR,monosomy rescue; PE, post-fertilization mitotic error; TR, trisomy rescue.
The data of the general population indicate the range of the mean parental ages at childbirth from the year 1970 to 2008.
[a]*P*-value=0.00017.
[b]Paternal age was not found in two old patients who had left our follow-up and whose hospital records had been discarded.
[c]Paternal age was not identified in one patient who was born after artificial insemination by donor.

satellite analysis at appropriate institutions. In this regard, considering the opportunity to receive detailed molecular studies, it is possible that upd(15)mat is overlooked more frequently in aged patients than in young patients. If so, this may be relevant to the significant difference in the relative frequency of TR/GC (M1) group between the two time periods ('since the year 2003' versus 'until the year 2002').

In summary, the results imply that the advanced maternal age at childbirth is a predisposing factor for the development of upd(15)mat because of increased M1 errors. This notion is applicable to maternal upd in general, as well as to trisomies. However, there are several caveats as discussed in the above, and the number of patients, especially those classified as TR/GC (M2) group, is small. Thus, further careful studies using a large number of patients are necessary in the future.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by Grants for Research on Intractable Diseases (H22-165) and for Health Research on Children, Youth and Families (H21-005) from the Ministry of Health, Labor and Welfare, and by Grants-in-Aid for Scientific Research (A) (22249010) and Grant-in-Aid for Young Scientists (B) (22791022) from the Japan Society for the Promotion of Science (JSPS).

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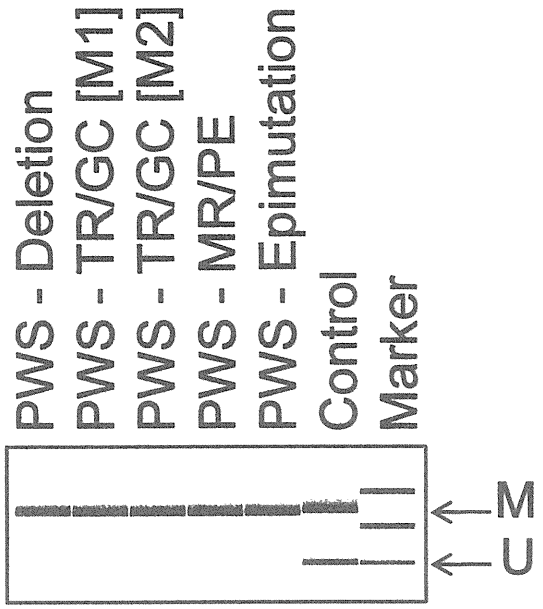
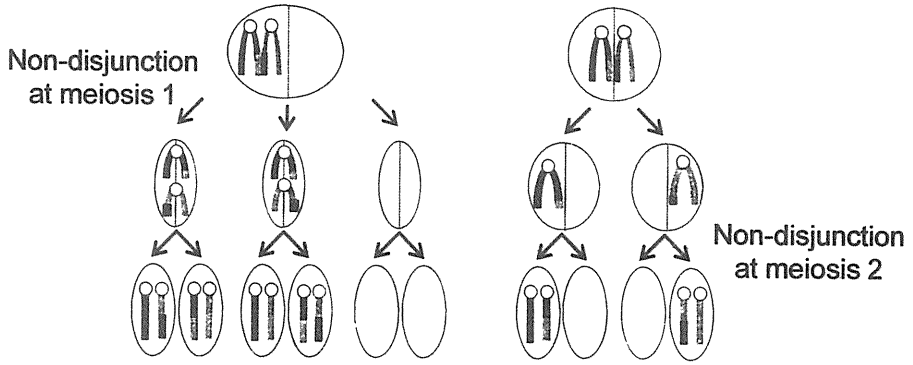
Supplementary Information accompanies the paper on Journal of Human Genetics website (http://www.nature.com/jhg)

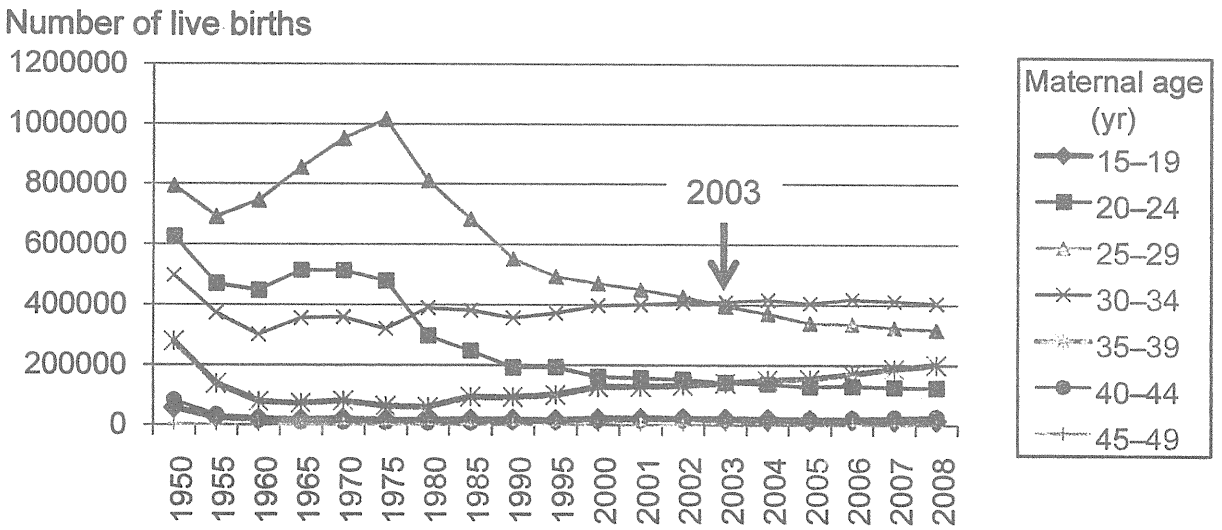


Supplementary Figure 1 Representative results of methylation analysis for the PWS-IC. Bisulfite-treated leukocyte genomic DNA was PCR-amplified for the PWS-IC, using methylated (M) and unmethylated (U) allele-specific primers (Kubota *et al.*, 1997). After PCR amplification, the PCR products were loaded onto LabChip with Gel-Dye Mix (Agilent, Santa Clara, CA). While both M and U alleles are delineated in a control subject, only M allele is identified in Prader-Willi syndrome (PWS) patients irrespective of the underlying causes. The PCR fragment size is 174 bp for the M allele and 100 bp for the U allele. The marker sizes are 100 bp, 150 bp, and 200 bp.

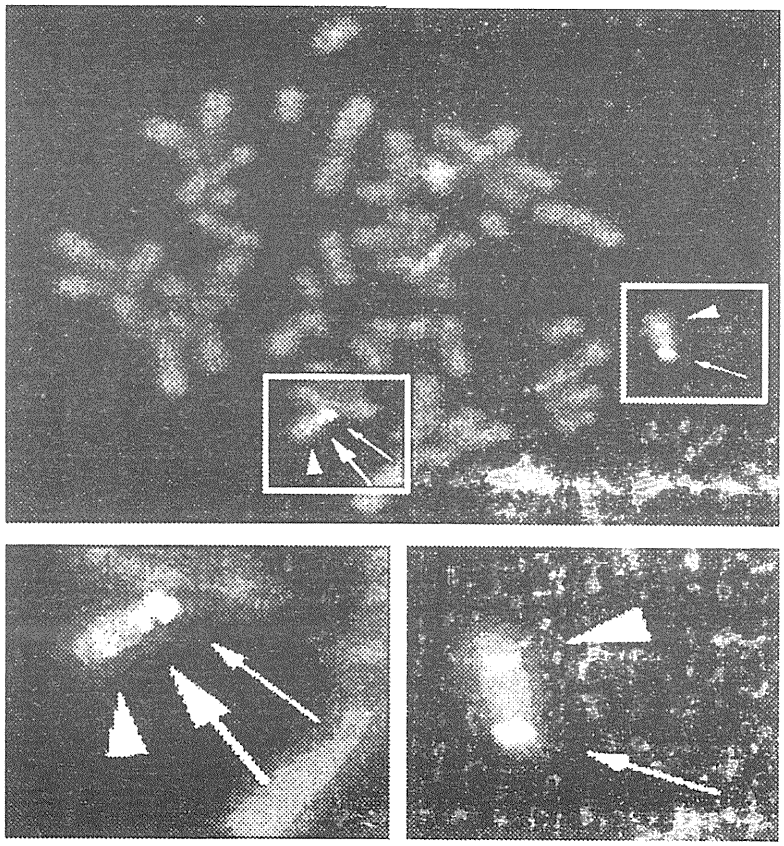
Locus	Position	Results		
D15S541	q11.2	Heterodisomy	Isodisomy	Isodisomy
D15S542	q11.2			
D15S1035	q11.2			
D15S11	q11.2	Heterodisomy or Isodisomy	Heterodisomy for at least one locus	
D15S128	q11.2			
D15S1048	q13.1			
D15S1007	q14			
D15S117	q21.1			
D15S131	q23			
D15S205	q25.2			
D15S127	q26.1	UPD by TR/GC (Meiosis 1)	UPD by TR/GC (Meiosis 2)	UPD by MR/PE
D15S120	q26.3			
D15S642	q26.3			



Supplementary Figure 2 Classification of upd(15)mat.
Upper part: Schematic representation of the methods utilized in this study. When at least one of the three pericentromeric loci is present in a heterodisomic condition, this indicates TR/GC [M1] type upd(15)mat. When the combination of isodisomy for the pericentromeric loci and heterodisomy for at least one locus in the middle to distal region is present, this indicates TR/GC [M2] type upd(15)mat. When all the loci are present in an isodisomic situation, this indicates MR/PE type upd(15)mat.
Lower part: Schematic representation showing that heterodisomy for centomeric loci is compatible with TR/GC [M1] type upd(15)mat, and that the combination of isodisomy for centromeric loci and heterodisomy for at least one middle to distal locus is consistent with TR/GC [M2] type upd(15)mat. Here, one recombination is assumed for 15q. When two recombinations take place, this can influence the status (isodisomy or heterodisomy) of middle to distal loci, but not that of centromeric loci. Thus, the status (isodisomy or heterodisomy) of centromeric loci is informative for the classification of upd(15)mat.



Supplementary Figure 3 Secular trend in the number of live-births according to maternal ages in Japan. Constructed by the authors on the basis of the Annual Nationwide Survey Data from the Ministry of Health, Labor and Welfare.



Supplementary Figure 4 FISH finding in a Prader-Willi syndrome patient with a microdeletion. Only a single red signal has been detected by a FISH probe for a region encompassing *SNRPN* (a thick arrow), whereas two green signals and two red signals have been identified by a CEP 15 probe for *D15Z1* (thin arrows) and by a probe for *PML* on 15q22 (arrowheads), respectively.

Supplementary Table 1 Primers utilized in this study.

	Forward primer	Reverse primer	PS	AT
<Methylation analysis of the *SNRPN*-DMR>[a]				
Methylated	TAAATAAGTACGTTTGCGCGGTC	AACCTTACCCGCTCCATCGCG	174	62
Unmethylated	GTAGGTTGGTGTGTATGTTTAGGT	ACATCAAACATCTCCAACAACCA	100	62
<Microsatellite analysis>[b]				
D15S541	GCATTTTTGGTTACCTGTATG	GTCTTCCAGGTTTATGGTTGTC	~150	60
D15S542	AGCAGACTCCGGAACCTCATC	CCTGCCTTCTTGCTGGGGCTG	~140	59
D15S1035	CACCCCCATGCAGAGT	AAGGCCAAGACCTGCC	172–262	60
D15S11	GACATGAACAGAGGTAAATTGGTGG	GCTCTCTAAGATCACTGGATAGG	~243	57
D15S128	GCTGTGTGTAAGTGTGTTTTATATC	GCAAGCCAGTGGAGAG	193–209	60
D15S1048	AGCCGTCTTTGTGCCA	TGCAGCCACTGTGGAA	197–233	57
D15S1007	GGGGAACCTACACTTCCG	CCAGGAATCTCAAATGGCTT	165–189	57
D15S117	GCACCAACAACTTATCCCAA	CCCTAAGGGGTCTCTGAAGA	132–150	60
D15S131	GAAAGGCACCTCATCTCG	TTAAAAACTCTGGAGCAGCG	238–274	60
D15S205	CTTAATGGTTTGGCAGGATA	AGCTTAAAANCAAAATCTCCC	128–170	60
D15S127	CCAACCACACTGGGAA	AACAGTTGCCCACGGT	~137	57
D15S120	TTTGTGATGGTCTTTTATAGGCATA	GGCTCAAAGTGTTTGCACTG	150–174	57
D15S642	CAGTTACCCAGGAAGCTGAA	AGATGCCGCCTGTACTAATG	195–218	60

PS: product size (bp), and AT: annealing temperature (°C).

[a] Kubota et al. (1997) Methylation-specific PCR simplifies imprinting analysis. *Nat. Genet.*, **16**, 16–17.

[b] NCBI Database (http://www.ncbi.nlm.nih.gov/unists).

Pediatr Radiol (2011) 41:1013–1019
DOI 10.1007/s00247-011-2132-1

ORIGINAL ARTICLE

Radiological evaluation of dysmorphic thorax of paternal uniparental disomy 14

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Received: 21 November 2010 / Revised: 31 January 2011 / Accepted: 1 February 2011 / Published online: 24 May 2011

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

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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

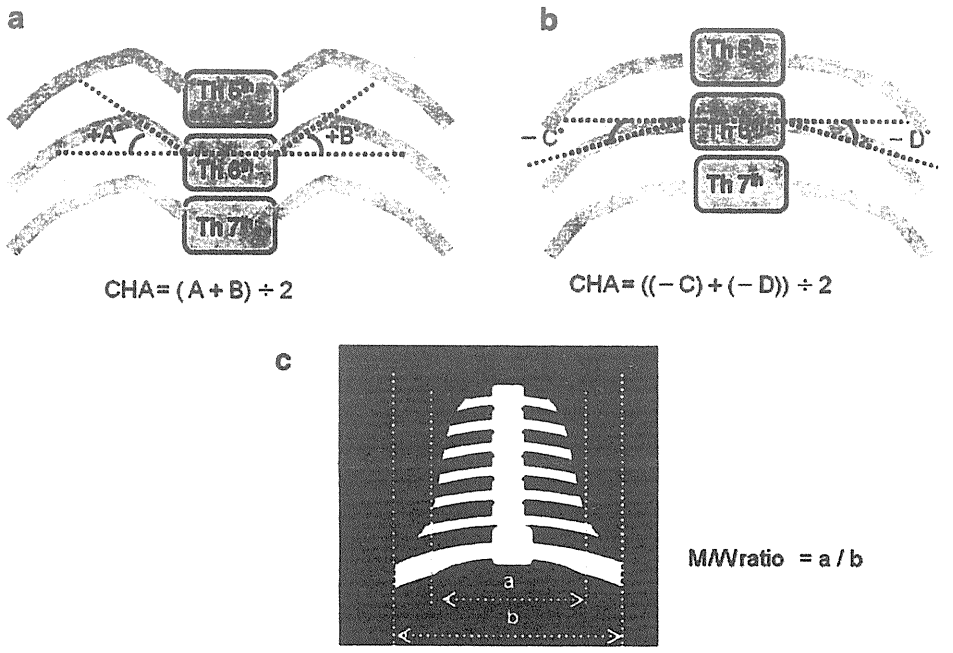

Fig. 1 **a, b** Diagram of coat-hanger angle (CHA) and mid/widest ratio. CHA refers to the average of the angles between the peak point of both 6th posterior ribs and the horizontal axis. If there is no peak point of the 6th posterior ribs, the center of the ribs is utilized instead. The horizontal axis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

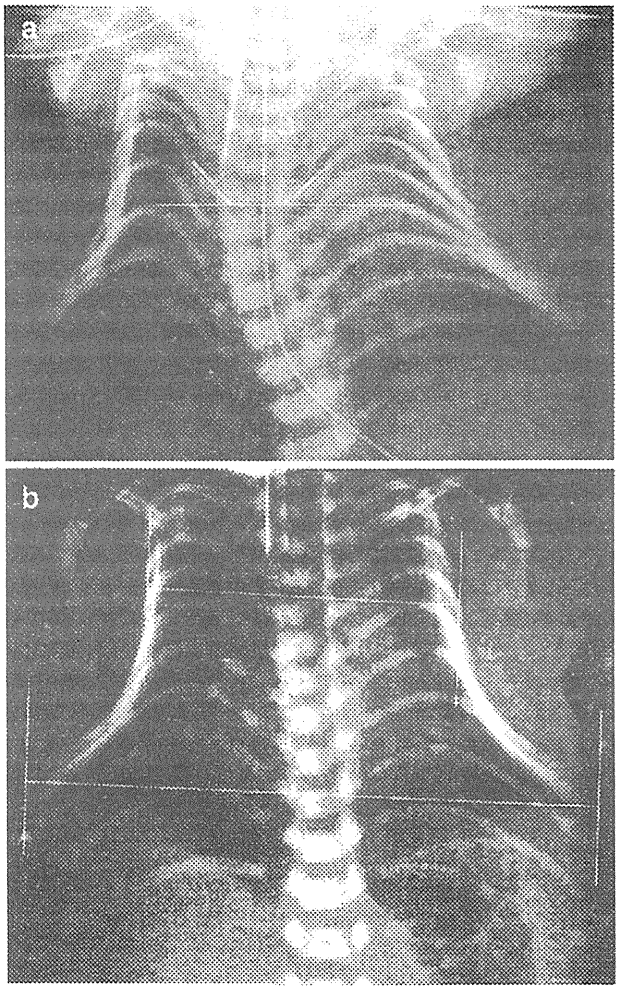

Fig. 2 Examples of CHA and M/W ratio. a The 6th posterior ribs show upward bowing that provides the coat-hanger sign. The CHA of this case (patient #7 in Table 1) was 45° (the measurement was 48° for the right and 42° for the left). b The M/W ratio was 58% in this case (patient #5 in Table 1). This is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

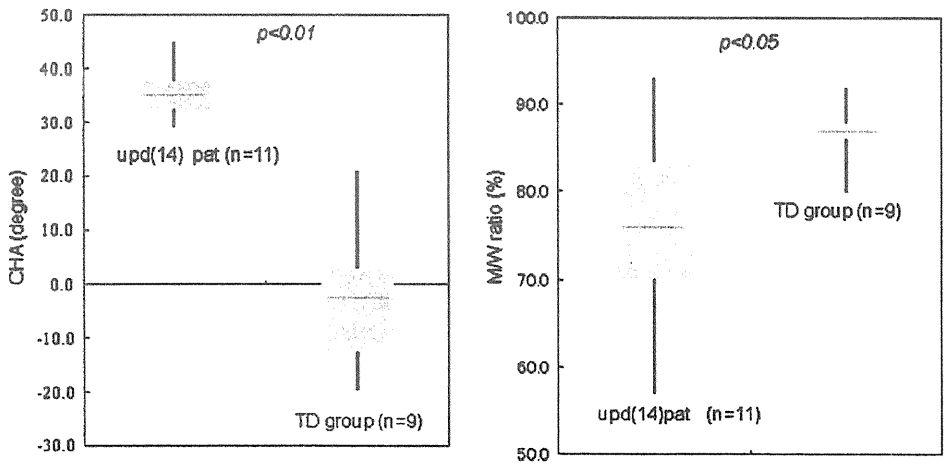

Fig. 3 Box plot of CHA and M/W ratio with the median, interquartile interval and range

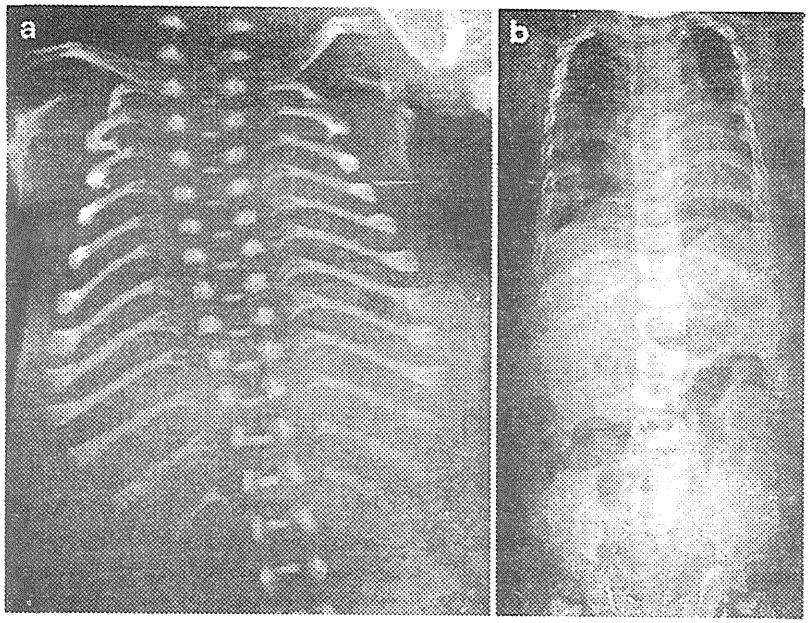

Fig. 4 Examples of the thoracic appearance and measurement of bone dysplasias with thoracic hypoplasia. **a** Thanatophoric dysplasia (TD) type 1 (stillbirth at 21 weeks of gestation). Note a narrow thorax with cupped anterior ends as well as short long bones with metaphyseal cupping. The posterior ribs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

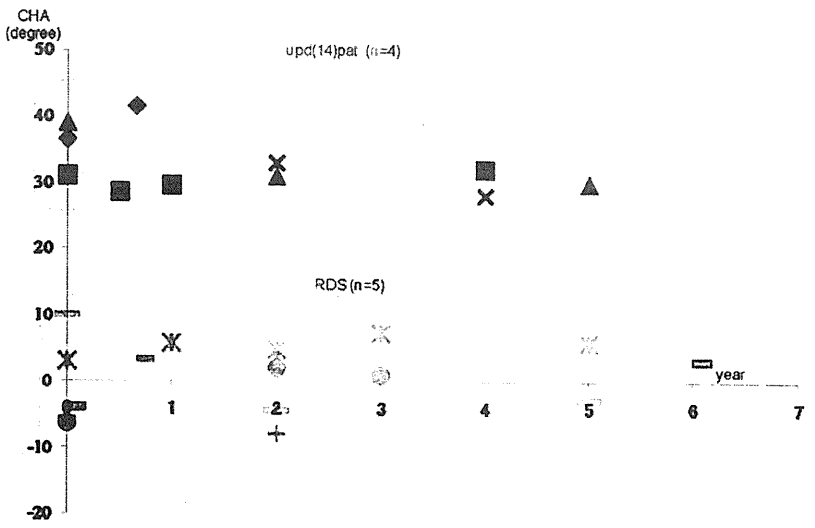

Fig. 5 Comparative observation of age-dependent transition of CHA between the upd(14)pat and respiratory distress syndrome (RDS) groups. Individual shapes represent individual patients