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Segmental Paternal Uniparental Disomy (patUPD) of 14q32 With Abnormal Methylation Elicits the Characteristic Features of Complete patUPD14

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Abstract

Uniparental disomy (UPD) for chromosome 14 is associated with well-recognized phenotypes, depending on the parent of origin. Studies in mouse models and human patients have implicated the involvement of the distal region of the long arm of chromosome 14 in the distinctive phenotypes. This involvement is supported by the identification of an imprinting cluster at chromosome 14q32, encompassing the differentially methylated regions (DMRs), IG-DMR and MEG3-DMR, as well as the maternally expressed genes *GTL2*, *DIO3*, and *RTL1* and the paternally expressed genes *DLK1*, *RTL1as*, and *MEG8*. Here we report on a preterm female infant with distal segmental paternal UPD14 (upd(14)pat) of 14q32-14q32.33, which resulted in thoracic deformity secondary to rib abnormalities ("coat-hanger" rib sign), polyhydramnios, and other congenital abnormalities characteristically described in cases of complete upd(14)pat. Microsatellite investigation demonstrated UPD of markers D14S250 and D14S1010, encompassing a ~3.5 Mb region of distal 14q and involving the imprinting cluster. This case provided insight into the etiology of the phenotypic effects of upd(14)pat, prompting methylation analysis of the *GTL2* promoter and the DMR between *GTL2* and *DLK1*. We compare the physical findings seen in this case with those of patients with other causes of abnormal methylation of 14q32, which consistently result in certain distinct clinical features, regardless of the cytogenetic and molecular etiology.

Keywords

segmental paternal uniparental disomy chromosome 14 (upd(14)pat); skeletal dysplasia; abnormal methylation; "coat-hanger" rib sign

INTRODUCTION

Imprinting disorders are known to result from different genetic mechanisms, such as uniparental disomy (UPD), microdeletions, mutations, and epimutations [Nicholls et al., 1998; Gicquel et al., 2005]. Different phenotypes result from UPD of paternal and of maternal origin. For paternal UPD of chromosome 14 (upd(14)pat) the genetic aberration has been described for the entire chromosome [Kurosawa et al., 2002; Offiah et al., 2003; Chu et al., 2004; Kagami et al., 2005; Curtis et al., 2006], including through chromosomal rearrangements [Wang et al., 1991; Papenhausen et al., 1995; Walter et al., 1996; Cotter et al., 1997; Kurosawa et al., 2002; McGowan et al., 2002; Murphy et al., 2003; Stevenson et al., 2004] and supernumerary markers [Mattes et al., 2007], for segments of chromosome 14 [Coveler et al., 2002; Kagami et al., 2005] and also for deletions and epimutations [Kagami et al., 2008]. Following the description of a number of case reports, a distinct clinical entity comprising rib abnormalities, defects of the abdominal wall, polyhydramnios, and dysmorphic features has been associated with upd(14)pat [Wang et al., 1991; Papenhausen et al., 1995; Walter et al., 1996; Cotter et al., 1997; Sutton and Shaffer, 2000; Coveler et al., 2002; Offiah et al., 2003; Chu et al., 2004; Stevenson et al., 2004; Kagami et al., 2005, 2008; Mattes et al., 2007]. Clinical severity can be variable but is usually severe. A different pattern of abnormalities, including pre- and postnatal growth retardation, precocious puberty, and other dysmorphic features results from maternal UPD14 (upd(14)mat) [Temple et al., 1991, 2007; Healey et al., 1994; Splitt and Goodship, 1997; Berends et al., 1999; Fokstuen et al., 1999; Hordijk et al., 1999; Sanlaville et al., 2000].

A cluster of imprinted genes is known to be located at 14q32 and evidence that this region contributes to the upd(14)pat phenotype comes from the observation that the characteristic phenotypic abnormalities seen in individuals with Robertsonian translocations involving chromosome 14, such as t(13;14) and t(14;14) [Wang et al., 1991; Papenhausen et al., 1995; Walter et al., 1996; Cotter et al., 1997; Kurosawa et al., 2002; McGowan et al., 2002] and full paternal UPD [Kurosawa et al., 2002; Offiah et al., 2003; Chu et al., 2004; Curtis et al., 2006] are also seen in segmental UPD, involving 14q12-14qter [Coveler et al., 2002] and the region distal to 14q23.3 [Kagami et al., 2005], as well as data from the conserved region in the mouse [Georgiades et al., 2000; Takada et al., 2002; Lin et al., 2003, 2007; Kaneko-Ishino et al., 2006]. The imprinted region includes the paternally expressed genes DLK1, DIO3, and RTL1 and the maternally expressed genes GTL2 (MEG3), MEG8 (RIAN), and antisense (as) RTL1 (RTL1as). This region is under regulatory control of the intergenic germline differentially methylated region (IG-DMR) [Lin et al., 2003], a *cis*-acting regulator on the maternally inherited chromosome. Ogata et al. [2008] have suggested that the pat UPD14 phenotype can be explained by decreased expression of both DLK1 and RTL1.

Here we describe an infant with distal segmental upd(14)pat. The affected infant had features characteristic of full upd(14)pat, including the characteristic “coat-hanger” rib sign of thoracic dystrophy. An abnormal methylation pattern was demonstrated at the two DMRs within the 14q32 imprinted region. This, in combination with other clinical features and a significant narrowing of the critical region, directly implicates abnormalities affecting the 14q imprinted cluster in causing the characteristic phenotype.

CLINICAL REPORT

The proband was the first-born child to her unrelated Caucasian parents. Her mother had previously experienced a first-trimester miscarriage. Both parents were healthy with no history of short stature or learning difficulties. Second trimester ultrasound scanning demonstrated polyhydramnios; tracheoesophageal fistula with esophageal atresia was suspected and maternal steroids were instituted. Amniocentesis revealed a normal female

karyotype (46,XX). Spontaneous onset of labor occurred at 28 weeks' gestation and a phenotypically female infant was delivered with a birth weight of 1.355 kg (91st centile). She required intubation for ventilatory support. Examination for esophageal atresia by positioning a nasogastric tube resulted in esophageal perforation and right-sided pneumothorax. Subsequent thoracotomy revealed normal mediastinal anatomy. Clinically she displayed a number of dysmorphic features: micrognathia, small ears with unfolded helices, prominent philtrum, rhizomesomelic shortening, long toes and fingers with adducted thumbs, scoliosis, and mild joint contractures (Fig. 1A,B). In addition, she had diastasis recti. Chest X-ray demonstrated normal lung parenchyma, but a narrow thorax with a "coat-hanger" appearance of the ribs bilaterally (Fig. 1C). On subsequent review, the scapular neck was hypoplastic, but no other radiological anomalies were detected on limited skeletal survey. Ultrasound imaging of the renal tract and head and echocardiogram did not reveal any other congenital anomalies. Nine weeks after birth attempts to wean her from ventilatory support failed and, in view of her severe respiratory problems, the decision was taken to withdraw intensive care. She died shortly thereafter. Autopsy was not undertaken.

METHODS AND RESULTS

Evaluation of Clinical Features

In order to conduct a diagnostic evaluation, the constellation of congenital abnormalities and dysmorphic features seen in the case was compared to those seen in cases with UPD14 caused by different mechanisms or with an abnormal methylation pattern of distal 14q resulting from microdeletion or epimutation (Table I). This demonstrated that the characteristic coat hanger rib radiological sign and other anomalies, including polyhydramnios, dysmorphic features, and diastasis recti, are consistently seen where there is an abnormality involving the paternal 14q32 allele, irrespective of the cause (full or segmental UPD, Robertsonian translocation, microdeletion, epimutation). However, it was also demonstrated that other clinical findings varied both between and within the same etiological group with no clear patterns to indicate the specific underlying mechanism. Since this infant also had dysmorphic features, such as long fingers and joint contractures, previously observed in some cases with upd(14)pat caused by different mechanisms, the cause of upd(14)pat in this case could not be further determined on clinical grounds.

Microsatellite Analysis

To investigate the possibility of upd(14)pat in this case, microsatellite markers throughout chromosome 14 were analyzed (Table II). DNA was extracted from 3 µl of blood using InstaGene Matrix (Bio-Rad, Hercules, CA) according to the instructions. Two PCR multiplexes were used with fluorescently labeled primers that amplify microsatellite markers on chromosome 14 (Table II). Amplification was achieved with a hot start Taq kit (Qiagen, Crawley, West Sussex, UK) using the following conditions: denaturation at 95°C for 15 min, followed by 25 cycles of 94°C for 30 sec, 58°C for 1 min 30 sec, 72°C for 1 min 30 sec, plus a final incubation at 72°C for 20 min. PCR products were analyzed on a 3100 genetic analyzer (Applied Biosystems, Life Technologies Corporation, Foster City, CA) and interpreted using Genotyper software (Applied Biosystems). In addition, two SNPs that map within the IG-DMR, *rs1884538* and *rs1884539*, were genotyped. Primers and methods of this assay have been previously published elsewhere [Buiting et al., 2008].

Analysis of microsatellite markers revealed upd(14)pat for two markers, D14S1010 and D14S250, with only one paternal allele present (Table II and Fig. 2). Delineation of the proximal extent of UPD is through marker D14S81, at which UPD was excluded, all distal markers either being uninformative or displaying uniparental inheritance (Table II). In addition, since the markers distal to D14S1010 were not informative, the extent of distal

involvement is unknown (Fig. 2). These results could not exclude deletion of the maternal allele.

Methylation-Specific PCR (MS-PCR) of the *GTL2* Promoter

Genomic DNA was bisulfite converted according to standard protocols. Bisulfite conversion changes unmethylated cytosines to thymines, but methylated cytosines are protected and remain unchanged providing sequence differences on which to design primers specific for each allele of a differentially methylated sequence. The region encompassing the *GTL2* promoter was PCR amplified using primers specific for the methylated and unmethylated alleles that have been published elsewhere [Dietz et al., 2003]. Touchdown PCR was used as follows: annealing temperatures were 5 cycles at 70°C for 30 sec, 5 cycles at 65°C for 30 sec, and 30 cycles for 60°C. Visualization of the PCR products by agarose gel electrophoresis (Fig. 3) demonstrates a complete absence of the maternally derived unmethylated amplicon in the patient. Both alleles were derived from the paternally inherited methylated product (Fig. 3), compatible with upd(14)pat or an imprinting defect or a deletion.

Methylation-Specific PCR for the IG-DMR

MS-PCR for the IG-DMR between *DLK1* and *GTL2* was performed to support the microsatellite analysis and provide further evidence that two paternal alleles are present and to be consistent with current diagnostic techniques [Temple et al., 2007]. Genomic DNA was bisulfite converted and MS-PCR analysis was performed with primers for the IG-DMR. The primers were designed with MethPrimer (<http://www.urogene.org/methprimer/>) to amplify methylated(M) and unmethylated(U) DNA, forward(F) and reverse(R).

Primer sequences for the IG-DMR were as follows:

IG-DMR-MF: 5'-GGTTTGTTAATTGTTAGCGATTTGTTAATTGC-3'.

IG-DMR-MR: 5'-AAAACCGAAAAACCTAAAAAACG-3' (5 FAM).

IG-DMR-UF: 5'-ATTGTTAGTGATTTGTTAATTGTGA-3'.

IG-DMR-UR: 5'-AAAAAACCAAAAAACCTAAAAACAA-3' (5 FAM).

PCR conditions were as follows: 95°C for 10 min, 35 cycles of 95°C for 30 sec, 58°C for 30 sec, 72°C for 30 sec, and 30 min at 72°C. The PCR products were visualized on a 2% agarose gel and then subjected to fragment analysis on an ABI 3100 Genetic Analyzer using the GeneScan software (Applied Biosystems; size standard: GeneScan-ROX 500) (Fig. 4).

IG-DMR MS-PCR on bisulfite-treated DNA from the patient at the *DLK1/GTL2* locus confirms the presence of a paternal methylated PCR product only and the absence of the maternal unmethylated PCR product (Fig. 4). This is compatible with either upd(14)pat or a deletion of the maternal allele.

Genomic Real-Time (TaqMan) PCR

To exclude a maternal deletion of the *GTL2/DLK1* locus, we performed a real-time PCR assay for the promoter region of *GTL2*. Probes and primers for this assay have been reported previously by Buiting et al. [2008]. The patient had normal dosage for the *GTL2* DMR (Fig. 5). This excludes a deletion of this locus on the maternal allele.

DISCUSSION

The patient described in this report had a phenotype characteristic of upd(14)pat with “coat-hanger” shaped ribs, specific dysmorphic features, diastasis recti, and polyhydramnios. Microsatellite marker analysis detected only paternal allele contribution at the segment of 14q32 (Fig. 2). Only paternally derived methylation patterns were detected in this region (Figs. 3 and 4) in the demonstrable absence of deletion of the maternal allele (Fig. 5), confirming segmental paternal isodisomy for 14q32.

The clinical features resemble those previously described in individuals with upd(14)pat arising from different mechanisms, (Table I). This is in support of the findings of Kagami et al. [2008] who also reported similar clinical features in individuals with a variety of genetic lesions including microdeletions encompassing this region and epimutations affecting the regulation of the paternally and maternally expressed genes. The finding of a variety of mechanisms resulting in a similar phenotype is in concordance with the situation with Prader–Willi and Angelman syndromes where a number of different lesions can generate the same phenotypic findings in different patients [Nicholls et al., 1998].

Certain consistent phenotypic similarities are observed in cases with abnormal imprinting of the chromosome 14 locus, regardless of whether the abnormality is the consequence of full or segmental paternal UPD14, a Robertsonian translocation resulting in paternal UPD14, a microdeletion of the imprinted region, or an epigenetic mutation resulting in an abnormal methylation pattern (Table I). In particular, the “coat-hanger” rib appearance remains the best indicator of abnormal imprinting at this locus, given that it was present in all but two cases (26/28), and that it has not been associated with other syndromes. Our case also displayed hypoplasia of the scapular neck, a radiographic finding also observed in previous cases [Sutton et al., 2003]. Some variation in phenotypic features exists, however, both between cases with the same abnormality affecting 14q32 and in those with a different etiology. For example, although small ears have been noted in all three cases of segmental upd(14)pat, they were also observed in some cases with different chromosomal and epigenetic abnormalities. Similarly, joint contractures were reported in 6/9 Robertsonian cases of upd(14)pat, 2/3 segmental and 3/6 full upd(14)pat cases, 2/5 microdeletion cases, and 2/3 with an epigenetic mutation (Table I). While certain findings point to an underlying abnormality of methylation, namely the “coat-hanger” rib sign with polyhydramnios and abdominal wall defects, the absence of a clear pattern of characteristic phenotypic abnormalities within each etiological subgroup makes it impossible to determine the exact causative mechanism based only on clinical grounds. Laboratory investigation requires consideration of all possible causes.

Segmental upd(14)pat is most likely the result of an early embryonic somatic recombination event in which a reciprocal exchange results in a region of isodisomy with subsequent loss of both the normal and alternative reciprocal maternal isodisomic cell lineages [Robinson, 2000]. This clinical picture has been previously reported [Coveler et al., 2002; Kagami et al., 2005]. However, our case allowed the critical region to be refined, directly implicating the region 14q32 in the phenotype. Given that there are consistent clinical features seen in all types of cases with no clear pattern of additional anomalies to help identify the etiology, any additional imprinted genes elsewhere on the long arm of human chromosome 14 would be unlikely to contribute to the characteristic features of upd(14)pat.

This case implicates abnormal methylation at the imprinted cluster in the cause of the upd(14)pat phenotype. The IG-DMR is located a few kilobase upstream of the *Gtl2* promoter and acquires a paternal methylation mark whereas the maternally inherited copy is transmitted in an unmethylated state. The IG-DMR has important *cis*-acting regulatory

functions on the maternally inherited chromosome. A targeted deletion in mouse of the IG-DMR inherited from the mother results in loss of imprinting of all genes in the cluster but paternal deletion results in no gross differences in expression across the domain suggesting that it is the maternally inherited unmethylated copy that is essential for maintaining imprinting control at this locus [Lin et al., 2003].

Here it has been demonstrated that upd(14) results in abnormal methylation, consistent with previous reports of segmental UPD involving 14q32 [Kagami et al., 2005], and that abnormal methylation of the associated imprinted genes can also result in the phenotype characteristic of upd(14)pat [Kagami et al., 2008]. In our cases of segmental UPD of 14q32, we have demonstrated hypermethylation of the *GTL2* promoter and the *DLK1/GTL2* IG-DMR and absence of the normally hypomethylated maternal alleles. In the case of upd(14)pat involving the chromosomal segment distal to 14q23.3 reported by Kagami et al. [2005] they showed absence of the maternal unmethylated allele following methylation-specific PCR of the *GTL2*-containing DMR. Methylation status at both the IG-DMR and the MEG3-DMR was analyzed in three cases with a upd(14)pat-like phenotype in the absence of UPD by Kagami et al. [2008], and epimutation was demonstrated through the detection of hypermethylation of the normally hypomethylated allele of maternal origin [Kagami et al., 2008].

Kagami et al. [2008] also reported five cases with the upd(14) pat-like phenotype in which these maternal alleles were deleted, three of whom showed milder clinical features. These reports emphasize the clinical importance of identifying whether the characteristic phenotype is secondary to a microdeletion, as there may be a high risk of recurrence of either the paternal or maternal UPD14-like phenotype when the proband has a deletion for the imprinted region. In the present case, exclusion of a microdeletion was undertaken through real-time PCR dosage analysis, demonstrating normal levels of *GTL2* transcription (Fig. 5 and Table II). Mice with deletions of the maternally derived IG-DMR display the paternal gene expression pattern with upregulation of the associated imprinted genes *Rtl1*, *Dlk1*, and *Dio3*; absence of expression of the maternally expressed genes; and hypermethylation of the *Gtl2*-DMR. The result is a paternal UPD14-like phenotype [Georgiades et al., 2000; Lin et al., 2003, 2007]. *Rtl1* is significantly expressed in mouse placental tissue, as well as in a number of fetal tissues, including the ribs and skeletal muscle. Levels of abnormal expression have been implicated in influencing phenotypic severity [Sekita et al., 2008]. Marked increase in expression of *RTL1* observed in human upd(14)pat patients is due to the fact that *RTL1* is usually silent on the maternal allele and a double dose is present in a upd(14)pat. In addition, evidence from mouse studies supports the hypothesis that loss of the micro-RNA containing *RTL1* s, which normally represses *RTL1*, exacerbates this and plays a significant role in the upd(14)pat phenotype [Lin et al., 2003; Kagami et al., 2008; Ogata et al., 2008].

Multiple isoforms of *DLK1* exist and these are related to members of the Notch/delta signaling pathway. Transmembrane-bound *DLK1* regulates Notch in *Drosophila* and inhibits expression of Notch-target genes, resulting in a reduced Notch function phenotype [Bray et al., 2008]. Notch signaling is fundamental to somitogenesis and abnormalities of this process result in abnormal segmentation, affecting the spine and ribs, for example, in conditions such as spondylocostal dysostosis [Turnpenny et al., 2007] and Alagille syndrome [Li et al., 1997; Oda et al., 1997; Yuan et al., 1997]. It is tempting therefore to implicate the involvement of paternally expressed *DLK1* in the etiology of the thoracic abnormality, although this remains to be substantiated.

In summary, it has been possible to narrow the critical region associated with the characteristic clinical phenotype to chromosome 14q32 through the segmental nature of

upd(14)pat and to demonstrate abnormal methylation patterns within the imprinting cluster at this locus. In keeping with other disorders resulting from abnormal methylation, such as Prader–Willi and Angelman syndromes, features of this condition are consistent, irrespective of the etiologic mechanism. Importantly from a diagnostic perspective, the “coat-hanger” rib sign remains an excellent clinical indicator of this specific abnormal methylation pattern, whereas other clinical features show variability and cannot be used to determine the underlying cause.

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FIG. 1.

A: Clinical photograph showing facial features. B: Clinical photograph showing long fingers and joint contractures of the fingers and wrist. C: AP chest radiograph showing a narrow thorax secondary to rib abnormalities ("coat-hanger" appearance), hypoplasia of the scapular neck, and scoliosis of the thoracic spine.

FIG. 2.

Illustration of the region of Chr 14 pertinent to this case. Top: Ideogram of whole chromosome 14 (from left to right: upper arm, centromere, lower arm) with cytogenetic banding. Distally, horizontal black lines mark the region of paternal UPD confirmed by MIT marker analysis (top line) and the potential extent of the UPD due to uninformative markers (lower line). A rectangular frame indicates the region of chromosome 14 that is shown in detail in the figure. Middle: Chromosome 14 from ~93 Mbp to ~103 Mbp (March 2006 NCBI genome assembly, build 36.1). A scale of physical coordinates is followed by the positions (vertical lines) of informative MIT markers D14S553 (heterozygous = biparental), D14S250 and D14S1010 (both homozygous indicating paternal UPD or maternal deletion). Additional vertical lines indicate the locations of *DLK1*, the IG-DMR, the MEG3-DMR and *MEG3 (GTL2)*. Quantitative methylation assays of the DMRs ruled out a maternal deletion as the cause of the MIT marker analysis results. Bottom: Focus on the ~150 kbp region delineated by *DLK1* and *MEG3* (vertical lines and bars: exons; horizontal lines with arrows: introns; arrows: direction of transcription). Two solid blocks represent the position and extent of the IG- and MEG3-DMRs.

FIG. 3.

Visualization by agarose gel electrophoresis of non-quantitative methylation-specific PCR, demonstrating that samples from parents and controls have paternally derived methylated and maternally derived unmethylated alleles, but the patient has only paternally derived methylated alleles, either due to UPD or a deletion. The sample from a patient with known maternal UPD14 shows only a maternally derived methylated allele.

FIG. 4.

Epigenotype analysis of the IG-DMR between *DLK1* and *MEG3/GTL2*. Bisulfite conversion and MS-PCR amplification of DNA from the patient and controls yield different sized products depending on the initial methylation status of CpGs in the starting material. The electropherogram shows the product sizes on the *x*-axis and peak height in fluorescence units on the *y*-axis. The maternally derived unmethylated product is absent in the patient sample but the methylated paternal product is present. This is consistent with the converse finding in the case of known maternal UPD14. The control sample has both maternally and paternally derived allele as expected.

FIG. 5.

Dosage analysis of the *GTL2* DMR by real-time PCR, which shows the case is not deleted for the maternal allele. DNA from a patient with a paternal deletion of the *DLK* was used as a deletion control.

TABLE I

A Summary of a Comparison of Features Seen in Cases With Abnormal Methylation of the Paternal 14q32 Allele by Genetic Mechanism

	Segmental paternal UPD14, including the imprinted region 14q32	Full paternal UPD14	Paternal UPD14 resulting from chromosomal rearrangements: Robertsonian translocations/dicentric chromosome 14/supernumerary marker 14	Deletion involving IG-DMR and MEG3-DMR	Epimutations—hypermethylation of maternal imprinted allele
References	Coveler et al. [2002] (14q12-14qter); Kagami et al. [2005] (14q23.3); current case (14q32)	Kurosawa et al. [2002], Offiah et al. [2003], Chu et al. [2004], Kagami et al. [2005], Curtis et al. [2006]	Wang et al. [1991], Papenhausen et al. [1995], Walter et al. [1996], Cotter et al. [1997], Klein et al. [1999], Yano et al. [2001], Kurosawa et al., [2002], McGowan et al. [2002], Stevenson et al. [2004], Mattes et al. [2007]	Kagami et al. [2008]	Kagami et al. [2008]
Number of cases	3	7	10	5	3
Polyhydramnios	3/3	7/7	9/10	4/5	3/3
Gestational age at delivery (weeks)	29–36	30–37	28–38	27–35	30–35
Birth weight centile	50–95th	25th–91st	10–99.6th	Normal range	Normal range
Characteristic facial features					
Prominent philtrum	3/3	6/7	5/10	4/5	3/3
Micro/retrognathia	3/3	5/7	4/10	4/5	3/3
Hirsute forehead	2/3	3/7	1/10	3/5	1/3
Small ears	3/3	0/7	4/10	0/5	1/3
Short/webbed neck	2/3	4/7	3/10	5/5	3/3
Skeletal anomalies					
Coat-hanger rib appearance	3/3	7/7	8/10	5/5	3/3
Joint contractures	2/3	3/7	6/10	2/5	2/3
Kyphoscoliosis	3/3	4/7	3/10	1/5	2/3
Short long bones	2/3	2/3	3/10	Not stated	Not stated
Congenital anomalies					
Diastasis recti	2/3	4/7	5/10	2/5	0/3
Congenital heart disease	1/3: ASD/VSD/PS	1/7: ASD	4/10: PDA/PFO; mild PS; aortic arch anomaly	1/5: PDA	0/3
Other	1/3: undescended testes	1/7: omphalocele; 1/7: hypospadias; 1/7: hypoplastic genitalia; 2/7: lax skin	2/10: HCM; 1/10: seizures with brain hypoplasia and subependymal calcification	2/5: laryngomalacia	1/3: laryngomalacia
Survival <1 year	1/3	4/7	4/10 (1 termination of pregnancy)	0/5	0/3

ASD, atrial septal defect; VSD, ventricular septal defect; PS, pulmonary stenosis; PDA, persistent ductus arteriosus; PFO, patent foramen ovale; HCM, hypertrophic cardiomyopathy; x/y: x=feature specifically described; y=total number of cases.

TABLE II

Markers Used to Interrogate UPD

Marker	Chromosome localization	Father	Mother	Case	Interpretation
<i>D14S122</i>	14q11.2	3,4	1,2	2,4	Biparental
<i>D14S1023</i>	14q11.2				
<i>D14S139</i>	14q13	1,4	2,3	1,2	Biparental
<i>D14S306</i>	14q21.1	1,2	2,3	1,3	Biparental
<i>D14S290</i>	14q21-23	1,2	1,3	1,1 or 1,-	Not informative
<i>D14S587</i>	14q22.2	1,2	1 or 1,1	1,2	Not informative
<i>D14S127</i>	14q31.2	1 or 1,1	2 or 2,2	1,2	Biparental
<i>D14S81</i>	14q32.13	1,2	1,3	1,3	Not informative
<i>D14S553</i>	14q32.13	1 or 1,1	1,2	1,2	Not informative
<i>D14S987</i>	14q32	1,3	1,2	1,3	Not informative
<i>D14S979</i>	14q32.2	1,2	2 or 2,2	2,2 or 2,—	Not informative
<i>D14S267</i>	14q32.2	1,2	2 or 2,2	2,2 or 2,—	Not informative
<i>D14S250</i>	14q32	2,3	1,2	3,3 or 3,—	Pat UPD or maternal deletion
<i>D14S78</i>	14q32	1,2	1,2	1,1 or 1,—	Not informative
<i>D14S985</i>	14q32.2	2 or 2,2	1,2	2,2 or 2,—	Not informative
<i>D14S1006</i>	14q32.2	1,2	2 or 2,2	2,2 or 2,—	Not informative
<i>rs1884538</i>	IG-DMR	C	C	C	Not informative
<i>rs1884539</i>	IG-DMR	C	C	C	Not informative
<i>D14S305</i>	14q32.2	1 or 1,1	1 or 1,1	1,1 or 1,—	Not informative
<i>D14S1010</i>	14q32.33	1,4	2,3	4,4 or 4,—	Pat UPD or maternal deletion
<i>D14S1007</i>	14q32.33	2 or 2,2	1,2	2,2 or 2,-	Not informative