Maternal Uniparental Disomy 14 Syndrome Demonstrates Prader-Willi Syndrome-Like Phenotype

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Objective To delineate the significance of maternal uniparental disomy 14 (upd(14)mat) and related disorders in patients with a Prader-Willi syndrome (PWS)–like phenotype.

Study design We examined 78 patients with PWS-like phenotype who lacked molecular defects for PWS. The *MEG3* methylation test followed by microsatellite polymorphism analysis of chromosome 14 was performed to detect upd(14)mat or other related abnormalities affecting the 14q32.2-imprinted region.

Results We identified 4 patients with upd(14)mat and 1 patient with an epimutation in the 14q32.2 imprinted region. Of the 4 patients with upd(14)mat, 3 had full upd(14)mat and 1 was mosaic.

Conclusions Upd(14)mat and epimutation of 14q32.2 represent clinically discernible phenotypes and should be designated "upd(14)mat syndrome." This syndrome demonstrates a PWS-like phenotype particularly during infancy. The *MEG3* methylation test can detect upd(14)mat syndrome defects and should therefore be performed for all undiagnosed infants with hypotonia. *(J Pediatr 2009;■:■-■).*

Maternal uniparental disomy 14 (upd(14)mat) is characterized by prenatal and postnatal growth retardation, neonatal hypotonia, small hands and feet, feeding difficulty, and precocious puberty.[1] Chromosome 14q32.2 contains several imprinted genes, and loss of expression of paternally expressed genes including *DLK1* and *RTL1* is believed to be responsible for upd(14)mat phenotype.[2] Thus far, 5 patients with epimutations and 4 patients with a microdeletion affecting the 14q32.2 imprinted region have been reported to have upd(14)mat-like phenotype.[2-4] Paternal uniparental disomy 14 (upd(14)pat) shows a distinct and much more severe phenotype characterized by facial abnormality, bell-shaped thorax and abdominal wall defects.[1] Initially, upd(14)mat was identified in patients with Robertsonian translocations involving chromosome 14, but increasing numbers of patients with a normal karyotype have been recognized.[1,5] Because maternal uniparental disomy 15 is responsible for the condition in more than 20% of patients with Prader-Willi syndrome (PWS), of which the overall prevalence is more than 1 in 15000 births,[6] one could suspect that upd(14)mat is underestimated. Phenotype of upd(14)mat is known to resemble that of PWS, which is characterized by neonatal hypotonia, small hands and feet, mental retardation, and hyperphagia resulting in obesity beyond infancy. Mitter et al[7] recently reported that upd(14)mat was detected in 4 of 33 patients who were suspected to have PWS and raised the question that upd(14)mat could be present in patients with PWS-like phenotype. Thus we examined patients who presented with PWS-like phenotype, but in whom PWS had been excluded.

Methods

The median age of the 78 patients enrolled in the study was 18.5 months, and the range was 1.4 to 324 months. Sex ratio was 1:1. All patients demonstrated PWS-like phenotype including hypotonia during infancy. We initially performed the *SNURF-SNRPN* DNA methylation test, and normal methylation results excluded the diagnosis of PWS.[8]

This study was approved by the Institutional Review Board Committees at Hokkaido University Graduate School of Medicine and National Center for Child Health and Development. The parents of the patients gave written informed consent.

DNA methylation status at the promoter region of imprinted *MEG3*, located in 14q32.2, was examined (**Figure 1**). Genomic DNA was extracted from leukocytes and treated with sodium bisulfite, and methylated allele– and unmethylated allele–specific primers were used to polymerase chain reaction amplify each allele, as described previously.[9] If aberrant DNA methylation was identified,

PWS	Prader-Willi syndrome
Upd(14)mat	Maternal uniparental disomy 14
Upd(14)pat	Paternal uniparental disomy 14

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we carried out microsatellite polymorphism analysis for 16 loci on chromosome 14 (ABI PRISM Linkage Mapping Set v2.5; Applied Biosystems, Foster City, California) with DNA from the patients and their parents (Figure 1). Polymerase chain reaction products were analyzed on an ABI310 automatic capillary genetic analyzer and with GeneMapper software (Applied Biosystems). If aberrant DNA methylation was identified but the patient demonstrated biparental origin of the chromosome 14s, we further examined the chromosomes for DNA methylation state, parental origin, and microdeletion in 14q32.2, as described previously.[2, 3]

Results

We identified abnormal hypomethylation at the *MEG3* promoter in 5 of 78 patients (Figure 2). Almost complete lack of methylation was found in 4 patients (case 1 to 4), but 1 patient (case 5) demonstrated faint methylation. Polymorphism studies demonstrated that 3 (cases 2 to 4) of the 4 patients with complete lack of *MEG3* promoter methylation had complete upd(14)mat, but 1 patient (case 1) had inherited both parental alleles (Table I; available at www.jpeds.com). We further examined the DNA methylation state and microdeletion or segmental upd at 14q32.3, and concluded that this patient (case 1) had an epimutation. The detailed data have been reported previously.[3] The patient (case 5) with faint *MEG3* methylation was demonstrated to have 2 maternal alleles, as well as 1 paternal allele with lower signal intensity. This indicated mosaicism of upd(14)mat (80%) and a normal karyotype (20%) (Figure 3; available at www.jpeds.com).

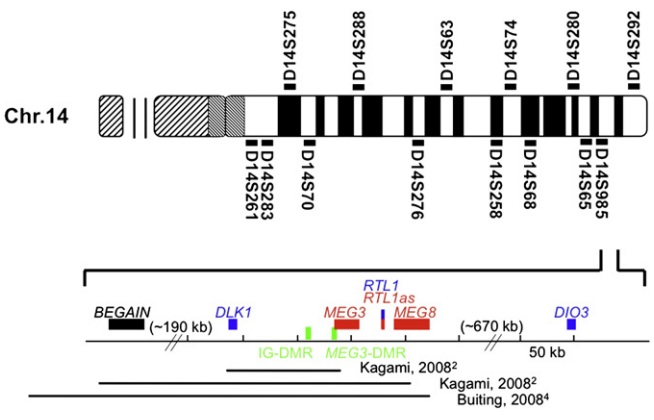

Figure 1. Schematic map of the 14q32.2 imprinted region. Loci on chromosome 14 represent markers used for microsatellite polymorphism analysis. Paternally expressed genes are shown in *blue*, maternally expressed genes in *red*, and nonimprinted genes are shown in *black*. Differentially methylated regions (DMRs) are shown in *green*. *IG-DMR,* Intergenic DMR. Reported microdeletions are demonstrated as *horizontal bars*.

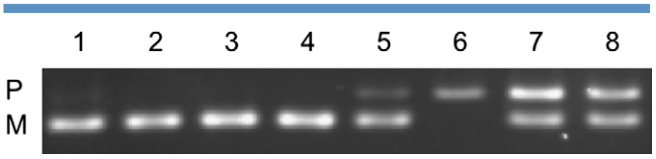

Figure 2. *MEG3* methylation test. *P,* Paternal methylated signal; *M,* maternal unmethylated signal; *1-5,* cases 1-5, respectively; *6,* paternal uniparental disomy 14; *7,* patient with PWS; *8,* normal control. Cases 1-4 show only the maternal unmethylated signal, and case 5 shows a faint paternal methylated signal.

The profiles of the patients with upd(14)mat or an epimutation are shown in Table II. We compared clinical features in these patients (Table III). All patients were referred to us during infancy because of hypotonia and motor developmental delay. Small hands and feet were also present in all patients. Prenatal growth retardation was present in all but 1 patient (case 1) who was later shown to have an epimutation. However, this patient had development of postnatal growth retardation, which was present in all patients. Premature onset of puberty was not evaluated in this study because the patients were too young. Apparent intellectual delay was only present in the patient who had upd(14)mat mosaicism (case 5). The clinical features of the patients with epimutation or with mosaic upd(14)mat were not distinct from those of the patients with full upd(14)mat.

Discussion

We detected 5 patients with upd(14)mat or epimutation at the 14q32.2-imprinted region in 78 subjects who had initially been suspected to have PWS. Mitter et al[7] reported that upd(14)mat was detected in 4 of 33 patients who were suspected to have PWS. However, Cox et al[10] reported that they did not find any upd(14)mat in 35 patients suspected to have PWS. Our study suggests that a significant number of patients with upd(14)mat are suspected to have PWS during infancy. To clarify how upd(14)mat and PWS share clinical features, we examined the clinical manifestations of our patients with upd(14)mat or an epimutation. All patients showed neonatal hypotonia and were referred to us during infancy. Feeding difficulty in the neonatal period and small hands and feet were also common to these patients and resembled features of PWS. It is noteworthy that all patients were referred during infancy, suggesting that upd(14)mat and PWS resemble each other, particularly during this period. Therefore upd(14)mat and related disorders, as well as PWS, should be important differential diagnoses for infants with hypotonia and feeding difficulty. Distinct features for upd(14)mat included less-specific facial characteristics, constant prenatal growth failure, and better intellectual development. Precocious puberty is not present in PWS; however, this was not evaluated in this study because the patients were not

Table II. Profiles of the patients with upd(14)mat and epimutation of 14q32.2

	Case 1	Case 2	Case 3	Case 4	Case 5
Molecular class	Epimutation	Upd(14)mat	Upd(14)mat	Upd(14)mat	Upd(14)mat (mosaic)
Age	2 y 2 m	4 y 2 m	2 y 7 m	1 y 9 m	3 y 4 m
Sex	Female	Male	Female	Female	Female
Karyotype	46,XX	46,XY	46,XX	46,XX	46,XX
Gestational age	41 w 5d	36 w 1 d	37 w 3 d	40 w 4 d	36 w
Birth weight g (SD)	3034 (0)	1955 (−2.6)	1680 (−3.3)	1858 (−2.8)	1434 (−3.9)
Birth length cm (SD)	50 (+0.7)	45.7 (−1.5)	40 (−4.0)	45 (−1.6)	39 (−3.9)
Birth OFC cm (SD)	Unknown	32 (−1.0)	30.4 (−2.0)	32 (−0.8)	30 (−2.2)
Present height cm (SD)	76.1 (−3.1)	89.5 (−2.8)	79 (−2.7)	72.5 (−3.4)	77.8 (−4.5)
Present weight kg (SD)	8.18 (−2.4)	11.6 (−2.1)	8.4 (−2.8)	6.4 (−3.7)	8.84 (−3.3)
Present OFC cm (SD)	45.2 (−1.5)	51.0 (+0.5)	48 (0)	44 (−1.8)	46.0 (−1.6)

old enough to demonstrate this feature. It is possible that when the patients get older, the clinical features of upd(14)mat may become more distinct from those of PWS.

We detected an epimutation in the 14q32.2-imprinted region, as well as upd(14)mat. The clinical features of the patient with the epimutation were grossly similar to those of patients with upd(14)mat. Thus far 5 patients with an epimutation in the paternal allele, including our patient, have been identified.[4,11] These patients exhibit clinical features indistinguishable from those with full upd(14)mat. Our patient with an epimutation demonstrated normal birth weight, but previously reported patients with an epimutation have shown intrauterine growth retardation. [4,11] Therefore normal birth weight is not a specific feature related to epimutation.

One of the patients with upd(14)mat was mosaic for upd(14)mat and normal karyotype. It is not easy to understand the pathogenesis of such a mosaic, but similar mosaicism of chromosome 15 has been reported.[12] Mosaicism for upd(15)mat and normal cell lines has been found in a patient with the PWS phenotype.[12] Similarly, our patient with mosaic upd(14)mat demonstrated typical clinical features of upd(14)mat. This could be explained by the small proportion of normal cell lines (less than 20%), or it could be that the level of mosaicism is different in each tissue. It is possible that the proportion of normal cells may be lower in the brain, which is most responsible for the phenotype of upd(14)mat.

As is clear in our series of patients, upd(14)mat phenotype can be caused by an epimutation of 14q32.2. Recently, Kagami et al[2] reported a microdeletion in 14q32.2 associated with a similar phenotype (**Figure 1**). Buiting et al[4] also reported a patient with a 1Mb deletion at 14q32.2 (**Figure 1**). Therefore upd(14)mat phenotype is associated with not only upd(14)mat but an epimutation or small deletion. This genetic complexity is similar to that of PWS. PWS is caused by paternal deletion of 15q11-q13, maternal uniparental disomy of chromosome 15, and epimutation (imprinting defect). A new name such as upd(14)mat syndrome would be appropriate to represent the entire upd(14)mat clinical features represented by upd(14)mat, epimutation of 14q32.2 and microdeletion in 14q32.2. Alternatively, Buiting et al[4] suggested the term, "Temple syndrome," because upd(14)mat was first described by Dr. I. K. Temple in 1991, who subsequently described an epimutation in 2007.[4,5,11]

Finally, it should be emphasized that the *MEG3* methylation test could detect not only upd(14)mat but an epimutation and small deletions involving *MEG3*.This is because the *MEG3* DMR that is used for the diagnostic DNA methylation test is involved in the shortest region of overlap of the microdeletions (**Figure 1**). It is therefore a powerful method for screening patients with upd(14)mat syndrome.

Table III. Clinical features in patients with upd(14)mat, epimutation and microdeletions of 14q32.2

	Present study					Previous studies		
	Case 1	Case 2	Case 3	Case 4	Case 5	Upd(14)mat (n = 35)	Epimutation (n = 4)	Microdeletion (n = 4)
Premature delivery	−	−	−	−	−	10/25	0/4	0/3
Prenatal growth failure	−	+	+	+	+	24/27	4/4	3/3
Postnatal growth failure	+	+	+	+	+	26/32	3/4	3/3
Somatic features	+	+	+	+	+	23/35	4/4	3/3
Frontal bossing	+	+	+	+	−	9/9		
High arched palate	−	+	+		+	7/9		
Micrognathia	+	+	−	+	+	5/5		
Small hands	+	+	+	+	+	24/27	4/4	3/3
Scoliosis	−	−	−	−	−	5/19		
Others								
Hypotonia	+	+	+	+	+	25/28	4/4	1/1
Obesity	−	−	−	−	−	14/34	3/4	1/4
Early onset of puberty	NA	NA	NA	NA	NA	14/16	3/4	2/3
Mental retardation	−	−	−	−	+	10/27	2/4	1/4

NA, Not applicable.
Previous studies are based on references 2, 3 and 4.

Upd(14)mat syndrome demonstrates PWS-like phenotype during infancy, and it should be considered when seeing a patient with hypotonia. The *MEG3* methylation test should be performed to identify this syndrome. ■

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Table I. Microsatellite polymorphism analyses for chromosome 14 in 6 families with aberrant MEG3 methylation

Locus	Region	Case 1 family			Case 2 family			Case 3 family			Case 4 family			Case 5 family		
		Patient	Father	Mother	Patient	Father	Mother	Patient	Father	Mother	Patient	Father	Mother	Patient	Father	Mother
D14S261	14q11.2	298, 298	274, 298	298, 298	297, 297	297, 299	297, 297	298, 298	296, 298	298, 298	297, 297	297, 297	275, 297	275, 297, 297	275, 299	273, 297
D14S283	14q11.2	147, 149	139, 149	137, 147	139, 139	137, 137	139, 139	137, 149	133, 137	137, 149	150, 150	142, 150	140, 150	139, 139	137, 139	139, 147
D14S275	14q12	146, 146	146, 156	146, 146	149, 149	145, 145	149, 151	148, 152	146, 146	148, 152	155, 155	149, 155	149, 155	146, 148, 152	152, 156	146, 148
D14S70	14q13.1	100, 102	102, 102	100, 104	101, 101	101, 103	101, 101	103, 103	99, 101	103, 103	104, 104	104, 106	104, 104	101, 101, 103	101, 103	101, 101
D14S288	14q21.2	191, 201	201, 203	191, 207	201, 201	203, 203	201, 201	193, 193	193, 203	193, 193	195, 195	213, 215	195, 197	190, 196, 204	188, 196	190, 204
D14S276	14q22.3	241, —	239, 241	247, —	242, 244	244, 246	242, 244	244, 244	242, 244	244, 244	245, 245	241, 241	245, 245	244, 246, 246	242, 244	246, 246
D14S63	14q23.2	187, 187	187, 187	187, 187	187, 193	183, 189	187, 193	183, 187	189, 191	183, 187	191, 191	185, 195	191, 195	187, 189, 193	187, 193	187, 189
D14S258	14q24.2	204, 206	196, 206	202, 204	196, 196	198, 202	196, 196	196, 196	200, 202	196, 196	202, 202	204, 204	202, 204	196, 196, 198	198, 200	196, 196
D14S74	14q24.3	299, 313	260, 299	303, 313	303, 303	303, 305	303, 303	299, 303	299, 301	299, 303	295, 295	305, 313	295, 301	299, 301, 305	299, 305	299, 301
D14S68	14q31.3	323, 323	323, 323	323, 323	321, 321	323, 323	321, 321	321, 323	323, 323	321, 323	323, 323	325, 325	321, 323	321, 321, 323	323, 323	321, 321
D14S280	14q32.12	246, 248	248, 248	246, 246	243, 243	243, 245	243, 243	247, 247	243, 247	247, 247	248, 248	244, 244	242, 248	241, 243, 247	241, 245	243, 247
D14S65	14q32.2	135, 141	135, 135	135, 141	145, 145	135, 149	135, 145	135, 147	137, 145	135, 147	150, 150	150, 150	150, 150	135, 147	147, 147	135, 147
D14S985	14q32.2	255, 255	251, 255	255, 257	250, 250	246, 254	250, 254	247, 247	249, 249	247, 247	248, 248	246, 248	248, 254	247, 249	247, 253	247, 249
D14S292	14q32.33	84, 86	84, 86	86, 86	92, 92	86, 88	88, 92	85, 87	83, 85	85, 87	92, 92	86, 92	88, 92	87, 89	89, 89	87, 89

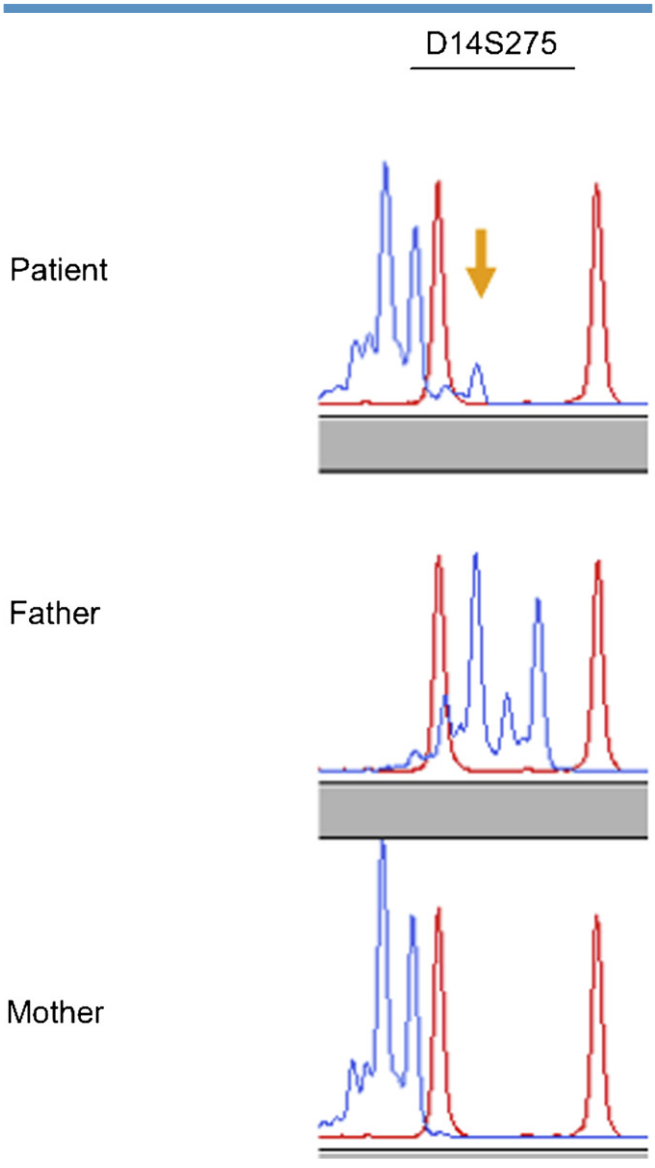

Figure 3. Microsatellite polymorphism analysis at D14S275 for the family of case 5. The patient demonstrates 3 peaks (146, 148, 152 bp), 2 (146, 148 bp) of which are transmitted from the mother, but 1 small peak (152 bp) indicated by the *arrow* is transmitted from the father. *Red peaks* depict size markers.