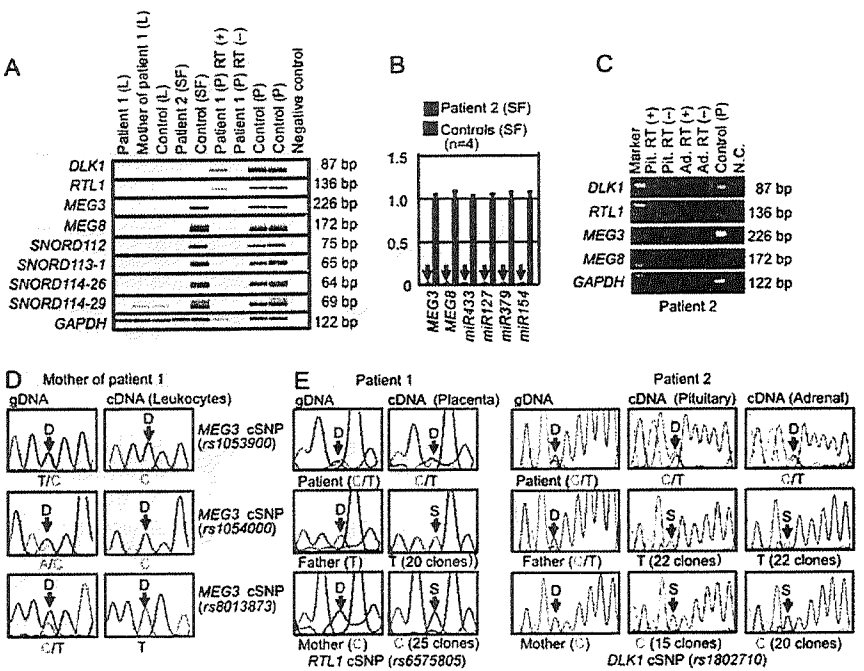
A
Patient 1 (L)
Mother of patient 1 (L)
Control (L)
Patient 2 (SF)
Control (SF)
Patient 1 (P) RT (+)
Patient 1 (P) RT (–)
Control (P)
Control (P)
Negative control
DLK1
RTL1
MEG3
MEG8
SNORD112
SNORD113-1
SNORD114-26
SNORD114-29
GAPDH
87 bp
136 bp
226 bp
172 bp
75 bp
65 bp
64 bp
69 bp
122 bp
B
Patient 2 (SF)
Controls (SF) (n=4)
1.5
1.0
0.5
0
MEG3
MEG8
miR433
miR127
miR379
miR154
C
Marker
Pit. RT (+)
Pit. RT (–)
Ad. RT (+)
Ad. RT (–)
Control (P)
N.C.
DLK1
RTL1
MEG3
MEG8
GAPDH
87 bp
136 bp
226 bp
172 bp
122 bp
Patient 2
D
Mother of patient 1
gDNA
cDNA (Leukocytes)
MEG3 cSNP (rs1053900)
T/C
C
MEG3 cSNP (rs1054000)
A/C
C
MEG3 cSNP (rs8013873)
C/T
T
E
Patient 1
gDNA
cDNA (Placenta)
Patient (C/T)
C/T
Father (T)
T (20 clones)
Mother (C)
C (25 clones)
RTL1 cSNP (rs6575805)
Patient 2
gDNA
cDNA (Pituitary)
cDNA (Adrenal)
Patient (C/T)
C/T
C/T
Father (C/T)
T (22 clones)
T (22 clones)
Mother (C)
C (15 clones)
C (20 clones)
DLK1 cSNP (rs1802710)

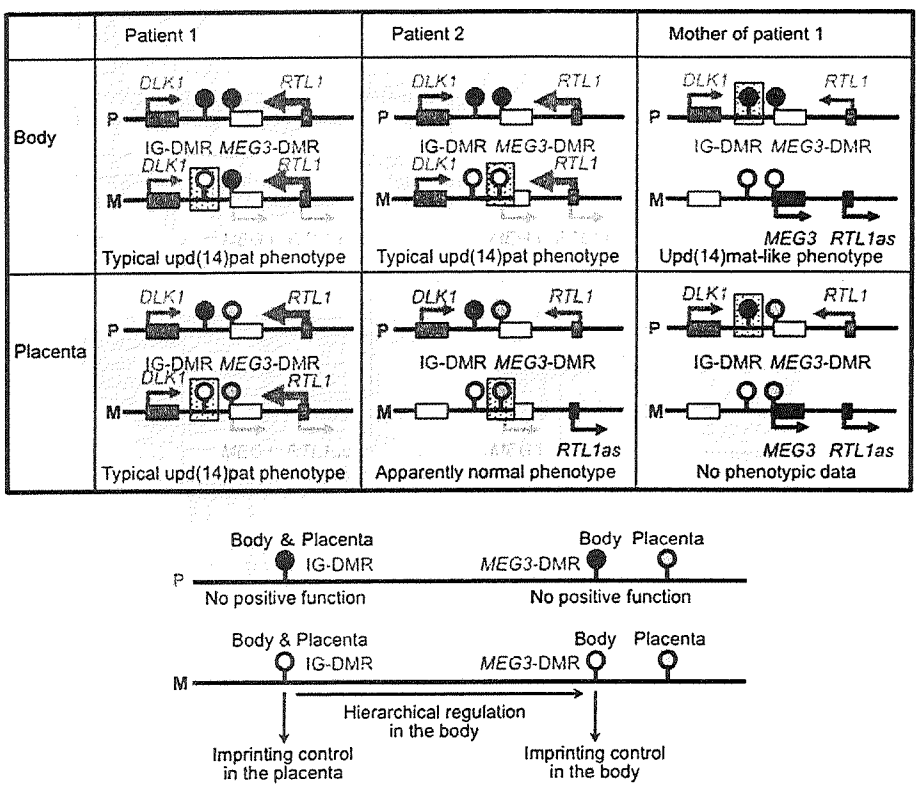
Patient 1
Patient 2
Mother of patient 1
Body
Placenta
DLK1
RTL1
P
M
IG-DMR
MEG3-DMR
RTL1as
MEG3
Typical upd(14)pat phenotype
Typical upd(14)pat phenotype
Upd(14)mat-like phenotype
Typical upd(14)pat phenotype
Apparently normal phenotype
No phenotypic data
Body & Placenta
Body
Placenta
No positive function
No positive function
Hierarchical regulation
in the body
Imprinting control
in the placenta
Imprinting control
in the body

Table S1. The Results of Microsatellite and SNP Analyses.

			Patient 1	Mother	Father	Patient 2	Mother	Father
<Microsatellite analysis>								
Locus	Position	Primer[a]						
D14S250	14q32.2	D14S250F/R	159	159	159	159/169	155/159	161/169
D14S1006	14q32.2	D14S1006F/R	126/138	126/140	138/140	136/138	138/144	136/138
D14S985	14q32.2	D14S985F/R	137	133/137	137	131/137	133/137	131/133
D14S1010	14q32.33	D14S1010F/R	137/141	133/137	141/145	143/145	143/147	135/145
D14S292	14q32.33	D14S292F/R	109/107	107/109	107	107/109	107/115	109/111
D14S1007	14q32.33	D14S1007F/R	119	109/119	119/123	121/125	121	121/125
<SNP analysis>								
NCBI No.	NT_026437 (bp)[b]	Primer[a]						
rs12435503	82191812	1F/1R	C	C	C			
rs3759556	82192052	1F/1R	A	A	A			
rs10139403	82194314	DLK1 99F/99R				A/G	A/G	G
rs13329039	82194517	DLK1 2F/2R	A	A	A	A	A	A
rs1135716	82195033	DLK1 2F/2R	C	C	C	C	C	C
rs34686110	82195112	DLK1 3F/3R	A	A	A	A	A	A
rs6575799	82198170	DLK1 4F/4R	G	G	G	G	G	G
rs2273607	82198179	DLK1 4F/4R	G	G	G	G	G	G
rs1058006	82198192	DLK1 4F/4R	A	A	A	A	A	A
rs2273608	82198494	DLK1 21F/21R	C/T	C	C/T	C	C/T	C
rs1757779	82199286	DLK1 100F/100R				C/T	C/T	T
rs1802710	82200398	DLK1cSNP F/R	C	C	C	C/T	C	C/T
rs34429112	82200437	DLK1 5F/5R	G	G	G	G	G	G
rs2295660	82200533	DLK1 6F/6R	T	T	T	T	T	T
rs1058009	82200613	DLK1 101F/101R	G	G	G	G	G	G
rs35339877	82200674	DLK1 8F/8R	G	G	G	G	G	G
rs12975	82200941	DLK1 8F/8R	C	C	C	C	C	C
rs3198687	82200983	DLK1 8F/8R	C	C	C	C	C	C
rs878110	82206242	22F/22R	C/T	C	T			
rs11627672	82238816	28F/28R	C	C	C			
rs10147396	82238817	28F/28R	C/G	C	G			
rs10147577	82238840	28F/28R	G	G	G			
rs10147404	82238853	28F/28R	A/C	C	A			
rs10138933	82244838	29F/29R	T	T	T			
rs10149782	82244962	29F/29R	A	A	A			
rs8019641	82245096	29F/29R	G	G	G			
rs2144820	82249282	30F/30R	T	C/T	T			
rs2180388	82269346	68F/68R	G	A/G	G			
rs12437020	82275703	CG4 F/R	G	G	G	G	G	G
rs10133627	82277327	CG6 F/R	C	C	C	C	C	C
rs1884538	82277410	4F/4R	G	G	A/G	G	G	G
rs1884539	82277539	4F/4R	A	A	A	G	A/G	A/G
rs12885923	82277562	4F/4R	A	A	A	A	A	A
rs12890188	82281176	7F/7R	A/G	A	A/G	A	A/G	A/G
rs12891580	82281189	7F/7R	C/T	T	CT	T	C/T	C/T
rs12889065	82287726	10F/10R				A	A	A
rs4906019	82287784	10F/10R				C	C	C
rs4906020	82289936	14F/14R				A	A	A
rs11627993	82290216	9F/9R (CG9)				C	C	C
rs12882497	82292169	MEG3 12F/12R				C	C	C
rs12882497	82292169	CTCF-D SNP F/R	C	C	C	C	C	C
Novel SNP	82292237	CTCF-D SNP F/R	G	G	G	G	G	G
rs11540030	82292300	CTCF-D SNP F/R	C	C	C	C	C	C
rs45546040	82292306	CTCF-D SNP F/R	C	C	C	C	C	C
rs11540030	82292300	MEG3 isoform 2 1F/1R		C		C	C	C
rs45546040	82292306	MEG3 isoform 2 1F/1R		C		C	C	C
rs10134980	82293281	MEG3 13F/13R				C	A/C	C
rs11624152	82294163	MEG3 16F/16R				G	G	G
rs11540029	82294855	MEG3 18F/18R		C		C	C	C
rs11540032	82294858	MEG3 18F/18R		T		T	T	T
rs11540031	82295128	MEG3 isoform 2 3F/3R	C	C	C	C	C	C
rs45518432	82295142	MEG3 isoform 2 3F/3R	T	T	T	T	T	T
rs11540028	82295153	MEG3 isoform 2 3F/3R	C	C	C	C	C	C
rs11540027	82295254	MEG3 isoform 2 3F/3R	A	A	A	A	A	A
rs45497397	82295376	MEG3 isoform 2 3F/3R	T	T	T	T	T	T
rs45470294	82295401	MEG3 isoform 2 3F/3R	T	T	T	T	T	T
rs45497097	82295482	MEG3 isoform 2 3F/3R	G	G	G	G	G	G
rs45617834	82295554	MEG3 isoform 2 3F/3R	C	C	C	C	C	C
rs45617834	82295554	MEG3 isoform 2 3F/3R	C	C	C	C	C	C
rs1053900	82301619	MEG3 cSNP(1F/1R)	C	C/T	C/T	C	C	C
rs1054000	82301735	MEG3 cSNP(1F/1R)	A/C	A/C	A	C	C	C
rs8013873	82301843	MEG3 cSNP(2F/2R)	C	C/T	T	C	C	C
rs7158663	82319177	MEG3 88F/88R				G	A/G	A/G
rs3742396	82319408	MEG3 88F/88R				A	A	A
rs3742379	82319439	MEG3 88F/88R				C	C/G	C/G
rs12884005	82347161	RTL1 8F/8R	G	G	G	G	G	G
rs35695758	82347239	RTL1 7F/7R	G	G	G	G	G	G
rs11623267	82348337	RTL1 4F/4R	C	C/G	C	C	C/G	C
rs6575805	82348770	RTL1 cSNP F/R	T/C	C	T	C	C	T/C
rs3825569	82350051	RTL1 cSNP (81F/81R)	C	C	C	C/T	C	T

[a] The primer sequences are shown in Table S3.
[b] The NCBI database (Genome Build 36.3).
Loci involved in microdeletions in patient 1 and her mother and in patient 2 are shaded in gray; SNP genotyping data are not informative for the parental origin of the microdeletion positive chromosomes.
cSNPs are underlined.

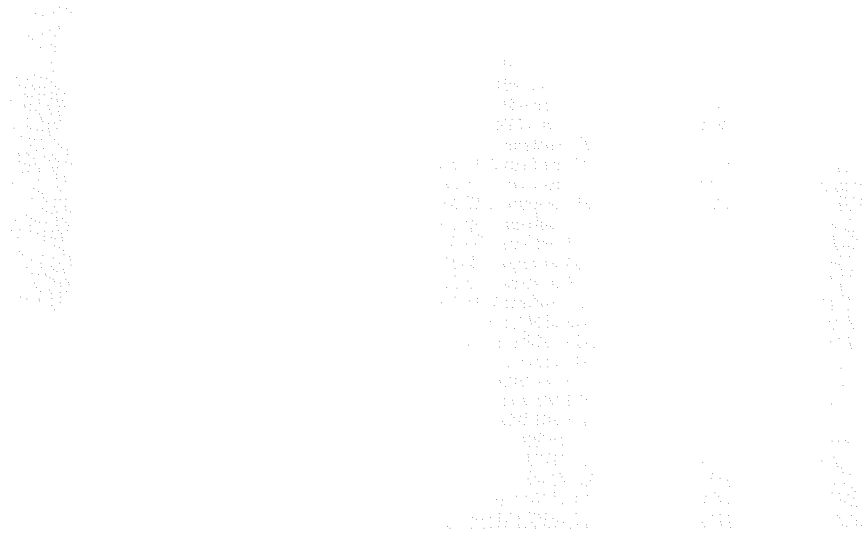

Table S2. Clinical Features in the Mother of Patient 1.

	The mother of patient 1	Upd(14)mat (n=35)[h] Sporadic
Age	27 years	0–30 years
Sex	Female	Male:Female=17:18
Karyotype	46,XX	
Pregnancy and delivery		
Premature delivery	No	10/25
Gestational age (weeks)	40	
Growth pattern		
Prenatal growth failure	No	24/27
Birth length (cm)	48.0 (–0.7 SD)[a]	
Birth weight (kg)	3.1 (–0.1 SD)[a]	
Postnatal growth failure	Yes	26/32
Present stature (cm)	146 (–2.2 SD)[b]	
Present weight (kg)	74.0 (+2.6 SD)[b]	
Pubertal development		
Early onset of puberty	No	14/16
Menarche (years)	12.0 (–0.2 SD)[c]	
Others		
Mental retardation	No	10/27
Obesity (BMI)	Yes (35)	14/34
Hypotonia	Equivocal[d]	25/28
Facial dysmorphism	Equivocal[e]	23/35
Small hands	Yes	24/27
Scoliosis	No	5/19
Remarks	Spontaneous abortions (3x)[f]	
Parental phenotype	Short stature[g]	

SD: standard deviation; BMI: body mass index.

[a] Assessed by the gestational age- and sex-matched Japanese reference data from the Ministry of Health, Labor, and Welfare (http://wwwdbtk.mhlw.go.jp/toukei/).

[b] Assessed by the age- and sex-matched Japanese reference data.

[c] The menarchial age in Japanese girls is 12.25±1.25 years.

[d] Allegedly, she had hypotonia during infancy.

[e] She exhibits mild frontal bossing and shallow orbits.

[f] Spontaneous abortions during the first trimester of the pregnancy; she also produced two normal boys.

[g] The paternal height is 155 cm (–3.0 SD), and the maternal height is 146 cm (–2.2 SD).

[h] In the column summarizing the clinical features of 35 cases with upd(14)mat, the denominators indicate the number of cases examined for the presence or absence of each feature, and the numerators represent the number of cases assessed to be positive for that feature; thus, the differences between the denominators and the numerators denote the number of cases evaluated to be negative for that feature (adopted from reference [2]).

Note: Possible repression of *DLK1* by the microdeletion involving the IG-DMR

The previous studies have indicated that upd(14)mat phenotype is primarily ascribed to loss of functional *DLK1* with an additional effect of loss of functional *RTL1* [2]. Thus, if the non-specific but upd(14)mat-like phenotype in the mother of patient 1 is related to the microdeletion on the paternally derived chromosome, the microdeletion might have affected a *cis*-acting regulatory element for the *DLK1* and/or *RTL1* expression. In this regard, although the data currently available argue against the

possibility that the microdeletion have impaired the *RTL1* expression, it remains tenable that the microdeletion might have affected the *DLK1* expression.

1. If the microdeletion in the mother of patient 1 affects the *DLK1* and/or *RTL1* expression on the paternally derived chromosome, it is predicted that the microdeletion also impairs the *DLK1* and/or *RTL1* expression on the paternalized imprinted region of maternal origin in patient 1. In this regard, expression analysis using cSNP showed clear biparental *RTL1* expression in the placenta of patient 1 (Figure 4E). This argues against the possibility that the microdeletion affects the *RTL1* expression. Unfortunately, although such expression analysis using cSNP was also performed for *DLK1* in patient 1, no informative cSNP was identified by direct sequencing of the entire coding region. Furthermore, although quantitative real time PCR was also attempted for *DLK1*, this was virtually impossible because of small quantity and poor quality of mRNA obtained from formalin-fixed and paraffin-embedded placental samples.
2. Patient 1 manifests typical upd(14)pat phenotype that has primarily been ascribed to the markedly elevated *RTL1* expression rather than to the doubled *DLK1* expression [2] (the markedly elevated *RTL1* expression has been shown previously [2], and this phenomenon is explained by the synergic effect of two active copies of *RTL1* and the absence of functional microRNA-containing *RTL1as* as a repressor for *RTL1* [12–15]). Thus, if the microdeletion affects the function of *DLK1* on the paternalized imprinted region of maternal origin in patient 1, this would still be capable of causing typical upd(14)pat phenotype in patient 1, as observed in case 2 reported by Kagami et al. [2] (Deletion-1 in Figure S3A). However, if the microdeletion affects the function of *RTL1* on the paternalized imprinted region of maternal origin in patient 1, this would lead to relatively mild upd(14)pat phenotype in patient 1, as observed in case 3–5 described by Kagami et al. [2] (Deletion-2 and Deletion-3 in Figure S3A).
3. In the mice, the paternally derived *Dlk1* mutation has produced several upd(14)mat-like features such as pre- and postnatal growth deficiency, obesity, and facial abnormalities [38], whereas the paternally inherited *Rtl1* deletion has caused mild growth deficiency only [13]. Thus, the clinical features in the mother of patient 1 (Table S2) are more similar to those of *Dlk1* knockout mice than to those of *Rtl1* knockout mice.
4. In the mouse, the targeted deletion for the IG-DMR (ΔIG-DMR) of paternal origin has permitted normal *Gtl2*-DMR methylation pattern, imprinting status, and phenotype in the body [12]. In this regard, since the deleted region is larger in the mother of patient 1 than in the mouse with ΔIG-DMR (especially at the centromeric region), a possible regulatory element might reside in the non-DMR sequence that is deleted from patient 1 and her mother and is preserved in the ΔIG-DMR mouse.

Table S3. Primers Utilized in the Present Study.

	Forward primer	Reverse primer	AT
<Microsatellite analysis>			
D14S250	GAAACTGGAACCACTGTGC	ACCCCTGCATTGTTTGAG	55
D14S1006	TTCCACAGGGCAAGCAGTA	TTCTGGCAAAACCCAACC	57
D14S985	CAGTGTGACCTTAAACAAGTCG	CCTGTGGGGTAGATACACGA	57
D14S1010	AGATTCTGGACTTGCCAAC	GTAGTAGTCAGGGCTTCCTAGAG	55
D14S292	CTGTGTGGTGCATCAATG	CATGAAGGCAGCCTCA	55
D14S1007	AGCTCCTATATGTCTTCACACAG	CTCCATTCCCATACGTCC	55
<SNP and insertion polymorphism analysis>			
1F/1R	CCACCCAAAGATTGGGA	CACACATACCCAGCTGA	60
DLK1 99F/99R	CCGCTGTTAGGAGGACTTGA	TGGCACACAGTAGGCACTTC	57
DLK1 2F/2R	CCTGGTGGGGTGAATTGTATA	TTCTGCGTGGCCCCTACAA	55
DLK1 3F/3R	TCTTCATATGTCCCCACCTTT	ACGCAGAGCTGAGGTGAACA	57
DLK1 4F/4R	CTCGTGTATGGAGAGGAAGCT	CGCATGAAAAGCAGCATTCA	57
DLK1 21F/21R	GTCACCCCGCAGATGTTC	ACGCATCGGGACTTGAGA	57
DLK1 100F/100R	TCTTCAGACGGGGTCAGAGT	GGGAAGAAGGGGCAGTAAAC	57
DLK1cSNP F/R (rs1802710)	AACCCATGCGAGAACGAC	GCAGGTCTTGTCGATGAAGC	57
DLK1 5F/5R	CCCTGAGGGCGTTTACTATGT	TGCTGGCGGAGTTGGTCA	57
DLK1 6F/6R	CAACCCATGCGAGAACGA	GCACTTGTTGAGGAAGACGAT	57
DLK1 101F/101R	CCCGCTTTGACTTGTCTTGT	GGCGCCAAATTAATGACAAT	57
DLK1 8F/8R	GATCGACATGACCACCTTCA	CCTGGTTCTGTTGCCTTGTTTT	57
22F/22R	CAGCAAAGCAGCCACATTT	TGGCTCCAGGGTTGTCTAGT	57
28F/28R	AGGCTGACCCACGTATTCC	AAGCAGAGGCACACTCCAAG	57
29F/29R	GCCTGGGGTACAGAGGAAGT	ACCGAGTGTGTCTGTGTGGA	57
30F/30R	GAGGGACAGACAGGTTTCCA	GCCCACCTCCTTGAGTCTTT	57
68F/68R	GCCTGAAAATGCTCTGGAAG	CTTCTGGGTCCAGCAAACTC	57
4F/4R	CTCACAGTTGCCCATGGCT	GCCCTTCCCACTTCTGTCTC	65
7F/7R	CTCGACTTGGCACAAAGG	TCCCTGGTGAGTGATTGG	60
10F/10R	AATGCCTCTGTCCAGGAATG	CCAGCCTGGGTGACATAATAA	57
14F/14R	GGCTCCTTAGGGACCCATCTT	CAAGGAAAAAGACCGTGGAA	57
9F/9R	CAAACCCGTGGTGCTTTG	AAGGGCATGAGTTGACGTT	60
MEG3 12F/12R	TCCCTTCTTTGCTGCAATCT	TGGGTGGGGTTTATATGGAG	57
CTCF-C region F/R[a]	GCTCATCCTCACCTGCTTTC	GTGGAGATGCCTGAGCTACC	57
CTCF-D region F/R[b]	CATGAGTTGTAAGCGGCAGA	AGGGTGAATTCAGGCACAAT	57
CTCF-D SNP F/R[c]	GTGCGGCTAGAGCAATTTGT	CGTCTTCCTTTTGCACATCC	57
MEG3 isoform 2 1F/1R	TCTGCGCCTCCATATAAACC	AGGATGGCCAACCACTCAC	57
MEG3 13F/13R	CTTTTGGTGAAATCGCCTTT	CCCTCCATCAGGAGAACAAA	57
MEG3 16F/16R	GTGGCCCACCTTCCTCTG	GAGGGAGGAGGGAGAAGAAA	57
MEG3 18F/18R	CCCTTTTGCTCTCTGCTGTT	TCTTCATCCTTTGCCATCCT	57
MEG3 isoform 2 3F/3R	CCTTTGATCAATTGCAGAGG	TTCCCCCAGAAAAGGATAGG	57
MEG3 cSNP(1F/1R)	ATCTGCAGGCTCTGCTTCTG	GCCAGGTGACCACAGGTATG	57
MEG3 cSNP(2F/2R)	GCCCTCCTGTGGTCTGAGTA	ACGATCACGAGGGGTCTCT	57
MEG3 88F/88R	GGATGCTGAGATTCGGGATA	TAGGAACACAACGGGACACA	57
RTL1 8F/8R	GTCAGAACCGCTACCTGGAG	GCTACCAAGGAATTCCAGGAC	57
RTL1 7F/7R	CAGCCATCCTCGTGCTACTG	CGCAAGACGACATCCTCATC	57
RTL1 4F/4R	CCAAAGGGGTGAAACTGAAC	GGAGATGTTGCGGGAGTAGA	57
RTL1 cSNP F/R (rs6575805)	CGTTTGGTTTGGAGCTTGA	TCCTGGCCATAAGAAAGCAC	57
RTL1 cSNP(81F/81R)	GGTGAACATGGCCTCTTCTG	AACGACCGTCTGAGAGTTGG	57
<Long PCR for FISH probe>			
FISH probe 1 (IG-DMR)	ATACACCTCCAGGGATTCATGTGAGGAT	GAGGGTTGCCTAAGCATCAAGATTCCAT	68
FISH probe 2 (*MEG3*-DMR)	CTGGAAGACATTTGACTCGCCTGATGTA	AGATTCTGCCCGCCACGTTGGTTATGAA	68
<Quantitative real-time PCR >[d]			
q-PCR-1	CATCTCGACTTGGCACAAAGG	AGGCAATGCACGGCAGAAA	60
q-PCR-2	TCACCCTGCACTGCCATGT	CGCTGTCTGACGCTGAAAGG	60
q-PCR-3	GGATTTCTGCTTTTCCCTGTAGCA	CCCCAGCCCCAGAGGAA	60
q-PCR-4	GGCCTGCTGCCCATCTACAC	CGCCACGTTGGTTATGAAATG	60

<Long PCR for breakpoint determination>			
Deletion analysis (patient 1 and her mother)	CAACAAATGAGAAAACAGCAGAGT	TCTTGAAAGTTTACATCCCCAAGT	60
Deletion analysis (patient 2)	GTGGCTAATAAACGTTCTCCTGTT	CACTTTCCACAGCAATTTACAAAG	60
<Direct sequence for breakpoint determination>			
Patient 1 and her mother	TCTTGAAAGTTTACATCCCCAAGT		55
Patient 2	GTGGCTAATAAACGTTCTCCTGTT		55
<MEG3 intron 5 including the inserted 66 bp sequence>			
Patient 2	CCATGCCCAGCCCAGCCCTATAGTA	GGGTGAGAAATGTCCAGAGC	57
<Direct sequence for mutation analysis>			
DLK1 1F/1R	GGAGGCGGTACGAAAAGG	GTTGGGGCTCACGAGACG	57
DLK1 2F/2R	CCTGGTGGGGTGAATTGTATA	TTCTGCGTGGCCCCTACAA	55
DLK1 3F/3R	TCTTCATATGTCCCCACCTTT	ACGCAGAGCTGAGGTGAACA	57
DLK1 4F/4R	CTCGTGTATGGAGAGGAAGCT	CGCATGAAAAGCAGCATTCA	57
DLK1 5F/5R	CCCTGAGGGCGTTTACTATGT	TGCTGGCGGAGTTGGTCA	57
DLK1 6F/6R	CAACCCATGCGAGAACGA	GCACTTGTTGAGGAAGACGAT	57
DLK1 7F/7R	AACCCCTCTCCTCACCGA	GCGAACACCACAAAGATTAGG	55
DLK1 8F/8R	GATCGACATGACCACCTTCA	CCTGGTTCTGTTGCCTTGTTTT	57
MEG3 isoform 2 1F/1R	TCTGCGCCTCCATATAAACC	AGGATGGCCAACCACTCAC	57
MEG3 isoform 2 2F/2R	CTCCCATGCCATAGGGTCT	GGAGGGTTGAAGTACCGTGA	55
MEG3 isoform 2 3F/3R	CCTTTGATCAATTGCAGAGG	TTCCCCCAGAAAAGGATAGG	57
MEG3 isoform 2 4F/4R	GCCCGACCTCTCTGAAGAT	GGTCTCTGCAAAGCCCCTAC	57
MEG3 isoform 2 5F/5R	CAGGTCCCTGCTGTCATCTT	GTGTCCTTGTGTCCATGAGC	57
MEG3 isoform 2 6F/6R	CTGGCGGTGTTTTTCAGTTT	GGCTTAAGAAGCCACATCG	57
MEG3 isoform 2 7F/7R	TGTTGCCTTCTTCCTCGTCT	CTGTGGTGGTCGAGTCCTTT	55
MEG3 isoform 2 8F/8R	CTTCGGTGTGTTTGGCTTTT	TCCTGGGTCTCAAGAAAGCA	57
MEG3 isoform 2 9F/9R	CGGCCTGAGTGAGGTTCTAC	AGGCCACATGTGTGTTCTTT	57
MEG3 isoform 2 10F/10R	GCCTTCATCTGCAACTGTGT	AGCACATCCATCAGTTTTTGTT	57
RTL1 1F/1R	CCCCGAAGTCGACTGGAT	TCCAGCCTGCTGAAGCTC	57
RTL1 2F/2R	CGGATCCGATGATCTTTCTG	AAACGGCTGGTAGCTCTTCA	57
RTL1 3F/3R	ACCGAAGATGTGTGGAAAGC	TCGATGAAGTTTCGCAGAGA	57
RTL1 4F/4R	CCAAAGGGGTGAAACTGAAC	GGAGATGTTGCGGGAGTAGA	57
RTL1 5F/5R	ATCCAAATCGACGACCAAAC	CGGCAGTGTTCCTCTGGA	57
RTL1 6F/6R	CTGCCACCTGTGAGAAACCT	ATCCAGGATGAGTCGTAGGG	57
RTL1 7F/7R	CAGCCATCCTCGTGCTACTG	CGCAAGACGACATCCTCATC	57
RTL1 8F/8R	GTCAGAACCGCTACCTGGAG	GCTACCAAGGAATTCCAGGAC	57
<Methylation analysis>			
CG4 F/R	TTTTATTATTGAATTGGGTTTGTTAGT	ACAATTCCTACTACAAAATTTCAACA	57
CG6 F/R	GTTAAGAGTTTGTGGATTTGTGAGAAATG	CTAAAAATCACCAAAACCCATAAAATCAC	57
CG7 F/R	TTGTGTTTGAATTTATTTTGTTT	CCCCAAATTCTATAACAAATTACT	57
CTCF-A F/R	AATGGTTTGAAAGAAAGGTTG	CAATACAAAATAAATACCACTCCA	55
CTCF-B F/R	GGAGAGTGGGGTTTATTGTGAA	AACCCTACAACCCCACAAAA	60
CTCF-C F/R	GAGGGTTTTTATTTGTTAGGGATT	TCCCCACACACATACCCTTT	55
CTCF-D F/R	GTTTATATTTGGGAATTAGTTATGT	TCAAACACAATATATAAAAAAAATC	55
CTCF-D allele-specific analysis[e]	GTTTATATTTGGGAATTAGTTATGT	CAACAACAAAACCCAAAATCAA	52
CTCF-E F/R	GTTTGGAGGATTGGGTTTTT	CCAACCACCACCAACCAA	50
CTCF-F & G F/R	GTTTGAGATTTGTTGGGTATTT	CAACTCAAAACCCAAAATAAC	55
<RT-PCR analysis >			
DLK1 cSNP F/R	AACCCATGCGAGAACGAC	GCAGGTCTTGTCGATGAAGC	57
MEG3 (exons 7–9, isoform 2)	TGCGGAAGAGGCCCTGAT	GTCCAGAGTCTCTGGGTCCA	54
RTL1 cSNP F/R[f]	CGTTTGGTTTGGAGCTTGA	TCCTGGCCATAAGAAAGCAC	57
MEG8 (exons 2–3)	CAGTGTTGCCTGGGTCTGA	ATCCCCTTGAAAGAGCAGGA	57
GAPDH (exons 2–5, isoform 1)	Taqman probe-primer mixture (Catalog No. 4326317E)		60
SNORD112	TGGACCAATGATGAGACAGTG	TGGACCTCAGTGTTTTGTGC	57
SNORD113-1	GAGTGATGAATAGTTCTGTGGCATA	GGACTTCAGAGTTTAGGGTTTAATCA	57
SNORD114-26	GATGATGAGCACTGGTGGAG	GACCTCAGAGTTCCAGACACG	57
SNORD114-29	TGGATCGATGATGACTACTGG	CAGACCTCAGAGTTCCAGACA	57

<RT-PCR direct sequencing>			
DLK1 cSNP (rs1802710)	AACCCATGCGAGAACGAC	GCAGGTCTTGTCGATGAAGC	57
MEG3 cSNPs (rs1053900)	ATCTGCAGGCTCTGCTTCTG	GCCAGGTGACCACAGGTATG	57
MEG3 cSNPs (rs1054000 & rs8013873)	GCCCTCCTGTGGTCTGAGTA	ACGATCACGAGGGGTCTCT	57
RTL1 cSNP (rs6575805)	CGTTTGGTTTGGAGCTTGA	TCCTGGCCATAAGAAAGCAC	57
<RTL1-specific q PCR>[g]			
RTL1 / Adaptor	TGCCACTTACCAGACTTGCACAGCAAA GAGGG	AGAGGTACCGGATCCGACTCGAGTCGA CATCG	70

[a] The primers were utilized to search a possible SNP in a 349 bp region encompassing the CTCF binding site C.
[b] The primers were utilized to search a possible SNP in a 356 bp region encompassing the CTCF binding site D.
[c] The primers were utilized to amplify a 300 bp segment harboring three known SNPs in the vicinity of the CTCF binding site D; the novel SNP detected in a single control subject was identified with these primers.
[d] Taqman probes used are: q-PCR-1, TAGAAGTCATTCAAACCC; q-PCR-2, CTGCCCTCTGTTTGATT; q-PCR-3, CCGCTGGGTGGCCCA; and q-PCR-4, TCACGGTACTTCAACCC.
[e] The primers amplify a 705 bp segment including the CTCF binding site D and the novel SNP identified in a single control subject.
[f] The cDNA synthesis for *RTL1* has been performed with *RTL1*-specifc primers that do not amplify *RTL1as*.
[g] The cDNA synthesis was performed using the following primer with adaptor and oligo(dt):
CTGATCTAGAGGTACCGGATCCGACTCGAGTCGACATCGTTTTTTTTTTTTTTTTTT.
The probe-primer mixtures utilized for Taqman real-time q-PCR are: assay No: Hs00171584 for *DLK1*, Hs00292028 for *MEG3*, and Hs00419701 for *MEG8*; and assay ID: 001028 for *miR433*, 000452 for *miR127*, 000568 for *miR379*, and 000477 for *miR154*.

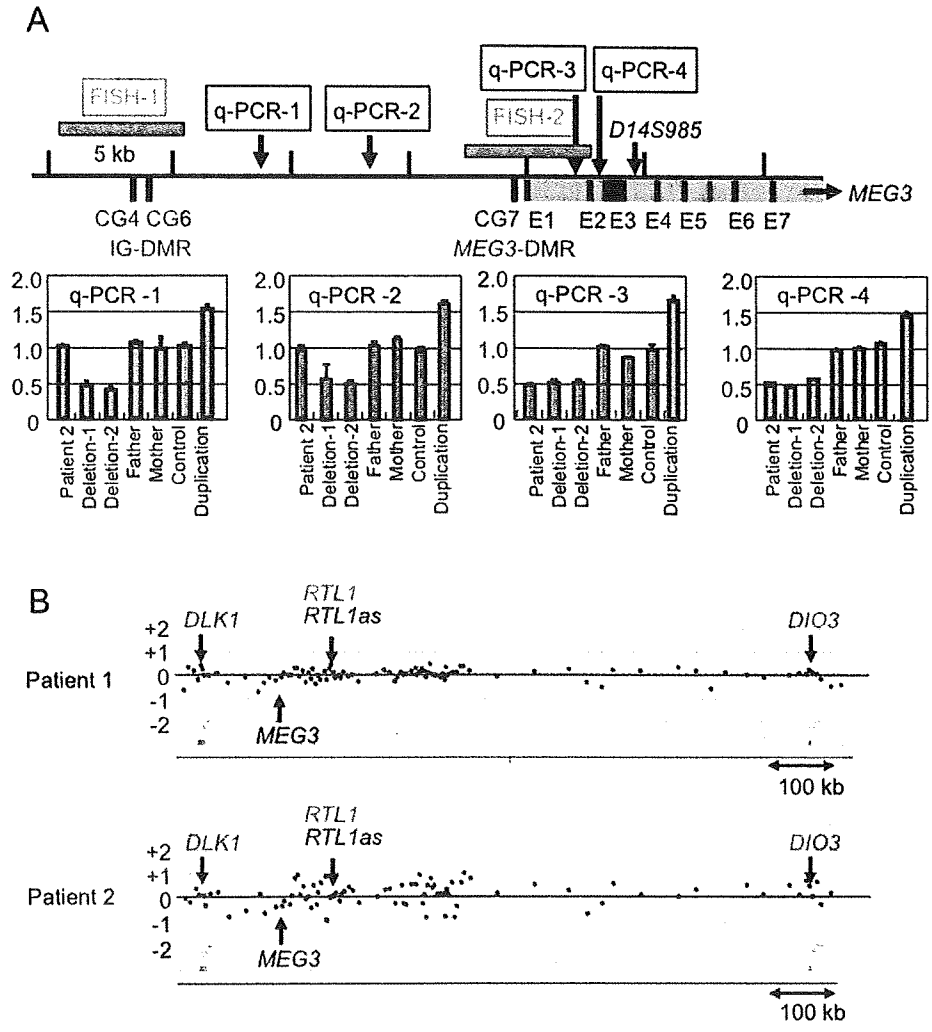
A
FISH-1
5 kb
q-PCR-1
q-PCR-2
q-PCR-3
q-PCR-4
FISH-2
D14S985
MEG3
CG4 CG6
IG-DMR
CG7 E1 E2 E3 E4 E5 E6 E7
MEG3-DMR
q-PCR -1
q-PCR -2
q-PCR -3
q-PCR -4
2.0
1.5
1.0
0.5
0
Patient 2
Deletion-1
Deletion-2
Father
Mother
Control
Duplication
B
Patient 1
Patient 2
DLK1
RTL1
RTL1as
DIO3
MEG3
+2
+1
0
-1
-2
100 kb

Figure S1

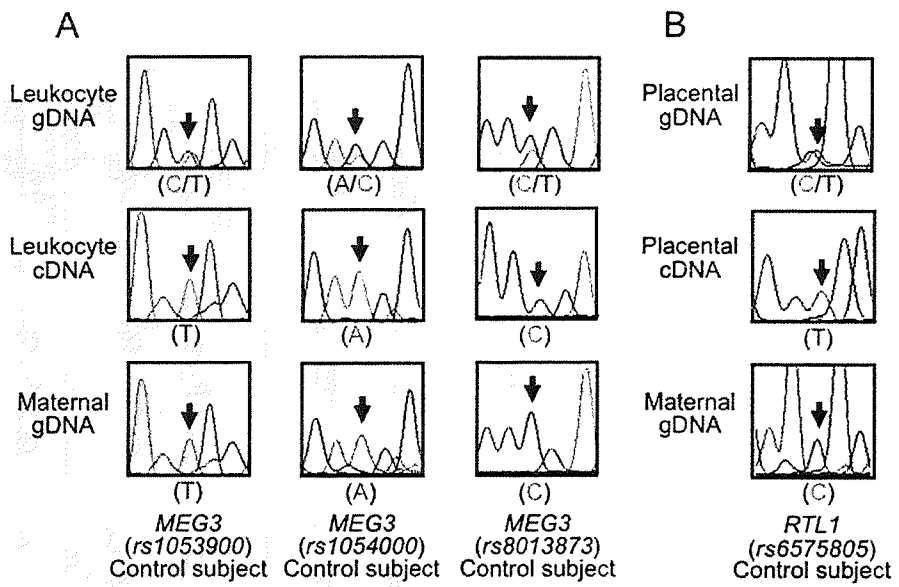
A
B
Leukocyte gDNA
Leukocyte cDNA
Maternal gDNA
Placental gDNA
Placental cDNA
Maternal gDNA
(C/T)
(A/C)
(C/T)
(T)
(A)
(C)
(T)
(A)
(C)
(C/T)
(T)
(C)
MEG3 (rs1053900) Control subject
MEG3 (rs1054000) Control subject
MEG3 (rs8013873) Control subject
RTL1 (rs6575805) Control subject

Figure S2

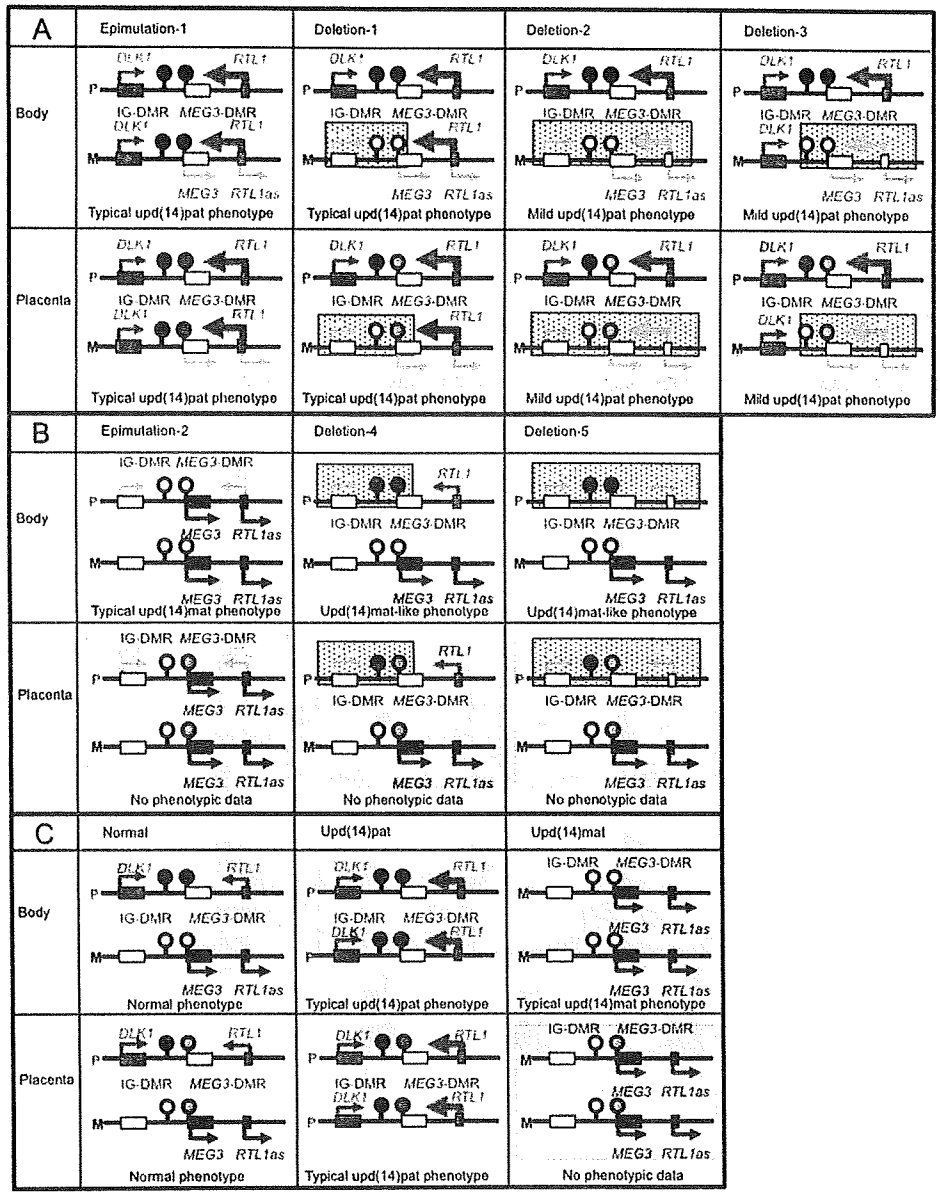
A
Epimutation-1
Deletion-1
Deletion-2
Deletion-3
Body
Placenta
DLK1
RTL1
IG-DMR
MEG3-DMR
MEG3
RTL1as
Typical upd(14)pat phenotype
Mild upd(14)pat phenotype
B
Epimutation-2
Deletion-4
Deletion-5
Typical upd(14)mat phenotype
Upd(14)mat-like phenotype
No phenotypic data
C
Normal
Upd(14)pat
Upd(14)mat
Normal phenotype

Figure S3

Legends for Supplementary Figures

The reference numbers correspond to those in the text.

Figure S1 Structural analysis.

(A) Quantitative real-time PCR analysis (q-PCR) for four regions (q-PCR-1~4) in patient 2. The q-PCR-1 and q-PCR-2 regions are present in two copies whereas q-PCR-3 and q-PCR-4 regions are present in a single copy in patient 2. The four regions are present in two copies in the parents and a control subject, in a single copy in the two previously reported patients with microdeletions involving the examined regions (Deletion-1 and Deletion-2 are case 2 and case 3 in Kagami et al. [2], respectively), and in three copies in a hitherto unreported case with 46,XX,der(17)t(14;17)(q32.2;p13)pat who have three copies of the 14q32.2 imprinted region. Since the microsatellite locus *D14S985* is present in two copies (Table S1) and the *MEG3*-DMR is deleted (Figure 2) in patient 2, this has served to localize the breakpoints.

(B) Oligoarray comparative genomic hybridization for a ~1 Mb imprinted region. All the signals remain within the normal range (–1 SD ~ +1 SD) (shaded in light blue) in patients 1 and 2.

Figure S2 Expression analysis.

(A) Maternal *MEG3* expression in the leukocytes of normal subjects. Genotyping has been performed for three cSNPs using genomic DNA (gDNA) and cDNA of leukocytes from control subjects and gDNA samples of their mothers, indicating that both maternally and non-maternally (paternally) derived alleles are delineated in the gDNA, whereas maternally inherited alleles alone are identified in cDNA. These three cSNPs have also been studied in the mother of patient 1 (Figure 4D).

(B) Paternal *RTL1* expression in the placenta of a normal subject. Genotyping has been carried out for *RTL1* cSNP using gDNA and cDNA samples of a fresh placenta and gDNA sample from the mother, showing that both maternally and non-maternally (paternally) derived alleles are delineated in the gDNA, whereas a non-maternally

(paternally) inherited allele alone is detected in cDNA. This cSNP has also been examined in the placenta of patient 1 (Figure 4E). Furthermore, the results confirm that the primers utilized in this study have amplified *RTL1*, but not *RTL1as*.

Figure S3 Schematic representation of the observed and predicted methylation and expression patterns in previously reported cases with upd(14)pat/mat-like phenotypes and in normal and upd(14)pat/mat subjects. For the explanations of the illustrations, see the legend for Figure 5. Previous studies have indicated that (1) Epimutation-1, Deletion-1, Deletion-2, and Deletion-3 lead to maternal to paternal epigenotypic alteration; (2) Epimutation-2 results in paternal to maternal epigenotypic alteration; and (3) Deletion-4 and Deletion-5 have no effect on the epigenotypic status [2,5–8,12].

(A) Cases with typical or mild upd(14)pat phenotype. Epimutation-1: Hypermethylation of the IG-DMR and the *MEG3*-DMR of maternal origin in the body, and that of the IG-DMR of maternal origin in the placenta (the *MEG3*-DMR is rather hypomethylated in the placenta) (cases 6–8 in Kagami et al. [2]). Deletion-1: Microdeletion involving *DLK1*, the two DMRs, and *MEG3* on the maternally inherited chromosome (case 2 in Kagami et al. [2]). Deletion-2: Microdeletion involving *DLK1*, the two DMRs, *MEG3*, *RTL1*, and *RTL1as* on the maternally inherited chromosome (cases 3 and 5 in Kagami et al. [2]). Deletion-3: Microdeletion involving the two DMRs, *MEG3*, *RTL1*, and *RTL1as* on the maternally inherited chromosome (case 4 in Kagami et al. [2]). These findings are explained by the following notions: (1) Epimutation (hypermethylation) of the normally hypomethylated IG-DMR of maternal origin directly results in paternalization of the imprinted region in the placenta and indirectly leads to paternalization of the imprinted region in the body via epimutation (hypermethylation) of the usually hypomethylated *MEG3*-DMR of maternal origin. Thus, the epimutation (hypermethylation) is predicted to have impaired the IG-DMR as the primary target, followed by the epimutation (hypermethylation) of the *MEG3*-DMR after fertilization; (2) Loss of the hypomethylated *MEG3*-DMR of maternal origin leads to paternalization of the imprinted region in the body; and (3) Loss of the hypomethylated IG-DMR of maternal origin results in

paternalization of the imprinted region in the placenta. Furthermore, epigenotype-phenotype correlations imply that the severity of upd(14)pat phenotype is primarily determined by the *RTL1* expression dosage rather than the *DLK1* expression dosage [2].

(B) Cases with upd(14)mat-like phenotype. Epimutation-2: Hypomethylation of the IG-DMR and the *MEG3*-DMR of paternal origin (Temple et al. [5], Buiting et al. [6], Hosoki et al. [7], and Zechner et al. [8]). Deletion-4: Microdeletion involving *DLK1*, the two DMRs, and *MEG3* on the paternally inherited chromosome (cases 9 and 10 in Kagami et al. [2]). Deletion-5: Microdeletion involving *DLK1*, the two DMRs, *MEG3*, *RTL1*, and *RTL1as* on the paternally inherited chromosome (case 11 in Kagami et al. [2] and patient 3 in Buiting et al. [6]). These findings are consistent with the following notions: (1) Epimutation (hypomethylation) of the normally hypermethylated IG-DMR of paternal origin directly results in maternalization of the imprinted region in the placenta and indirectly leads to maternalization of the imprinted region in the body through epimutation (hypomethylation) of the usually hypermethylated *MEG3*-DMR of paternal origin. Thus, epimutation (hypomethylation) is predicted to have affected the IG-DMR as the primary target, followed by the epimutation (hypomethylation) of the *MEG3*-DMR after fertilization; and (2) Loss of the hypermethylated DMRs of paternal origin has no effect on the imprinting status [2,12], so that upd(14)mat-like phenotype is primarily ascribed to the additive effects of loss of functional *DLK1* and *RTL1* from the paternally derived chromosome (the effects of loss of *DIO3* appears to be minor, if any [2,10]). Although the *MEGs* expression dosage is predicted to be normal in Deletion-4 and Deltion-5 and doubled in Epimutation-2 as well as in upd(14)mat, it remains to be determined whether the difference in the *MEGs* expression dosage has major clinical effects or not.

(C) Normal and upd(14)pat/mat subjects.