Figure 5. Schematic representation of the observed and predicted methylation and expression patterns. Deleted regions in patients 1–3 are indicated by stippled rectangles. P: paternally derived chromosome; and M: maternally derived chromosome. Representative imprinted genes are shown; these genes are known to be imprinted in the body and the placenta [2,14] (see also Figure S2). Placental samples have not been obtained in patients 2 and 3 (highlighted with light green backgrounds). Thick arrows for *RTL1* in patients 1 and 3 represent increased *RTL1* expression that is ascribed to loss of functional microRNA-containing *RTL1as* as a repressor for *RTL1* [18,20,21]; this phenomenon has been indicated in placentas with upd(14)pat and an epimutation and a microdeletion involving the two DMRs (Figure S3A and S3C) [2]. *MEG3* and *RTL1as* that are disrupted or predicted to have become silent on the maternally derived chromosome are written in gray. *DLK1* that may have been affected due to impairment of a *cis*-acting regulatory element (shown in purple circles) on the paternally derived or paternalized chromosome is indicated in black. Filled and open circles represent hypermethylated and hypomethylated DMRs, respectively; since the *MEG3*-DMR is rather hypomethylated and regarded as non-DMR in the placenta [2] (see also Figure 3), it is painted in gray.

Table 1. Clinical Features in Patients 1 and 3.

	Patient 1	Patient 3	Upd(14)pat (n=20)[c]
Present age	5.5 months	Deceased at 4 days	0–9 years
Sex	Female	Female	Male:Female=9:11
Karyotype	46,XX	46,XX	
Pregnancy and delivery			
Gestational age (weeks)	33	28	28–37
Delivery	Caesarean	Vaginal	Vaginal:Caesarean=6:7
Polyhydramnios	Yes	No	20/20 (<28)[d]
Amnioreduction (weeks)	2x (28, 30)	No	6/6
Placentomegaly	Yes	No	10/10
Growth pattern			
Prenatal growth failure	No	No	1/13
Birth length (cm)	43 (WNR)[a]	34 (WNR)[a]	
Birth weight (kg)	2.84 (>90 centile)[a]	1.32 (WNR)[a]	
Postnatal growth failure	Yes	…	5/6
Present stature (cm)	56.3 (–3.0 SD)[b]	…	
Present weight (kg)	5.02 (–3.0 SD)[b]	…	
Characteristic face			
Frontal bossing	No	Yes	5/7
Hairy forehead	Yes	Yes	9/10
Blepharophimosis	Yes	No	14/15
Depressed nasal bridge	Yes	Yes	13/13
Anteverted nares	Yes	No	6/10
Small ears	Yes	Yes	11/12
Protrudinphiltrum	Yes	No	15/15
Puckered lips	No	No	3/10
Micrognathia	Yes	Yes	11/12
Thoracic abnormality			
Bell-shaped thorax	Yes	Yes	17/17
Mechanical ventilation	Yes	Yes	17/17
Abdominal wall defect			
Diastasis recti	…	…	15/17
Omphalocele	Yes	Yes	2/17[e]
Others			
Short webbed neck	Yes	Yes	14/14
Cardiac disease	No	Yes (PDA)	5/10
Inguinal hernia	No	No	2/6
Coxa valga	Yes	No	3/4
Joint contractures	Yes	No	8/10
Kyphoscoliosis	No	No	4/7
Extra features		Hydronephrosis (bilateral)	

WNR: within the normal range; SD: standard deviation; and PDA: patent ductus arteriosus.

[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 [10].

[c] In the column summarizing the clinical features of 20 patients with upd(14)pat, 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]).

[d] Polyhydramnios has been identified by 28 weeks of gestation.

[e] Omphalocele is present in two cases with upd(14)pat and in two cases with epimutations [2].

Table 2. Clinical Features in Patient 2.

	Patient 2 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 [10].

[c] The menarchial age in Japanese girls is 12.25±1.25 years [11].

[d] Allegedly, she had hypotonia during infancy.

[e] Patient 2 exhibits mild frontal bossing and shallow orbits.

[f] Spontaneous abortions during the first trimester of the pregnancy; patient 2 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]).

Table 3. Clinical and molecular findings in mice with maternally inherited ΔIG-DMR and Δ*Gtl2*-DMR

and those with PatDi(12).

	Wildtype	PatDi(12)	ΔIG-DMR (~4.15 kb)[a]	Δ*Gtl2*-DMR (~10 kb)[b] Neomycin cassette (+)
<Body>				
Phenotype	Normal	Abnormal[c]	PatDi(12) phenotype[c]	Normal at birth Lethal by 4 weeks
Methylation pattern				
IG-DMR	Differential	Methylated	Methylated[d]	Differential
Gtl2-DMR	Differential	Methylated	Epimutated[e]	Methylated[d]
Expression pattern				
Pegs	Monoallelic	Increased (~2x)	Biparental Increased (2x or 4.5x)[f]	Grossly normal
Megs	Monoallelic	Absent	Absent	Decreased (<0.2~0.5x)[g]
<Placenta>				
Phenotype	Normal	Placentomegaly	Apparently normal	Not determined
Methylation pattern				
IG-DMR	Differential	Methylated	Not determined	Not determined
Gtl2-DMR	Non-DMR	Non-DMR	Not determined	Not determined .
Expression pattern				
Pegs	Monoallelic	Not determined	Increased (1.5~1.8x)[g]	Decreased (0.5~0.85x)[g]
Megs	Monoallelic	Not determined	Decreased (0.6~0.8x)[g]	Decreased (<0.1~1.0)[g]
Remark			Paternal transmission[h]	Paternal transmission[i] Biparental transmission[j]

[a] The deletion size is smaller than that of patients 1 and 2 in this study, especially at the centromeric region. Thus, it might be possible that a *cis*-acting regulatory element for *DLK1* expression exists in a region that is deleted in patients 1 and 2 and is preserved in the ΔIG-DMR mice.

[b] The microdeletion also involves *Gtl2*; in addition, the deletion size is larger than that of patient 3 in this study, so that some essential element(s) might have been deleted or disrupted in the Δ*Gtl2*-DMR mouse, but not in patient 3.

[c] Body phenotype includes bell-shaped thorax with rib anomalies, distended abdomen, and short and broad neck.

[d] Hemizygosity for the methylated DMR of paternal origin.

[e] Hypermethylation of the maternally derived DMR.

[f] 2x *Dlk1* and *Dio3* expression levels and 4.5x *Rtl1* expression level. The markedly elevated *Rtl1* expression level is ascribed to a synergic effect between activation of the usually silent *Rtl1* of maternal origin and loss of functional microRNA-containing *Rtl1as* as a repressor for *Rtl1* [21,23–25].

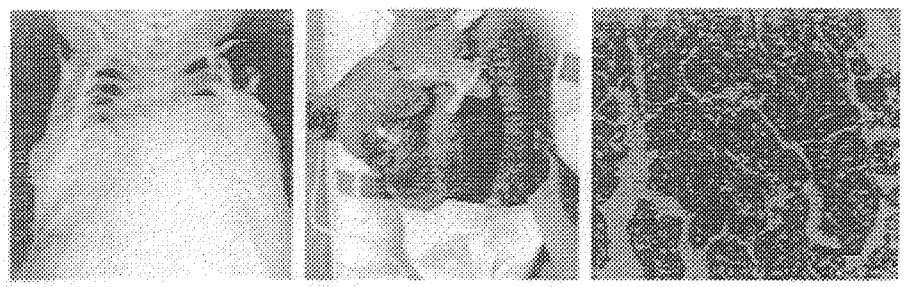
[g] The expression level is variable among examined tissues and examined genes.

[h] The ΔIG-DMR of paternal origin has permitted normal *Gtl2*-DMR methylation pattern, intact imprinting status, and normal phenotype in the body (no data on the placenta).

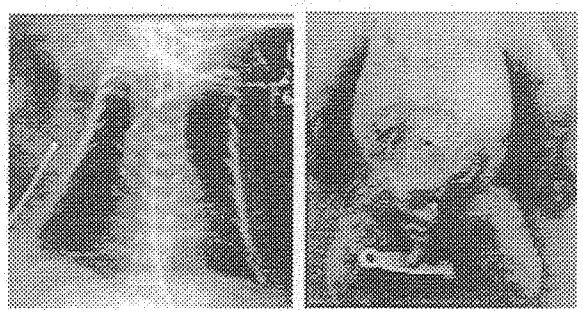
[i] The Δ*Gtl2*-DMR of paternal origin is accompanied by normal methylation pattern of the IG-DMR and variably reduced *Pegs* expression and increased *Megs* expression in the body, and has yielded severe growth retardation accompanied by perinatal lethality.

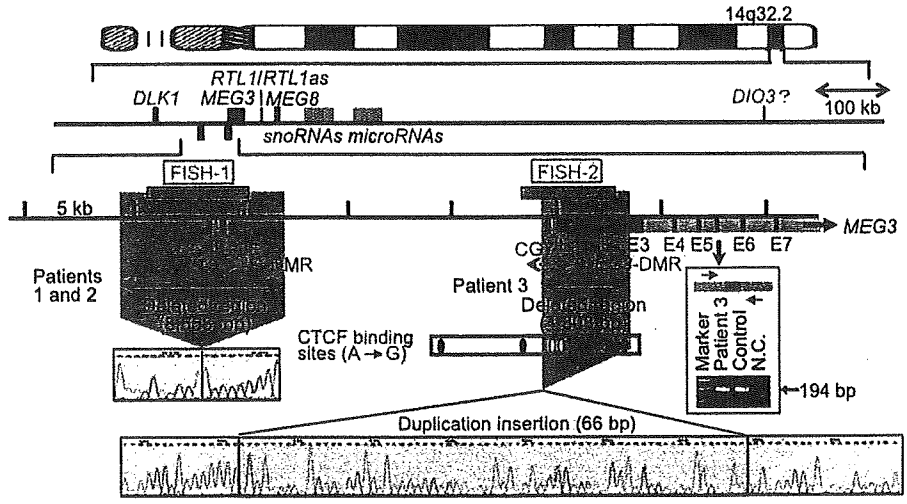
[j] The homozygous mutants have survived and developed into fertile adults, despite rather altered expression patterns of the imprinted genes.

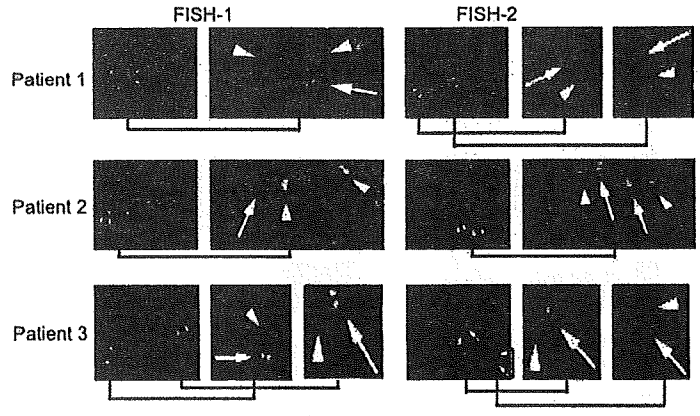
Patient 1

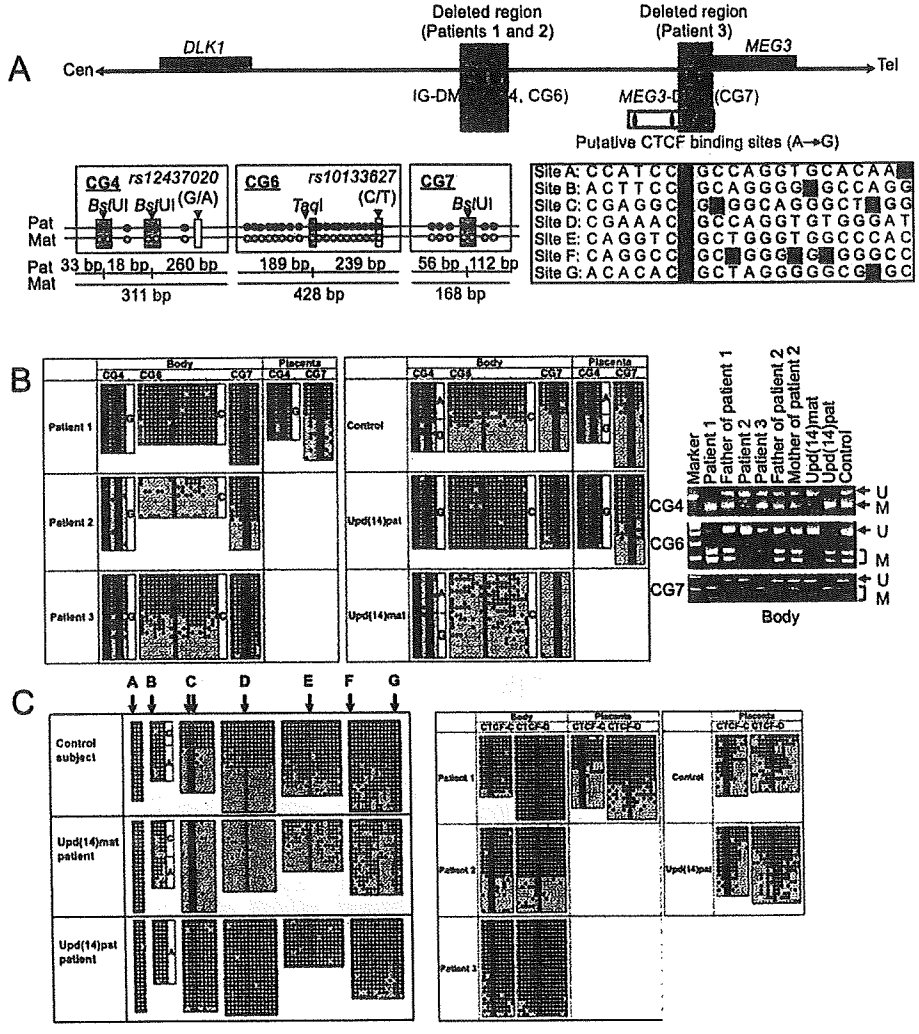


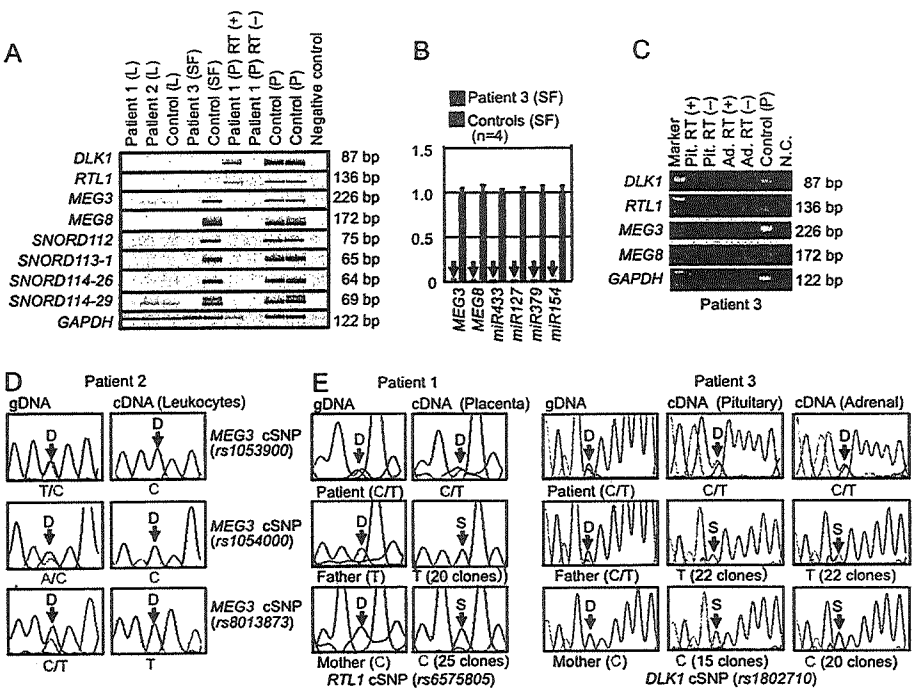
Patient 3

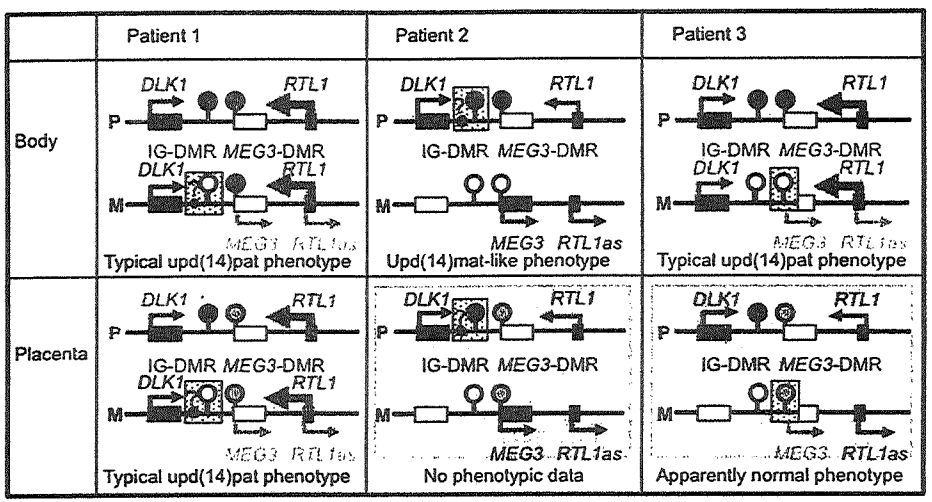


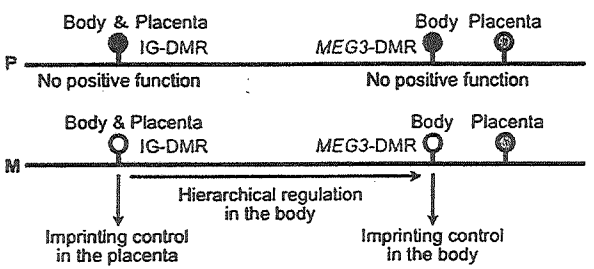

14q32.2
RTL1/RTL1as
DLK1
MEG3
MEG8
DIO3?
100 kb
snoRNAs microRNAs
FISH-1
FISH-2
5 kb
MEG3
E3 E4 E5 E6 E7
Patients
1 and 2
Patient 3
CTCF binding
sites (A→G)
Marker
Patient 3
Control
N.C.
194 bp
Duplication insertion (66 bp)


FISH-1
FISH-2
Patient 1
Patient 2
Patient 3


A
Deleted region (Patients 1 and 2)
Deleted region (Patient 3)
DLK1
MEG3
Cen
Tel
IG-DM
4, CG6)
MEG3-D
(CG7)
Putative CTCF binding sites (A→G)
CG4 rs12437020
BsfUI BsfUI (G/A)
CG6 rs10133627
TaqI (C/T)
CG7
BsfUI
Pat
Mat
33 bp 18 bp 260 bp
189 bp 239 bp
56 bp 112 bp
311 bp
428 bp
168 bp
Site A: C C A T C C G C C A G G T G C A C A A
Site B: A C T T C C G C A G G G G G C C A G G
Site C: C G A G G C G G G C A G G G C T G G
Site D: C G A A A C G C C A G G T G T G G G A T
Site E: C A G G T C G C T G G G T G G C C C A C
Site F: C A G G C C G C G G G G G G G C C C
Site G: A C A C A C G C T A G G G G G C G G C
B
Body
Placenta
CG4
CG6
CG7
Patient 1
Patient 2
Patient 3
Control
Upd(14)pat
Upd(14)mat
Marker
Patient 1
Father of patient 1
Patient 2
Patient 3
Father of patient 2
Mother of patient 2
Upd(14)mat
Upd(14)pat
Control
U
M
Body
C
A B C D E F G
Control subject
Upd(14)mat patient
Upd(14)pat patient
CTCF-C CTCF-D


A
Patient 1 (L)
Patient 2 (L)
Control (L)
Patient 3 (SF)
Control (SF)
Patient 1 (P) RT (+)
Patient 1 (P) RT (–)
Control (P)
Control (P)
Negative control
DLK1 87 bp
RTL1 136 bp
MEG3 226 bp
MEG8 172 bp
SNORD112 75 bp
SNORD113-1 65 bp
SNORD114-26 64 bp
SNORD114-29 69 bp
GAPDH 122 bp
B
Patient 3 (SF)
Controls (SF) (n=4)
1.5
1.0
0.5
0
MEG3
MEG8
miR433
miR127
miR379
miR154
C
Marker
Plt. RT (+)
Plt. RT (–)
Ad. RT (+)
Ad. RT (–)
Control (P)
N.C.
DLK1 87 bp
RTL1 136 bp
MEG3 226 bp
MEG8 172 bp
GAPDH 122 bp
Patient 3
D
Patient 2
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 3
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
Patient 3
Body
Placenta
DLK1
RTL1
P
M
IG-DMR
MEG3-DMR
MEG3
RTL1as
Typical upd(14)pat phenotype
Upd(14)mat-like phenotype
Typical upd(14)pat phenotype
Typical upd(14)pat phenotype
No phenotypic data
Apparently normal phenotype


Body & Placenta
IG-DMR
Body Placenta
MEG3-DMR
P
No positive function
No positive function
Body & Placenta
IG-DMR
Body Placenta
MEG3-DMR
M
Hierarchical regulation
in the body
Imprinting control
in the placenta
Imprinting control
in the body