Review Article

Molecular Mechanisms Leading to the Phenotypic Development in Paternal and Maternal Uniparental Disomy for Chromosome 14

Tsutomu Ogata[1] and Masayo Kagami[1]

[1]*Department of Endocrinology and Metabolism, National Research Institute for Child Health and Development, Tokyo, Japan*

Abstract. Human chromosome 14q32.2 carries a cluster of imprinted genes. They include paternally expressed genes (*PEGs*) such as *DLK1* and *RTL1*, and maternally expressed genes (*MEGs*) such as *GTL2* (alias, *MEG3*), *RTL1as* (*RTL1* antisense), and *MEG8*. Consistent with this, paternal and maternal uniparental disomies for chromosome 14 (upd(14)pat and upd(14)mat) cause distinct phenotypes. In this review, we summarize the current knowledge about the underlying factors for the development of upd(14)pat and upd(14)mat phenotypes. The data available suggest that the *DLK1-GTL2* intergenic differentially methylated region (IG-DMR) plays an important role in the maternal to paternal epigenotypic switch, and that excessive *RTL1* expression and decreased *DLK1* and *RTL1* expression play a major role in the development of upd(14)pat-like and upd(14)mat-like phenotypes, respectively.

Key words: uniparental disomy, imprinting, chromosome 14, DMR, epimutation

---

Introduction

Human chromosome 14q32.2 carries a cluster of imprinted genes including paternally expressed genes (*PEGs*) such as *DLK1* and *RTL1*, and maternally expressed genes (*MEGs*) such as *GTL2* (alias, *MEG3*), *RTL1as* (*RTL1* antisense), and *MEG8* (Fig. 1) (1, 2). The parent-of-origin specific monoallelic expression patterns are tightly related to the methylation status of differentially methylated regions (DMRs) (3).

For the 14q32.2 imprinted region, the *DLK1-GTL2* intergenic DMR (IG-DMR) and the *GTL2*-DMR are extensively hypermethylated after paternal transmission and grossly hypomethylated after maternal transmission (4–7).

Consistent with these findings, both paternal and maternal uniparental disomies for chromosome 14 (upd(14)pat and upd(14)mat) cause distinct phenotypes. Upd(14)pat results in a unique phenotype characterized by facial abnormality, small bell-shaped thorax, abdominal wall defects, and polyhydramnios (7). In particular, the bell-shaped thorax is pathognomonic and may be lethal or requires long-term mechanical ventilation, and polyhydramnios usually needs repeated amniocentesis before 30 wk of gestation. Upd(14)mat leads to clinically discernible features
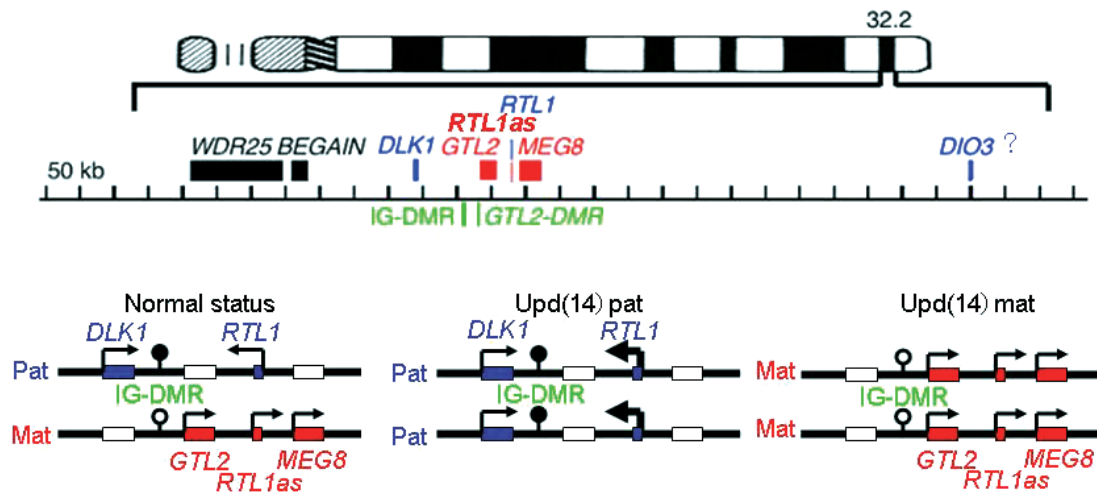
Fig. 1 The human chromosome 14q32.2 imprinted region and the expression patterns of the imprinted genes. *PEGs* are shown in blue, *MEGs* in red, and biparentally expressed genes in black. The IG-DMR and the *GTL2*-DMR are depicted in green. In the normal status, all the *PEGs* and *MEGs* show monoallelic expression patterns, in association with the parental origin specific methylation patterns of the DMRs. In upd(14)pat, *PEGs* are expressed from both chromosomes, with severely increased *RTL1* expression because of the absence of the functional *RTL1as*. In upd(14)mat, *MEGs* are expressed from both chromosomes.

such as pre- and postnatal growth failure, hypotonia, mild facial abnormalities, small hands, and early onset of puberty (7). The phenotypic spectrum is wide and ranges from nearly normal phenotype to severe phenotype reminiscent to that of Prader-Willi syndrome (8, 9). Furthermore, in agreement with the pivotal role of imprinted genes in placental growth and development (10, 11), upd(14)pat is associated with placentomegaly (12), while placental size has not been examined in upd(14)mat.

Such phenotypic development is ascribed to perturbed expression of imprinted genes, i.e., increased expression of *PEGs* and absent expression of *MEGs* in upd(14)pat and increased expression of *MEGs* and absent expression of *PEGs* in upd(14)mat. In this regard, we have recently proposed the major factors for the development of upd(14)pat/mat phenotypes, on the basis of (epi)genotype-phenotypes correlations in a total of 12 patients with microdeletions and epimutations affecting the imprinted region as

well as the mouse data available (7, 13). Here, we first summarize the current knowledge about the mouse homologous region and subsequently explain the underlying mechanisms leading to the development of human upd(14)pat/mat phenotypes. We also refer to the placental data in comparison with the body data.

Mouse Data

Uniparental disomy for chromosome 12

The human 14q32.2 imprinted region is highly conserved on the distal part of the mouse chromosome 12 (14). The imprinted genes on the distal chromosome 12 show monoallelic expression in both the normal embryos and placentas (10, 14). Thus, paternal uniparental disomy for chromosome 12 (PatDi(12)) results in distinct clinical phenotype such as prenatal lethality, cartilage defects, abdominal distension, and placentomegaly, whereas MatDi(12) leads to characteristic phenotype such as perinatal

lethality, growth failure (~60%), and placental hypoplasia (15). Since these clinical features are grossly reminiscent of those of human upd(14) pat/mat, this suggests the involvement of similar (epi)genetic mechanisms in both human and mouse disomies for the conserved imprinted region.

Targeted deletion of the IG-DMR (ΔIG-DMR)

This experiment has shown that the germ-line derived IG-DMR functions as a *cis*-acting regulator for the imprinted region of maternal origin in the bodies (5, 16). Namely, ΔIG-DMR causes paternalization of a maternally derived imprinted region and a unique phenotype comparable to that of PatDi(12) in embryos, with ~4.5 times of *Rtl1* expression and ~2 times of *Dlk1* and *Dio3* expression as well as nearly absent *Megs* expression (5, 15, 16). The marked *Rtl1* expression is ascribed to a synergic effect between activation of the usually silent maternally derived *Rtl1* and loss of functional *Rtl1as* as a repressor for *Rtl1* (5, 17). The doubled *Dlk1* and *Dio3* expression is simply due to the activation of *Pegs* of maternal origin (5). The absent *Megs* expression is associated with hypermethylation of the *Gtl2*-DMR (5), consistent with the notion that methylation pattern of the *Gtl2*-DMR is established after fertilization depending on the methylation pattern of the IG-DMR (18). By contrast, the ΔIG-DMR has no imprinting or clinical effect after paternal transmission (5).

Knockout mouse experiments

Knockout mouse experiments have successfully been performed for *Dlk1*, *Rtl1/Rtl1as*, and *Dio3*. The *Dlk1* mutation causes several upd(14)mat-like features such as pre- and postnatal growth deficiency (~80%), obesity, and facial abnormalities only after paternal transmission (19). The paternally inherited *Rtl1* deletion results in mild growth deficiency (~80%) and perinatal lethality (20), and the maternally derived *Rtl1as* deletion leads to placentomegaly and dilated fetal capillaries in the labyrinth zone in association with 2.5–3.0 times of *Rtl1* expression (20). *Dio3* knockout mice show reduced enzyme activities and some phenotypic effects after paternal transmission (21). Furthermore, *Gtl2^lacZ* mice with dysregulated imprinting status caused by a transgene insertion have a normal phenotype with at least 60–80% reduction of all the *Megs* (22).

Placental analysis

Placental analyses have revealed different expression patterns and phenotypes between the PatDi(12) mice and the ΔIG-DMR mice. While mice with PatDi(12) have placentomegaly (15), those with maternally derived ΔIG-DMR have normal placentas with mildly increased *Pegs* expression and considerably preserved *Megs* expression (16). In addition, while the IG-DMR methylation pattern is comparable between the normal mouse embryo and placenta, the *Gtl2*-DMR in the embryo does not behave as a DMR in the placenta, with the ratio of methylated to unmethylated clones being ~50%:50% after paternal transmission and ~25%:75% after maternal transmission (16). These findings suggest the differential imprinting control and resultant phenotypic consequences between the embryos and placentas with ΔIG-DMR.

Human Data

Identification of the IG-DMR and the *GTL2*-DMR

We and other investigators have identified the human IG-DMR (5, 7). In particular, we found two regions with the property of the IG-DMR, and designated CG4 and CG6. We have also identified the human *GTL2*-DMR, and designated CG7; here, although the *GTL2*-DMR has been reported (6), CG7 is the first region that was confirmed to be the *GTL2*-DMR by bisulfite sequencing (7).

Proposed hypothesis

We have proposed that the IG-DMR plays an important role in the maternal to paternal epigenotypic switch, and that excessive *RTL1* expression and decreased *DLK1* and *RTL1* expression play a major role in the development of upd(14)pat-like and upd(14)mat-like phenotypes, respectively. This hypothesis assumes that the functions of the IG-DMR and the imprinted genes within this domain are primarily similar between the human and the mouse (5, 7). We present here how this hypothesis can explain the development of upd(14)pat/mat-like phenotypes in non-disomic patients. We do not refer to *DIO3* and *MEGs* other than *RTL1as*; although the relevance of *DIO3* and the total absence of *MEGs* still remains tenable at this time, upd(14)pat/mat patients are apparently free from thyroid dysfunction (7), despite the primary function of *DIO3* being thyroid hormone metabolism (21), and biological functions remains totally unknown for *MEGs* other than *RTL1as*.

Familial microdeletions (Family A) (Fig. 2)

This unique three-generation family contains two sibs (III-1 and III-3) with typical upd(14)pat phenotype and the mother (II-2) and the maternal grandfather (I-3) with upd(14)mat-like phenotype including mild short stature (–2.2 SD in the mother and –2.9 SD in the grandfather) (7). Methylation analysis showed hypermethylated DMRs in case III-3 and hypomethylated DMRs in cases II-2 and I-3. Deletion analysis revealed ~109 kb deletion involving *DLK1*, the IG-DMR, the *GTL2*-DMR, and *GTL2* in cases with upd(14) pat/mat-like phenotypes. Thus, the deletion has caused typical upd(14)pat phenotype after maternal transmission and upd(14)mat-like phenotype after paternal transmission.

The results are well explained by the above notion. In the two sibs (III-1 and III-3) with typical upd(14)pat phenotype, since the loss of IG-DMR is derived from the mother, this would have caused paternalization of the imprinted domain, resulting in the expression of *PEGs* from both chromosomes. However, since *DLK1* is deleted from the maternally inherited chromosome, *DLK1* should be present in a single active copy, as in normal individuals. By contrast, since *RTL1* is present in two copies in the absence of functional *RTL1as*, the expression dosage of *RTL1* should be increased markedly (4–5 times), as in upd(14)pat patients. Thus, it is likely that severely increased *RTL1* dosage has played a critical role in the development of typical upd(14) pat phenotype.

In the mother (II-2) and the maternal grandfather (I-3) with upd(14)mat-like phenotype, since the loss of IG-DMR is of paternal origin, this would have no effect on the imprinting status. Thus, the upd(14)mat-like phenotype would simply be ascribed to the loss of *DLK1* from the paternally derived chromosome.

Familial microdeletions (family B) (Fig. 3)

This two-generation family contains the daughter (III-1) with relatively mild upd(14) pat-like phenotype in terms of the duration of respiratory duration, abdominal defects, and the degree of polyhydramnios, and the mother (II-2) with upd(14)mat-like phenotype including severe short stature (–4.4 SD) (7). Methylation analysis showed hypermethylated DMRs in case III-1 and hypomethylated DMRs in case II-2. Deletion analysis revealed a ~411 kb deletion involving *WDR25*, *BEGAIN*, *DLK1*, the IG-DMR, the *GTL2*-DMR, *GTL2*, *RTL1*, *RTL1as*, and *MEG8* in cases with upd(14)pat/mat-like phenotypes. Thus, the deletion has caused relatively mild upd(14)pat phenotype after maternal transmission and upd(14)mat-like phenotype with severe short stature after paternal transmission.

The results are similarly explained by the above notion. In case III-1, loss of IG-DMR from the maternally derived chromosome would have caused paternalization of the imprinted domain. However, since *DLK1* is deleted from the
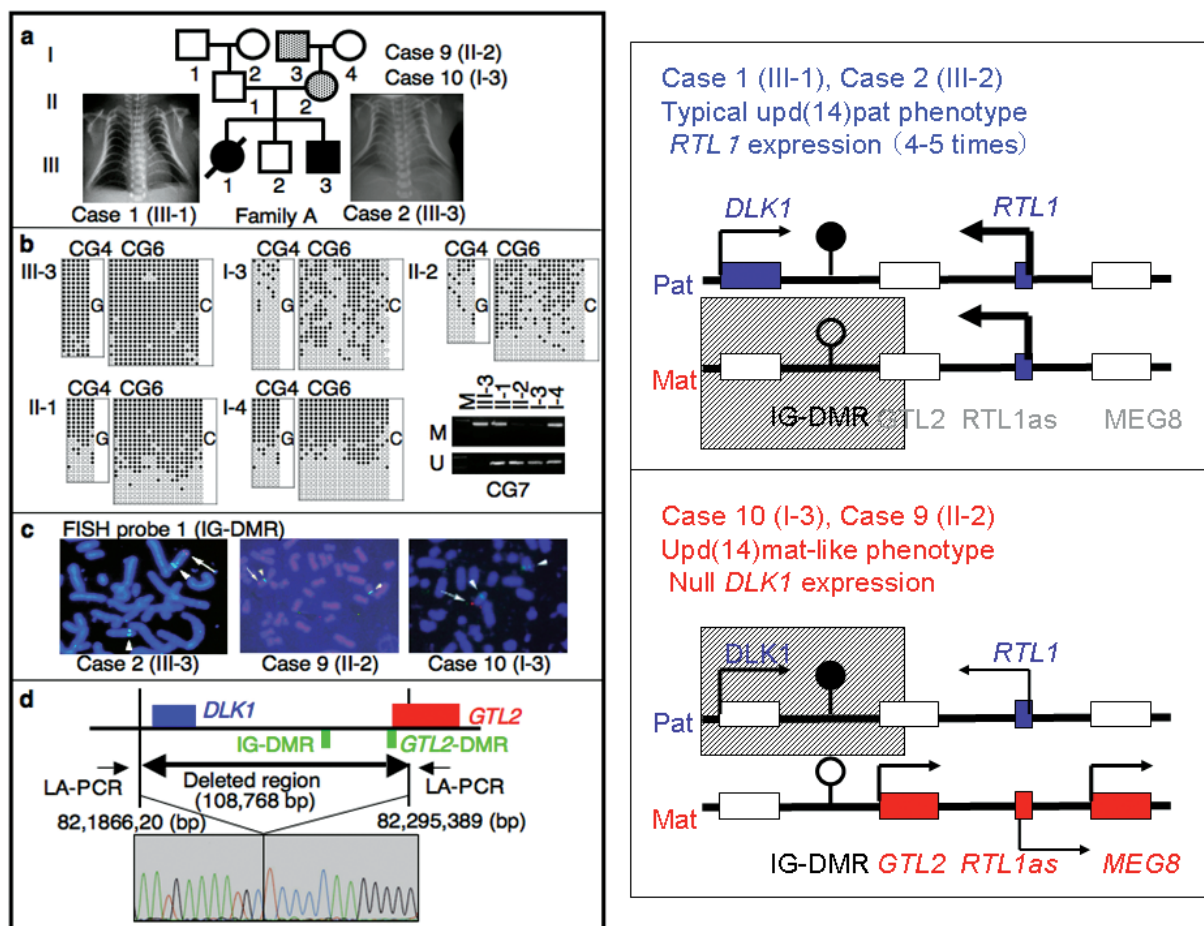
Fig. 2 Family A.
   a. The pedigree. Cases III-1 (deceased) and III-3 have typical upd(14)pat phenotype, and cases I-3 and II-2 exhibit upd(14)mat-like phenotype.
   b. Methylation analysis of the DMRs. The IG-DMR (CG4 and CG6) and the *GTL2*-DMR (CG7) are severely hypermethylated in case III-3 and grossly hypomethylated in cases I-3 and II-2. For the IG-DMR, each lane indicates a single clone, and each circle denotes a CpG island; filled and open circles represent methylated and unmethylated cytosines, respectively. For the *GTL2*-DMR (CG7), methylated allele-specific primers (M) and unmethylated allele-specific primers (U) have been utilized.
   c. FISH analysis for the IG-DMR. Heterozygous microdeletion is identified in cases III-3, II-2, and I-3.
   d. Direct sequencing for a long and accurate (LA)-PCR product, demonstrating a ~109 kb deletion in cases III-1, II-2, and I-3.
   The predicted gene dosages are indicated on the right part.

maternally inherited chromosome, *DLK1* should be present in a single active copy, as in normal individuals. By contrast, while *RTL1* is also present in a single copy, the expression dosage of *RTL1* should be 2.5–3.0 times higher than the normal individuals because of the absence of functional *RTL1as*. Thus, it appears that moderately increased *RTL1* dosage is essential for the development of relatively mild upd(14) pat phenotype.
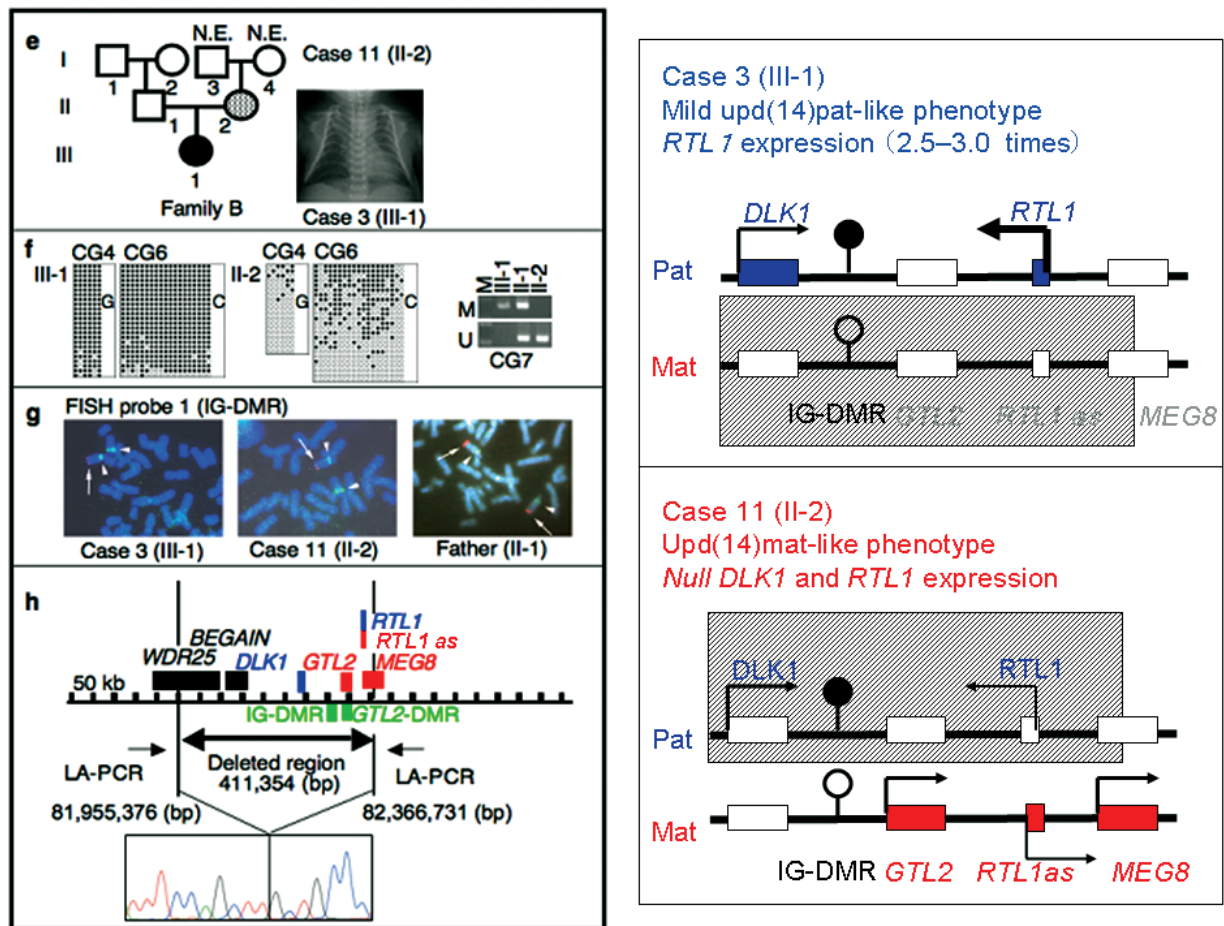
Fig. 3 Family B.
  e. The pedigree. Cases III-1 show upd(14)pat-like phenotype, and cases I-3 and II-2 exhibit upd(14)mat-like phenotype.
  f. Methylation analysis of the DMRs. The IG-DMR (CG4 and CG6) and the *GTL2*-DMR (CG7) are severely hypermethylated in case III-3 and grossly hypomethylated in case II-2.
  g. FISH analysis for the IG-DMR. Heterozygous microdeletion is identified in cases III-1 and II-2.
  h. Direct sequencing for an LA-PCR product, demonstrating a ~411 kb deletion in cases III-1 and II-2.
  The predicted gene dosages are indicated on the right part.

In the mother (II-2), there should be no alteration of the imprinting status because of the loss of IG-DMR from the paternally derived chromosome. Thus, the upd(14)mat-like phenotype with severe short stature would simply be ascribed to the loss of *DLK1* and *RTL1* from the paternally derived chromosome.

Sporadic microdeletions

We have also identified two deletions of maternal origin, i.e., a ~475 kb deletion involving the IG-DMR, the *GTL2*-DMR, *GTL2*, *RTL1*, *RTL1as*, and *MEG8* and a ~6.5 Mb deletion involving the whole imprinted region in patients with relatively mild upd(14)pat-like phenotype (7). This would also be explained by the

moderately increased *RTL1* dosage, as in the case III-1 of family B.

Epimutations (hypermethylated DMRs)

We have identified three patients with typical upd(14)pat phenotype and hypermethylated DMRs (epimutations) (7). In particular, genotyping analysis for a SNP within the IG-DMR (CG4) confirmed hypermethylation of the usually hypomethylated DMRs of maternal origin. The results are explained by assuming that the hypermethylation of the maternally inherited IG-DMR has caused paternalization of the imprinted region, as in the loss of IG-DMR of maternal origin. In this case, since the expression pattern of the imprinted domain would be comparable to those in upd(14)pat, with 4–5 times of *RTL1* expression dosage, this explains the development of typical upd(14)pat phenotype in these patients.

Epimutations (hypomethylated DMRs)

We have also found a patient with fairly typical upd(14)mat-like phenotype and hypomethylated DMRs (epimutations) (13). The results suggest that the hypomethylation of the paternally inherited IG-DMR has resulted in maternalization of the imprinted region, leading to the development of upd(14)mat-like phenotype.

Placental analysis

Since virtually all the imprinted genes studied to date are expressed in the placenta (10, 14), we examined placental samples obtained from case III-3 in family A with the microdeletion and from one case with epimutation (hypermethylation), as well as from a upd(14) pat patient and a nearly gestational age-matched control subject (7). We could also obtain three sets of samples consisting of cDNA and genomic DNA of normal fresh placenta and leukocyte genomic DNA of the mother.

Consequently, we have shown the following: [1] monoallelic paternal *DLK1* expression and maternal *GTL2* expression in the placentas (the genotyping results were not informative for other imprinted genes); [2] paternalization of the maternally inherited imprinted region with markedly elevated *RTL1* expression dosage in case III-3 of family A and one case with epimutation; [3] parental origin dependent differential methylation pattern of the IG-DMR and grossly hypomethylated *GTL2*-DMR (the results are consistent with the IG-DMR being the germline derived DMR and the *GTL2*-DMR being the secondary DMR, because the germline derived DMRs are delineated as DMRs in the placentas as well as in the bodies, whereas the secondary DMRs, though they behave as DMRs in the bodies, are rather hypomethylated irrespective of the parental origin in the placentas) (7, 23–25); and [4] characteristic histological findings such as proliferation of dilated and congested chorionic villi. These findings imply that the phenotypic development is closely associated with altered expression dosage of the imprinted genes in both the body and the placenta, and that the epigenetic control is different between the bodies and the placentas in the human as well as in the mouse and between the human and the mouse placentas with maternally derived deletion of the IG-DMR.

Perspectives

Despite the above progress, many matters remain to be clarified. They include: the precise mechanisms involved in the imprinting regulation, the clinical and molecular consequences caused by the *GTL2*-DMR deletion alone, the repressor function of *RTL1as* for *RTL1* in the human, the biological functions of most *MEGs/Megs*, the presence or absence of multiple microRNAs in the human imprinted region, the imprinting status of human *DIO3*, the mechanisms leading to epimutations, and the mechanisms involved in the placental imprinting regulation. These matters await further investigations.

References

1. Cavaille J, Seitz H, Paulsen M, Ferguson-Smith AC, Bachellerie JP. Identification of tandemly-repeated C/D snoRNA genes at the imprinted human 14q32 domain reminiscent of those at the Prader-Willi/Angelman syndrome region. Hum Mol Genet 2002;11:1527–38.

2. Charlier C, Segers K, Wagenaar D, Karim L, Berghmans S, Jaillon O, et al. Human-ovine comparative sequencing of a 250-kb imprinted domain encompassing the callipyge (clpg) locus and identification of six imprinted transcripts: DLK1, DAT, GTL2, PEG11, antiPEG11, and MEG8. Genome Res 2001;11:850–62.

3. Li E, Beard C, Jaenisch R. Role for DNA methylation in genomic imprinting. Nature 1993; 366:362–65.

4. Paulsen M, Takada S, Youngson NA, Benchaib M, Charlier C, Segers K, et al. Comparative sequence analysis of the imprinted Dlk1-Gtl2 locus in three mammalian species reveals highly conserved genomic elements and refines comparison with the Igf2-H19 region. Genome Res 2001;11:2085–94.

5. Lin SP, Youngson N, Takada S, Seitz H, Reik W, Paulsen M, et al. Asymmetric regulation of imprinting on the maternal and paternal chromosomes at the Dlk1-Gtl2 imprinted cluster on mouse chromosome 12. Nat Genet 2003;35:97–102.

6. Murphy SK, Wylie AA, Coveler KJ, Cotter PD, Papenhausen PR, Sutton VR, et al. Epigenetic detection of human chromosome 14 uniparental disomy. Hum Mutat 2003;22:92–7.

7. Kagami M, Sekita Y, Nishimura G, Irie M, Kato F, Okada M, et al. Deletions and epimutations affecting the human 14q32.2 imprinted region in individuals with paternal and maternal upd(14)-like phenotypes. Nat Genet 2008;40:237–42.

8. Gunay-Aygun M, Schwartz S, Heeger S, O'Riordan MA, Cassidy SB. The changing purpose of Prader-Willi syndrome clinical diagnostic criteria and proposed revised criteria. Pediatrics 2001;108:E92.

9. Bittel DC, Butler MG. Prader-Willi syndrome: clinical genetics, cytogenetics and molecular biology. Expert Rev Mol Med 2005;7:1–20.

10. Coan PM, Burton GJ, Ferguson-Smith AC. Imprinted genes in the placenta. Placenta 2005;26(Suppl A):S10–20.

11. Fowden AL, Sibley C, Reik W, Constancia M. Imprinted genes, placental development and fetal growth. Horm Res 2006;65(Suppl 3):50–8.

12. Kagami M, Yamazawa K, Matsubara K, Matsuo N, Ogata T. Placentomegaly in paternal uniparental disomy for human chromosome 14. Placenta (in press).

13. Hosoki K, Ogata T, Kagami M, Tanaka T, Saitoh S. Epimutation (hypomethylation) affecting the chromosome 14q32.2 imprinted region in a girl with upd(14)mat-like phenotype. Eur J Hum Genet 2008; [Epub ahead of print].

14. Kaneko-Ishino T, Kohda T, Ishino F. The regulation and biological significance of genomic imprinting in mammals. J Biochem 2003;133:699–711.

15. Georgiades P, Watkins M, Surani MA, Ferguson-Smith AC. Parental origin-specific developmental defects in mice with uniparental disomy for chromosome 12. Development 2000;127:4719–28.

16. Lin SP, Coan P, da Rocha ST, Seitz H, Cavaille J, Teng PW, et al. Differential regulation of imprinting in the murine embryo and placenta by the Dlk1-Dio3 imprinting control region. Development 2007;134:417–26.

17. Seitz H, Youngson N, Lin SP, Dalbert S, Paulsen M, Bachellerie JP, et al. Imprinted microRNA genes transcribed antisense to a reciprocally imprinted retrotransposon-like gene. Nat Genet 2003;34:261–2.

18. Takada S, Paulsen M, Tevendale M, Tsai CE, Kelsey G, Cattanach BM, et al. Epigenetic analysis of the Dlk1-Gtl2 imprinted domain on mouse chromosome 12: implications for imprinting control from comparison with Igf2-H19.Hum Mol Genet 2002;11:77–86.

19. Moon YS, Smas CM, Lee K, Villena JA, Kim KH, Yun EJ, et al. Mice lacking paternally expressed Pref-1/Dlk1 display growth retardation and accelerated adiposity. Mol Cell Biol 2002;22:5585–92.

20. Sekita Y, Wagatsuma H, Nakamura K, Ono R, Kagami M, Wakisaka N, et al. Role of

retrotransposon-derived imprinted gene, Rtl1, in the feto-maternal interface of mouse placenta. Nat Genet 2008;40:243–8.

21. Hernandez A, Martinez ME, Fiering S, Galton VA, St Germain D. Type 3 deiodinase is critical for the maturation and function of the thyroid axis. J Clin Invest 2006;116:476–84.

22. Sekita Y, Wagatsuma H, Irie M, Kobayashi S, Kohda T, Matsuda J, *et al.* Aberrant regulation of imprinted gene expression in Gtl2lacZ mice. Cytogenet Genome Res 2006;113:223–9.

23. Monk D, Arnaud P, Apostolidou S, Hills FA, Kelsey G, Stanier P, *et al.* Limited evolutionary conservation of imprinting in the human placenta. Proc Natl Acad Sci USA 2006;103:6623–8.

24. Lewis A, Mitsuya K, Umlauf D, Smith P, Dean W, Walter J, *et al.* Imprinting on distal chromosome 7 in the placenta involves repressive histone methylation independent of DNA methylation. Nat Genet 2004;36:1291–5.

25. Umlauf D, Goto Y, Cao R, Cerqueira F, Wagschal A, Zhang Y, *et al.* Imprinting along the Kcnq1 domain on mouse chromosome 7 involves repressive histone methylation and recruitment of Polycomb group complexes. Nat Genet 2004;36:1296–300.