European Journal of Human Genetics (2008) 16, 1019–1023

www.nature.com/ejhg

SHORT REPORT

Epimutation (hypomethylation) affecting the chromosome 14q32.2 imprinted region in a girl with upd(14)mat-like phenotype

Kana Hosoki[1], Tsutomu Ogata*[,2], Masayo Kagami[2], Touju Tanaka[3] and Shinji Saitoh[1]

[1]*Department of Pediatrics, Hokkaido University Graduate School of Medicine, Sapporo, Japan;* [2]*Department of Endocrinology and Metabolism, National Research Institute for Child Health and Development, Tokyo, Japan;* [3]*Division of Clinical Genetics and Molecular Medicine, National Center for Child Health and Development, Tokyo, Japan*

Maternal uniparental disomy for chromosome 14 (upd(14)mat) causes clinically discernible features such as pre- and/or postnatal growth failure, hypotonia, obesity, small hands, and early onset of puberty. The monoallelic expression patterns at the 14q32.2 imprinted region are tightly related to methylation status of the *DLK1–MEG3* intergenic differential methylation region (DMR) and the *MEG3*-DMR that are severely hypermethylated after paternal transmission and grossly hypomethylated after maternal transmission. We examined this imprinted region in a 2 2/12-year-old Japanese patient who was born with a normal birth size (length, +0.2 SD; weight, −0.5 SD) and showed postnatal growth failure (height, −3.1 SD; weight, −3.4 SD), hypotonia, frontal bossing, micrognathia, and small hands. Methylation analysis, genotyping analysis, and deletion analysis were performed with blood samples of the patient and the parents, showing that the DMRs of this patient were grossly hypomethylated in the absence of upd(14)mat and deletion of the DMRs. The results indicate the occurrence of an epimutation (hypomethylation) affecting the normally methylated DMRs of paternal origin, and imply that epimutations should be examined in patients with upd(14)mat-like phenotype.
European Journal of Human Genetics (2008) **16,** 1019–1023; doi:10.1038/ejhg.2008.90; published online 14 May 2008

Keywords: epimutation; growth failure; imprinting; differentially methylated region; upd(14)mat

Introduction

Maternal uniparental disomy for chromosome 14 (upd(14)mat) results in clinically discernible features such as pre- and postnatal growth failure, hypotonia, obesity, small hands, and early onset of puberty.[1] Phenotypic development is consistent with chromosome 14q32.2 region harboring several paternally expressed genes (*PEGs*) such as *DLK1* and *RTL1* and maternally expressed genes (*MEGs*) such as *MEG3* (alias *GTL2*), *RTL1as* (*RTL1* antisense), and *MEG8*.[2,3] The parent-of-origin-specific monoallelic expression patterns are tightly related to methylation status of differential methylation regions (DMRs).[4] For the 14q32.2 imprinted region, the previous studies have identified the intergenic DMR (IG-DMR) between *DLK1* and *MEG3* and the *MEG3*-DMR that are severely hypermethylated after paternal transmission and grossly hypomethylated after maternal transmission.[5–7] In particular, the germline-derived IG-DMR plays a pivotal role in the imprinting regulation, because methylation pattern of the secondary *MEG3*-DMR is dependent on that of the IG-DMR.[8]

The upd(14)mat-like phenotype has also been exhibited by non-disomic patients. Temple *et al*[9] described a patient

*Correspondence: Dr T Ogata, Department of Endocrinology and Metabolism, National Research Institute for Child Health and Development, 2-10-1 Ohkura, Setagaya, Tokyo 157-8535, Japan.
Tel: +81 2 5494 7025; Fax: +81 2 5494 7026;
E-mail: tomogata@nch.go.jp
Received 10 January 2008; revised 25 March 2008; accepted 3 April 2008; published online 14 May 2008

with upd(14)mat-like phenotype and an epimutation (hypomethylation) of the normally methylated DMR of paternal origin. Kagami *et al*[5] reported three patients with upd(14)mat-like phenotype and microdeletions affecting the 14q32.2 imprinted region including the DMRs of paternal origin. In this regard, the IG-DMR deletion from the paternally derived chromosome has no effect on the imprinting status, although that from the maternally derived chromosome results in a maternal to paternal epigenotypic alteration.[5,7] Thus, simple genotype–phenotype correlations can be applied for the three patients with the microdeletions, implying that loss of paternally derived *DLK1* and *RTL1* constitutes primary additive underlying factors for the development of upd(14)mat-like phenotype, although the perturbation of other imprinted genes could also have some effects.[5]

Here, we report an epimutation identified in a patient with upd(14)mat-like phenotype.

Patient and methods

Case report

This Japanese girl was born at 41 weeks of gestation after natural conception, with a history of mild oligohydramnios in the third trimester. At birth, her length was 50.0 cm (+0.2 SD), her weight 3.03 kg (−0.5 SD), and her head circumference 34.5 cm (+0.8 SD). The non-consanguineous parents were clinically normal, and the height was 161.0 cm (−1.7 SD) for the father and 154.5 cm (−0.7 SD) for the mother.

At 5 months of age, she was referred to us, because she was unable to control her head. Physical examination revealed generalized hypotonia without palsy and abnormal tendon reflex, and several somatic features such as frontal bossing, micrognathia, and small hands (Supplementary Figure 1). In addition, her length became below −2 SD of the mean from 10 months of age, while hypotonia was gradually ameliorated. She controlled her head at 7 months of age, sat without support at 11 month, and walked without support at 19 months. Repeatedly performed biochemical studies for hypotonia and growth failure were normal, as were skeletal roentgenograms and brain magnetic resonance imaging. The karyotype was 46XX in all the 30 lymphocytes examined. With a provisional diagnosis of Prader–Willi syndrome (PWS) that is primarily based on hypotonia and growth deficiency, fluorescence *in situ* hybridization (FISH) analysis for *SNRPN* and methylation analysis for the DMR at the *SNRPN* promoter region were performed,[10] showing normal findings. In addition, hypomethylation of the *H19*-DMR and upd(7)mat, which can cause growth failure, were also excluded by previous methods.[11,12] On the last examination at 2 2/12 years of age, her height was 76.1 cm (−3.1 SD), her weight 7.9 kg (−3.4 SD), and her head circumference 44.9 cm (−1.9 SD). Her mental development appeared age appropriate.

Methylation analysis

This study was approved by the Institutional Review Board Committees at Hokkaido University Hospital and National Center for Child Health and Development. After obtaining written informed consent, we examined the IG-DMR (CG4 and CG6) and the *MEG3*-DMR (Figure 1a), using bisulfite-treated leukocyte genomic DNA. For the IG-DMR, bisulfite sequencing was performed as reported previously,[5] and the SNPs (*rs12437020* for CG4 and *rs10133627* for CG6) were also genotyped. For the *MEG3*-DMR, PCR amplification was performed with methylated and unmethylated allele-specific primers, as described previously.[5,6] A hitherto unreported upd(14)mat patient and the previously reported upd(14)pat patient[5] were similarly analyzed with permission.

Genotyping analysis

We performed microsatellite analysis for 16 loci on chromosome 14 and SNP analysis for 39 loci around the DMRs (Supplementary Table 1). The primers used were as reported previously.[5]

Deletion analysis

Lymphocyte metaphase spreads were hybridized with a long and accurate (LA)-PCR product encompassing the IG-DMR and that spanning the *MEG3*-DMR (Figure 1a), together with an RP11-566I2 probe for 14q12 used as an internal control. Furthermore, the two LA-PCR products were also obtained from the patient and a control subject, and subjected to fragment size comparisons after restriction enzyme digestions, to detect a possible tiny deletion in the patient. The detailed methods for the deletion analysis have been reported previously.[5]

Results

Methylation analysis

The results are shown in Figure 1b. For the IG-DMR, CG4 and CG6 were grossly hypomethylated in the patient and the upd(14)mat patient, severely methylated in the upd(14)pat patient, and delineated in apparently mosaic patterns in the parents. In addition, the CG4 SNP typing indicated parental origin-dependent methylation patterns in the parents, and heterodisomy for the this region in the upd(14)mat patient. The CG6 SNP typing data were not informative. For the *MEG3*-DMR, PCR products were obtained with an unmethylated allele-specific primer pair alone in the patient and the upd(14)mat patient, with a methylated allele-specific primer pair alone in the upd(14)pat patient, and with both primer pairs in the parents.

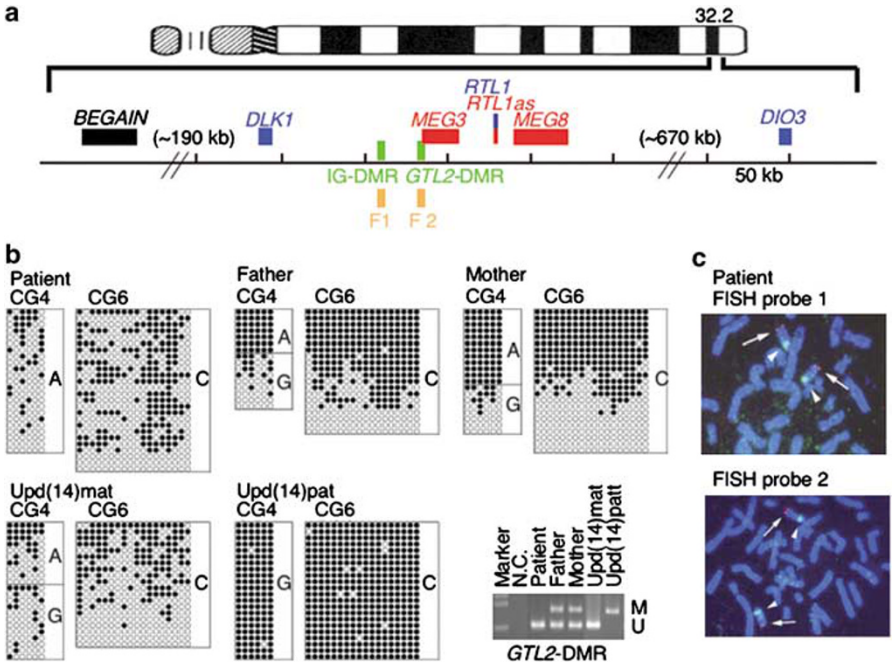

Figure 1 Summary of the molecular studies. (**a**) The regional physical map of the human chromosome 14q32.2 imprinted region. *PEGs* are shown in blue and *MEGs* in red; although it remains to be clarified whether *DIO3* is a *PEG*, mouse *Dio3* is known to be preferentially but not exclusively expressed from a paternally derived chromosome in embryos.[13] *WDR25* and *BEGAIN* appear biparentally expressed genes. The IG-DMR and the *MEG3*-DMR are depicted in green, and the FISH probes 1 and 2 covering the DMRs indicated in orange. The physical distance is ~190 kb between *BEGAIN* and *DLK1*, ~170 kb between *DLK1* and *MEG8*, and ~670 kb between *MEG8* and *DIO3*. (**b**) Methylation patterns of the IG-DMR (CG4 and CG6) and the *MEG3*-DMR. Bisulfite sequencing has been performed for CG4 and CG6. Each line indicates a single clone and each circle denotes a CpG island; filled and open circles represent methylated and unmethylated cytosines, respectively. The SNP typing data for CG4 and CG6 are also shown. Methylated (M) and unmethylated (U) allele-specific PCR amplification has been performed for the *MEG3*-DMR. NC: negative control. (**c**) FISH analysis using FISH probe 1 (F1) for the IG-DMR and FISH probe 2 (F2) for the *MEG3*-DMR. The red signals (arrows) have been detected by the two FISH probes and the green signals (arrowheads) have been identified by an RP11-566I2 probe for 14q12 used as an internal control.

Genotyping analysis

Microsatellite analysis demonstrated biparental origin of the two chromosome 14 homologs, and SNP analysis indicated lack of a segmental upd(14)mat around the DMRs (Supplementary Table 1).

Deletion analysis

FISH probes 1 and 2 detected two signals in the patient (Figure 1c). The fragment size comparison after enzyme digestions showed no abnormal bands suggestive of a tiny deletion in the patient.

Discussion

This patient had hypomethylated DMRs in the absence of discernible maternal disomy affecting the DMRs or loss of the paternally derived DMRs. This implies the occurrence of an epimutation (hypomethylation) affecting the normally methylated DMRs of paternal origin. To our knowledge, such an epimutation (hypomethylation) has previously been identified only in a patient reported by Temple *et al*.[9] Actually, the DMR examined in that patient appears to be a part of the *MEG3*-DMR rather than the IG-DMR on the basis of its position. It is likely, however, that the IG-DMR is also hypomethylated in that patient, because the *MEG3*-DMR can stay hypomethylated only in the presence of the hypomethylated IG-DMR.[8]

Clinical features of the two patients with epimutation are summarized in Table 1, together with those of upd(14)mat patients. Notably, clinical features are grossly similar in epimutation patients and upd(14)mat patients. Although our patient had no prenatal growth failure, lack of prenatal and/or postnatal growth failure has been described in several upd(14)mat patients,[14–16] and this would be due to body growth being a multifactorial trait subject to multiple genetic and environmental factors.[17] In this regard, it has been reported that clinical features are comparable between patients with paternal upd(14) and those with epimutations (hypermethylation) affecting the normally hypomethylated DMRs of maternal origin.[5] Taken together, the methylation patterns of the DMRs appear to be closely related to the expression patterns of virtually all the imprinted genes on 14q32.2.

It is noteworthy that the patient was initially suspected as having PWS. Indeed, growth deficiency, hypotonia, and small hands are shared by upd(14)mat and PWS,[18,19] and

Table 1 Clinical phenotypes in patients with epimutations and upd(14)mat

	Epimutations		*Upd(14)mat*
	This report	*Temple* et al[9]	(n = *35*)[a]
Age	2 2–12 years	10 7–12 years	0–30 years
Sex	Female	Male	M:F = 17:18
Premature delivery	–	–	10/25
Prenatal growth failure	–	+	24/27
Postnatal growth failure	+	+	26/32
Somatic features	+	+	23/35[b]
Frontal bossing	+	+	9/9
High arched palate	–	+	7/9
Micrognathia	+	–	5/5
Small hands	+	+	24/27
Scoliosis	–	+	5/19
Others			
Hypotonia	+	+	25/28
Obesity	–	–	14/34
Early onset of puberty	Unknown	Borderline	14/16
Mental retardation	–	–	10/27
Thyroid dysfunction	–	–	ND

ND: not described.
In the column summarizing the clinical features of 35 patients with upd(14)mat, the denominators indicate the number of patients examined for the presence or absence of each feature, and the numerators represent the number of patients assessed to be positive for that feature; thus, the differences between the denominators and the numerators denote the number of patients evaluated to be negative for that feature.
[a]Patients with maternal uniparental disomy for chromosome 14 reported in the literature, several upd(14)mat patients with no phenotypic description have not been included. The references for the 35 upd(14)mat patients are summarized in Kagami *et al*.[5]
[b]The ratio of patients with at least one somatic feature.

upd(14)mat has occasionally been identified in patients referred for molecular examination of PWS.[19,20] Thus, upd(14)mat and epimutations should be considered in patients with PWS-like phenotype.[18,19]

In summary, we observed an epimutation (hypomethylation) of the paternally derived DMRs in a patient with upd(14)mat-like phenotype. Further studies will identify epimutations in patients with upd(14)mat-like phenotype, thereby contributing to clarify the relevance of epimutations in human imprinted disorders.

Acknowledgements

This study was supported by grants for Child Health and Development (20C-2) and for Research on Children and Families (H18-005) from the Ministry of Health, Labor, and Welfare, and by Grants-in-Aid for Scientific Research (Priority Areas: 16086215) from the Ministry of Education, Culture, Sports, Science and Technology.

Disclosure

The authors have reported no conflicts of interest.

References

1 Kotzot D: Maternal uniparental disomy 14 dissection of the phenotype with respect to rare autosomal recessively inherited traits, trisomy mosaicism, and genomic imprinting. *Ann Genet* 2004; **47**: 251–260.
2 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**: 1527–1538.
3 Charlier C, Segers K, Wagenaar D *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**: 850–862.
4 Li E, Beard C, Jaenisch R: Role for DNA methylation in genomic imprinting. *Nature* 1993; **366**: 362–365.
5 Kagami M, Sekita Y, Nishimura G *et al*: Deletions and epimutations affecting the human chromosome 14q32.2 imprinted region: implications for the phenotypic development in paternal and maternal uniparental disomy for chromosome 14. *Nat Genet* 2008; **40**: 237–242.
6 Murphy SK, Wylie AA, Coveler KJ *et al*: Epigenetic detection of human chromosome 14 uniparental disomy. *Hum Mutat* 2003; **22**: 92–97.
7 Lin SP, Youngson N, Takada S *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.
8 Takada S, Paulsen M, Tevendale M *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.
9 Temple IK, Shrubb V, Lever M, Bullman H, Mackay DJ: Isolated imprinting mutation of the DLK1/GTL2 locus associated with a clinical presentation of maternal uniparental disomy of chromosome 14. *J Med Genet* 2007; **44**: 637–640.
10 The ASHG/ACMG Test and Technology Transfer Committee: Diagnostic testing for Prader–Willi and Angelman syndromes. *Am J Hum Genet* 1996; **58**: 1085–1088.

11 Kagami M, Nagai T, Fukami M, Yamazawa K, Ogata T: Silver-Russell syndrome in a girl born after *in vitro* fertilization: partial hypermethylation at the differentially methylated region of PEG1/MEST. *J Assist Reprod Genet* 2007; **24**: 131–136.
12 Yamazawa K, Kagami M, Ogawa M, Horikawa R, Ogata T: Placental hypoplasia in maternal uniparental disomy for chromosome 7. *Am J Med Genet A* 2008; **146**: 514–516.
13 Tsai CE, Lin SP, Ito M, Takagi N, Takada S, Ferguson-Smith AC: Genomic imprinting contributes to thyroid hormone metabolism in the mouse embryo. *Curr Biol* 2002; **12**: 1221–1226.
14 Rosa AL, Wu YQ, Kwabi-Addo B, Coveler KJ, Reid Sutton V, Shaffer LG: Allele-specific methylation of a functional CTCF binding site upstream of MEG3 in the human imprinted domain of 14q32. *Chromosome Res* 2005; **13**: 809–818.
15 Aretz S, Raff R, Woelfle J *et al*: Maternal uniparental disomy 14 in a 15-year-old boy with normal karyotype and no evidence of precocious puberty. *Am J Med Genet A* 2005; **135**: 336–338.
16 Takahashi I, Takahashi T, Utsunomiya M, Takada G, Koizumi A: Long-acting gonadotropin-releasing hormone analogue treatment for central precocious puberty in maternal uniparental disomy chromosome 14. *Tohoku J Exp Med* 2005; **207**: 333–338.
17 Vogel F, Motulsky AG: *Human Genetics: Problems and Approaches*. Heidelberg: Springer-Verlag, 1986.
18 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.
19 Berends MJ, Hordijk R, Scheffer H, Oosterwijk JC, Halley DJ, Sorgedrager N: Two cases of maternal uniparental disomy 14 with a phenotype overlapping with the Prader–Willi phenotype. *Am J Med Genet* 1999; **84**: 76–79.
20 Mitter D, Buiting K, von Eggeling F *et al*: Is there a higher incidence of maternal uniparental disomy 14 [upd(14)mat]? Detection of 10 new patients by methylation-specific PCR. *Am J Med Genet A* 2006; **140**: 2039–2049.

Supplementary Information accompanies the paper on European Journal of Human Genetics website (http://www.nature.com/ejhg)