

A maternal hypomethylation syndrome presenting as transient neonatal diabetes mellitus

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Abstract The expression of imprinted genes is mediated by allele-specific epigenetic modification of genomic DNA and chromatin, including parent of origin-specific DNA methylation. Dysregulation of these genes causes a range of disorders affecting pre- and

post-natal growth and neurological function. We investigated a cohort of 12 patients with transient neonatal diabetes whose disease was caused by loss of maternal methylation at the TNDM locus. We found that six of these patients showed a spectrum of methylation loss, mosaic with respect to the extent of the methylation loss, the tissues affected and the genetic loci involved. Five maternally methylated loci were

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affected, while one maternally methylated and two paternally methylated loci were spared. These patients had higher birth weight and were more phenotypically diverse than other TNDM patients with different aetiologies, presumably reflecting the influence of dysregulation of multiple imprinted genes. We propose the existence of a maternal hypomethylation syndrome, and therefore suggest that any patient with methylation loss at one maternally-methylated locus may also manifest methylation loss at other loci, potentially complicating or even confounding the clinical presentation.

Abbreviations

BWS	Beckwith–Wiedemann syndrome
DMR	differentially methylated region
IUGR	Intra-uterine growth retardation
LOM	Loss of methylation
MS-PCR	Methylation-specific PCR
TNDM	Transient neonatal diabetes mellitus
UPD	Uniparental disomy
WRGL	Wessex Regional Genetics Laboratory

Introduction

Imprinting is the process whereby certain chromosomal loci are differentially marked in parental germlines in a heritable fashion, resulting in differential gene expression in the offspring (reviewed in Reik and Walter 2001; Allegrucci et al. 2005). Imprinted genes comprise only a small proportion of human genes, but many are regulators of foetal or placental growth and development, or have neurological or behavioural roles (Tycko and Morison 2002). Deregulation of imprinted genes has been implicated in a number of sporadic and inherited genetic disorders (Robertson 2005). For example, deregulation of genes at 11 p15 gives rise not only to Beckwith–Wiedemann syndrome (BWS; Weksberg et al. 2005), an overgrowth disorder, but to Silver–Russell syndrome (SRS; Gicquel et al. 2005; Eggermann et al. 2005; see also Blik et al. 2006) which is a growth retardation disorder. Growth retardation is also characteristic of transient neonatal diabetes mellitus (TNDM), which is attributable to deregulation of the imprinted locus at 6 q24 (Temple and Shield 2002).

Most, though not all, imprinted genes are characterised by differentially methylated regions (DMRs) where CpG-rich regions of genomic DNA are methylated typically, though not universally, on the non-expressed allele. Recently Arima et al. (2005) showed that some patients with TNDM and loss of methylation (LOM) at the maternally-methylated TNDM DMR

also lacked maternal methylation at KvDMR in the BWS locus; they hypothesised that the gene products of the two regions formed a functional network. When we set out to confirm their findings in our own TNDM cohort, we noticed that several of the patients with LOM at TNDM DMR had clinical features at variance with the rest of the cohort, particularly with respect to birth weight. When we found that two of these patients did manifest partial LOM at KvDMR (Mackay et al. 2006), we hypothesised that it might have arisen as part of a general hypomethylation syndrome, and set out to assay the epigenotypes of our patients at several imprinted loci, by quantifying the methylation at the DMRs.

Materials and methods

Patients

The patient cohort (a subset of that described in Mackay et al. 2005) comprised 12 individuals with a clinical diagnosis of TNDM including low birth weight and hyperglycaemia developing within 6 weeks of birth and resolving within 18 months. Ethical approval was granted by the local Research Ethics Committee for this study on TNDM patients, the patients' DNA samples being anonymised but remaining associated with the clinical data given at referral. For the purposes of birth weight calculations, only term births were included, 'term' being defined as 38–42 weeks of gestation.

Molecular genetic diagnosis of LOM at 6 q24 was made by methylation-specific PCR analysis as described in Mackay et al. (2005), and paternal UPD6 was excluded by microsatellite analysis of chromosome 6. Five of these patients have been reported before (Gardner et al. 2000; Kant et al. 2005; Mackay et al. 2006). The TNDM control cohort comprised other neonatal diabetics, including ten with paternal UPD6 and ten with mutations in KCNJ11 (Gloyn et al. 2005). An anonymised cohort of age-matched normal control samples was drawn from the sample base maintained within WRGL for such purposes. In addition, positive control samples with known imprinting mutations or uniparental disomies of chromosomes 6, 7, 11, 14 and 15 were anonymised and employed as positive controls in MS-PCR assays. Parents of TNDM cases were routinely included in MS-PCR experiments, partly to exclude inherited imprinting defects, but chiefly because the parental samples, generally obtained at the same time and extracted in the same manner as the proband samples, provided a valuable control for variations in sample quality in DNA from many different centres.

Bisulphite treatment

Genomic DNA derived from peripheral blood leukocytes (2 µg) was bisulphite treated using the EZ DNA Methylation kit (Zymo Research) and resuspended in a final volume of 50 µl, of which 1 µl was used in each PCR reaction.

Methylation-specific PCR (MS-PCR) and pyrosequencing

MS-PCR reactions for chromosomes 6 and 11, and pyrosequencing reactions for KvDMR, were performed as described in Mackay et al. (2006). MS-PCR primers for other loci were designed on the same principles (Supplementary Table 1). Primer sequences for GRB10 were derived from Arnaud et al. (2003); for PEG1 from Moore et al. (2003); for PEG3 from Murphy et al. (2001); for DLK1 from Wylie et al. (2000), and for H19 from Vu et al. (2000). Primer sequences for SNRPN are those of Zeschnick et al. (1997). Pyrosequencing reactions for PEG3 were performed according to the same principles as those for KvDMR; details of reaction conditions are available on request.

Methylation ratiometry at each maternal locus was demonstrated by two independent methods. For KvDMR and PEG3, MS-PCR data was confirmed by pyrosequencing of bisulphite-induced polymorphisms, interrogating CpG dinucleotides in the same DMRs. For GRB10 and PEG1/MEST, two sets of MS-PCR primers were employed, interrogating adjacent groups of CpG dinucleotides in the PEG1 DMR; in this way the experiments were controlled against spurious results arising from sequence variation in MS-PCR primer binding-sites.

Results

We undertook further DNA methylation analysis on 12 individuals from the TNDM cohort, who had previously been shown to exhibit total loss of maternal methylation at the TNDM DMR on 6 q24, and for whom DNA was available for study. In all cases, leukocyte-derived DNA was analysed, and in some cases, DNA was also available from skin fibroblasts or buccal swabs. MS-PCR was performed on the maternally-methylated DMRs of GRB10 (7 p11–12), PEG1/MEST (7 q32), KvDMR (11 p15), SNRPN (15 q11–13) and PEG3 (19 q13), and on the paternally methylated DMRs of H19 (11 p15.5) and DLK1 (14 q32). Control reactions were performed on 52 age-matched, anonymised normal control DNAs, and on 50 samples from patients referred for TNDM

testing, but without LOM at chromosome 6 (including 10 with paternal uniparental disomy of chromosome 6 and 7 with duplication of 6 q24).

Table 1 summarises the results of MS-PCR analysis. Out of 12 individuals with LOM 6 q24, six exhibited partial or total LOM at the other imprinted loci investigated. There are several noteworthy features of these findings. Firstly, methylation change could be observed at one or more of the GRB10, PEG1, KvDMR and PEG3 DMRs. An example of this is patient 4, who showed partial methylation loss at KvDMR and PEG1 (Fig. 1). Secondly, methylation change was mosaic in those cases where different tissues were available. For example, in patient 3, fibroblast-derived DNA exhibited a twofold reduction of methylation at the PEG3 DMR, whereas blood-derived DNA exhibited almost total methylation loss at this locus (Fig. 2). Thirdly, some patients exhibited complete or near-complete loss of methylation at loci other than 6 q24. As an example, PEG3 DMR methylation in patient 1 approached zero, as shown by both MS-PCR and pyrosequencing (Supplementary Fig. 1).

Methylation loss was seen only in maternally-methylated loci; there was no evidence of methylation change in the paternally-methylated H19 or DLK1 DMRs in any case or control sample; nor, interestingly, was it observed at the maternally-methylated SNRPN locus (Table 1). No methylation change was observed in the parents of the probands, nor among 52 age-matched controls, nor among 50 other referrals for growth retardation and neonatal diabetes (Table 1).

The phenotypes of the cohort are summarised in Table 2. The mean birth weight of the patients with multiple LOM (excluding the multiple birth) is slightly higher than that of the remainder of the cohort, but the difference was not statistically significant ($P = 0.71$). However, these patients did have higher average birth weight than TNDM patients of other aetiologies. A combined cohort of patients with UPD6 ($n = 10$) and duplication of 6 q24 ($n = 7$) had mean birth weight 1963 ± 297 g, median 1,850 g. The birth weight difference between patients with multiple LOM and other TNDM patients was 500 g and narrowly achieved statistical significance ($P = 0.0498$). Interestingly, the median birth weight of all the LOM6 q24 patients (excluding multiple and preterm births) was 2,260 g, 410 g higher than the non-LOM TNDM patients, and was significant at $P = 0.02$ (Fig. 3).

Discussion

We found evidence of a spectrum of maternal hypomethylation at imprinted loci in six out of 12 patients with

Table 1 Results of methylation-specific PCR of imprinted loci

Patient number	Tissue	ZAC 6 q24	GRB10 7 p21	PEG1 7 q32	KvDMR 11 p15	PEG3 19 q13	SNRPN 15 q11	H19 11 p15	DLK1 14 q32
1	B	∞	∞	2	4	∞	1	1	1
	MB	∞	∞	2	1	∞	1	1	1
2	B	∞	∞	2	1	2	1	1	1
	FIB	∞	∞	5	1	5	1	1	1
3	B	∞	∞	1	2	2	1	1	1
	FIB	∞	25	1	1	13	1	1	1
4	B	∞	1	4	3	1	1	1	1
	MB	∞	1	3	3	1	1	1	1
5	B	∞	1	∞	1	1	1	1	1
6	B	∞	1	5	1	1	1	1	1
7	B	∞	1	1	1	1	1	1	1
8	B	∞	1	1	1	1	1	1	1
9	B	∞	1	1	1	1	1	1	1
10	B	∞	1	1	1	1	1	1	1
11	B	∞	1	1	1	1	1	1	1
12	B	∞	1	1	1	1	1	1	1
Normal controls ^a (mean ± SD)	B	1.00 ± 0.16 ^c	1.00 ± 0.21	1.00 ± 0.20	1.00 ± 0.17 ^c	1.00 ± 0.22 ^c	1.00 ± 0.16	ND	1.00 ± 0.30
Neonatal diabetes ^b controls (mean ± SD)	B		1.00 ± 0.17	1.00 ± 0.14		1.00 ± 0.08	1.00 ± 0.16		1.00 ± 0.20

Numbers presented represent unme/me ratios as exemplified in Fig. 1, and represent duplicate results obtained in at least two experiments each for two independent bisulphite treatments, and confirmed by the use of two MS-PCR primer sets or MS-PCR corroborated by pyrosequencing

B blood; MB mouthbrush; FIB fibroblast

^a Normal control cohort of 52 anonymised age-matched individuals

^b Neonatal diabetes cohort of 50 individuals referred for TNDM testing with a clinical diagnosis of neonatal diabetes and intrauterine growth retardation, including 10 with uniparental disomy of chr6 and 7 with paternal duplication of 6 q24

^c Control data drawn from previous publications; Mackay et al. (2005, 2006)

TNDM and loss of maternal methylation at TNDM DMR. This is the first report of a hypomethylation syndrome affecting more than two loci, and we hypothesise that similar hypomethylated epigenotypes may be identified among other patient cohorts. In the cohort described here, the primary presentation was TNDM, with total LOM at 6 q24 and partial or total LOM at other loci. It therefore seems plausible that any individual with maternal LOM (for example, a BWS patient) may exhibit a similar spectrum of methylation change.

Patients exhibiting maternal hypomethylation had significantly higher median birth weight than TNDM patients of other aetiologies, and, intriguingly, this difference was present both in patients with multiple LOM and in all TNDM patients with LOM6 q24. We therefore hypothesise that other imprinted loci, as yet unidentified, contribute to the phenotypes of this whole cohort, and we are currently working to develop further imprinting assays in order to investigate these individuals further.

There was evidence that methylation loss at multiple loci modified the clinical presentation of TNDM in individual patients. For example, the two individuals in this cohort with the most marked LOM at KvDMR

(patients 1 and 4) are also those with anterior abdominal wall defect, a clinical feature of BWS (Mackay et al. 2006). We propose that in patients with other hypomethylation syndromes, methylation loss at additional loci may modify the primary clinical presentation. However, TNDM is perhaps unusual in that LOM, when detected, is always complete; this epigenotype may present a unique and extreme backdrop for the presentation of other methylation changes. Only further investigation of other disease cohorts will resolve this question.

In no case did we detect methylation loss at the H19, DLK1 or SNRPN DMRs. The former two are paternally-methylated loci, and their sparing suggests that the defect in these individuals is one of maternal methylation only. The sparing of the maternal methylation of SNRPN DMR is more surprising. It may be a chance occurrence in this relatively small cohort, or reflect the ascertainment bias of these individuals. Alternatively, the mechanism(s) responsible for this demethylation may discriminate between the SNRPN mark and the others described here; El-Maarri et al. (2001) have proposed that SNRPN methylation develops later than other marks in oocyte development, though Lucifero

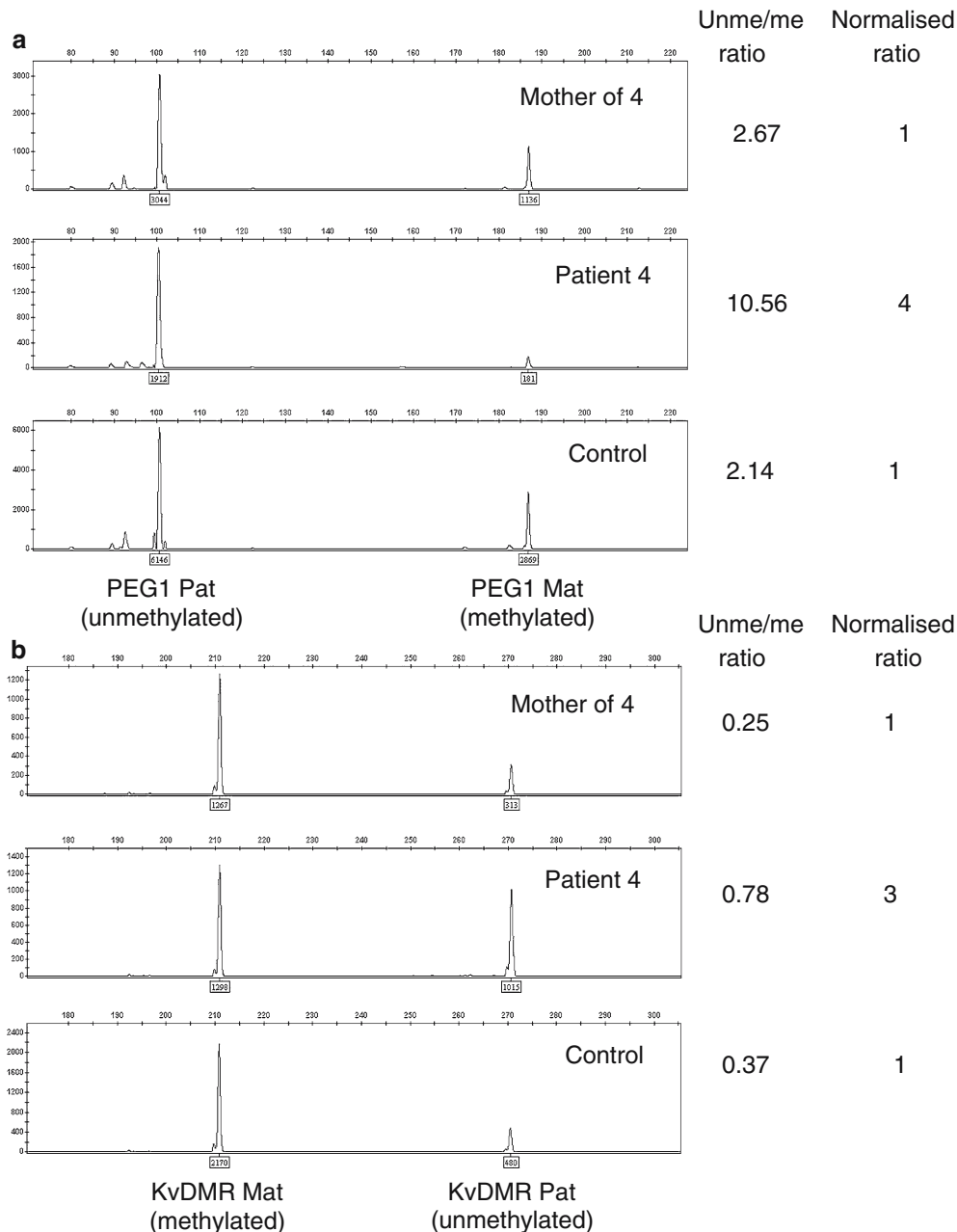


Fig. 1 Methylation-specific PCR of PEG1 DMR and KvDMR: patient 4, showing loss of methylation at two loci. In this figure, as in all the MS-PCR figures, a common layout is used. *X*-axis scale represents calculated product size (bp), *Y*-axis indicates peak height (arbitrary units), as do the figures under each peak. Peaks corresponding to methylated and unmethylated products are identified under the *bottom panel* of each Figure, as is the DMR template. The ratio (unme/me) was calculated as the peak height ratio of unmethylated versus methylated amplification products, therefore, in all figures, numbers greater than 1 indicate loss of maternal methylation, with an infinite ratio indicating loss of all

detectable maternal methylation. Ratios were normalised to the average of at least six control samples per experiment, though for the purposes of the figures presented, not all the controls are shown and the ratiometry is therefore approximated. All DNA templates are blood-derived unless otherwise stated. Electropherograms are representative examples of results obtained for at least two duplicated experiments on at least two independent bisulphite reactions. **a** PEG1 DMR; **b** KvDMR. **a, b** *First panel* the mother of patient 4; *second panel* patient 4; *third panel* normal control

et al. (2002) do not observe this distinction. We are continuing to investigate these possibilities.

In all cases, methylation loss was complete at 6 q24, reflecting the clinical presentation of the patients.

Beside 6 q24, hypomethylation was mosaic in almost all cases where multiple samples were available, and in general was more marked in non-leukocyte samples than leukocyte samples. From eight cases where only

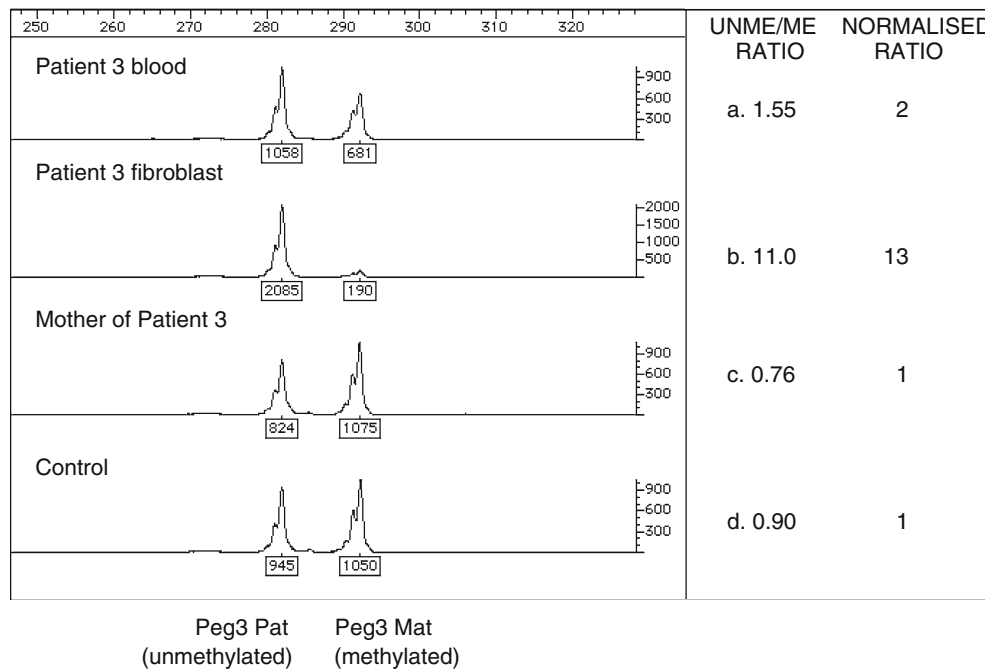


Fig. 2 Methylation-specific PCR of PEG3 DMR: patient 3, showing mosaic loss of methylation in blood-derived and fibroblast DNA. *Top panel*, patient 3, blood-derived DNA; *second*

panel, patient 3, primary fibroblast-derived DNA; *third panel*, mother of 3; *fourth panel*, normal control

Table 2 Phenotypic characteristics of TNDM patients

	LOM noted at multiple loci	LOM noted at 6 q24 only
<i>N</i>	6	6
Mean term birth weight (<i>N</i> = term single births)	2342 ± 420 g (5)	2060 ± 273 g (3)
Median term birth weight	2,350 g	2,253 g
Multiple births	1/6	1/6
Macroglossia	4/6	1/4
Anterior abdominal wall defects	2/6	0/4
Reported placental defects	2/6	1/4
Reported developmental delay	2/6	1/4
Reported growth delay	1/6	0/4
Reported maternal age > 35 year	3/6	0/4
Reported maternal infertility or IVF	1/6	1/4

Regarding clinical features, the table indicates the number of individuals in which each given feature was observed, and the number for which those data were recorded at referral

leukocyte DNA was available, methylation change was seen in only two. It is perfectly possible that other tissues from any of these individuals would have manifested further methylation changes. The generally less marked variations in leukocyte DNA may reflect ongoing clonal selection in this lineage.

In two cases, we observed loss of all detectable methylation at a site other than TNDM DMR, in one case at PEG1 DMR and in another at PEG3 DMR. The individuals concerned did not present as infants with radically different clinical features from the rest of the cohort, except that their birth weights were the two highest of the whole TNDM cohort. The published

data concerning knockout mice suggest that mutations at these loci may have behavioural more than physical phenotypes (Lefebvre et al. 1998; Li et al. 1999). To our knowledge no data exist concerning loss of methylation at PEG1 or PEG3 DMRs in liveborn humans (though it has been described in hydatidiform moles; El-Maarri et al. 2003; Judson et al. 2002). Further investigations will be required to determine the phenotypes, if any, associated with these epigenotypes. Regarding the effects of the less dramatic methylation changes found in some patients, clearly somatic mosaicism exerted some effects over the phenotype seen, limiting the clarity of the phenotypic picture; it should

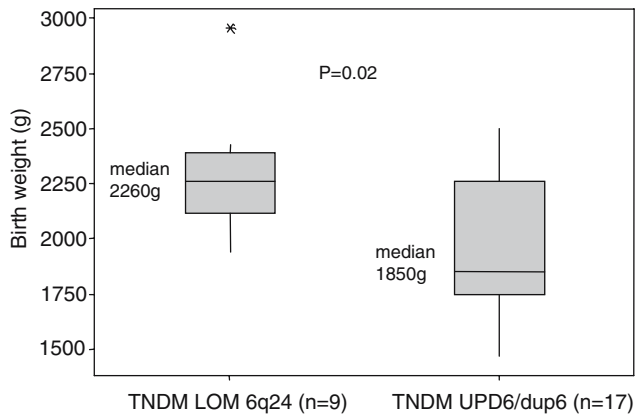


Fig. 3 Birth weight comparison between TNDM patients with loss of methylation at 6 q24 and those with other aetiologies. Birth weights of singleton term births are represented as a *box plot* defining the 25th and 75th centiles of the data sets. The median birth weights are marked, and *vertical lines* indicate the full birth weight range, with one outlier marked by an *asterisk*

also be remembered that some of these patients had placental trauma or insufficiency, so imprinting changes may also have been present in placenta. We anticipate that other patients with atypical presentation of a methylation disorder will manifest a spectrum of methylation changes, and indeed that novel syndromes may be described arising from this epigenetic spectrum.

The underlying cause of this syndrome is not clear. The mosaicism of these cases is consistent with a failure of maintenance methylation in the early zygote, similar to the stochastic epigenetic mutations described by Howell et al. (2001) on disruption of *Dnmt1*o activity in mice. In humans, failure of maintenance methylation has been proposed to underlie discordant monozygotic twinning in BWS (Weksberg et al. 2002; Bestor 2003), and the presence of two sets of discordant multiple births in this cohort also is highly suggestive of a mechanistic link; by contrast, discordant twinning has not been described as a feature of Angelman syndrome.

However, a maternal-effect mutation may also be postulated. Arnaud et al. (2006) used a mouse model to demonstrate stochastic and mosaic maternal methylation in the progeny of *Dnmt3L*-knockout mothers, indicating that this gene, which is active at the reprogramming of germ cells, can manifest its effects at post-fertilisation stages. In this context it should be noted that two of the mothers in this cohort were known to have experienced a period of infertility, which is suggestive of problems with oocyte number or maturation; additionally, maternal age over 35 years was noted in three cases, suggesting that oocyte maturation may

have been affected in these women for genetic or purely environmental reasons. Given the observed connection between diet and imprinted methylation at some loci (Waterland et al. 2006) it also remains possible that the maternal imprinting was compromised by dietary deficiency at oocyte maturation, or even at germline development.

To conclude, we describe a series of patients with a spectrum of maternal methylation loss at multiple imprinted loci. We will broaden our investigations to survey further imprinted loci, and also explore other patient cohorts, to determine the prevalence and the phenotypic consequences of these anomalies.

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References

- Allegrucci C, Thurston A, Lucas E, Young L (2005) Epigenetics and the germline. *Reproduction* 129:137–149
- Arima T, Kamikihara T, Hayashida T, Kato K, Inoue T, Shirayoshi I, Oshimura M, Soejima H, Mukai T, Wake N (2005) *ZAC*, *LIT1* (*KCNQ1OT1*) and *p57^{KIP2}* (*CDKN1C*) are in an imprinted gene network that may play a role in Beckwith-Wiedemann syndrome. *Nucl Acids Res* 33:2650–2660
- Arnaud P, Monk D, Hitchins M, Gordon E, Dean W, Beechey CV, Peters J, Craigen W, Preece M, Stanier P, Moore GE, Kelsey G (2003) Conserved methylation imprints in the human and mouse *GRB10* genes with divergent allelic expression suggests differential reading of the same mark. *Hum Mol Genet* 12:1005–1019
- Arnaud P, Hata K, Kaneda M, Li E, Sasaki H, Feil R, Kelsey G (2006) Stochastic imprinting in the progeny of *Dnmt3L*^{-/-} females. *Hum Mol Genet* 15:589–598
- Blik J, Terhal P, van den Bogaard M-J, Maas S, Hamel B, Salieb-Beugelaar G, Simon M, Letteboer T, van der Smagt J, Kroes H, Mannens M (2006) Hypomethylation of the *H19* gene causes not only Silver–Russell syndrome (SRS) but also isolated asymmetry or an SRS-like phenotype. *Am J Hum Genet* 78:604–614
- Bestor T (2003) Imprinting errors and developmental asymmetry. *Philos Trans R Soc Lond B* 358:1411–1415
- El-Maarri O, Buiting K, Peery EG, Kroisel PM, Balaban B, Wagner K, Urman B, Heyd J, Lich C, Brannan CI, Walter J, Horsthemke B (2001) Maternal methylation imprints on human chromosome 15 are established during or after fertilisation. *Nature Genet* 27:341–344
- El-Maarri O, Seoud M, Coullin P, Herbiniaux U, Oldenburg J, Rouleau G, Slim R (2003) Maternal alleles acquiring paternal methylation patterns in biparental complete hydatidiform moles. *Hum Mol Genet* 12:1405–1413
- Eggermann T, Meyer E, Schönherr N, Obermann C, Mavany M, Eggermann K, Ranke MB, Wollmann HA (2005) Epigenetic mutations in 11 p15 in Silver–Russell syndrome are restricted

- to the telomeric imprinting domain. *J Med Genet* 10.1136/jmg.2005.038687
- Gardner RJ, Mackay DJ, Mungall AJ, Polychronakos C, Siebert R, Shield JP, Temple IK, Robinson DO (2000) An imprinted locus associated with transient neonatal diabetes mellitus. *Hum Mol Genet* 9:589–596
- Gicquel C, Rossignol S, Cabrol S, Houang M, Steunou V, Barbu V, Danton F, Thibaud N, Le Merrer M, Burglen L, Bertrand A, Netchine I, Le Bouc Y (2005) Epimutation of the telomeric imprinting center region on chromosome 11 p15 in Silver–Russell syndrome. *Nat Genet* 37:1003–1007
- Gloyn AL, Reimann F, Girard C, Edghill EL, Proks P, Pearson ER, Temple IK, Mackay DJ, Shield JP, Ellard S, Ashcroft FM, Gribble FM, Hattersley AT (2005) Relapsing diabetes can result from moderately activating mutations in *KCNJ11*. *Hum Mol Genet* 14:925–934
- Howell C, Bestor TH, Ding F, Latham KE, Mertineit C, Trasler JM, Chaillet JR (2001) Genomic imprinting disrupted by a maternal-effect mutation in the *Dnmt1* gene. *Cell* 104:829–838
- Judson H, Hayward BE, Sheridan E, Bonthron DT (2002) A global disorder of imprinting in the human female germ line. *Nature* 416:539–42
- Kant SG, van der Weij AM, Oostdijk W, Wit JM, Robinson DO, Temple IK, Mackay DJG (2005) Monozygous triplets discordant for transient neonatal diabetes mellitus and for imprinting of the *TNDM* DMR. *Hum Genet* 117:298–401
- Lefebvre L, Viville S, Barton SC, Ishino F, Keverne EB, Surani MA (1998) Abnormal maternal behaviour and growth retardation associated with loss of the imprinted gene *Mest*. *Nat Genet* 20:163–169
- Li L, Keverne EB, Aparicio SA, Ishino F, Barton SC, Surani MA (1999) Regulation of maternal behavior and offspring growth by paternally expressed *Peg3*. *Science* 284:330–333
- Lucifero D, Mertineit C, Clarke HJ, Bestor TH, Trasler JM (2002) Methylation dynamics of imprinted genes in mouse germ cells. *Genomics* 79:530–538
- Mackay DJG, Temple IK, Shield JPH, Robinson DO (2005) Bisulphite sequencing of the transient neonatal diabetes mellitus DMR facilitates a novel diagnostic test but reveals no methylation anomalies in patients of unknown aetiology. *Hum Genet* 116:255–261
- Mackay DJG, Hahnemann JMD, Boonen SE, Poerksen S, Bunyan DJ, White HE, Durston VJ, Thomas NS, Robinson DO, Shield JPH, Clayton-Smith J, Temple IK (2006) Epimutation of the *TNDM* locus and the Beckwith–Wiedemann Syndrome centromeric locus in individuals with transient neonatal diabetes mellitus. *Hum Genet* 119:179–184
- Moore MW, Dietz LG, Tirtorahardjo B, Cotter PD (2003) A multiplex methylation PCR assay for identification of uniparental disomy of chromosome 7. *Hum Mut* 21:645–648
- Murphy SK, Wylie AA, Jirtle RL (2001) Imprinting of *PEG3*, the human homologue of a mouse gene involved in nurturing behavior. *Genomics* 7:110–7
- Reik W, Walter J (2001) Genomic Imprinting: parental influence on the genome. *Nat Rev Genet* 2:21–32
- Robertson KD (2005) DNA methylation and human disease. *Nat Rev Genet* 6:597–610
- Temple IK, Shield JPH (2002) Transient neonatal diabetes, a disorder of imprinting. *J Med Genet* 39:872–875
- Tycko B, Morison IM (2002) Physiological functions of imprinted genes. *J Cell Physiol* 192:245–258
- Vu TH, Li T, Nguyen D, Nguyen BT, Yao X-M, Hu J-F, Hoffman AR (2000) Symmetric and asymmetric DNA methylation in the human *IGF2-H19* imprinted region. *Genomics* 64:132–143
- Waterland RA, Lin J-R, Smith CA, Jirtle RL (2006) Post-weaning diet affects genomic imprinting at the insulin-like growth factor 2 (*Igf2*) locus. *Hum Mol Genet* 15:705–716
- Weksberg R, Shuman C, Caluseriu O, Smith AC, Fei Y, Nishikawa J, Stockley TL, Best L, Chitayat D, Olney A, Ives E, Schneider A, Bestor TH, Li M, Sadowski P, Squire D (2002) Discordant *KCNQ1OT1* imprinting in sets of monozygotic twins discordant for Beckwith–Wiedemann syndrome. *Hum Mol Genet* 11:1317–1325
- Weksberg R, Shuman C, Smith AC (2005) Beckwith–Wiedemann syndrome. *Am J Med Genet* 137C:12–23
- Wylie AA, Murphy SK, Orton TC, Jirtle RL (2000) Novel imprinted *DLK1/GTL2* domain on human chromosome 14 contains motifs that mimic those implicated in *IGF2/H19* regulation. *Genome Res* 10:1711–1718
- Zeschnigk M, Lich C, Buiting K, Doerfler W, Horsthemke B (1997) A single-tube PCR test for the diagnosis of Angelman and Prader–Willi syndrome based on allelic methylation differences at the *SNRPN* locus. *Eur J Hum Genet* 5:94–98