Isolated imprinting mutation of the DLK1/GTL2 locus associated with a clinical presentation of maternal uniparental disomy of chromosome 14

I K Temple, V Shrub, M Lever, H Bullman, D J G Mackay


Since the first reports of Wang et al.1 and Temple et al.2 in 1991, a well-characterised clinical phenotype has emerged for both paternal and maternal uniparental disomy of chromosome 14 (UPD14). Maternal UPD14, the inheritance of both chromosome homologues from the mother with no contribution from the father, is characterised by prenatal and postnatal growth retardation, hypotonia, joint laxity, motor delay, early onset of puberty, and minor dysmorphic features of the face, hands and feet.3 Paternal UPD14 has a more severe presentation, with polyhydramnios, thoracic and abdominal-wall defects, growth retardation, severe developmental delay and characteristic dysmorphism.3 The constancy of features has pointed to aberrant imprinting as the likely cause of the phenotypes. Cases of segmental UPD14 have established distal 14q as the critical region for this phenotype.4 5

An imprint locus exists at 14q32 under the control of a paternally methylated intergenic differentially methylated region (IG-DMR).7 The imprinted genes in this region include the paternally expressed DLK1 (delta, Drosophila homologue-like 1) a transmembrane signalling protein and growth regulator homologous to proteins in the Notch/delta pathway.5 Genes for RNA species are also found within the imprinted domain, including the maternally expressed GTL2 (gene trap locus 2), 15 kb distal to the IG-DMR, and a large microRNA cluster.9

The functional hemizygosity of imprinted genes means that a single imprinting disorder can arise from multiple mechanisms, such as UPD, copy-number change in imprinted genes, disruption of regulatory sequences, or mutation of the single active allele.10 We describe a patient referred to a joint clinical-genetics community child-health clinic with features overlapping those of maternal UPD14, and with isolated methylation deficit at the IG-DMR.

CASE REPORT

The proband was the third child of non-consanguineous parents, conceived normally and born after a normal pregnancy, with a birth weight of 2.04 kg. He has three sisters. The early neonatal period was complicated by poor feeding and hypotonia and at 1 week of age, a ventricular septal defect was diagnosed but did not require treatment. The patient’s head was noted to be on the 75th centile with normal fontanelles and no clinical evidence of hydrocephalus. By 3 months, he had developed mild scoliosis, treated with a spinal brace. No vertebral abnormalities were detected on skeletal radiography.

All motor milestones were delayed; the child did not walk until 3 years of age, when fine motor delay was also noted. Other developmental skills were assessed as normal for age. Although nonverbal comprehension and communication were assessed as normal, he had delayed speech with poor intelligibility, which led to a referral for palatal movement assessment. He was noted to have a high palate but no movement abnormality. There was no submucous cleft. He presented clinically with features of oromotor dyspraxia because of his slow eating and poor pronunciation. He required supplements to maintain his weight, and a programme to improve speech and language. He was investigated for muscle disease, including a muscle biopsy, but power was assessed as normal and muscle histology was also normal. An abdominal ultrasound showed a large single cyst of his left kidney but kidney function was normal.

At the age of 4 years, the patient’s height was on the second centile, weight was less than the 40th centile and head circumference on the 25th centile for age. He was noted to have frontal bossing, maxillary hypoplasia with malocclusion and crowded upper teeth. He had a low posterior hairline and a short neck with slight, bilateral webbing, more marked on the left. He had a prominent philtrum and a small mouth with full lips. His hands and feet were noted to be small, but were normal in shape except for fifth-finger clinodactyly. The genitalia were normal. Neurological examination demonstrated hypotonia but no reduction in muscle power. A skull radiograph showed absence of the sphenoid bones.

At 8 years the patient’s height was measured on the 0.4th centile, but growth velocity was normal. Weight continued on the 0.4th centile. Endocrine studies showed a normal level of insulin-like growth factor 1 and normal thyroid function. There was no evidence of premature puberty.

By 10 years 7 months, progression of the scoliosis to 60° required continued spinal-jacket usage and corrective surgery using spinal rods that require lengthening every 9 months. The patient had made considerable progress educationally and was at normal school, performing within the average range, according to his teachers and grades. He was generally healthy. He was reported as having ongoing difficulties with fine-motor

Abbreviations: DLK1, delta, Drosophila homologue-like 1; GTL2, gene trap locus 2; IG-DMR, intergenic differentially methylated region; IOM, loss of methylation; MS-PCR, methylation-specific PCR; UPD14, uniparental disomy of chromosome 14
coordination, particularly with handwriting, using cutlery and tying shoelaces. He was diagnosed as having organisational motor dyspraxia. His head circumference was on the 10th centile and his height on the 0.4th centile. Hand measurements were 11 cm (palm and finger length) with a middle finger measurement of 3.8 cm (0.4th centile). His feet were small (UK size 11/European size 30). He was in early puberty, assessed at Tanner stage 2/3. He had enlarged testes, pubic and upper-lip hair but no axillary hair. His voice had lowered in character during the 2 months prior to the clinic appointment. He was diagnosed with malocclusion of the teeth, with the secondary dentition having erupted behind the lower set so that the maxilla was not free to grow forward. His facial features are shown in fig 1.

Initial investigations found a normal male karyotype and normal inheritance of microsatellite markers on chromosome 14. However, the combination of facial features, particularly frontal bossing and prominent philtrum, marked hypotonia and scoliosis in the presence of normal muscle power, dyspraxia, normal intelligence and small hands and feet, indicated that further analysis at 14q32 was warranted.

**METHODS AND RESULTS**

**Methylation-specific PCR of the 14q32 IG-DMR**

Methylation-specific PCR (MS-PCR) uses the divergent sequence changes deriving from bisulphite treatment of differentially methylated DNA, yielding differently sized products in a ratio reflecting that of the starting material. The reaction uses lymphocyte-derived DNA purified by standard methods. The reaction contained a forward primer and divergent reverse primers, encompassing 5CpG dinucleotides, from the IG-DMR within 14q32. The amplicon corresponds to chr14:100362206-100362432 of the Human Genome Sequence (derived from primer sequences on http://genome.ucsc.edu; release March 2006):

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GTL2b-fam CTCCAACAACAAAAACCCAAAATCAAAACACCTCTC;
GTL2b-unmeth GTTGTAGATGGTGGAGAGTAGAGAGGGAGTGTG;
GTL2b-meth CCGCTTTTGTTCGTTTGTGGCGGCGG). The primer set was validated using 120 normal controls, for whom mean methylation ratios of 1.0 were obtained, and patient controls with maternal (n = 2) and paternal (n = 2), UPD14 in whom paternal and maternal amplicons, respectively, were not detected (fig 2 and data not shown).
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The amplification reaction contained 1 µl DNA, 0.2 mmol/l dNTPs, 5 pmol of each primer, Taq polymerase (HotStar; Qiagen, Valencia, California, USA) and buffer containing 1.5 mmol/l Mg²⁺, in a final volume of 10 µl. Reaction conditions were 95°C for 15 min, followed by 28 cycles at 95°C for 20 seconds, 60°C for 20 seconds and 72°C for 20 seconds, then a final cycle at 72°C for 5 min. Methylated (paternal) and unmethylated (maternal) product sizes were 193 and 221 bp respectively. PCR products were visualised on a genetic analyser (ABI 3130; Applied Biosystems, Foster City, CA, USA). Peaks were inspected and peak heights <100 or > 8000 pixels were discarded, and the degree of methylation calculated as paternal/maternal peak heights and normalised against normal controls (n = 6/experiment).

MS-PCR of the proband demonstrated a complete absence of the methylated (paternal) product, giving an epigenotype indistinguishable from maternal UPD14 (fig 2). An identical epigenotype was obtained with primer set GTL2a (amplifying hg18 chr14:100362206-100362432; data not shown). This finding eliminated the possibility of a primer binding site mutation causing artefactual amplification failure of the paternal product.
Microsatellite analysis

To determine whether the absence of the paternal amplicon was due to a small region of segmental maternal UPD14, microsatellite analysis was carried out on proband and parental samples according to standard methods, using primer sets throughout chromosome 14q (table 1). The results indicated biallelic inheritance of chromosome 14 (table 1 and supplementary fig 1; available at http://jmg.bmj.com/supplemental). Notably, the biallelically inherited markers D14S1006 and D14S985 closely flanked the IG-DMR, so that segmental UPD of that region was unlikely and, if present, would be <117 kb in extent.

Long-range PCR

Finally, it was possible that the paternal product was absent because of a microdeletion within the paternal allele of the IG-DMR. Such microdeletions within the H19 DMR have been detected in association with familial Beckwith–Wiedemann syndrome.12 To test this possibility, long-range PCR primers were designed, which would amplify a 4.3-kb genomic DNA sequence spanning the IG-DMR (hg18:chr14:100360151-100364428: primer sequences LR-DMR-F: GACAGGAGAGACTGGACATTA-GGTG and LR-DMR-R: GGGAGGGGGTAAGGATGATTGAC). Amplification was performed using a commercial kit (Roche Expand PCR Kit; Roche, Basel, Switzerland) according to the manufacturer’s instructions, and products were separated on 1% agarose gel (supplementary fig 2; available at http://jmg.bmj.com/supplemental). Comparing the proband with normal controls, there was no evidence of a microdeletion in the IG-DMR.

Table 1

<table>
<thead>
<tr>
<th>D14S</th>
<th>Cytogenetic position</th>
<th>Size (Mb)</th>
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<th>Father</th>
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<td>100.4</td>
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<td>1.3</td>
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</table>

Methylation mutation at chromosome 14q32

The changes in gene expression resulting from the proband’s methylation mutation have not been determined. However, reduced expression of DLK1 is predicted. Murine studies indicate that Dlk1 is a regulator of somatic growth; Moon et al13 showed that Dlk1 null mice have poor postnatal growth and accelerated fat deposition, a phenotype more severe than, but consistent with, that seen in this individual.

It has recently been shown that maternal loss of methylation (LOM) at one imprinted locus may be associated with LOM at other loci and that an overarching mechanism may be responsible for generalised LOM in some patients with imprinting disorders.11 14 We examined the proband’s DNA for evidence of LOM at both the paternally methylated H19 DMR and maternally methylated DMRs including TNDM and SNRPN but found no evidence of any methylation abnormality at these loci (data not shown). The cause of the LOM in the proband therefore remains unknown.

In conjunction with this case study, we analysed IG-DMR methylation in 35 further patients referred to the Wessex Genetics Service with clinical features of UPD14 analysis but no molecular evidence of UPD14. We failed to identify other cases with paternal LOM (data not shown), and so at present must conclude that a methylation mutation is an uncommon cause of the phenotype. However, with methylation-based diagnostic tests for maternal UPD14 now in routine use, it is likely that further cases will be recognised. This will enable more extensive
clinical documentation of this disorder, a more precise
comparison of genotype-phenotype correlation between this
disorder and maternal UPD14, and potentially the dissection
of the genetic causes of this syndrome.

ACKNOWLEDGEMENTS
We thank Dr DO Robinson at WRGL Salisbury for helpful discussions.

Supplementary material is available on the JMG website at http://jmg.bmj.com/supplemental

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Competing interests: None declared.

Parental informed consent was obtained for the publication of this case
report.

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Received 3 April 2007
Revised 13 June 2007
Accepted 14 June 2007
Published Online First 29 June 2007

REFERENCES
1 Wang JCC, Passage MB, Yen PH, Shapiro LJ, Mohandas TK. Uniparental
heterodisomy for chromosome 14 in a phenotypically abnormal familial
balanced 13/14 robertsonian translocation carrier. Am J Hum Genet
2 Temple IK, Cocksall A, Hassold T, Pettay D, Jacobs P. Maternal uniparental
3 Sutton VR, Shaffer LG. Search for imprinted regions on chromosome 14:
Comparison of maternal and paternal UPD cases with cases of chromosome 14
4 Cavelier KJ, Yang SP, Sutton VR, Milstein JM, Wu YQ, Knox-Du Bais C,
Beischel LS, Johnson JP, Shaffer LG. A case of segmental paternal isodisomy of
5 Kagami M, Nishimura G, Okuyama T, Hayashidani M, Takeuchi T, Tanaka S,
Ishino F, Kurosawa K, Ogata T. Segmental and full paternal isodisomy for
chromosome 14 in three patients: Narrowing the critical region and implication
6 Wylie AA, Murphy SK, Orton TC, Jirfe RL. Novel imprinted DLK1/GTL2 domain
on human chromosome 14 contains motifs that mimic those implicated in IGF2/
7 Geuns E, De TN, Hilven P, Van SA, Liebaers I, De RM. Methylation analysis of the
intergenic differentially methylated region of DLK1-GTL2 in human. Eur J Hum
8 Baladron V, Ruiz-Hidalgo MJ, Nueda ML, az-Guerra MJ, Garcia-Ramirez JJ,
Bonvini E, Gubina E, Laboarda J. dlk acts as a negative regulator of Notch1
activation through interactions with specific EGF-like repeats. Exp Cell Res
9 Seitz H, Roya H, Bortolin ML, Lin SP, Ferguson-Smith AC, Cavaille J. A large
imprinted microRNA gene cluster at the mouse Dlk1-Gtl2 domain. Genome Res
10 Delaval K, Fell R. Epigenetic regulation of mammalian genomic imprinting. Curr
11 Mackay DJ, Boonen SE, Clayton-Smith J, Goadship J, Hahnenmann JM, Kant SG,
Njostad PR, Robin NH, Robinson DO, Siebert R, Shield JP, White HE, Temple IK.
A maternal hypomethylation syndrome presenting as transient neonatal diabetes
12 Sparago A, Cerrato F, Vernucci M, Ferrero GB, Silengo MC, Riccio A.
Microdeletions in the human H19 DMR result in loss of IGF2 imprinting and
13 Moon YS, Kim K, Villena JA, Kim KH, Yun EJ, Sul HS. Mice lacking
paternally expressed Pref-1/Dlk1 display growth retardation and accelerated
14 Rosignol S, Steunou V, Chatac C, Kerjean A, Rigoté M, Vieges-Paquier E,
Jouannet P, Le BY, Giracle C. The epigenetic imprinting defect of patients with
Beckwith-Wiedemann syndrome born after assisted reproductive technology is

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*J Med Genet* 2007 44: 637-640 originally published online June 29, 2007
doi: 10.1136/jmg.2007.050807

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