Paternal uniparental disomy in monozygotic twins discordant for hemihypertrophy

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Hemihypertrophy, or hemihyperplasia, is a condition in which there may be asymmetrical overgrowth of the cranium, face, trunk, and/or limbs on one side of the body. There may also be asymmetrical visceromegaly on the ipsilateral or contralateral side. Hemihypertrophy may occur in isolation, hence the term “isolated hemihypertrophy (IH)”, or as part of a number of overgrowth syndromes in which other characteristic clinical features are present. Such syndromes include neurofibromatosis, Klippel-Trenaunay-Weber syndrome, McCune-Albright syndrome, and Beckwith-Wiedemann syndrome (BWS). 1

We propose that isolated hemihypertrophy is in fact part of the spectrum of phenotypes of BWS. In addition, we propose that postzygotic recombination resulting in mosaic paternal uniparental disomy for 11p15 in the affected twin.

Table 1
Serial kidney measurements (length) for twin 2

<table>
<thead>
<tr>
<th>Age</th>
<th>Right kidney (cm)</th>
<th>Left kidney (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 days</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>5 months</td>
<td>6.1</td>
<td>5.4</td>
</tr>
<tr>
<td>1 year 4 months</td>
<td>7.0</td>
<td>5.5</td>
</tr>
<tr>
<td>3 years 2 months</td>
<td>7.7</td>
<td>5.8</td>
</tr>
<tr>
<td>3 years 9 months</td>
<td>9.1</td>
<td>7.0</td>
</tr>
</tbody>
</table>

Key points

- Isolated hemihypertrophy is a syndrome of asymmetrical peripheral and visceral overgrowth, and tumour predisposition. Asymmetrical overgrowth also occurs in Beckwith-Wiedemann syndrome (BWS), where it is associated with other features including macrosomia, macroglossia, abdominal wall defects, visceromegaly, and increased risk of embryonal tumours.
- Here we describe a pair of female monozygotic twins discordant for isolated hemihypertrophy, and show mosaic paternal uniparental disomy for 11p15 in the affected twin.
- We propose that isolated hemihypertrophy is in fact part of the spectrum of phenotypes of BWS. In addition, we propose that postzygotic recombination resulting in uniparental disomy for 11p15 is one mechanism responsible for discordance of phenotype between monozygotic twins.

Features of BWS (other than hemihypertrophy in twin 2) were excluded on examination by two dysmorphologists.

METHODS

Genomic DNA was extracted from peripheral blood by standard methods. DNA profiling using PCR amplification was performed for 15 STR loci: FES/FPS, TH01, vWA31, D18S51, D21S11, FGA, D8S1179, D3S1359, D13S317, D16S539, D5S818, D7S820, CSF1PO, F13A1, and TPOX; these loci are located throughout the human genome. Primers and PCR conditions were used according to the manufacturers' recommendations (Applied Biosystems and Promega Corporation,
The hybridisation patterns from five single locus probes, MS31, MS43A, MS621, YNH24, and g3, together with two multilocus probes, 33.15 and 33.6, were also examined following the manufacturer's protocol (Cellmark Diagnostics, UK).

In the case of singleplex PCR amplification, the following primers were used: locus THO1: forward primer 5'- GTG ATT CCC ATT GGC CTG TTC CTC - 3', reverse primer 5'-Fam - GTG GGC TGA AAA GCT CCC GAT TAT - 3'; locus D11S4088: forward primer 5'-Hex -GGG CAG AGG CAG TGG AG - 3', reverse primer 5'- GCA TGT TTC GGG GGT G - 3'; locus D11S1363: forward primer 5'-fluorescein - GAA AAT GGT ATT TA GA A AC C AA-3'; reverse primer 5'- CCC AAG GGC TTA C A AC-3'.

Figure 1  Allele analysis at 11p15 loci. (A) Primers against chromosome 11p15 polymorphic loci (D11S1363, D11S4088, and THO1) were used to amplify DNA from twins 1 and 2, together with DNA from the father (F) and mother (M). Edited electropherograms are shown. The arrows indicate the paternal (F) and maternal (M) alleles in twin 2, while the sizes of relevant paternal and maternal alleles are shown at the bottom of each dashed vertical line. (B) The approximate positions of 11p15 loci are shown in centimorgans (Genethon genetic map) and distances in base pairs (indicated in millions) from 11pter; data were obtained from the NCBI and Human Genome Browser Gateway databases. The locations of several genes are also shown above the horizontal line; the diagram is not drawn to scale. The loci indicated in bold are those used to achieve the data presented in (A).
The reaction mixes consisted of approximately 10 ng genomic DNA, 1 x reaction buffer (20 mol/l Tris-HCl (pH 8.4), 50 mmol/l KCl, 1.5-3 mol/l MgCl2, 0.2 mmol/l dNTPs, 0.2 µmol/l (THO1) or 0.4 µmol/l (D11S4088 and D11S1363) of each of forward and reverse primers, and 0.25 U of Taq DNA polymerase in a 10 µl reaction. The amplification conditions involved 93°C for three minutes, 28-30 cycles of 94°C for 45 seconds, 54°C (THO1 and D11S1363) or 65°C (D11S4088) for 60 seconds, and 72°C for 60 seconds with a final 10 minutes at 72°C.

One microlitre of each amplification reaction was added to 12 µl of deionised formamide and denatured by boiling, before electrophoresis of the PCR products in an Applied Biosystems Prism 310 Genetic Analyzer. GeneScan and Genotyper Version 3.7 software were used for allele analysis.

RESULTS
Initially the twins were examined to determine if they were monozygotic. This analysis involved determining their DNA profile, together with their parents', at 15 STR and five VNTR loci. The twins gave identical profiles at all loci except the 11p15 STR locus, THO1, where unbalanced amplification of alleles was observed in twin 2. In order to investigate this imbalance further, two loci (D11S4088 and D11S1363) located more telomeric than THO1 were amplified for all family members using fluorescently labelled primers. Unbalanced allele amplification was observed for both loci in twin 2, with the apparent maternal allele being only marginally present (fig 1). In contrast, there was amplification of both maternal and paternal alleles at all loci in twin 1.

While the intensity of the maternal allele for D11S4088 in twin 1 was relatively smaller than the intensity seen in the corresponding 209 bp allele of her mother (fig 1), this is thought to represent preferential amplification of the smaller allele in twin 1, rather than low level mosaicism of UPD for this locus. A similar preferential amplification of the smaller allele is seen in the amplification of D11S4088 using paternal DNA, in which the larger allele yields a peak area on the electrophorogram that is 77% of that of the smaller allele. In addition, there are approximately equal peak areas for both maternal and paternal alleles at the flanking loci in twin 1, which precludes an extensive area of UPD as seen in twin 2.

DISCUSSION
We have examined a series of STR loci in a pair of monozygotic twins discordant for the phenotype of hemihypertrophy, and have shown uniparental disomy of the paternal allele at 11p15 in the affected twin.

This genetic phenomenon has been previously reported, occurring in 20 to 28% of patients in a series of sporadic cases of BWS. The mechanism of uniparental disomy is compatible with hypotheses that this overgrowth syndrome with variable expression occurs as a result of overexpression of paternally derived growth factors and/or underexpression of maternally expressed growth suppressors.

The variability of phenotypic expression of BWS raises difficulty in defining diagnostic criteria. However, it should be stressed that the affected patient described here does not fit the generally used criteria for BWS, and that the finding of mosaic UPD supports the view that IH may be part of a spectrum of phenotypes encompassing BWS. Interestingly, in a study of 49 BWS patients, Slatter et al suggested that those patients with UPD were significantly more likely to have hemihypertrophy than those in which UPD had been ruled out (6/9 v 1/23, p < 0.001). In addition, the association of BWS with monozygotic twins of discordant phenotype is well reported, with the majority of these twins being female.

BWS has a frequency of 1/13 700 live births and is sporadic in most cases. The most consistent features of the syndrome are macrosomia, macroglossia, abdominal wall defects, hemihypertrophy, and increased risk of embryonal tumours including Wilms tumour. BWS is linked to the 11p15 locus, where a role of imprinting has been postulated in its aetiology. A cluster of candidate genes, with roles in development and neoplasia, has been identified at 11p15. One of these, IGF2, is an imprinted gene with normal monoallelic paternal expression. There is evidence for uniparental disomy of the expressed paternal IGF2 allele or abnormal expression from the normally silent maternally derived allele in some cases. Morison et al reported biallelic expression of IGF2 in children with overgrowth who do not meet criteria for BWS. They proposed that the manifestations of BWS and "incomplete" forms of BWS represent disorders along a spectrum of "IGF2 overgrowth disorders". In accordance with the observation of mosaicism as a common feature of overgrowth disorders, Morison et al suggested that BWS represents an extreme manifestation of IGF2 overexpression, reflecting the extent and location of cells involved. Accordingly, somatic loss of IGF2 imprinting could be expected to produce features along the spectrum of BWS, but insufficient to meet diagnostic criteria.

Other candidate genes in the 11p15 region include H19, a biologically active non-translated mRNA expressed from the maternal allele, with a postulated role in tumour suppression. Mutations affecting the maternally expressed cyclin dependent kinase inhibitor encoded by the CDKN1C gene have been identified in sporadic and familial cases of BWS.

Two imprinting centres in 11p15 have been proposed, based on methylation studies in BWS patients. A distal imprinting centre regulating IGF2 and H19 imprinting, and a more centromeric imprinting centre are proposed. KvDMR1 is a CpG island upstream of KCNQ1OT1, which is maternally imprinted in normal subjects. Loss of maternal imprinting at KvDMR1 has been reported in 50% of BWS patients, resulting in biallelic expression of KCNQ1OT1.

Based on a consistent finding of loss of imprinting in samples of twin pairs discordant for BWS, Weksberg et al proposed that the mechanism of monozygotic twinning in BWS is related to the presence of this epigenetic alteration. The finding of UPD for the imprinted region 11p15 in monozygotic twins discordant for hemihypertrophy is in agreement with this hypothesis. Altered gene expression, through loss of the maternally imprinted region in a proportion of cells, may contribute to the formation of two distinct cell clones in the early embryo. The resulting asymmetry of the embryo may play a role in promoting twinning. This is aligned with mechanisms previously proposed.

It has been suggested that the mechanism of the epigenetic change associated with twinning is the failure of maintenance of methylation at or before the twinning event. Here, we expand the proposed genetic mechanism to include a postzygotic recombination event resulting in a clone of cells with UPD for 11p15. The high prevalence of monozygotic twins in BWS relative to the normal population makes a pre-separation recombination event a plausible explanation for the association of these phenotypes. It also raises UPD as a further mechanism responsible for discordant hemihypertrophy/BWS in monozygotic twins.

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REFERENCES


