

Paternal uniparental disomy in monozygotic twins discordant for hemihypertrophy

P M H West, D R Love, P M Stapleton, I M Winship

J Med Genet 2003;**40**:223–226

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.¹ There may also be asymmetrical visceromegaly on the ipsilateral or contralateral side.² 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

The incidence of IH is ~1/86 000 live births,³ with a male:female ratio of 1:2.¹ The aetiology of IH is unknown. A number of different chromosomal anomalies, including diploid-triploid mosaicism and trisomy 18 mosaicism, have been identified, and the causes of IH are likely to be heterogeneous.² It has been suggested that IH may be one end of the spectrum of phenotypes of BWS, linked to the chromosomal locus 11p15.¹

Here we report a case of apparently isolated hemihypertrophy in one of a pair of monozygotic twins, with paternal uniparental disomy in an area of the 11p15 locus associated with BWS.

CASE REPORT

The subjects of the study are a pair of female twins, one of whom (twin 2) has hemihypertrophy. Twin 1 has a normal physical appearance.

The twins were born at 36 weeks of gestation by normal vaginal delivery. Twin 1 weighed 2730 g and twin 2 weighed 3205 g. Apgar score at birth was 10 for each girl. Twin 1 fed well and gained weight as expected with no neonatal problems. Twin 2 initially failed to pass meconium and was intolerant of feeds. She was noted to have hemihypertrophy affecting the right leg and arm and an ultrasound scan showed enlargement of the right kidney. She was given a glycerine suppository and passed meconium at 46 hours. A rectal biopsy was undertaken to exclude Hirschsprung's disease. Twin 2 received phototherapy for jaundice on day 6. She was discharged from hospital on day 12, at which time she was breast feeding well and gaining weight. Both children reached developmental milestones at expected ages.

Twin 2 has been followed with serial ultrasound scans of her kidney, with measurements of the kidney lengths as shown in table 1. This twin's general asymmetry has necessitated continuing orthopaedic care. Orthopaedic scanogram at the age of 15 months showed a femur length of 17.1 cm on the right and 16.1 cm on the left. Tibia lengths were 13.5 cm (right) and 12.8 cm (left) with a total leg length discrepancy of 1.7 cm. Further scanograms at the age of 6 years 3 months showed a leg length discrepancy of 4.5 cm, and at 8 years 3 months showed leg lengths of 64 cm (right) and 59 cm (left). The leg length discrepancy will be treated with distal femoral and proximal tibial epiphysiodesis at maturity.

On examination at the age of 9 years 10 months, both twins were noted to have heart murmurs that were thought to be

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.

Table 1 Serial kidney measurements (length) for twin 2

Age	Right kidney (cm)	Left kidney (cm)
5 days	4.0	4.0
5 months	6.1	5.4
1 year 4 months	7.0	5.5
3 years 2 months	7.7	5.8
3 years 9 months	9.1	7.0

innocent in nature. At that time twin 1 weighed 29.55 kg (25th centile) and her height was 135.2 cm (75th centile). Twin 2 weighed 35.45 kg (just below the 90th centile) and her height was 137.6 cm (between the 75th and 90th centile). Twin 2 had asymmetry of the skull. Neither twin had dysmorphic features. Both girls are progressing academically at an age appropriate level.

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, THO1, 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,

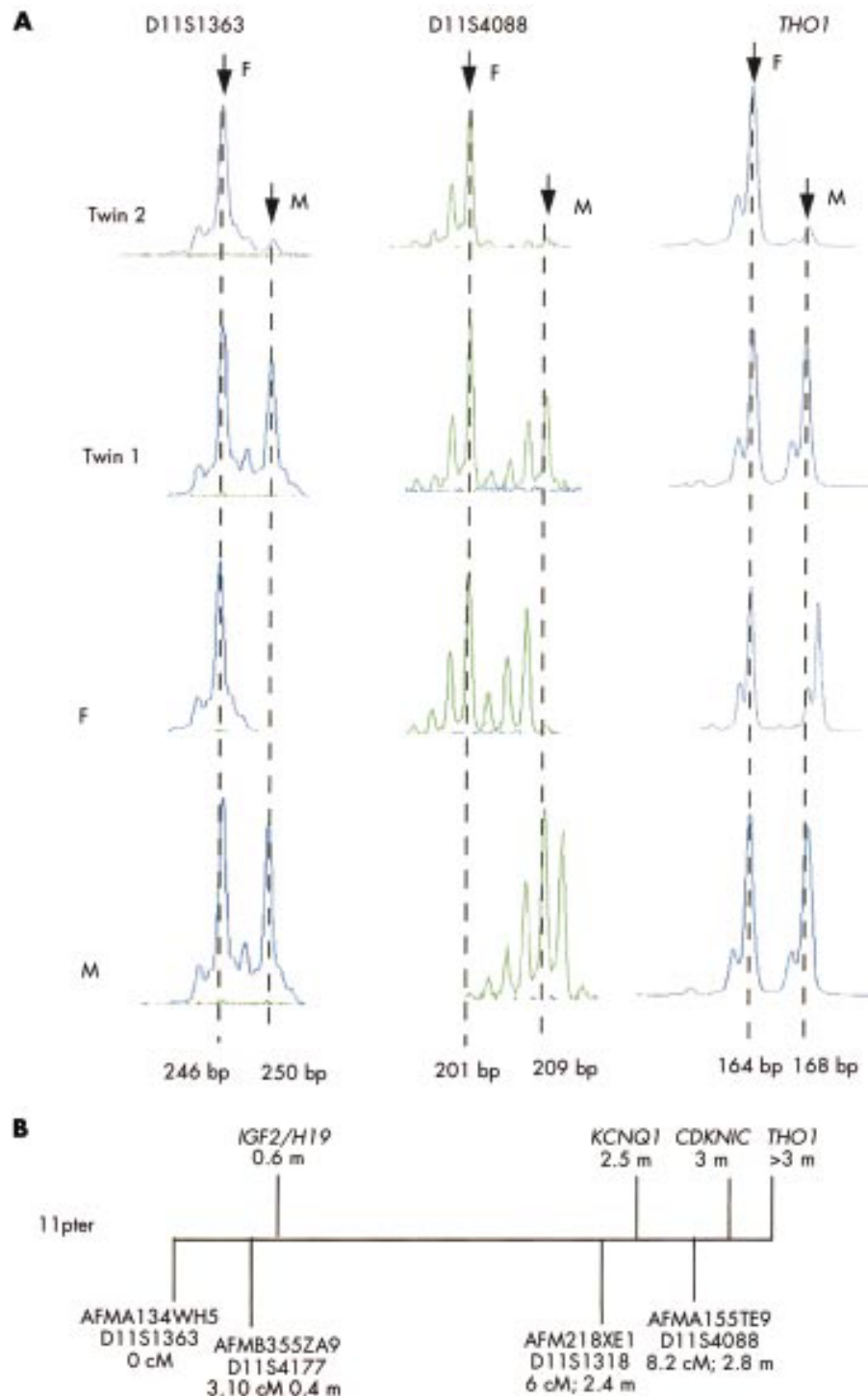


Figure 1 Allele analysis at 11p15 loci. (A) Primers against chromosome 11p15 polymorphic loci (D11S1363, D11S4088, and THO1) were used to amplify DNA from twins 1 and 2, together with DNA from the father (F) and mother (M). Edited electropherograms are shown. The arrows indicate the paternal (F) and 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).

USA). 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 TAG AAA CCA A - 3'; reverse primer 5' - CCC AAG GGC TTA CAA C - 3'.

The reaction mixes consisted of approximately 10 ng genomic DNA, 1 × reaction buffer (20 mol/l Tris-HCl (pH 8.4), 50 mmol/l KCl), 1.5–3 mmol/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 electropherogram 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 is 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,^{4–6} 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 derived 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,⁷ 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*⁸ 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.^{8–13}

BWS has a frequency of 1/13 700 live births and is sporadic in most cases.¹⁴ The most consistent features of the syndrome

are macrosomia, macroglossia, abdominal wall defects, hemihypertrophy, and increased risk of embryonal tumours including Wilms tumour.¹⁵ 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, have been identified at 11p15.¹ One of these, *IGF2*, is an imprinted gene with normal monoallelic paternal expression.¹⁶ 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.^{17–18} Morison *et al*¹⁹ 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*¹⁹ 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.²⁰ Mutations affecting the maternally expressed cyclin dependent kinase inhibitor encoded by the *CDKN1C* gene have been identified in sporadic and familial cases of BWS.²¹

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.²² KvDMR1 is a CpG island upstream of *KCNQ1OT1*, which is maternally imprinted in normal subjects.^{23–24} Loss of maternal imprinting at KvDMR1 has been reported in 50% of BWS patients, resulting in biallelic expression of *KCNQ1OT1*.^{23–25}

Based on a consistent finding of loss of imprinting in samples of twin pairs discordant for BWS, Weksberg *et al*²⁵ 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.^{25–26}

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.²⁵ 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.

ACKNOWLEDGEMENTS

We thank the family who participated in this study, and the Northern Regional Genetics Service, Auckland Hospital, for patient recruitment and access to resources. The research was supported by the Faculty of Medical and Health Sciences of the University of Auckland.

Authors' affiliations

P M H West, I M Winship, Faculty of Medical and Health Sciences, The University of Auckland, Auckland 1001, New Zealand

D R Love, School of Biological Sciences, University of Auckland, Auckland 1001, New Zealand
P M Stapleton, DNA Diagnostics Ltd, Auckland 1001, New Zealand

Correspondence to: Dr I M Winship, Faculty of Medical and Health Sciences, The University of Auckland, Private Bag 92019, Auckland 1001, New Zealand; i.winship@auckland.ac.nz

REFERENCES

- Hoyme HE**, Seaver LH, Jones KL, Procopio F, Crooks W, Feingold M. Isolated hemihyperplasia (hemihypertrophy): report of a prospective multicenter study of the incidence of neoplasia and review. *Am J Med Genet* 1998;**79**:274-8.
- Cohen MM Jr**. Asymmetry: molecular, biologic, embryopathic, and clinical perspectives. *Am J Med Genet* 2001;**101**:292-314.
- Tomooka Y**. Congenital hemihypertrophy with adrenal adenoma and medullary sponge kidney. *Br J Radiol* 1988;**61**:851-3.
- Slatter RE**, Elliott M, Welham K, Carrera M, Schofield PN, Barton DE, Maher ER. Mosaic uniparental disomy in Beckwith-Wiedemann syndrome. *J Med Genet* 1994;**31**:749-753.
- Henry I**, Puech A, Riesewijk A, Ahnine L, Mannens M, Beldjord C, Bitoun P, Tournade MF, Landrieu P, Junien C. Somatic mosaicism for partial paternal isodisomy in Wiedemann-Beckwith syndrome: a post-fertilization event. *Eur J Hum Genet* 1993;**1**:19-29.
- Schneid H**, Suerin D, Vazquez MP, Gourmelen M, Cabrol S, Le Bouc Y. Parental allele specific methylation of the human insulin-like growth factor II gene and Beckwith-Wiedemann syndrome. *J Med Genet* 1993;**30**:353-62.
- Soleto-Avila C**, Gonzalez-Crussi F, Fowler JW. Complete and incomplete forms of Beckwith-Wiedemann syndrome: their oncogenic potential. *J Pediatr* 1980;**96**:47-50.
- Clayton-Smith J**, Read AP, Donnai D. Monozygotic twinning and Wiedemann-Beckwith syndrome. *Am J Med Genet* 1992;**42**:633-7.
- Franceschini P**, Guala A, Vardeu MP, Franceschini D. Monozygotic twinning and Beckwith-Wiedemann syndrome. *Am J Hum Genet* 1993;**46**:353-4.
- Leonard NJ**, Bernier FP, Rudd N, Machin GA, Bamforth F, Bamforth S, Grundy P, Johnson C. Two pairs of male monozygotic twins discordant for Wiedemann-Beckwith syndrome. *Am J Med Genet* 1996;**61**:253-7.
- Litz CE**, Taylor KA, Qiu JS, Pescovitz OH, de Martinville B. Absence of detectable chromosomal and molecular abnormalities in monozygotic twins discordant for the Wiedemann-Beckwith syndrome. *Am J Med Genet* 1988;**30**:821-33.
- Berry AC**, Belton EM, Chantler C. Monozygotic twins discordant for Wiedemann-Beckwith syndrome and the implications for genetic counselling. *J Med Genet* 1980;**17**:136-8.
- Olney AH**, Buehler BA, Waziri M. Wiedemann-Beckwith syndrome in apparently discordant monozygotic twins. *Am J Med Genet* 1988;**29**:491-9.
- Cohen MM Jr**. Overgrowth syndromes: an update. *Adv Pediatr* 1999;**46**:441-91.
- Li M**, Squire JA, Weksberg R. Overgrowth syndromes and genomic imprinting: from mouse to man. *Clin Genet* 1998;**53**:165-70.
- DeChiara M**, Robertson E, Efstratiadis A. Parental imprinting of the mouse insulin-like growth factor II gene. *Cell* 1991;**64**:849-59.
- Ogawa O**, Eccles MR, Szeto J, McNoe LA, Yun K, Maw MA, Smith PJ, Reeve AE. Relaxation of insulin-like growth factor gene imprinting implicated in Wilms tumour. *Nature* 1993;**362**:749-51.
- Mannens M**, Hoovers JM, Redeker E, Veriaal M, Feinberg AP, Little P, Boavida M, Coad N, Steenman M, Blik J, Niikawa N, Tonoki H, Hakamura Y, de Bo E, Slater RM, John R, Cowell JK, Junien C, Tommerrup N, Weksberg R, Poeschel SM, Leshot NJ, Westerveld I. Parental imprinting of human chromosome region 11p15.4-pter involved in the Wiedemann-Beckwith syndrome and various human neoplasia. *Eur J Hum Genet* 1994;**2**:3-23.
- Morison IM**, Becroft DM, Taniguchi T, Woods CG, Reeve AE. Somatic overgrowth associated with overexpression of insulin-like growth factor II. *Nat Med* 1996;**2**:311-16.
- Li M**, Squire JA, Weksberg R. Molecular genetics of Wiedemann Beckwith syndrome. *Am J Med Genet* 1998;**79**:253-9.
- Hatada I**, Nabetani A, Morisaki H, Xin Z, Ohishi S, Tonoki H, Niikawa N, Inoue M, Komoto Y, Okada A, Steichen E, Ohashi S, Fukushima Y, Nakayama M, Mukai T. New p57KIP2 mutations in Beckwith-Wiedemann syndrome. *Hum Genet* 1997;**100**:681-3.
- Maher ER**, Reik W. Beckwith-Wiedemann syndrome: imprinting in clusters revisited. *J Clin Invest* 2000;**105**:247-52.
- Lee MP**, BeBaun MR, Mitsuya K, Galonek HL, Brandenburg S, Oshimura M, Feinberg AP. Loss of imprinting of a paternally expressed transcript, with antisense orientation to KvLQT1 locus identified by screening to differentially expressed transcripts using monochromosomal hybrids. *Hum Mol Genet* 1999;**8**:1209-17.
- Smilnich NJ**, Day CD, Fitzpatrick GV, Caldwell GM, Lossie AC, Cooper PR, Smallwood AC, Joyce JA, Schofield PN, Reik W, Nicholls RD, Weksberg R, Driscoll DJ, Maher ER, Shows TB, Higgins MJ. A maternally methylated CpG island in KvLQT1 is associated with an antisense paternal transcript and loss of imprinting in Beckwith-Wiedemann syndrome. *Proc Natl Acad Sci USA* 1999;**96**:8064-9.
- Weksberg R**, Shuman C, Caluseriu O, Smith AC, Fei YL, Nishikawa J, Stockley LT, Best L, Chitayat D, Olney A, Ives E, Schneider A, Bestor TH, Li M, Sadowski P, Squire J. Discordant *KCNQ1OT1* imprinting in sets of monozygotic twins discordant for Beckwith-Wiedemann syndrome. *Hum Mol Genet* 2002;**11**:1317-25.
- Burn J**, Povey S, Boyd Y, Munro EA, West L, Harper K, Thomas D. Duchenne muscular dystrophy in one of monozygotic twin girls. *J Med Genet* 1986;**23**:494-500.