Mosaic uniparental disomy in Beckwith-Wiedemann syndrome

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Abstract

Beckwith-Wiedemann syndrome (BWS) is a congenital overgrowth syndrome with variable expression. The major features are anterior abdominal wall defects, macroglossia, and gigantism and less commonly neonatal hypoglycaemia, organomegaly, congenital renal anomalies, hemihypertrophy and embryonal tumours occur. BWS is a genetically heterogeneous disorder; most cases are sporadic but approximately 15% are familial and a small number of BWS patients have cytogenetic abnormalities involving chromosome 11p15. Genomic imprinting effects have been implicated in familial and non-familial BWS, and uniparental disomy (UPD) for chromosome 11 has been reported in sporadic cases. We investigated the incidence, pathogenesis, and clinical associations of UPD in 49 patients with non-familial BWS and a normal karyotype. UPD for chromosome 11p15 was detected in nine of 32 (28%) informative patients. A further two patients appeared to be disomic at the WT1 locus in chromosome 11p13, but were uninformative at chromosome 11p15.5 loci tested. In all cases with UPD the affected person was mosaic for a paternal isodisomy and a normal cell line indicating that UPD had arisen as a postzygotic event. Compared to cases in which paternal isodisomy for chromosomes 11p15.5 had been excluded (n=23), BWS patients with UPD was more likely to have hemihypertrophy (6/9 versus 1/23, $p<0.001$) and less likely to have exomphalos (0/9 versus 13/23, $p<0.01$), but there were no significant differences between disomic and non-disomic cases in the incidence of hypoglycaemia, nephromegaly, neoplasia, and developmental delay. The detection of UPD in BWS patients allows accurate genetic counselling to be provided and provides an insight into the molecular pathogenesis of BWS.

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Beckwith-Wiedemann syndrome (BWS), a congenital overgrowth syndrome with an estimated incidence of 1/13 700, is characterised by the association of gigantism, macroglossia, and visceromegaly with a variety of developmental anomalies: abdominal wall defects (including exomphalos), hemihypertrophy,

genitourinary abnormalities and mental retardation.¹⁻³ Approximately 7% of patients develop embryonal tumours.^{4,5} Most cases of BWS appear to be sporadic. However familial BWS occurs in 15% of patients and is inherited as an autosomal dominant trait with variable expression and incomplete penetrance. In familial BWS penetrance is more complete when the mother is the transmitting parent and examples of transmitting males with affected children are very rare.⁶⁻⁸ The BWS gene has been mapped to chromosome 11p15 by genetic linkage studies.^{7,9} In addition, a small number of BWS patients have chromosomal abnormalities of chromosome 11p15. In BWS cases with duplications of chromosome 11p15, the duplicated material is paternally derived in all cases in which the origin of the duplication has been determined.¹⁰ In BWS patients with balanced translocations and inversions of chromosome 11 the chromosomal aberration has been maternally inherited.¹¹ These findings suggest that the BWS gene(s) is imprinted. Further evidence for genomic imprinting effects in BWS was reported by Henry *et al*¹² and Nystrom *et al*¹³ who detected paternal uniparental disomy in 3/8 and 1/14 children with sporadic BWS respectively. We have investigated 49 persons with non-familial BWS to determine the incidence, pathogenesis, and clinical associations of UPD in BWS.

Methods

PATIENTS

Forty-nine persons (26 male, 23 female, mean age 6.1 years, range 5 months to 31 years) with sporadic BWS were ascertained by contacting clinical geneticists and paediatricians throughout the United Kingdom and the patient support group. A diagnosis of BWS was based on the following criteria: (1) three major features (anterior abdominal wall defects, macroglossia, and pre/postnatal growth >90th centile), or (2) two major features plus three or more of: characteristic ear signs (ear lobe creases or posterior helical ear pits), facial naevus flammeus, hypoglycaemia, nephromegaly, and hemihypertrophy.³ No patients had any abnormality on cytogenetic analysis or a family history of BWS. Peripheral blood for DNA analysis was obtained from the affected child and parents.

MOLECULAR GENETIC INVESTIGATION

High molecular weight DNA was isolated from peripheral blood and tissue by standard methods.¹⁴ To screen for UPD, each affected

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Figure 1 Mosaic paternal isodisomy in four patients with Beckwith-Wiedemann syndrome at the TH locus. In each affected child (C) the maternal allele is less intense than the paternal allele. C=child, F=father, M=mother.

Table 1 Uniparental disomy in BWS

Patient	Locus			Estimated % of cells with UPD
	Chromosome 11p15.5		Chromosome 11p13	
	TH	IGF2	WT1	
BWS 3	UPD	UPD	UPD	43
BWS 7	UPD	UPD	N	47
BWS 12	UPD	UPD	UPD	63
BWS 32	UPD	—	—	23
BWS 35	UPD	—	UPD	23
BWS 37	UPD	—	N	79
BWS 38	UPD	—	—	46
BWS 41	UPD	—	—	43
BWS 47	UPD	—	N	49

UPD = mosaic paternal isodisomy, N = no UPD, — = uninformative.

Table 2 Comparison of clinical features of BWS patients with (UPD) and without (No UPD) mosaic paternal isodisomy for chromosome 11p15.5

Clinical features	UPD	No UPD	Statistical significance*
Male:female	5:4	11:13	NS
Anterior abdominal wall defect	4/9	19/23	NS
Exomphalos	0/9	13/23	$\chi^2 = 6.4$, $p < 0.01$
Birth weight >90th centile	6/9	11/23	NS
Postnatal growth >90th centile	8/9	19/23	NS
Hemihypertrophy	6/9	1/23	$\chi^2 = 11.3$, $p < 0.001$
Hypoglycaemia	6/9	12/23	NS
Nephromegaly	6/9	11/23	NS
Developmental delay	1/9	5/23	NS
Wilms' tumour	1/9	0/23	NS

*NS = not significant ($p > 0.1$)

child and parent was genotyped for microsatellite polymorphisms at the TH locus in chromosome 11p15.5 and the WT1 locus (primers WTA-400 and WTA-401) in chromosome 11p13.¹⁵ A microsatellite polymorphism at the insulin-like growth factor II locus (IGF2)¹⁶ was investigated in patients with evidence of UPD using previously described methods and procedures. For analysis of the tetranucleotide polymorphism at TH, DNA was amplified either using previously published primers¹⁷ and the products digested with *HinfI*, or a set of redesigned primers to produce a smaller product (TH-F 5' CTG GGC TCT GGG GTG ATT CC 3'; TH-R 5' CCG AGT GCA GGT CAC AGG GA 3'). DNA (50 ng) was amplified in 25 μ l reactions containing standard PCR buffer (10 mmol/l Tris-Cl, pH 8.8, 50 mmol/l KCl, 0.01% gelatin, 1.5 mmol/l $MgCl_2$), 10 pmol each primer, 0.1 pmol end labelled primer, 200 μ mol/l each of dATP, dCTP, dGTP, and dTTP, and 0.5 U *Taq* polymerase. The samples were subjected to 30 PCR amplification cycles of one minute denaturation at 94°C, one minute annealing at 60°C, and one minute extension at 72°C.

In cases in which there appeared to be allelic imbalance suggestive of mosaic UPD, we attempted to confirm mosaicism and to quantify the proportion of disomic cells in the DNA

sample by determining the ratio of paternal to maternal alleles at the TH locus. To do this, fluorescently labelled (short) primers for the TH locus were prepared and used to amplify DNA from BWS patients with UPD for 25 cycles of PCR. The PCR products (1 μ l) were mixed with 1 μ l GS2500 markers and 2 μ l formamide, and the samples denatured for two minutes at 95°C. The mixture was then loaded onto a 6% standard sequencing gel and analysed using an automated ABI 373 DNA sequencer. The data obtained were analysed with the fragment analysis part of the ABI Genescan 672 software package.

STATISTICAL ANALYSIS

Intergroup differences were compared using the χ^2 test with Yates's correction. Statistical significance was taken at the 5% level.

Results

MOLECULAR GENETIC ANALYSIS

Thirty-two and 29 of 49 patients were informative at the TH locus in chromosome 11p15.5 for the assessment of paternal isodisomy and heterodisomy respectively. No patients displayed evidence of paternal heterodisomy, but nine of 32 (28%) informative cases had evidence of paternal isodisomy for chromosome 11p15.5 (fig 1). In each case there was evidence of allelic imbalance with the paternal allele more intense than the maternal allele. These findings suggested that the nine patients were mosaic for disomic and normal cell lines. Six of the nine patients with UPD at TH were informative for the assessment of UPD at the WT1 locus in chromosome 11p13. Three cases also had UPD at chromosome 11p13 but in three patients there was segmental isodisomy with no UPD at WT1 (BWS 7, BWS 37, and BWS 47) (table 1). The ratio of the paternal to maternal allele at the TH locus was determined in the nine patients with apparent mosaic UPD. The calculated proportion of disomic cells varied between 23% (BWS 32 and BWS 35) and 79% (BWS 37) (table 1).

CLINICAL AND MOLECULAR CORRELATION

The clinical features of the nine patients with mosaic paternal isodisomy for chromosome 11p15.5 were compared to the 23 patients in whom this was excluded (table 2). There was a clear association between mosaic UPD and hemihypertrophy such that six of the seven informative subjects with hemihypertrophy had mosaic UPD. The one patient with hemihypertrophy and no evidence of UPD was one of a pair of monozygotic female twins discordant for BWS. Three patients with mosaic UPD did not have hemihypertrophy, including the patient (BWS 37) with the highest proportion of disomic cells in peripheral blood (fig 2, table 1). Exomphalos was less common in the UPD group than the non-UPD group ($p < 0.01$), but there were no statistically significant differences between the UPD and non-UPD groups in the frequency of all anterior

Figure 2 Quantification of paternal and maternal alleles at the TH locus in a child (BWS37) with mosaic paternal isodisomy (see methods section for details). Both parents are heterozygous. The child is heterozygous but the paternal allele peak is greater than the maternal allele peak. C=child, F=father, M=mother.

abdominal wall defects, pre- or postnatal gigantism, hypoglycaemia, nephromegaly, or developmental delay. Wilms' tumour occurred in three of the 49 patients, but only one of these was informative for UPD analysis (BWS 47) and was found to have mosaic paternal isodisomy at chromosome 11p15.5, but not at WT1.

Discussion

Our finding of mosaicism for paternal isodisomy of chromosome 11p15.5 in 28% of informative sporadic BWS patients is similar to that found in the previous studies by Henry *et al.*¹⁸ and Schneid *et al.*¹⁹ who detected UPD

in five of 25 (20%) and four of 16 (25%) French cases respectively. All our cases with UPD were mosaic, as were eight of the nine French cases.^{18,19} Thus in most BWS patients UPD appears to have arisen as a postzygotic event. In addition to mosaicism, the finding of segmental isodisomy in three of our cases and in four French cases^{18,19} is compatible with UPD resulting from a mitotic recombination error. UPD for chromosome 15 occurs in the Prader-Willi and Angelman syndromes. Maternal UPD (isodisomy or heterodisomy) produces Prader-Willi syndrome and most cases appear to result from non-disjunction in meiosis I.²⁰ Paternal UPD is seen in Angelman syndrome but appears rarely to result from errors in meiosis I. This may reflect the increased frequency of aneuploidy in oocytes compared to sperm, and possibly also post-spermatogenesis selection.²⁰ In Angelman syndrome complete paternal isodisomy is typically found and presumably results from maternal chromosome 15 loss and reduplication of the paternal homologue, or "correction" of a trisomic conceptus.²⁰ This differs from the post-zygotic origin of paternal UPD in BWS, but it is not known whether this difference reflects the relative frequency of trisomy 15 conceptions, or whether embryos with complete isodisomy or heterodisomy of chromosome 11 would have a low viability. In mice, paternal disomy for chromosome 7 (distal 7p is homologous to human chromosome 11p15.5) is lethal and only chimaeric embryos survive.²¹ In humans the presence of imprinted genes on chromosome 11q (the gene for familial glomus tumours is imprinted and maps to chromosome 11q23-qter²²) might also influence the viability of embryos with complete hetero- or isodisomy.

The association of BWS with paternal isodisomy of chromosome 11p is compatible with the hypothesis that BWS is caused by an excess of a paternally expressed growth promoter (a deficiency of a maternally expressed growth suppressor could also be implicated). From our results and those of Henry *et al.*¹⁸ and Schneid *et al.*¹⁹ it is clear that the critical region for UPD is telomeric to chromosome 11p13 and includes the region (chromosome 11p15.5) to which the familial BWS gene maps. Two imprinted candidate genes are contained within chromosome 11p15.5, IGF2 and H19. IGF2 is a growth promoter which is expressed from the paternal allele in man and mice,^{21,23,24} and chimaeric mouse embryos with paternal disomy of distal chromosome 7p (which is homologous to human chromosome 11p15) are larger than controls.²¹ H19 is widely expressed during embryonal development, is closely linked to IGF2, but is expressed from the maternal allele in man and mouse.²⁵⁻²⁷ Although H19 may not encode a protein product,²⁸ a recent study suggests that H19 expression can suppress growth in an embryonal tumour cell line.²⁹ Persons with paternal disomy of chromosome 11p15 would be expected to have increased expression of IGF2 and reduced expression of H19. Recently Weksberg *et al.*³⁰ have reported four non-disomic BWS patients in which there was disruption of maternal repression of IGF2

resulting in biallelic IGF2 expression. While these findings clearly implicate IGF2 overexpression in the pathogenesis of the BWS phenotype, other factors may also be involved, as suggested by reports of a patient with gigantism, Wilms' tumour, and biallelic expression of IGF2, but no other evidence of BWS³¹ and of a BWS patient with normal imprinting of IGF2.²⁴

The strong association between hemihypertrophy and mosaic UPD in BWS presumably reflects differences in the proportion of disomic cells between the hypertrophied and non-hypertrophied sides. In our study the only informative patient with hemihypertrophy without UPD was one of monozygotic twins discordant for BWS. It is possible that sharing of blood in utero with the unaffected twin might have masked mosaic UPD in the affected twin and analysis of a skin biopsy from the hypertrophied side might have been a more useful sample for analysis. Monozygotic twins discordant for BWS are well recognised and 13 of 14 reported cases with normal chromosomes are female.³ A postzygotic mitotic recombination event would represent a plausible explanation for BWS discordance in such cases but mosaic UPD has yet to be detected in these cases. The delayed development of female embryos compared to male embryos might result in embryos with mosaic UPD twinning more frequently if the embryo is female. Not all BWS patients with hemihypertrophy will have mosaic UPD; Weksberg *et al*³⁰ reported three BWS patients with hemihypertrophy and relaxation of IGF2 imprinting in whom UPD was excluded.

Wiedemann⁴ reported an association between hemihypertrophy and Wilms' tumour in BWS. Henry *et al*¹⁸ and Schneid *et al*¹⁹ both found there was a high incidence of Wilms' tumour in BWS patients with UPD (40% and 50% respectively). Our own data did not show a statistically significant difference between the incidence of Wilms' tumour in UPD and non-UPD patients. However, the numbers are small and combining our data with that of other studies^{13,18,19} the incidence of Wilms' tumour in BWS patients with UPD is 26%. This is higher than the incidence of Wilms' tumour in unselected BWS patients, but lower than the risk based on the French studies alone.^{18,19} The finding of increased IGF2 expression in some sporadic Wilms' tumours and of localised renal tissue mosaicism for paternal UPD on chromosome 11 in some patients with sporadic Wilms' tumour³² would be compatible with the concept that overexpression of IGF2 and a reduction in H19 expression in BWS patients with UPD would predispose to the development of Wilms' tumour. It is of interest that Grundy *et al*^{33,34} reported a case of Wilms' tumour, hemihypertrophy, and paternal isodisomy of chromosome 11p15, but found that 11p paternal UPD was uncommon in Wilms' tumour patients without BWS associated anomalies.

The absence of exomphalos in patients with mosaic UPD is compatible with the suggestion that they may have less severe complications

because of their mosaicism. Alternatively the difference could result from fundamental differences in the aetiology of the BWS phenotype in UPD and non-UPD patients. Some patients with mild BWS signs that do not satisfy the criteria for the diagnosis of BWS could have a low proportion of disomic cells.

Genetic counselling is difficult in BWS because the clinical features become less apparent with age and the expression of BWS in a transmitting parent is extremely variable so that familial cases may be underdiagnosed.⁵ The identification of UPD in BWS patients allows accurate genetic counselling to be provided to these cases. Although the case for routine ultrasound screening for Wilms' tumour in BWS is not proven, if an association between UPD and Wilms' tumour is confirmed, screening could be targeted to a high risk subgroup. Thus detection of UPD can enhance the management of BWS families. In contrast to previous studies of UPD in BWS,^{18,19} we used PCR based microsatellite markers instead of RFLPs and Southern analysis. We found the former approach advantageous because of the increased informativeness of microsatellite markers and the ability to type small amounts of DNA. The incidence of UPD was similar with both approaches^{18,19} and we found the PCR based approach to be reliable and reproducible (we and others have used a similar approach to detect allele loss in tumour DNA³⁵⁻³⁷). Furthermore, Reik (personal communication) independently investigated seven of the BWS patients we identified as having UPD. Three of these cases were informative for the *AvaII* RFLP at IGF2 and UPD was confirmed on Southern analysis. In addition, all seven cases showed increased methylation at H19 and IGF2 consistent with UPD, and no cases in which we excluded UPD had hypermethylation (Reik, personal communication). Schneid *et al*¹⁹ have previously reported IGF2 hypermethylation in BWS patients with UPD and the findings of Reik confirm the validity of a PCR based approach to detecting UPD.

BWS is genetically heterogeneous and the molecular pathogenesis may also be heterogeneous. Careful correlation of the clinical phenotype with the molecular pathology may provide an insight into the aetiology of the variable expression of BWS. Definition of the critical region of chromosome 11p for UPD in BWS will provide a basis for understanding the aetiology of BWS, and the detailed comparison of the clinical features of BWS patients with variable lengths of segmental isodisomy may identify target regions for other imprinted genes on chromosome 11.

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