

ARTICLE

Molecular subtypes and phenotypic expression of Beckwith–Wiedemann syndrome

Wendy N Cooper¹, Anita Luharia², Gail A Evans¹, Hussain Raza¹, Antonita C Haire¹, Richard Grundy^{3,4}, Sarah C Bowdin^{1,2}, Andrea Riccio⁵, Gianfranco Sebastio⁶, Jet Bliiek⁷, Paul N Schofield⁸, Wolf Reik⁹, Fiona Macdonald² and Eamonn R Maher^{*,1,2}

¹Medical and Molecular Genetics Section, Institute of Biomedical Research, University of Birmingham, Birmingham, UK; ²West Midlands Regional Genetics Service, Birmingham Women's Hospital, Edgbaston, Birmingham, UK; ³Department of Paediatrics, Birmingham Children's Hospital, University of Birmingham, Birmingham, UK; ⁴Department of Paediatric Oncology, Birmingham Children's Hospital, University of Birmingham, Birmingham, UK; ⁵Dipartimento di Scienze Ambientali, Seconda Università di Napoli, Caserta, Italy; ⁶Department of Pediatrics, Federico II University of Naples, Naples, Italy; ⁷Department of Clinical Genetics, Academic Medical Center, Amsterdam, The Netherlands; ⁸Department of Anatomy, University of Cambridge, Cambridge, UK; ⁹Laboratory of Developmental Genetics and Imprinting, The Babraham Institute, Cambridge, UK

Beckwith–Wiedemann Syndrome (BWS) results from mutations or epigenetic events involving imprinted genes at 11p15.5. Most BWS cases are sporadic and uniparental disomy (UPD) or putative imprinting errors predominate in this group. Sporadic cases with putative imprinting defects may be subdivided into (a) those with loss of imprinting (LOI) of *IGF2* and *H19* hypermethylation and silencing due to a defect in a distal 11p15.5 imprinting control element (IC1) and (b) those with loss of methylation at KvDMR1, LOI of *KCNQ10T1* (*LIT1*) and variable LOI of *IGF2* in whom there is a defect at a more proximal imprinting control element (IC2). We investigated genotype/epigenotype–phenotype correlations in 200 cases with a confirmed molecular genetic diagnosis of BWS (16 with *CDKN1C* mutations, 116 with imprinting centre 2 defects, 14 with imprinting centre 1 defects and 54 with UPD). Hemihypertrophy was strongly associated with UPD ($P < 0.0001$) and exomphalos was associated with an IC2 defect or *CDKN1C* mutation but not UPD or IC1 defect ($P < 0.0001$). When comparing birth weight centile, IC1 defect cases were significantly heavier than the patients with *CDKN1C* mutations or IC2 defect ($P = 0.018$). The risk of neoplasia was significantly higher in UPD and IC1 defect cases than in IC2 defect and *CDKN1C* mutation cases. Kaplan–Meier analysis revealed a risk of neoplasia for all patients of 9% at age 5 years, but 24% in the UPD subgroup. The risk of Wilms' tumour in the IC2 defect subgroup appears to be minimal and intensive screening for Wilms' tumour appears not to be indicated. In UPD patients, UPD extending to *WT1* was associated with renal neoplasia ($P = 0.054$). These findings demonstrate that BWS represents a spectrum of disorders. Identification of the molecular subtype allows more accurate prognostic predictions and enhances the management and surveillance of BWS children such that screening for Wilms' tumour and hepatoblastoma can be focused on those at highest risk.

European Journal of Human Genetics (2005) 13, 1025–1032. doi:10.1038/sj.ejhg.5201463;
published online 6 July 2005

Keywords: imprinting; Beckwith; Wiedemann

*Correspondence: Professor ER Maher, Section of Medical and Molecular Genetics, Institute of Biomedical Research, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK. Tel: +44 121 627 2741;

Fax: +44 121 414 2538; E-mail: E.R.Maher@bham.ac.uk

Received 23 September 2004; revised 26 May 2005; accepted 26 May 2005; published online 6 July 2005

Introduction

Beckwith–Wiedemann Syndrome (BWS) is a congenital overgrowth disorder with an incidence of about one in 13 000. Phenotypic expression of BWS is variable, but the three major features are pre- and/or postnatal overgrowth, macroglossia and anterior abdominal wall defects ranging from diastasis recti to exomphalos.¹ Less frequent (minor) features include ear creases and helical pits, neonatal hypoglycaemia, hemihypertrophy, facial naevus flammeus and a predisposition to embryonal tumours, particularly Wilms' tumour. The frequency of embryonal neoplasms in BWS is generally considered to be 5–10%, but there are no standard clinical diagnostic criteria^{1,2} and estimates of tumour frequency have varied between studies. Hence, molecular genetic diagnosis would facilitate the diagnosis of BWS and comparison of different BWS cohorts.

The genetics of BWS are complex, but all causes to date are associated with alterations in the expression or function of one or more imprinted genes in the 11p15.5 imprinted gene cluster.³ Chromosome 11p15.5 was first implicated by the finding of paternally derived duplications of 11p15.5 in BWS patients.^{4–7} Subsequently, maternally inherited balanced rearrangements of 11p15 were also demonstrated to be associated with BWS.^{8,9} In contrast, maternally derived 11p15.5 duplication was associated with growth retardation.¹⁰ Overall, it is estimated that up to 3% of BWS patients have a duplication (BWS^{DUP11}) or a balanced rearrangement (inversion BWS^{INV11}; translocation BWS^{TRANS11}). The finding of chromosome 11 paternal uniparental disomy (BWS^{UPD}) in a subset of sporadic BWS patients provided further evidence that BWS is an imprinting disorder.^{11,12} About 20% of sporadic BWS patients have UPD that is invariably a mosaic paternal isodisomy and includes the 11p15.5 gene cluster.^{13,14} This cluster contains more than eight imprinted genes, but those most strongly linked to BWS include the paternally expressed growth promoter *IGF2*, and the maternally expressed candidate tumour suppressor genes *CDKN1C* (*p57^{KIP2}*, a cyclin-dependent kinase inhibitor) and *H19* (an untranslated RNA). Whereas BWS^{UPD} cases are predicted to have increased *IGF2*, reduced *H19* and reduced *CDKN1C* expression, only the expression of paternally expressed genes such as *IGF2* should be altered in BWS^{DUP11} patients. Nevertheless, *CDKN1C* was unequivocally implicated in the pathogenesis of BWS by the finding of germline *CDKN1C* mutations in a subset of patients (BWS^{MUTCDKN1C} subtype^{15–17}). Approximately 15% of BWS cases are familial, and germline *CDKN1C* mutations are found in about half of familial cases and 5% of sporadic cases.^{16,17} Extensive analysis of potential imprinting control elements for the reciprocally imprinted *IGF2* and *H19* genes led to the definition of a paternally methylated intergenic CTCF binding region that is proposed to function as an imprinting control centre (IC1) regulating imprinting of *IGF2* and *H19*, but not more

centromeric genes such as *CDKN1C*.^{18–20} Approximately 5% of BWS patients (BWS^{ICD1}) are proposed to have a defect of this distal imprinting centre as the maternal *IGF2* and *H19* alleles display a paternal epigenotype (hypermethylation and silencing of *H19* and biallelic *IGF2* expression). Recently, some patients with IC1 defects have been demonstrated to have germline deletions within the intergenic CTCF binding region.²¹ Finally, a second more centromeric imprinting centre (IC2) has also been proposed.^{3,22,23} IC2 is associated with a maternally methylated differentially methylated region (KvDMR1) and a paternally expressed untranslated RNA (*KCNQ1OT1* (*LIT1*)).²² Loss of maternal allele methylation at KvDMR1 (and biallelic *KCNQ1OT1* expression) occurs in 50–60% of BWS patients (BWS^{ICD2}),^{23,24} and is associated with down-regulation *in cis* of *CDKN1C* expression²⁵ and variable loss of imprinting (LOI) of *IGF2*.²³ However, *H19* imprinting and methylation is unchanged consistent with IC1 and IC2 as two distinct imprinting centres within the 11p15.5 gene cluster. Most patients with IC2 defects appear to have epimutations, but germline deletions may occur.²⁶

Previously, we looked for 'genotype–phenotype' correlations in BWS and reported associations between hemihypertrophy and BWS^{UPD}, and exomphalos and BWS^{MUTCDKN1C} and BWS^{ICD2} subtypes.^{16,24} In addition, we noted that embryonal tumours occurred in the BWS^{UPD} and BWS^{ICD1} but not BWS^{ICD2} subgroups, but these differences were not statistically significant. Subsequently, DeBaun *et al*²⁷ confirmed the associations with anterior abdominal wall defects and demonstrated significant associations of BWS^{UPD} with hemihypertrophy, cancer and hypoglycaemia. However, Weksberg *et al*²⁸ found that cancer was not limited to the BWS^{UPD} and BWS^{ICD1} groups, but non-Wilms' tumours were frequent in their BWS^{ICD2} group. The identification of clear genotype–phenotype correlations, which led to different management strategies according to the underlying molecular diagnosis, would have important clinical implications. Hence, we have undertaken further analysis on a large ($n=200$) group of BWS cases with a defined molecular subtype. This analysis demonstrates that BWS should be considered as a heterogeneous disorder with different molecular subgroups of BWS posing different genetic and clinical management problems.

Methods

Patients

In all, 193 patients were ascertained via a national research study or following referral to the West Midlands Regional Genetics Service for diagnostic testing (a minority were included in previous publications^{16,24}). In order to avoid ascertainment bias, all patients referred for molecular genetic diagnosis on suspicion of BWS underwent molecular analysis. In order to obtain meaningful genotype–

phenotype correlations, we then analysed only those cases with a proven molecular diagnosis of BWS. Having ascertained our cohort of 193 patients with a confirmed molecular genetic diagnosis of BWS, we recruited into the study an additional seven BWS^{ICD1} cases in order to provide sufficient power for statistical analysis of the BWS^{ICD1} subgroup.²¹ Sufficient DNA was available for analysis of the extent of UPD in 48 cases, and in order to increase statistical power, DNA samples from a further two patients with UPD and Wilms' tumour were studied to evaluate more fully whether the extent of segmental disomy influenced Wilms' tumour risk.²⁹ However, these two additional BWS^{UPD} patients were excluded from the genotype–phenotype analysis, as their tumour status was known upon enrolment. Clinical information was collected by a standard questionnaire, inspection of hospital notes or direct examination. Patients were included in the study following a molecular diagnosis of BWS irrespective of the original indication for referral, but we excluded patients from multiple pregnancies for the prenatal growth analyses.

Molecular analysis

DNA was extracted from peripheral blood lymphocytes or tissue samples by standard procedures. *CDKN1C* was sequenced as described previously.¹⁶ UPD analysis was performed on 25 ng of genomic DNA in a 10 μ l total reaction volume containing fluorescently labelled forward primer (0.4 μ M), unlabelled reverse primer (0.4 μ M), dNTP (0.16 mM) Ampli *Taq* (0.75 U) in 1 \times Ampli *Taq* buffer containing 1.5 mM MgCl₂. After an initial denaturation step (5 min at 94°C), 24 cycles of amplification were performed (94°C 1 min, T_A °C 1 min, 72°C 1 min) followed by a final extension for 5 min at 72°C. The microsatellite markers tested were those at tyrosine hydroxylase,¹¹ $T_A = 62^\circ\text{C}$, D11S1318 ($T_A = 57^\circ\text{C}$) and D11S1984 ($T_A = 57^\circ\text{C}$). Alleles were resolved through a 6% polyacrylamide gel on an ABI377, and the ratio of paternal to maternal allele intensity was determined using the Genotyper software (ABI), a ratio of greater than 1.3:1 in favour of the paternal allele indicated UPD.

Methylation analysis was performed either by Southern blotting as described previously (IC1),³⁰ (IC2)²² or following bisulphite conversion of unmethylated cytosines to uracil. Bisulphite modification of DNA was according to Agathangelou *et al*,³¹ the modified DNA was amplified by PCR (using a fluorescently labelled forward primer); for H19 methylation analysis, the primers used were: forward GTA GGG TTT TTG GTA GGT ATA GAG; and reverse CTT AAA TAA CCC RAA ACR TTT CCA C ($T_A = 55^\circ\text{C}$, and a hot start was required). For KvDMR1 methylation analysis the primers used were W2 GTT ATT TTA TAT TTA GTT AGT GTT TTA TG and W4 TCT TAC TAA AAA ACT CCC TAA AAA TC ($T_A = 53^\circ\text{C}$). The PCR products were then digested with the restriction enzyme *Bst*U1, which has the recogni-

tion site CGCG and hence will cut only modified DNA derived from previously methylated DNA. The fragments were separated on an ABI377 or 3730 and the ratio of methylated to unmethylated DNA determined.

Statistical analysis

Fisher's exact testing, Wilcoxon–Rank sum, *t*-testing and Kaplan–Meier analysis were used as appropriate. Statistical significance was taken at the 5% level.

Results

In all, 200 BWS patients with a defined molecular subtype (BWS^{MUTCDKN1C} $n = 16$ (from seven families), BWS^{ICD2} $n = 116$, BWS^{ICD1} $n = 14$ and BWS^{UPD} $n = 54$) were analysed for genotype–phenotype correlations. Although we did not restrict molecular analysis to patients satisfying specific clinical criteria, 97% of patients had at least two 'major' features of BWS (eg macrosomia; macroglossia; exomphalos or umbilical hernia; visceromegaly, anterior linear ear lobe creases/posterior helical ear pits; embryonal tumour, hemihypertrophy, adrenocortical cytomegaly or renal anomaly or one or more family members with a clinical diagnosis of BWS; see <http://www.geneclinics.org/>). Interestingly, of two patients with hemihypertrophy and an ICD2 defect, there were no other features of BWS in one case and only neonatal hypoglycaemia in the other.

Considering all patients together, there were significant associations between the presence of ear pits and/or creases and facial naevus flammeus ($P = 0.015$), macroglossia and facial naevus flammeus ($P = 0.022$), hemihypertrophy and embryonal tumour ($P = 0.044$), and inverse relationships between the presence of exomphalos and hemihypertrophy ($P < 0.001$) and hemihypertrophy and macroglossia ($P = 0.006$).

The severity of *anterior abdominal wall defects* differed significantly between subgroups (see Figure 1a). Thus, the frequency of exomphalos was much higher in the BWS^{MUTCDKN1C} and BWS^{ICD2} subtypes than in the BWS^{ICD1} and BWS^{UPD} subtypes (BWS^{MUTCDKN1C} vs BWS^{ICD1} $P = 0.006$, BWS^{MUTCDKN1C} vs BWS^{UPD} $P = 0.001$, BWS^{ICD2} vs BWS^{ICD1} $P \leq 0.001$, BWS^{ICD2} vs BWS^{UPD} $P \leq 0.001$). In contrast, BWS^{UPD} and BWS^{ICD1} subtypes were more likely to have no defect or an umbilical hernia (see Figure 1a).

Birth weight and gestation data were used to calculate birth weight centiles and these were compared for the four molecular subgroups. BWS^{ICD1} were the heaviest (mean 99.8 ± 0.45 centile), followed by BWS^{UPD} (mean 90.7 ± 21.4 centile), BWS^{ICD2} (mean 85.6 ± 28.0 centile) and BWS^{MUTCDKN1C} (mean 82.9 ± 27 centile). The birth weight centile differences between the BWS^{ICD1} and BWS^{MUTCDKN1C} groups, and the BWS^{ICD1} and BWS^{ICD2} groups were statistically significant ($P = 0.018$ and 0.018 , respectively). All other comparisons were $P > 0.09$.

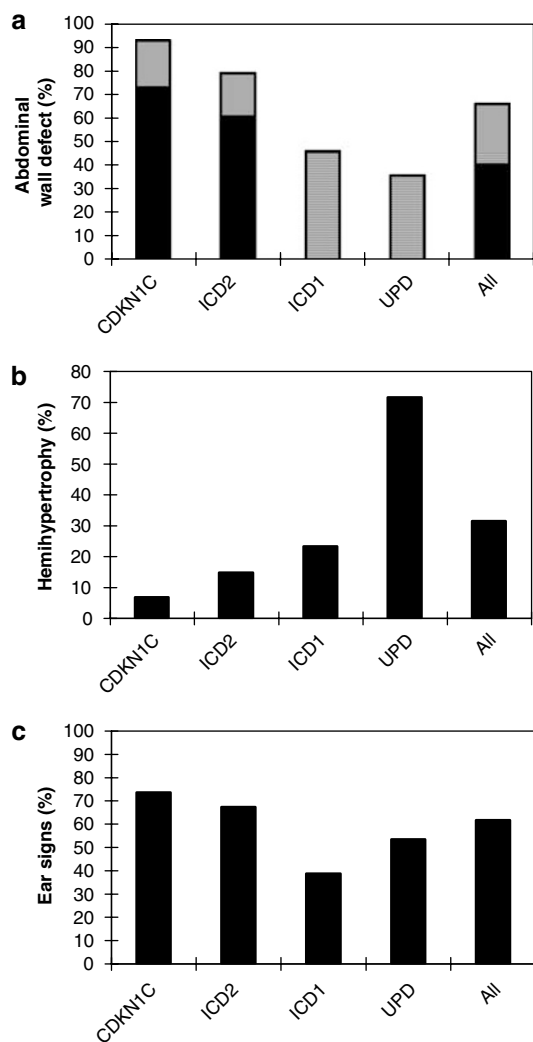


Figure 1 The frequencies of clinical features in BWS stratified by molecular pathology. The frequency of umbilical hernia and exomphalos (a), (the more severe defect, exomphalos, is depicted by a black bar whereas umbilical hernias are depicted by grey bars), hemihypertrophy (b) and ear creases or pits (c) was determined in individuals with BWS and a known molecular defect.

Hemihypertrophy was present in 31% of the patients analysed, but whereas the majority of the BWS^{UPD} group (71%) had hemihypertrophy, it was detected in only a minority of the other subtypes (Figure 1b): BWS^{MUTCDKN1C} = 7%, BWS^{ICD2} = 15%, BWS^{ICD1} = 23% (BWS^{UPD} vs BWS^{MUTCDKN1C} $P=0.06$, BWS^{UPD} vs BWS^{ICD2} $P<0.001$, BWS^{UPD} vs BWS^{ICD1} $P=0.001$).

The overall frequency of *ear creases and pits* was 61% (BWS^{MUTCDKN1C} = 73%, BWS^{ICD2} = 67%, BWS^{ICD1} = 38%, BWS^{UPD} = 53%), but these were significantly more common in BWS^{ICD2} cases than BWS^{ICD1} cases ($P=0.035$) (see Figure 1c). There were no significant differences between BWS subgroups for the frequencies of macroglossia (overall frequency 91%, BWS^{MUTCDKN1C} = 93%, BWS^{ICD2} = 92%, BWS^{ICD1} = 100%, BWS^{UPD} = 86%), *facial naevus flammeus* (overall frequency 43%, BWS^{MUTCDKN1C} = 33%, BWS^{ICD2} = 48%, BWS^{ICD1} = 31%, BWS^{UPD} = 38%) or *neonatal hypoglycaemia* (overall frequency 54%, BWS^{MUTCDKN1C} = 56%, BWS^{ICD2} = 51%, BWS^{ICD1} = 62%, BWS^{UPD} = 55%).

In all, 5% of patients in our cohort developed a neoplastic lesion (see Table 1) including one BWS^{UPD} patient who had both a hepatoblastoma and nephroblastoma (Wilms' tumour), this is similar to the frequency observed by Wiedemann in his study of nearly 400 patients.³² Neoplasia was more frequent in individuals with BWS^{ICD1} or BWS^{UPD} (13%) than in those with BWS^{MUTCDKN1C} or BWS^{ICD2} (1%) ($P=0.007$, Figure 2). In order to perform more accurate subgroup analysis, we combined our data with those reported by others.^{27–29,33} The results are depicted in Figure 2. Neoplasia was observed in all molecular subgroups, and the overall frequency of tumours was 11%; however, the frequencies in patients with BWS^{UPD} or BWS^{ICD1} were 22 and 28%, respectively, which is significantly greater than the 2.9% and 9.5% observed in patients with BWS^{ICD2} and BWS^{MUTCDKN1C} cases (BWS^{UPD} vs BWS^{ICD2} $P<0.0001$; BWS^{ICD1} vs BWS^{ICD2} $P<0.0001$; ICD1 vs BWS^{MUTCDKN1C} $P<0.05$; BWS^{UPD} vs BWS^{MUTCDKN1C} $P=0.06$). Wilms' tumours were not observed in BWS^{MUTCDKN1C} or BWS^{ICD2} subgroups; however,

Table 1 Clinical information on BWS patients in whom neoplasia was observed. BWS121 and BWS361 were initially diagnosed as Wilms' tumour (nephroblastoma)

Molecular subgroup	Age at diagnosis	Tumour type	Patient
BWS ^{MUTCDKN1C}	33 years	Mesoblastic nephroma	BWS070B
BWS ^{ICD1}	7 months	Nephroblastoma	R1
BWS ^{ICD1}	1 year	Nephroblastoma	R5
BWS ^{UPD}	1 year	Nephroblastoma	BWS430B
	2.5 years	Hepatoblastoma	
BWS ^{UPD}	1 month	Nephroblastomatosis	BWS121B
BWS ^{UPD}	2.2 years	Nephroblastoma	5724
BWS ^{UPD}	1 year	Hepatoblastoma	BWS283B
BWS ^{UPD}	4 years	Adrenal adenoma	BWS185B
BWS ^{UPD}	2 years	Nephroblastomatosis	BWS361B

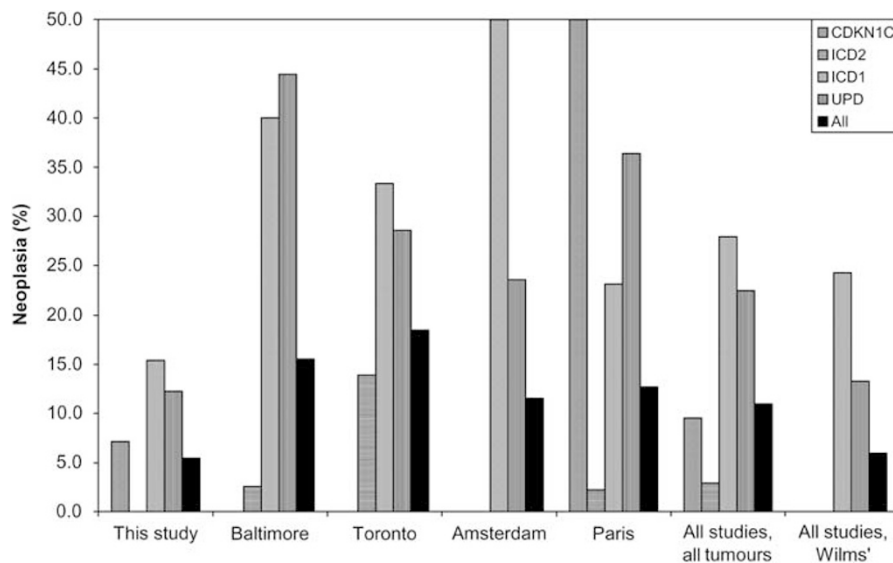


Figure 2 The frequencies of neoplasia observed in this study and four smaller studies. The frequency of neoplasia was determined in this study, and four smaller studies. Toronto indicates the study of 65 patients by Weksberg *et al*,²⁸ Baltimore refers to the study of 58 patients by DeBaun *et al*,²⁷ Amsterdam refers to the study of 52 patients by Blik *et al*,²⁹ and France refers to the study of 71 patients by Gaston *et al*.³³ 'All studies, Wilms'' refers to the data for Wilms' tumours (WT) taken from this study, the Canadian, Dutch and French studies. *CDKN1C* mutation analysis was not carried out on the American and Dutch cohorts. In total, 45 tumours were reported from 411 children with BWS (21 WT from 353 BWS children), 24 tumours from 107 children (13 WT from 98) with UPD, 12 tumours from 43 children (eight WT from 33) with imprinting centre 1 defects, seven tumours from 240 children (0 WT from 201) with imprinting centre 2 defect and two tumours from 21 children (0 WT from 21) with a germline *CDKN1C* mutation.

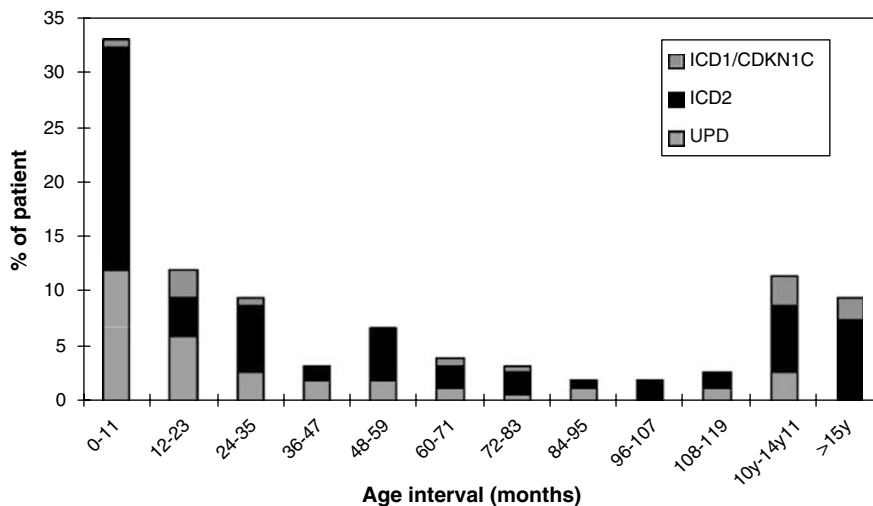


Figure 3 The age intervals of children at latest clinical follow-up or first tumour incidence. The proportion of the cohort falling into different age groups is indicated.

they are the most frequently reported tumour in BWS^{ICD1} and BWS^{UPD} subgroups (compare Figure 2; 'All studies, all tumours' with 'All studies, Wilms'). The risk of neoplasia in our series was lower than in some other series.^{27-29,33} This might reflect ascertainment bias in some series or differences in age distributions of various cohorts. In our series, most children were aged <8 years (see Figure 3). To

allow for this, we performed a Kaplan–Meier plot for all patients and the BWS^{ICD2} and BWS^{UPD} subgroups. The age-related risks of neoplasia in the three groups at 5 years were 9, 0 and 24%, respectively (see Figure 4).

In view of the evidence of imprinted transcripts at the Wilms' tumour suppresser gene, *WT1* locus, 11p13,³⁴⁻³⁷ we investigated whether the extent of segmental UPD

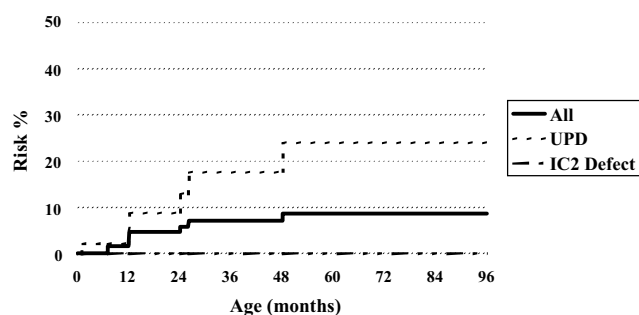


Figure 4 Kaplan–Meier plot of risk of neoplasia in all patients with BWS, and in BWS^{UPD} and BWS^{ICD2} subgroups. For details of neoplastic lesions see Table 1.

influenced the risk of renal neoplasia in BWS^{UPD} patients. Of 50 BWS^{UPD} cases analysed, disomy extended to *WT1* in 28 patients. Disomy at *WT1* was present in 7/8 patients with renal neoplasia (six with Wilms' tumour and two with severe nephroblastomatosis) and in 21/42 not known to have renal neoplasia ($P=0.054$). UPD included *WT1* in 5/6 patients with Wilms' tumour.

Discussion

We analysed genotype–phenotype correlations in a series of 200 BWS patients with defined molecular abnormalities. There are no generally agreed diagnostic criteria for BWS. By focusing on patients with a molecular genetic diagnosis of BWS, we were able to consider patients for inclusion irrespective of whether they satisfied 'strict' or 'loose' clinical diagnostic criteria (see <http://www.geneclinics.org>). A molecular diagnosis is possible in at least 70% of BWS children, so although in some cases a diagnosis of BWS has to be made on clinical findings, in most cases diagnosis is possible by a combination of clinical and molecular genetic findings. Some patients with molecular findings characteristic of BWS have an incomplete phenotype (eg we found two patients with hemihypertrophy and an IC2 defect in which there were no other features of BWS in one patient and only neonatal hypoglycaemia in the other), but in our series such cases were uncommon. Nevertheless, Martin *et al*³⁸ have reported five patients with hemihypertrophy and an IC2 defect with no or minimal additional features of BWS (eg single episode of hypoglycaemia, glabellar naevus flammeus). These findings suggest that some patients presenting primarily with hemihypertrophy form part of the BWS spectrum and further follow-up is required to see how the natural history and prognosis of these patients differs from other patients with hemihypertrophy and no clinical or molecular features of BWS.

We found that there were significant differences between molecular subtypes of BWS in tumour risk, severity of

anterior abdominal wall defect, birth weight, hemihypertrophy and ear signs of BWS. Differences in tumour risk are important for the clinical management of BWS. We have studied the largest cohort of BWS patients with defined molecular pathology yet analysed, and the overall frequency of neoplasia was 5%, which is lower than in other large studies from USA, Canada and Europe.^{27–29,33} These differences probably reflect differences in ascertainment procedures (eg we tried to avoid preferentially ascertaining patients with tumours and did not use cancer registries) but might also reflect differences in the ages of children in the different cohorts. Kaplan–Meier analysis allows age-related risks of neoplasia to be calculated and we found that the risk for all patients was 9% at age 5 years but was higher in BWS^{UPD} (24%) and lower in BWS^{ICD2} (0% subgroups). Overall, we found a significantly higher risk of tumours in BWS^{ICD1} and BWS^{UPD} cases than in those with *CDKN1C* mutations or IC2 defects. While this is consistent with previous studies,^{27–29,33} in most individual studies statistical significance was not reached. Therefore, to obtain the best estimates of tumour risks in different molecular subtypes of BWS, we combined our data with those of other centres. This confirmed that overall risk of tumour is highest in the BWS^{ICD1} and BWS^{UPD} groups and lowest in the BWS^{MUTCDKN1C} and BWS^{ICD2} groups. Although we did not identify any tumours in our BWS^{ICD2} group, Weksberg *et al*²⁸ observed a 16% tumour risk in such individuals, however, these were hepatoblastomas, rhabdomyosarcomas and a gonadoblastoma, and not Wilms' tumour, which is the most frequently occurring BWS associated tumour in Caucasian populations. Tumours also appear to be relatively uncommon in germline *CDKN1C* mutation carriers. We have identified a mesoblastic nephroma in one patient and two cases of neuroblastoma have been reported previously.^{17,33}

Although all children with BWS should be considered at increased risk of tumours compared to the general population, the value of routine tumour surveillance programmes is controversial.^{39,40} While many authorities have suggested that all children with BWS should be offered 3-monthly renal ultrasound scans to age 7 years, no effect of screening on survival from Wilms' tumour has been detected. Another controversial question is whether α -fetoprotein monitoring should be instigated for early detection of hepatoblastoma. Our findings suggest that, among children with a molecular diagnosis of BWS, screening for Wilms' tumour should be targeted to those at greatest risk, the BWS^{UPD} and BWS^{ICD1} subgroups. In contrast, screening for hepatoblastoma would be of most benefit in BWS^{ICD2} cases and intensive screening for Wilms' tumour is not indicated for this subgroup. The high risk of Wilms' tumours in the BWS^{ICD1} and BWS^{UPD} groups compared to the BWS^{MUTCDKN1C} group suggests that increased IGF2 or decreased H19 expression (rather than *CDKN1C* inactivation) has a key role in the patho-

genesis of these tumours. Accordingly, LOI of IGF2 and H19 hypermethylation and repression are frequently observed in sporadic Wilms' tumours.^{41,42} LOI of IGF2 may also occur in patients with IC2 defects, but there is no information as to whether tumour risk in BWS^{ICD2} cases correlates with IGF2 imprinting status. As only a subset of BWS^{UPD} patients develop tumours, we investigated whether the extent of UPD, in particular, whether disomy extended to *WT1*, influenced the risk of neoplasia. Intriguingly the frequency of Wilms' tumour and severe nephroblastomatosis was higher in those with *WT1* disomy, although it did not reach statistical significance. While *WT1* is not thought to be imprinted, it has been suggested that an alternative *WT1* transcript (*AWT1*) and the antisense *WT1* transcript (*WT1-AS* which overlaps the 5'-end of *WT1*) may be imprinted.^{35,36,43} In foetal tissues, transcription of *WT1-AS* occurs from both alleles; however, in adult tissues, the maternal allele has been silenced by methylation, whereas in Wilms' tumours the foetal state is retained.³⁶ Paternal UPD for 11p13 would also have the effect of maintaining the chromosome in the foetal state associated with biallelic expression of *WT1-AS*. *WT1* protein levels are high in foetal kidney and Wilms' tumours, but low in adult kidney and it has been shown by Moorwood *et al*³⁵ that *WT1-AS* transcription can elevate *WT1* protein levels *in vitro*. This may imply that individuals who have UPD extending beyond 11p13 and whose mosaicism encompasses the cells of the kidney are those at highest risk of developing Wilms' tumour.

We confirmed our earlier finding that exomphalos is much more frequent in BWS^{MUTCDKN1C} and BWS^{ICD2} cases than BWS^{ICD1} or BWS^{UPD} children, and in mouse models of BWS, mice overexpressing *Igf2* exhibit overgrowth without exomphalos,⁴⁴ whereas exomphalos in the absence of organ overgrowth is observed in the *Cdkn1c* knockout mouse.^{45,46} The phenotypic similarity between patients with *CDKN1C* mutations and those with BWS^{ICD2} (ie loss of methylation (LOM) of KvDMR1) suggested that the unmethylated DMR may act to silence *CDKN1C* expression, and consistent with this, patients with KvDMR1 LOM do indeed show reduced fibroblastic *CDKN1C* expression in culture.²⁵ Our novel observation that mean birth weight centile was higher in the BWS^{ICD1} group than in the BWS^{MUTCDKN1C} and BWS^{ICD2} groups is also consistent with the results of mouse models of BWS (see above) and suggests that although *IGF2* and *CDKN1C* may regulate a common growth control pathway, there are subtle differences in the phenotypic consequences of *IGF2* overexpression and *CDKN1C* inactivation/downregulation. Although not all cases of BWS will have a detectable molecular abnormality, molecular diagnosis is possible in most cases. As the results of molecular analysis influence both genetic counselling and surveillance of BWS children, optimum medical management should include molecular genetic analysis.

Acknowledgements

We thank the many referring clinicians and the patients and their families for their help with this study. We thank SPARKS and the MRC for financial support.

References

- 1 Elliott M, Maher ER: Beckwith–Wiedemann syndrome. *J Med Genet* 1994; **31**: 560–564.
- 2 Martinez RMY: Clinical features in the Wiedemann–Beckwith syndrome. *Clin Genet* 1996; **50**: 272–274.
- 3 Maher ER, Reik W: Beckwith–Wiedemann syndrome: imprinting in clusters revisited. *J Clin Invest* 2000; **105**: 247–252.
- 4 Turleau C, Degrouchy J, Chavincolin F, Martelli H, Voyer M, Charlas R: Trisomy 11p15 and Beckwith–Wiedemann syndrome – a report of 2 cases. *Hum Genet* 1984; **67**: 219–221.
- 5 Brown KW, Gardner A, Williams JC, Mott MG, McDermott A, Maitland NJ: Paternal origin of 11p15 duplications in the Beckwith–Wiedemann syndrome – a new case and a review of the literature. *Cancer Genet Cytogenet* 1992; **58**: 66–70.
- 6 Nystrom A, Cheetham JE, Engstrom W, Schofield PN: Molecular analysis of patients with Wiedemann–Beckwith syndrome 2 paternally derived disomies of chromosome-11. *Eur J Pediatr* 1992; **151**: 511–514.
- 7 Slavotinek A, Gaunt L, Donnai D: Paternally inherited duplications of 11p15.5 and Beckwith–Wiedemann syndrome. *J Med Genet* 1997; **34**: 819–826.
- 8 Norman A, Read A, Clayton-Smith J, Andrews T, Donnai D: Recurrent Wiedemann–Beckwith syndrome with inversion of chromosome (11)(p11.2p15.5). *Am J Med Genet* 1992; **42**: 638–641.
- 9 Tommerup N, Brandt C, Pedersen S, Bolund L, Kamper J: Sex-dependent transmission of Beckwith–Wiedemann syndrome – associated with a reciprocal translocation t(9,11)(p11.2,p15.5). *J Med Genet* 1993; **30**: 958–961.
- 10 Fisher AM, Thomas NS, Cockwell A *et al*: Duplication of chromosome 11p15 of maternal origin result in a phenotype that includes growth retardation. *Hum Genet* 2002; **111**: 290–296.
- 11 Slatter RE, Elliott M, Welham K *et al*: Mosaic uniparental disomy in Beckwith–Wiedemann syndrome. *J Med Genet* 1994; **31**: 749–753.
- 12 Henry I, Bonaitipellie C, Chehensse V *et al*: Uniparental paternal disomy in a genetic cancer-predisposing syndrome. *Nature* 1991; **351**: 665–667.
- 13 Dutley F, Baumer A, Kayserili H *et al*: Seven cases of Wiedemann–Beckwith syndrome including the first reported case of mosaic paternal isodisomy along the whole of chromosome 11. *Am J Med Genet* 1998; **79**: 347–353.
- 14 Catchpoole D, Lam WWK, Valler D *et al*: Epigenetic modification and uniparental inheritance of H19 in Beckwith–Wiedemann syndrome. *J Med Genet* 1997; **34**: 353–359.
- 15 Hatada I, Ohashi H, Fukushima Y *et al*: An imprinted gene p57KIP2 is mutated in Beckwith–Wiedemann syndrome. *Nat Genet* 1996; **14**: 171–173.
- 16 Lam WWK, Hatada I, Ohishi S *et al*: Analysis of germline *CDKN1C* (p57KIP2) mutations in familial and sporadic Beckwith–Wiedemann syndrome (BWS) provides a novel genotype–phenotype correlation. *J Med Genet* 1999; **36**: 518–523.
- 17 Lee M, Debaun M, Randhawa G, Reichard B, Elledge S, Feinberg A: Low frequency of p57KIP2 mutation in Beckwith–Wiedemann syndrome. *Am J Hum Genet* 1997; **61**: 304–309.
- 18 Hark A, Schoenherr C, Katz D, Ingram R, Levorse J, Tilghman S: CTCF mediates methylation-sensitive enhancer-blocking activity at the H19/Igf2 locus. *Nature* 2000; **405**: 486–489.
- 19 Bell A, Felsenfeld G: Methylation of a CTCF-dependent boundary controls imprinted expression of the *Igf2* gene. *Nature* 2000; **405**: 482–485.

- 20 Nakagawa H, Chadwick RB, Peltomaki P, Plass C, Nakamura Y, de la Chapelle A: Loss of imprinting of the insulin-like growth factor II gene occurs by biallelic methylation in a core region of H19-associated CTCF-binding sites in colorectal cancer. *Proc Natl Acad Sci USA* 2001; **98**: 591–596.
- 21 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 Beckwith–Wiedemann syndrome. *Nat Genet* 2004; **36**: 958–960.
- 22 Smilnich NJ, Day CD, Fitzpatrick GV *et al*: A maternally methylated CpG island in *KCNQ1* is associated with an antisense paternal transcript and loss of imprinting in Beckwith–Wiedemann syndrome. *Proc Natl Acad Sci USA* 1999; **96**: 8064–8069.
- 23 Lee M, DeBaun M, Mitsuya K *et al*: Loss of imprinting of a paternally expressed transcript, with antisense orientation to *K(V)LQT1*, occurs frequently in Beckwith–Wiedemann syndrome and is independent of insulin-like growth factor II imprinting. *Proc Natl Acad Sci USA* 1999; **96**: 5203–5208.
- 24 Engel JR, Smallwood A, Harper A *et al*: Epigenotype–phenotype correlations in Beckwith–Wiedemann syndrome. *J Med Genet* 2000; **37**: 921–926.
- 25 Diaz-Meyer N, Day C, Khatod K *et al*: Silencing of *CDKN1C* (*p57*(*KIP2*)) is associated with hypomethylation at *KvDMR1* in Beckwith–Wiedemann syndrome. *J Med Genet* 2003; **40**: 797–801.
- 26 Niemitz EL, DeBaun MR, Fallon J *et al*: Microdeletion of *LIT1* in familial Beckwith–Wiedemann syndrome. *Am J Hum Genet* 2004; **75**: 844–849.
- 27 DeBaun MR, Niemitz EL, McNeil DE, Brandenberg SA, Lee MP, Feinberg AP: Epigenetic alterations of *H19* and *LIT1* distinguish patients with Beckwith–Wiedemann syndrome with cancer and birth defects. *Am J Hum Genet* 2002; **70**: 604–611.
- 28 Weksberg R, Nishikawa J, Caluseriu O *et al*: Tumor development in the Beckwith–Wiedemann syndrome is associated with a variety of constitutional molecular 11p15 alterations including imprinting defects at *KCNQ1OT1*. *Hum Mol Genet* 2001; **10**: 2989–3000.
- 29 Blik J, Maas SM, Ruijter JM *et al*: Increased tumour risk for BWS patients correlates with aberrant *H19* and not *KCNQ1OT1* methylation: occurrence of *KCNQ1OT1* hypomethylation in familial cases of BWS. *Hum Mol Genet* 2001; **10**: 467–476.
- 30 Reik W, Brown KW, Slatter RE, Sartori P, Elliott M, Maher ER: Allelic methylation of *H19* and *IGF2* in the Beckwith–Wiedemann syndrome. *Hum Mol Genet* 1994; **3**: 1297–1301.
- 31 Agathangelou A, Honorio S, Macartney D *et al*: Methylation associated inactivation of *RASSF1A* from region 3p21.3 in lung, breast and ovarian tumours. *Oncogene* 2001; **20**: 1509–1518.
- 32 Wiedemann HR: Tumors and hemihypertrophy associated with Wiedemann–Beckwith syndrome. *Eur J Pediatr* 1983; **141**: 129.
- 33 Gaston V, Le Bouc Y, Soupre V *et al*: Analysis of the methylation status of the *KCNQ1OT1* and *H19* genes in leukocyte DNA for the diagnosis and prognosis of Beckwith–Wiedemann syndrome. *Eur J Hum Genet* 2001; **9**: 409–418.
- 34 Ward A, Dutton JR: Regulation of the Wilms' tumour suppressor (*WT1*) gene by an antisense RNA: a link with genomic imprinting? *J Pathol* 1998; **185**: 342–344.
- 35 Moorwood K, Charles AK, Salpekar A, Wallace JI, Brown KW, Malik K: Antisense *WT1* transcription parallels sense mRNA and protein expression in foetal kidney and can elevate protein levels *in vitro*. *J Pathol* 1998; **185**: 352–359.
- 36 Malik K, Salpekar A, Hancock A *et al*: Identification of differential methylation of the *WT1* antisense regulatory region and relaxation of imprinting in Wilms' tumor. *Cancer Res* 2000; **60**: 2356–2360.
- 37 Malik KT, Wallace JI, Ivins SM, Brown KW: Identification of an antisense *WT1* promoter in intron 1: implications for *WT1* gene regulation. *Oncogene* 1995; **11**: 1589–1595.
- 38 Martin RA, Grange DK, Zehnbauser B, Debaun MR: *LIT1* and *H19* methylation defects in isolated hemihyperplasia. *Am J Med Genet* 2005; **134**: 129–131.
- 39 Craft AW, Parker L, Stiller C, Cole M: Screening for Wilms-tumor in patients with aniridia, Beckwith–Syndrome, or hemihyper trophy. *Med Pediatr Oncol* 1995; **24**: 231–234.
- 40 Choyke PL, Siegel MJ, Craft AW, Green DM, DeBaun MR: Screening for Wilms tumor in children with Beckwith–Wiedemann syndrome or idiopathic hemihypertrophy. *Med Pediatr Oncol* 1999; **32**: 196–200.
- 41 Moulton T, Crenshaw T, Hao Y *et al*: Epigenetic lesions at the *H19* locus in Wilms-tumor patients. *Nat Genet* 1994; **7**: 440–447.
- 42 Ogawa O, Eccles MR, Szeto J *et al*: Relaxation of insulin-like growth factor II gene imprinting implicated in Wilms' tumour. *Nature* 1993; **362**: 749–751.
- 43 Dallosso AR, Hancock AL, Brown KW, Williams AC, Jackson S, Malik K: Genomic imprinting at the *WT1* gene involves a novel coding transcript (*AWT1*) that shows deregulation in Wilms' tumours. *Hum Mol Genet* 2004; **13**: 405–415.
- 44 Sun F-L, Dean WL, Kelsey G, Allen ND, Reik W: Transactivation of *Igf2* in a mouse model of Beckwith–Wiedemann syndrome. *Nature* 1997; **389**: 809–815.
- 45 Yan Y, Lee M, Massague J, Barbacid M: Ablation of the CDK inhibitor *p57*(*Kip2*) results in increased apoptosis and delayed differentiation during mouse development. *Genes Dev* 1997; **11**: 973–983.
- 46 Zhang P, Liégeois NJ, Wong C *et al*: Altered cell differentiation and proliferation in mice lacking *p57*(*KIP2*) indicates a role in Beckwith–Wiedemann syndrome. *Nature* 1997; **387**: 151–158.