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Molecular subtypes and phenotypic expression of Beckwith–Wiedemann syndrome

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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 *KCNQ1OT1 (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

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 suppresser genes *CDKN1C* (, 57^{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^{DUPL11} patients. Nevertheless, *CDKN1C* was unequivocally implicated in the pathogenesis of BWS by the finding of germline *CDKN1C* mutations in a subset of patients (BWS^{MUT}

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

H[illegible] was present in 31% of the patients

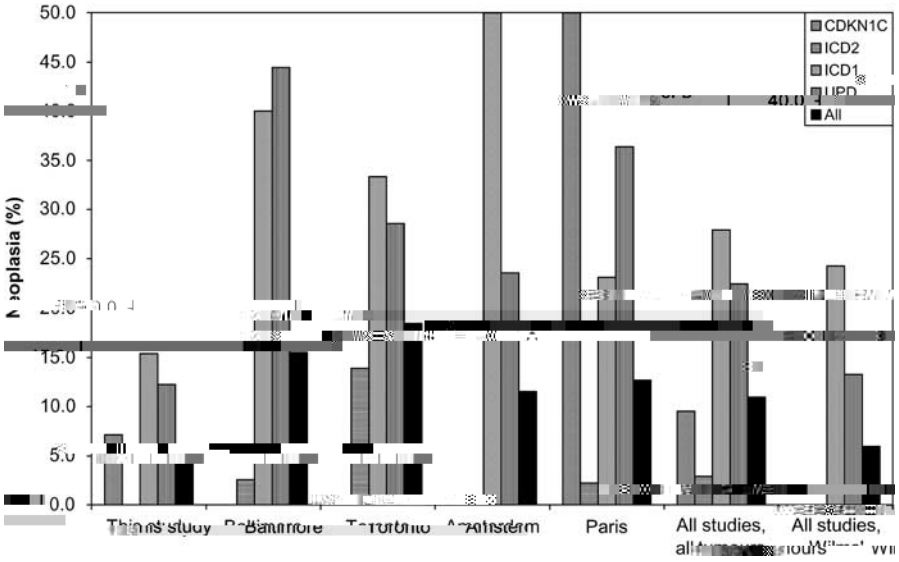

Figure 2 The frequencies of neoplasia observed in this study and four smaller studies. The frequency of neoplasia was determined in this study, and four smaller studies. Toronto indicates the study of 65 patients by Weksberg [28] Baltimore refers to the study of 58 patients by DeBaun [27] Amsterdam refers to the study of 52 patients by Bliek [29] and France refers to the study of 71 patients by Gaston [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

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

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

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 [illegible]. 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 $BWS^{MUT\textit{CDKN1C}}$ 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 *C[illegible]1* 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 $BWS^{MUT\textit{CDKN1C}}$ 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* over expression 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.

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