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LETTER TO JMG

Beckwith-Wiedemann syndrome and assisted reproduction technology (ART)

E R Maher, L A Brueton, S C Bowdin, A Luharia, W Cooper, T R Cole, F Macdonald, J R Sampson, C L Barratt, W Reik, M M Hawkins

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Beckwith-Wiedemann syndrome (BWS) is a model imprinting disorder resulting from mutations or epimutations affecting imprinted genes on chromosome 11p15.5.¹ The classical clinical features of BWS are macroglossia, pre- and/or postnatal overgrowth, and anterior abdominal wall defects (umbilical hernia or exomphalos). Additional more variable features include hemihypertrophy, neonatal hypoglycaemia, facial naevus flammeus, ear pits and creases, renal anomalies, and an increased risk of embryonal tumours.² Most cases of BWS are sporadic and ~20% of these have uniparental disomy (paternal isodisomy) for a variable region of chromosome 11 which always includes the 11p15.5 imprinted gene cluster.³⁻⁵ Up to 60% of sporadic BWS patients have epigenetic changes at differentially methylated regions within 11p15.5 that are associated with alterations in the imprinting or expression of paternally expressed genes, such as *IGF2* and *KCNQ1OT*, or maternally expressed genes, such as *H19* and *CDKN1C*.¹ Thus, 5-10% have epigenetic alterations at the *IGF2/H19* loci (the maternal *H19* and *IGF2* alleles display paternal allele methylation and expression patterns with biallelic *IGF2* expression and silencing of *H19* expression),⁶ and 40-50% have loss of maternal allele methylation at a differentially methylated region (KvDMR1) within an intron of *KCNQ1*. KvDMR1 loss of methylation is associated with biallelic expression of *KCNQ1OT*.⁷⁻⁹ The epigenetic alterations at *H19/IGF2* or KvDMR1 are thought to result from defects at two putative imprinting control centres (BWSIC1 and BWSIC2, respectively).¹ The precise nature of the putative BWSIC2 is unknown and therefore the origin of these putative BWSIC2 defects is unknown. Weksberg *et al*¹⁰ showed a clear association between monozygotic twinning and BWS with KvDMR1 loss of methylation and suggested two possible explanations: (1) that discordance for BWS in monozygotic twins is caused by unequal splitting of the inner cell mass during twinning resulting in differential maintenance of imprinting at KvDMR1, or (2) that loss of imprinting associated with KvDMR1 demethylation predisposes to twinning as well as to discordance for BWS.

Recently, a possible association between another human imprinting disorder, Angelman syndrome, and intracytoplasmic sperm injection (ICSI) was reported.¹¹ Angelman syndrome occurs in ~1 in 15 000 newborns and most cases have a deletion of 15q.¹² Although only a minority of cases (<5%) of Angelman syndrome are caused by an imprinting defect,^{13 14} both of the two cases associated with ICSI that were described by Cox *et al*¹¹ had an imprinting defect. This led to the suggestion that ICSI may be associated with an increased susceptibility to imprinting errors. To investigate this hypothesis further, we have determined whether there is evidence of an association between BWS and a history of assisted conception techniques.

METHODS AND RESULTS

We reviewed the notes of 149 BWS patients who had been referred to the BWS Research Group at the Birmingham Uni-

versity Section of Medical Genetics and/or the West Midlands Molecular Genetics Service (for uniparental disomy analysis) and for whom detailed clinical information had been collected. A history of assisted conception techniques was recorded for six cases (4%) (table 1). To estimate whether this was likely to be a significantly increased proportion, we compared the frequency of in vitro fertilisation (IVF) and ICSI births in the BWS cohort with that in the general population. The first ART associated BWS case was born in 1989 and the most recent in 2002. Data for the number of children born after ART are available for 1995, 1996, 1997, 1998, 1999, and 2000 (www.hfea.gov.uk) and during these years there was a total of 43 074 births after IVF or ICSI to UK residents. The corresponding number of total births in the UK was 4 320 482, so that 0.997% of births in the general population were after IVF or ICSI. Based on these data, if the proportion of births after IVF and ICSI in BWS patients and in the general population were similar, we would have expected 1.7252 of the 149 BWS patients studied to have been born as a result of IVF or ICSI. To test the significance of the observed and expected frequencies we used a Poisson approximation to the binomial distribution and obtained a two tailed p value of 0.018. Thus, the observed frequency (n=6) of IVF and ICSI births in the BWS series is significantly greater than the expected (1.7252), with an associated 95% confidence interval on the excess risk

Key points

- Beckwith-Wiedemann syndrome (BWS) is a model imprinting disorder resulting from mutations or epimutations affecting imprinted genes on chromosome 11p15.5.
- Recently a possible association of Angelman syndrome, another human imprinting disorder, and intracytoplasmic sperm injection (ICSI) has been described.
- To determine if there might be an association between ICSI and BWS births, we reviewed the incidence of assisted reproduction technology (ART) births in a cohort of 149 sporadic BWS births.
- Six of 149 (4%) BWS children were born after ART (three after ICSI and three after in vitro fertilisation (IVF)) compared to ~1.2% in the general population (p=0.009).
- These observations support an association between ART and human imprinting disorders.
- As both IVF and ICSI procedures were associated with BWS, loss of maternal allelic methylation at differentially methylated regions within imprinted gene clusters associated with in vitro embryo culture may be an important factor in the pathogenesis of ART associated imprinting disorders.

Table 1 Clinical features of six patients diagnosed with Beckwith-Wiedemann syndrome after ICSI and IVF therapy

Patient ID	ART history	Clinical features	Genetic investigations
1	Twin 1 of dichorionic, diamniotic male twins conceived by ICSI (twin 2 was unaffected)	Macroglossia, umbilical hernia, ear pits, facial naevus flammeus, neonatal hypoglycaemia	Normal chromosome analysis, UPD excluded, hypomethylation KvDMR1
2	Male singleton pregnancy conceived by ICSI	Macroglossia, umbilical hernia, unilateral inguinal hernia, birth weight 3515 g, postnatal weight >97th centile, naevus flammeus, single posterior helical pit	Normal male karyotype, molecular genetic investigations not yet performed
3	Singleton male infant conceived by ICSI	Macroglossia, macrosomia (birth weight 4030 g, 137 cm cord), facial naevus flammeus	Normal male karyotype, UPD excluded, hypomethylation KvDMR1
4	IVF conception, non-identical twin pregnancy (twin unaffected)	Macroglossia, macrosomia, hepatomegaly, ear lobe creases, facial naevus flammeus	Normal male karyotype, UPD excluded
5	IVF conception, single pregnancy	Exomphalos, adrenal cytomegaly, nephromegaly. Died of prematurity related complications (born at 27 weeks' conception)	Normal chromosome analysis, UPD excluded
6	IVF conception, twin 2 of monozygotic female twins. Twin 1 unaffected	Macroglossia, diastasis recti, neonatal hypoglycaemia, helical ear pits, hemihypertrophy, facial naevus flammeus	Normal cytogenetic analysis, no molecular genetic results available

of 1.5, 8.8. It should be noted that (1) although these calculations do not take account of maternal age, there is no reported evidence that maternal age in BWS births differs from that in the general population and (2) data on the frequency of IVF and ICSI births are not available for years before 1995. Had such data been available, then this would have very probably reduced the expected number of BWS births after IVF or ICSI, as the birth rate in the general population has been declining since 1989 and the annual number of ART births before 1995 would have been less than during the five years included in the comparison. Therefore our comparison is likely to be conservative in relation to calendar year.

Of the six BWS cases, three occurred after ICSI treatment and three after IVF. Molecular genetic studies for uniparental disomy were performed in four cases and were negative in each case. Two cases were assessed for KvDMR1 methylation status and both cases showed loss of methylation on the maternal allele. One of the IVF associated cases (case 6) occurred in a discordant pair of monozygotic twins, which is of interest in the light of the report of Weksberg *et al.*,¹⁰ who showed an association between monozygotic twinning in BWS and KvDMR1 loss of methylation. The KvDMR1 methylation status of case 6 is not known (DNA was not available), but it is interesting to note that Weksberg *et al.*¹⁰ speculated that KvDMR1 is vulnerable to demethylation at a critical stage of preimplantation development and that this loss of imprinting predisposes to twinning and discordance for BWS.

DISCUSSION

We have described an apparent increased frequency in children born with the aid of assisted reproductive technology (ART) in patients with the BWS imprinting disorder. Furthermore, as a detailed reproductive history had not been specifically requested, we may have overlooked additional cases. Our findings are compatible with those of Cox *et al.*,¹¹ who reported an apparently increased risk of Angelman syndrome after ICSI. We note that in both the ART associated BWS cases in which a molecular alteration was identified, we detected loss of maternal allele methylation at KvDMR1. This is consistent with the loss of maternal allele methylation at *SNRPN* in ART associated Angelman syndrome cases. However, whereas such imprinting defects are uncommon in Angelman syndrome, KvDMR1 loss of methylation is the most common molecular abnormality in sporadic BWS (~40-50% of cases).⁷⁻⁹ We are not aware of any other surveys of the incidence of ART among a series of BWS cases, but we note that in two reports of children born after ART the frequency of BWS was 1 in 73 and 1 in 91 children, respectively.^{15, 16} As the (minimal) incidence of BWS has been estimated recently at 0.13 per 10 000 liveborn

infants,¹⁷ these findings would further support our hypothesis of a causal link between ART and disordered genomic imprinting.

Although Cox *et al.*¹¹ linked Angelman syndrome with ICSI, in our series the association of ART with BWS was not limited to ICSI. The occurrence of BWS after in vitro fertilisation without ICSI suggests that a common feature of the ICSI and IVF procedures might predispose to abnormal imprinting. We note that in animals in vitro culture of embryos and ES cells can affect DNA methylation and imprinting¹⁸⁻²⁰ and in vitro culture of sheep pre-embryos may be associated with fetal overgrowth and hypomethylation of a differentially methylated region of the maternal *Igf2r* allele.²¹ This raises the possibility that in vitro embryonal cell culture per se might predispose to maternal allele demethylation and imprinting errors. Further studies are required to determine the precise relationship between human imprinting disorders and ART, but with the trends towards increasing use of ICSI and for extending in vitro culture times in ART,²² it will be increasingly important to address these questions in large scale studies of children born after ART.

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