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

beckwith-Wiedemann syndrome (BWS) is a model imprinting disorder resulting from mutations or epimutations affecting imprinted genes on chromosome 11p15.5. The classical clinical features of BWS are macrosomia, pre- and/or postnatal overgrowth, and anterior abdominal wall defects (umbilical hernia or exomphalos). Additional more variable features include hemihypertrophy, neonatal hypoglycaemia, facial naeve flammae, ear pits and creases, renal anomalies, and an increased risk of embryonal tumours. 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. 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 KCNQ1OT1, or maternally expressed genes, such as H19 and CDKN1C. 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), 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 KCNQ1OT1. The epigenetic alterations at H19/IGF2 or KvDMR1 are thought to result from defects at two putative imprinting control centres (BWS1C1 and BWS1C2, respectively). The precise nature of the putative BWS1C2 is unknown and therefore the origin of these putative BWS1C2 defects is unknown. Weihsberg et al. showed a clear association between monzygotic 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. Angelman syndrome occurs in ∼1 in 15 000 newborns and most cases have a deletion of 15q. Although only a minority of cases (<5%) of Angelman syndrome are caused by an imprinting defect, both of the two cases associated with ICSI that were described by Cox et al. 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 University 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 of births after IVF and ICSI. 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.

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.
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., 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. 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).17 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 17 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 dispersed 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.

**ACKNOWLEDGEMENTS**

We thank SPARKS for financial support to WC and EM and Cancer Research UK to WR.

**Authors’ affiliations**

E R Maher, L A Bruton, W Cooper, T R Cole, F Macdonald, Section of Medical and Molecular Genetics, University of Birmingham, Birmingham B15 2TT, UK

E R Maher, L A Bruton, S C Bowdin, A Luhario, T R Cole, F Macdonald, West Midlands Genetics Service, Birmingham Women’s Hospital, Edgbaston, Birmingham B15 2TG, UK

J R Sampson, Institute of Medical Genetics for Wales, University of Wales College of Medicine, Cardiff CF14 4XN, UK

C L Barratt, Reproductive Biology and Genetics Group, University of Birmingham, The Medical School, Birmingham B15 2TT, UK

W Reik, Laboratory of Developmental Genetics and Imprinting, The Babraham Institute, Babraham Hall, Babraham, Cambridge CB2 4AT, UK

M M Hawkins, Centre for Childhood Cancer Survivor Studies, Department of Public Health & Epidemiology, The University of Birmingham, Edgbaston, Birmingham B15 2TT, UK

Correspondence to: Professor E R Maher, Section of Medical and Molecular Genetics, University of Birmingham, Birmingham B15 2TT, UK; e.r.maher@bham.ac.uk

**REFERENCES**


Want to extend your search?

Cross journal searching

If you can't find what you are looking for in the Journal of Medical Genetics you can extend your search across many of the more than 200 journals available for selection. You can restrict your search to specific subject areas (eg, clinical medicine, basic research), or select specific journals, or search all available titles.

www.jmedgenet.com
In the May 2002 issue of the journal, in the paper by Evans et al on “Malignant peripheral nerve sheath tumours in neurofibromatosis 1”, the Kaplan Meier curve published in the article was an analysis from birth to current age or death rather than from diagnosis. The p value attached to the curve related to the analysis from diagnosis. The correct figure of survival from diagnosis is shown below.

In the letter by Maher et al on “Beckwith-Wiedemann syndrome and assisted reproduction technology (ART)” in the January 2003 issue of the journal (vol 40, pp 62-64), there were four errors in the first paragraph of the Methods and result section and in the Key points. The correct paragraphs are reproduced below with the errors noted in bold.

**METHODS AND RESULTS**

We reviewed the notes of 149 BWS patients who had been referred to the BWS Research Group at the Birmingham University 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 (“http://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 \( \frac{149}{0.997} \) 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 0.009. Thus, the observed frequency (n=6) of IVF and ICSI births in the BWS series is significantly greater than the expected (1.4855), with an associated 95% confidence interval on the excess risk 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.

**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 \( \sim 1.0\% \) 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.