Wiedemann-Beckwith syndrome: Epigenetics, Imprinting and growth

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Wiedemann-Beckwith syndrome

11p15.5 imprinted gene cluster and human disease

Importance of humans as model organism for imprinting and epigenetics

Beckwith-Wiedemann syndrome (BWS): mechanisms of disease

Genotype-phenotype correlations in BWS

Genetic and environmental causes of epimutations in human imprinting disorders (BWS)
Mouse Imprinted Genes, Regions and Phenotypes
Beckwith-Wiedemann syndrome (BWS)

- Mostly sporadic
- pre- and/or postnatal overgrowth, macroglossia and anterior abdominal wall defects
- additional variable features (hypoglycaemia, ear creases, FNF, organomegaly, hemihypertrophy)
- ~5% of cases embryonal tumours
- 11p15.5 epigenetic and genetic abnormalities
Natural history

Prenatal and perinatal
  Up to 50% incidence of polyhydramnios, premature birth, and foetal macrosomia

Neontal morbidity and mortality
  Complications of prematurity associated with exomphalos, macroglossia or neonatal hypoglycemia

Growth
  Usually rapid growth in early childhood but rate usually slows at 7-8 years

Hemihypertrophy (hemihyperplasia)
  Approx 25% of cases

Hypoglycemia
  Neonatal hypoglycaemia usually mild and transient but occasionally is severe and prolonged
Natural history

**Congenital anomalies**
- Anterior abdominal wall defects
- Renal anomalies e.g. medullary dysplasia, duplicated collecting system, nephrocalcinosis, medullary sponge kidney, cystic changes

**Neoplasia**
- Most frequently Wilms tumour and hepatoblastoma, but also neuroblastoma, adrenocortical carcinoma, and rhabdomyosarcoma
- Approx 5-10% of cases

**Neurodevelopment**
- Usually normal unless cytogenetic duplication or H/O untreated hypoglycaemia

**Adults**
- No specific complications
- About 15% of cases familial
- Features of BWS less apparent with age
Beckwith-Wiedemann syndrome

**Major**
- Anterior abdominal wall defect
- Macroglossia
- Pre- or postnatal overgrowth (>90th centile)

**Minor**
- Ear creases and ear pits
- Facial naevus flammeus
- Neonatal hypoglycaemia
- Nephromegaly
- Hemihypertrophy
- Embryonal tumours

**Diagnosis:**
**Strict:** 3 major features or 2 major plus 3 minor
**Moderate:** 2 major plus 1 minor
Beckwith-Wiedemann syndrome

- **Major**
  - Anterior abdominal wall defect
  - Macroglossia
  - Pre- or postnatal overgrowth (>90th centile)
  - Molecular abnormality

- **Minor**
  - Ear creases and ear pits
  - Facial naevus flammeus
  - Neonatal hypoglycaemia
  - Nephromegaly
  - Hemihypertrophy
  - Embryonal tumours

**Diagnosis:**
Molecular abnormality + one major or two minor
Imprinting region 11p15
BWS Candidate Genes

Two imprinting centres/DMRs:

IC1: distal between IGF2 and H19

IC2: centromeric within KCNQ1 (KvDMR1)

Expressed
Silenced
11p15.5 duplications
Uniparental disomy
IC1 epimutations
CDKN1C mutation
IC2 epimutations
IC1 CNVs
IC2 deletions

\[ \text{IGF2} \]
\[ \text{CDKN1C} \]

\[ \text{Growth} \]
<table>
<thead>
<tr>
<th>IGF2</th>
<th>CDKN1C (p57KIP2)</th>
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<tbody>
<tr>
<td>1. <em>Paternally</em> expressed growth promoter</td>
<td><em>Maternally</em> expressed growth <em>suppressor</em> cyclin-dependent kinase inhibitor</td>
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<td>2. Correlation between the tissue type affected in BWS and the most abundant sites of IGF2 expression</td>
<td>(related to p27\textsuperscript{KIP1} p21\textsuperscript{CIP1} )</td>
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<td>3. In mice IGF2 overexpression results in overgrowth and loss of paternal expression to growth retardation</td>
<td>Germline CDKN1C mutations in BWS</td>
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<td>4. IGF2 imprinting reciprocally linked to H19 imprinting</td>
<td>~50% of familial cases</td>
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<td>~5% sporadic cases</td>
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<td><em>CDKN1C inactivation in mice = anterior abdominal wall defects</em></td>
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Parent of origin of CDKN1C mutations
Imprinting Centres

- Key elements involved in *in cis* control of imprinted gene clusters
- Imprinting centres coincide with germ line differential methylation marks (DMR)
- Imprinting centre inactivation by deletion or epimutation results in altered epigenotype *in cis* and disrupts imprinting
H19-IGF2 imprinting Model

Pat
IGF2  
CH3

Mat
CTCF

Insulator  

H19  
Enhancer

BWS ~5%
1% CNV
Silver Russell Syndrome

- Pre and/or postnatal growth restriction
- Facial phenotype
- Asymmetry

- Maternal UPD chromosome 7 ~5%
- Maternal 11p15.5 duplication <1%

- Hypomethylation H19 in ~40% of SRS patients

- H19 hypoCH3 classical SRS phenotype

Epimutation of the telomeric imprinting center region on chromosome 11p15 in Silver-Russell syndrome

Christine Gicquel1, Sylvie Rossignol1, Sylvie Cabrol1, Muriel Houang1, Virginie Steunou1, Véronique Barbu2, Fabienne Danton1, Nathalie Thibaud1, Martine Le Merrer3, Lydia Burdgen4, Anne-Marie Bertrand5, Irene Netchine4 & Yves Le Bouc1
Imprinting Centre 2 Defect in BWS
Anterior abdominal wall defects in BWS

UPD vs ICD2 and CDKN1C = p<0.0001 for exomphalos
UPD vs ICD2    p=0.02 for umbilical hernia
Molecular Genetics Enhances Diagnosis and Management of BWS

IC1 defect and UPD associated with high risk of Wilms tumour

CDKN1C mutations and IC2 defect low risk of Wilms tumour = no screening
Germline IC1 Defects and Wilms Tumour

Scott et al 2008

437 children without features of overgrowth syndromes from an unselected series cohort with WT

Constitutional 11p15 defects in 13 children (3%)

WT more often bilateral if 11p15 defect (4/13 vs 29/424, P<0.001) accounting for 12% of bilateral cases

Expression of 11p15 defects variable: BWS/non-syndromic Wilms Tumour

Constitutional 11p15 abnormalities, including heritable imprinting center mutations, cause nonsyndromic Wilms tumor
Aetiology of “Epimutations” in Imprinting Disorders

• Genetic
  - in cis
  - trans

• Environmental:
  Assisted Reproductive Technologies and Imprinting Disorders
Family BWS IC2 Epimutation

- two BWS children: loss of methylation at KvDMR1 – no evidence of deletion
- opposite parental alleles of 11p15.5
- Assumption: epimutation resulting from trans imprinting defect
- no H/O ART
- Consanguinity = Recessive disorder
## Genes with *trans* effect

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<th>Conditions</th>
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Autozygosity mapping analysis

- **Linkage study:**
  - No linkage to *ZFP57* *(and no mutation)*
  - Five regions of homozygosity (>2 Mb)
  - Maternal genotypes: ~8 Mb homozygous region on 19q13.4

- **Sequencing:**
  - *NLRP7* no pathogenic variants
Sequencing of *NLRP2*

- homozygous 2bp deletion in exon 6 (c.1479delAG) in mother
- *Prediction:* truncated protein (p.Arg493SerfsX32) lacking LRR domain
- not in 271 control and 11 BWS patients
NLRP2 mutation analysis

Heterozygous

Homozygous

Homozygous

Heterozygous

Heterozygous

HM
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NLRP7 and reproductive outcomes

- at least three families with FHM: affected women liveborn offspring
- one family: affected women → homozygous splice mutation in \textit{NLRP7} (Murdoch et al. 2007)
Methylation analysis

To determine if the *trans* imprinting defect extended beyond 11p15.5:

Methylation analysis undertaken on the 2 affected siblings and the 1 unaffected sibling:

- *PEG1* @ 7q32
- *PLAGL/ZAC* @ 6q24
- *SNRPN* @ 15q11-13

Methods: MS-PCR & Pyrosequencing

Partial LOM at *PEG1* in one child
NLRP/NALP genes

(Nucleotide-binding, Leucine-rich-Repeat [LRR], and Pyrin domains [PYD]-containing protein)

• Subfamily of CATERPILLER protein family
• Some NLRPs: component of inflammasome
• NLRP2: modulator of inflammatory response →
  – no evidence of immune/autoinflammatory disorder
• Widely expressed; many in human oocytes and embryos
  – ? Work in stage-dependant manner during early human development
Summary: *NLRP2* mutation

- rare cause of familial BWS
- **Inheritance:** similarities to FHM
- clinical heterogeneity/incomplete penetrance
- further investigation: role of *NLRP2/7* in genomic imprinting
BWS and ART

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

Association of In Vitro Fertilization with Beckwith-Wiedemann Syndrome and Epigenetic Alterations of LIT1 and H19
Michael R. DeBaun, Emily L. Niemitz, and Andrew P. Feinberg

In Vitro Fertilization May Increase the Risk of Beckwith-Wiedemann Syndrome Related to the Abnormal Imprinting of the KCNQ1OT Gene
Christine Gicquel, Véronique Gaston, Jacqueline Mandelbaum, Jean-Pierre Siffroi, Antoine Flahault, and Yves Le Bouc

Beckwith-Wiedemann Syndrome and IVF: A Case-Control Study
Jane Halliday, Kay Oke, Sue Breheny, Elizabeth Algar, and David J. Amor
BWS and Assisted Reproductive Technologies (ART)

- Increased relative risk of BWS after ART (e.g. ART in 4% BWS vs 1% in UK population)
- However absolute risk is low (<0.1%)
- Both ICSI and IVF associated
- 24/25 BWS ART patients have KvDMR1 LOM (IC2 epimutation) (expected 50%; P< 0.0001)

- Two theories
  - associated with infertility/superovulation therapy
  - associated with in vitro embryo culture

Evidence from mouse studies that *in vitro* culture of ES cells might be associated with epigenetic changes

In cattle and sheep, *large offspring syndrome* is an extensively documented phenomenon occurring as a consequence of in-vitro culture of ruminant embryos
Significance of BWS and ART Association

If ART is associated with susceptibility to epigenetic errors then

- Epigenetic changes may not be restricted to KvDMR1?
- Epigenetic changes at non-11p15.5 loci modify BWS phenotype in post-ART cases
- Post ART epigenetic changes could predispose to disorders not currently recognised as being related to imprinting/epigenetics?
Germline methylation in BWS IC2 epimutations at non-11p15.5 loci

- Methylation analysis performed on 55 IC2 epimutation BWS patients at imprinted DMRs at 6q24, 7q32 15q11-13
- 8 patients post ART
- LOM at 11p15.5 loci occurred in 37.5% of ART and 6.4% of non-ART BWS IC2 defect cases (P= 0.03)
- LOM at 11p15.5 is more frequent, but not exclusive to post-ART BWS
- LOM occurs at DMRs with maternal allele methylation not paternal allele methylation or satellite repeat regions (Rossignol et al 2006)
Methylation profiling

- 6 of 55 cases had LOM+ (3 ART, 3 non-ART)
  - 2 of 55 at ZAC (TNDM)
  - 4 of 58 at PEG1
  - 1 of 55 at SNRPN

KvDMR1 LOM N=55

Non-ART N=47

ART N=8

LOM+ N=3 (38%)

PEG1

PEG1 & SNRPN

ZAC

P=0.03
# Phenotype of post ART and non-ART BWS

<table>
<thead>
<tr>
<th>Feature of BWS</th>
<th>All BWS % (n=193) Cooper et al</th>
<th>UK ART % (n=25)</th>
<th>ICD2 non-ART % (n=116)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypoglycaemia</td>
<td>54</td>
<td>44</td>
<td>50</td>
</tr>
<tr>
<td>Macroglossia</td>
<td>91</td>
<td>90</td>
<td>87</td>
</tr>
<tr>
<td>Ear anomaly</td>
<td>61</td>
<td>56</td>
<td>65</td>
</tr>
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</table>
| Exomphalos    | 42                            | 43             | 69                     | P=0.029 *
| Macrosomia    | 75                            | 70*            | 79*                    |
| Hemihypertrophy | 31                       | 13             | 16                     |
| Embryonal tumour | 6                         | 13             | 0                      |

*singletons
Risk of neoplasia in ICD2 cases

**embryonal tumours in ART group**
- Hepatoblastoma
- Rhabdomyosarcoma

Log rank $P = 0.0014$
LOM at non-11p15.5 loci influence post-ART clinical phenotypes

ICSI pregnancy
Born 41 weeks BW 25th centile (7lb 8oz)
Large Tongue with apnoeas. Subsequently required tracheostomy.
Umbilical Hernia
Facial Naevus Flammeus, Bilateral Ear creases

Age 4 months: Weight 75th centile
Subsequently increased to >90th centile

Clinical diagnosis of post-ART BWS

MS-MLPA analysis: demonstrated LOM at H19

Subsequently demonstrated LOM at PEG1 and ZAC
Survey of imprinting disorders after ART

- Hypothesis: Imprinting disorders may be an underdiagnosed cause of morbidity after ART births
- Survey of 2492 children born after ART in the Ireland /England
- Questionnaire completed by 61%, 70 children detailed clinical assessment
- In entire cohort one case of BWS and no cases of Angelman syndrome. No evidence of a significant group of ART children with unrecognised milder forms of AS or BWS.
- Absolute risk of recognisable imprinting disorders in children conceived by ART is small (<1%)

Bowdin et al 2007
Conclusions

Studies of Rare Imprinting Disorders such as BWS can inform the role of epimutations in human disease.

Environmental and genetic causes may predispose to epimutations.

Epimutations in BWS (particularly post-ART) are not limited to 11p15.5.

Characterisation of epigenetic changes in post-ART BWS may provide novel epigenotype-phenotype correlations.

Provide insights into the pathogenesis of ART related complications e.g. growth and developmental defects?
Acknowledgements

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Collaborators:
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Wellcome Trust

WellChild
the national charity for sick children