Human Molecular Genetics, 2003, Vol. 12, Review Issue 1 **R61–R68**
DOI: 10.1093/hmg/ddg067

Beckwith–Wiedemann syndrome demonstrates a role for epigenetic control of normal development

Rosanna Weksberg[1,3–6,*], Adam C. Smith[1,6], Jeremy Squire[2,7] and Paul Sadowski[3]

[1]Institute of Medical Sciences, [2]Department of Laboratory Medicine and Pathobiology, [3]Department of Medical Genetics and Microbiology and [4]Department of Paediatrics, University of Toronto, Toronto, Ontario, Canada, [5]Division of Clinical and Metabolic Genetics and [6]The Research Institute Programme in Genetics and Genomic Biology, The Hospital for Sick Children, Toronto, Ontario, Canada and [7]Ontario Cancer Institute, Toronto, Ontario, Canada

Received January 3, 2003; Revised January 10, 2003; Accepted January 15, 2003

The Beckwith–Wiedemann syndrome (BWS) is characterized by somatic overgrowth and a predisposition to pediatric embryonal tumors. It is associated with genetic or epigenetic abnormalities in a cluster of imprinted genes found within a genomic region of approximately one megabase on human chromosome 11p15. Imprinted genes are expressed preferentially or exclusively from either the paternal or maternal allele. The 11p15 region is organized into two imprinted domains in which genomic imprinting is controlled by separate 'imprinting control regions'. Twenty-five to 50% of BWS patients have biallelic rather than monoallelic expression of the insulin-like growth factor 2 (*IGF2*) gene. Another 50% of patients have an epigenetic mutation resulting in loss of imprinting of a transcript called *KCNQ1OT1*. Each of these genes resides in one of the two imprinted domains that appear to be subject to developmental dysregulation in BWS. In this review, we discuss the insights that the study of BWS have contributed to our understanding of the mechanisms of growth control, oncogenesis and genomic imprinting. Specifically, methylation and chromatin modification may coordinate the expression of closely linked imprinted genes. Finally, we discuss how knowledge of epigenetic mechanisms associated with the early stages of embryogenesis suggest caution in the current debate surrounding assisted reproductive and cloning technologies.

INTRODUCTION

Beckwith–Wiedemann syndrome (BWS) was first described four decades ago as a disorder of growth regulation manifesting as somatic overgrowth, congenital malformations and tumor predisposition. Although the majority of cases are sporadic, a small number of pedigrees with autosomal dominant inheritance demonstrated linkage to 11p15 (1,2). The involvement of genomic imprinting in the phenotype was suggested by the preferential loss of maternal alleles in BWS-related tumors (3) and the maternal inheritance of the autosomal dominant forms of the condition (4). In fact, BWS and its related tumors provide a unique opportunity to investigate the role of genomic imprinting in normal growth and development.

BWS is a clinically heterogeneous disorder. The presenting findings may include macrosomia (prenatal and/or postnatal gigantism), hemihyperplasia, macroglossia, abdominal wall defects, embryonal tumors, ear anomalies, visceromegaly, renal abnormalities and neonatal hypoglycemia. Additional supportive findings may include polyhydramnios and prematurity, enlarged placenta, cardiomegaly, hemangiomata, cleft palate, advanced bone age and characteristic facies with midfacial hypoplasia and infraorbital creases. The characteristic facial appearance tends to regress over time (5).

WHAT IS GENOMIC IMPRINTING?

While most autosomal genes are expressed from both alleles, there is a subset of genes that are imprinted, i.e. they are expressed from only one allele (the paternal or maternal copy) (for recent reviews see 6–10). The molecular basis of imprinting is said to be 'epigenetic': two alleles that are

*To whom correspondence should be addressed at: Clinical and Metabolic Genetics, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, Canada M5G 1X8. Tel: +1 4168136386; Email: rweksb@sickkids.ca

identical in nucleotide sequence but of opposite parental origin are regulated differently in the same nucleus. This process is reversible. The silent, imprinted allele can be reactivated when passed through the germ line of the opposite parental sex and the active allele is silenced. Imprinted genes tend to occur in large clusters of greater than 1 Mb in length. One such cluster is located on 11p15 where dysregulation of imprinted genes is causally related to BWS.

Imprinting of the genes within these domains is regulated in *cis* by one or more imprinting center(s). Imprinting centers are thought to generate parent-of-origin-specific chromatin states that are propagated bidirectionally over several hundred kilobases of DNA to regulate the resetting of the imprint in the germline (11). The imprinting centers are likely also involved in maintaining imprints in somatic cells. Imprinting center mutations, therefore, should result in the failure to reset imprints, leading to inheritance of an inappropriate 'epigenotype' across an interval of up to one megabase (12).

Imprinted regions have been found to show a number of common characteristic features. These include differential DNA methylation, allele-specific RNA transcription, antisense transcripts, histone modifications, as well as differences in replication timing. Recent work has focused on the regulatory role of the parent of origin-specific differentially methylated regions (DMRs). A differentially methylated region is a DNA sequence that bears a CpG methylation mark that is specific to the chromosomal parent-of-origin. Such DMRs may be maternally or paternally methylated. Usually, although not always, the methylated allele is the silenced (imprinted) allele. Changes in the parent-of-origin-specific methylation of the DMR constitute an epigenetic lesion since they do not involve a change in the nucleotide sequence.

IMPRINTED GENES ON 11p15

The imprinted cluster of genes on 11p15 contains at least 12 imprinted genes (13–18). The 11p15 region has been divided into two distinct domains that are thought to be regulated by two imprinting centers separated by a non-imprinted region (Fig. 1).

Domain 1

Domain 1 contains the imprinted genes insulin-like growth factor 2 (*IGF2*) and *H19*, and a differentially methylated region, 'DMR1' postulated to be an imprinting center. The maternally expressed *H19* gene encodes an apparently untranslated polII transcript, and the *IGF2* gene encodes a paternally expressed fetal growth factor. Up-regulation of *IGF2* is thought to be important in the pathogenesis of BWS (19–27) and a variety of tumors (28,29). The expression profile of *IGF2* in normal development parallels the spectrum of organs/systems affected in patients with BWS (30). Increased expression of *IGF2* may be caused by paternal chromosome duplications of chromosome 11p15, paternal uniparental disomy (two copies of the paternal chromosome region), or alterations to differential methylation (3,31–33). Significantly, transgenic mice that overexpress *IGF2* exhibit some but not all of the features of BWS (overgrowth, macroglossia) (20).

The *H19* and *IGF2* genes compete for a common set of downstream enhancers located 3′ of the *H19* gene (34–36). DMR1 is located 2 kb upstream of the mouse *H19* gene and regulates the reciprocal imprinted expression of *H19* and *IGF2* gene in domain 1 by functioning as a chromatin boundary element or insulator (36–43) (Fig. 1). On the maternal chromosome, DMR1 is unmethylated, permitting the binding of a zinc finger protein called CTCF. Binding of CTCF blocks access of the *IGF2* promoter to the downstream enhancers. Thus, the maternal copy of *H19* is activated by these enhancers and is transcribed. Methylation of the paternal copy of DMR1 and the *H19* promoter are thought not only to silence the *H19* promoter but also to prevent binding of the CTCF protein to DMR1. As a result, the *IGF2* promoter can access the downstream enhancers and *H19* is silenced (42). Rarely, cases of BWS have hypermethylation of the paternal H19 promoter that causes biallelic *IGF2* expression (44).

Domain 2

In Domain 2, there are six known imprinted genes including *CDKN1C* ($p57^{KIP2}$), a maternally expressed gene that encodes a cyclin-dependent kinase inhibitor and negatively regulates cell proliferation (45). In tumors, *CDKN1C* shows aberrant methylation associated with cell cycle dysregulation (46–48); however, this gene is rarely mutated in tumors (49,50). Interestingly, mutations in *CDKN1C* do cause BWS (51–53) and are often associated with autosomal dominant inheritance of the syndrome. *TSSC3* (*IPL*) (54) is a maternally expressed gene that shows homology to *Tdag51*, a gene involved in Fas-mediated apoptosis. Mice lacking the *Impt1* gene have placental overgrowth but are otherwise normal (55). *SLC22A1L* (*IMPT1*) (56,57) is a maternally expressed gene encoding a possible organic cation transporter. Mutations of this gene have been reported in breast cancer and a rhabdomyosarcoma cell line (58). The maternally expressed *KCNQ1* gene product forms part of a potassium channel. Six known translocation sites spanning the length of this gene (14,59) are strongly associated with BWS. Intron 10 of the *KCNQ1* gene contains another DMR called *KvDMR1* or 'DMR2'. The paternal allele is non-methylated, permitting the paternal expression of a long transcript called *KCNQ1OT1*, also known as *LIT1* (14,60). This transcript originates near DMR2 and is transcribed in an antisense direction to the *KCNQ1* gene in which it originates. Maternal methylation of DMR2 is thought to silence maternal expression of *KCNQ1OT1* and to allow expression of a number of maternally expressed genes including *KCNQ1* and *CDKN1C* (14,60). Furthermore, a targeted deletion of the paternal *KCNQ1OT1* DMR2 caused diminished expression of the *KCNQ1OT1* transcript and activation of expression of the *KCNQ1* and *CDKN1C* genes (61,62). This suggests that this antisense transcript negatively regulates in *cis* the expression of several genes at long-range. These data suggest that the paternally-expressed *KCNQ1OT1* transcript and/or DMR2 itself can function as mediators of imprinting in domain 2. Recent evidence suggests that DMR2 has insulator activity in the mouse (63) and insulator and silencer activity in the human (Du and Sadowski, in preparation).

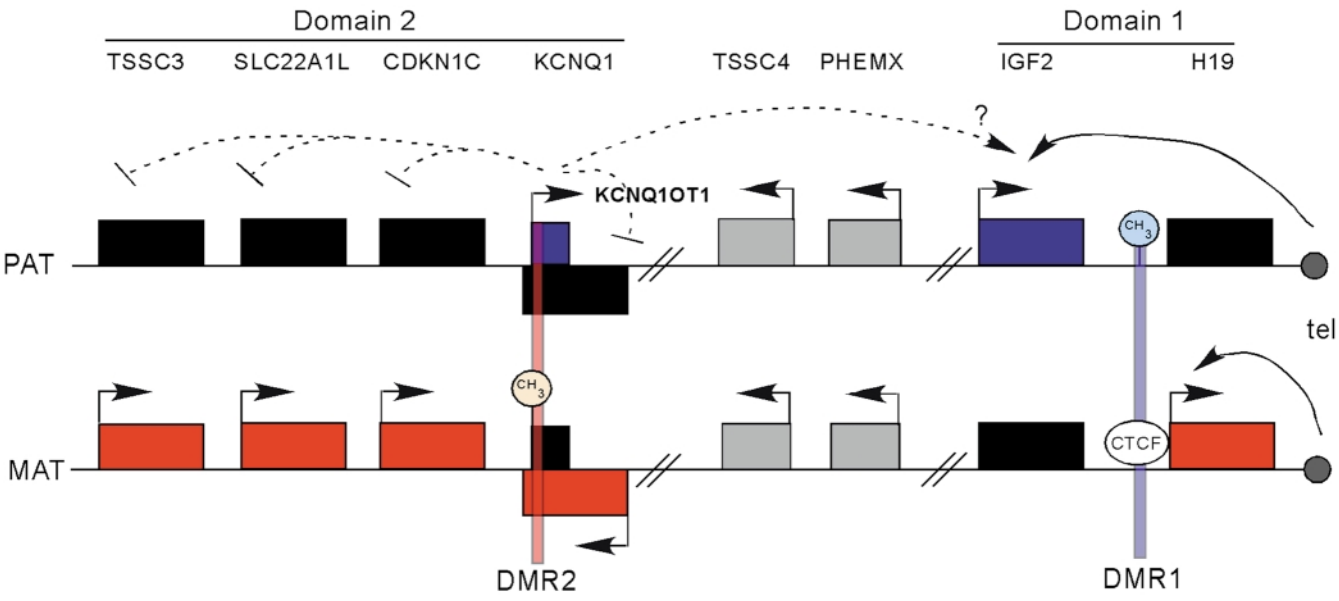

Figure 1. Map of the 11p15 imprinted region. Maternally expressed genes are shown in red and paternally expressed genes are shown in blue. Genes shown in gray are not imprinted. The direction of transcription is indicated by the square arrows. Hatch marks indicate regions of 11p15 not shown. Model of imprint regulation for domains 1 and 2 on 11p15. Known regulatory mechanisms shown by arced arrows and dashed arrows indicate proposed regulatory pathways. DMR1 and DMR2 locations are indicated by blue and red colored boxes, respectively. Methylation is indicated by a circle containing a methyl-group (CH3).

Table 1. BWS genetic and epigenetic subgroups

	DNA	RNA	Karyotype	Frequency[a]	Inheritance
A. Regional	Paternal 11p15 UPD		Normal	10–20%	Sporadic
			11p15 Duplication	1%	Sporadic[b]
	Disruption of *KCNQ1OT1*		11p15 Translocation/Inversion	1%	Sporadic[b]
B. Domain 1	*H19* hypermethylation	*IGF2* LOI	Normal	2%	Sporadic
	Normal *H19* methylation	*IGF2* LOI	Normal	25–50%	Sporadic
C. Domian 2	CDKN1C mutation		Normal	5–10%	Sporadic
	CDKN1C mutation		Normal	25%	Autosomal dominant
	KvDMR1 LOM	*KNQ1OT1* LOI	Normal	50%	Sporadic
D. Other	Unknown		Normal	5%	Autosomal dominant
	Unknown	Unknown	Normal	10–20%	Sporadic

LOM: loss of methylation, LOI: loss of imprint, UPD: uniparental disomy, CH3: methylation.
[a]These molecular abnormalities are not always mutually exclusive.
[b]May present as sporadic or recurrent cases in families.

MOLECULAR GENETICS OF BWS

Current data indicate that BWS is a multigenic disorder with clear parent-of-origin effects, and that BWS and its related tumors result from dysregulation of several closely linked genes associated with cell cycle and growth control on chromosome 11p15. Imprinted genes implicated in the etiology of BWS map to the 11p15 imprinted region (Fig. 1) and include the paternally expressed genes *IGF2* and *KCNQ1OT1*, and the maternally expressed genes *H19, CDKN1C* and *KCNQ1*. Table 1 shows estimated frequencies of known genetic/epigenetic BWS subgroups. Some affect both domains; others are limited to one domain or the other. However, the interaction of signals between the two domains has not yet been explored.

Genetic lesions

The majority of BWS cases occur sporadically and have no identifiable genetic lesion; only a minority of BWS cases have a demonstrable constitutional DNA sequence alteration. Rarely there are 11p15 chromosome abnormalities (33) such as chromosome 11 duplications and translocations. The majority of the identified mutations are in the *CDKN1C* gene (51,64,65). Notably, *CDKN1C* mutations are found more commonly in autosomal dominant pedigrees (51,64,65) than in sporadic cases. Mice with a targeted disruption of the *CDKN1C* gene (66,67) exhibit some abnormalities (such as omphalocele) similar to BWS, although overgrowth is absent. Mice with a *CDKN1C* mutation and increased *IGF2* expression exhibit many of the signs of BWS (68).

In most BWS cases, the primary molecular change is either epigenetic or unknown. The most common epigenetic alteration associated with BWS (50% of cases) is the loss of methylation at DMR2 (60) associated with loss of imprinting of *KCNQ1OT1* (14,44). Paternal 11p15 uniparental disomy occurs in 10–20% of cases (3,33). Expression of the normally silent maternal allele of *IGF2* occurs in 25–50% of BWS cases (69) and for most of these cases the cause, whether genetic or epigenetic, is not known. A few of them are associated with hypermethylation of the *H19* promoter and loss of maternal *H19* expression, which is referred to as *H19*-dependent loss of imprinting of *IGF2*. However most cases of loss of imprinting of the *IGF2* gene are associated with normal monoallelic maternal expression of the *H19* gene. This is referred to as H19-independent loss of imprinting of *IGF2*. Unlike the mouse gene, the human *IGF2* gene is thought to be differentially methylated on the maternal allele. Change in the methylation status of the *IGF2* gene has not been reported for BWS. Some BWS cases with loss of imprinting for DMR2/*KCNQ1OT1* also show loss of imprinting of the *IGF2* gene (14,59). These data support the possibility of regulatory interactions between the two imprinted domains (Fig. 1).

Recent observations suggest that regulation of imprinting at 11p15 could be even more complex than the data already presented suggests (70). Several BWS-associated translocations disrupting *KCNQ1* do not affect the methylation of DMR2 or *KCNQ1OT1* expression (unpublished data), but still lead to altered *IGF2* imprinting (71) and replication timing (72). The translocations may have separated some genes (e.g. *IGF2*, *H19*) from their cognate enhancers or other regulatory elements and disrupt imprinting in ways that are presently poorly understood. It is noteworthy that mice carrying a targeted chromosomal translocation whose breakpoint is between the *CDKN1C* and *KCNQ1* genes lose expression and imprinting of *Cdkn1c*, *Tssc3* and *Slc22A1L* genes (73).

IMPRINTING AND TUMORIGENESIS

Altered genomic imprinting may also have a role in the inactivation of tumor suppressor genes or the overexpression of oncogenes in certain types of cancers (28). It is well established that mutations in tumour suppressor genes contribute to the malignant process when both normal alleles have lost their function. Most commonly, an initial mutation occurs in one allele; the second event may be a chromosomal deletion or a mitotic recombination between the chromosome carrying the mutant allele and the chromosome carrying the normal allele. Such changes are detected in tumors when closely linked regions of the genome undergo loss of constitutional heterozygosity (LOH). By convention, geneticists usually refer to such changes presenting in somatic cells as UPD, and the equivalent genetic change in tumors is called LOH. Some types of tumor have been shown to undergo LOH, in a parent-of-origin specific manner, so that either a paternal or a maternal chromosomal region is retained (28). Such parental bias is seen when a tumor suppressor gene maps to an imprinted chromosomal region. If a tumor suppressor gene is imprinted, 'one-hit' rather than 'two hit' kinetics could lead to complete gene inactivation. Conversely, it follows that mitotic recombination affecting a chromosomal region containing one or more imprinted oncogenes could lead to two copies of a chromosome region derived from one parent and a net increase in expression of any growth-promoting sequences mapping to such a disomic region. Thus, such parental bias in tumors exhibiting LOH may help identify the genomic locations of tumor suppressor genes or oncogenes involved in the malignant process, and may also provide clues concerning their imprinting status in normal cells.

11p15 AND INCREASED TUMOR RISK

As described above, the molecular alterations and epigenetics of BWS and some of the embryonal tumors associated with this syndrome, such as Wilms' tumor and rhabdomyosarcoma, have provided useful insights concerning the role of imprinted genes in cancer. Just as in embryonal tumors there is often preferential retention of paternal genes from 11p15, so paternal disomy of this same region is also seen frequently in BWS. Dysregulation of 11p15 cell cycle proteins and growth factors seem to be strongly associated with embryonal tumorigenesis and overgrowth, and our analysis of the molecular genetics of domains 1 and 2 in BWS and associated tumors has focused on the negative and positive regulators of growth *CDKN1C* ($p57^{kip2}$), and *IGF2* (44,69). Epigenetic changes of the control mechanisms (DMR 1 and 2) outlined above can impact regional regulation of constitutional gene expression of such genes, and lead to tumor predisposition. Similarly acquired alterations to these pivotal control mechanisms in domains 1 and 2 can take place in a single somatic cell, and can lead to sporadic tumors. We recently reported that children with BWS who develop embryonal tumors such as rhabdomyosarcoma and hepatoblastoma have epigenetic changes in domain 2 (44). In contrast, Wilms' tumor is more strongly associated with epigenetic alterations in domain 1 (74–76). Taken together these data demonstrate that in BWS cases, embryonal tumors have two distinct 11p15 epigenetic tumorigenic pathways that may also reflect similar tumorigenic pathways in sporadic embryonal tumors. Thus understanding how coordinate dysregulation of imprinted genes takes place will be important in determining the reasons for the variable expression of tumors in BWS and in the causation of sporadic embryonal tumors subject to 11p15 epigenetic and molecular lesions.

Epigenetic changes have been shown to occur in many pediatric and adult cancers (77–82). The epigenetic changes such as methylation at *H19* seen in patients with BWS who develop Wilms' tumor are also seen in patients who develop Wilms' tumor without BWS. This indicates that the timing of epigenetic change may determine whether one develops a syndrome like BWS or a cancer. For example such a change in an early embryo can present as BWS and cancer predisposition whereas such a change in an embryonic precursor for an organ generates predisposition to cancer in that organ. Finally, common pediatric embryonal tumor types can also be associated with BWS including rhabdomyosarcoma, adrenal cortical carcinoma, gonadoblastoma and neuroblastoma. We have recently shown that BWS cases with these tumors exhibit loss of methylation at DMR2 and not at DMR1 (44). These data indicate that there are likely to be two distinct cancer-predisposing regions on 11p15.

IMPRINTING OF 11p15 IN EARLY DEVELOPMENT

BWS provides a unique opportunity to study genomic imprints in early development. It appears that the preimplantation embryo is a critical period in development with respect to establishment and maintenance of imprints (83–86). Interestingly, we have recently shown an early developmental epigenetic error relating twinning and BWS (83). We studied 10 monozygotic twin pairs who were discordant for BWS and found loss of methylation at DMR2 (KvDMR) and biallelic expression of *KCNQ1OT1* in all of the affected twins. The unaffected twins did not share the loss of methylation at DMR2 nor did these twin pairs have any other detectable genetic or epigenetic defects on 11p15. Further, we found a significant excess of monozygotic twinning in BWS estimated at 8% compared with the general population occurrence of 0.3–0.4%, whereas the occurrence of dizygotic twins in BWS did not differ significantly from the general population. In addition there was a significant excess of females over males in monozygotic twin pairs (16:4). Our data suggest that there is a critical time period in preimplantation development when disturbance of imprinting is associated with increased likelihood of monozygotic twinning. It is thought that two-thirds of all monozygotic twinning occurs between embryonic days 5–9. In fact, the process of monozygotic twinning and other environmental disturbances in the preimplantation embryo may increase the risk for epigenetic lesions.

ASSISTED REPRODUCTIVE TECHNOLOGIES

The high incidence of monozygotic twinning associated with BWS and disruption of imprinting suggests that careful monitoring of assisted reproductive technologies is warranted. Assisted reproductive technologies expose the preimplantation embryo to a variety of manipulations that could affect both rates of monozygotic twinning and maintenance of genomic imprints. Assisted reproductive technologies include *in vitro* fertilization, intra-cytoplasmic sperm injection and ooplasm donation, all of which could disrupt the correct maintenance of imprints. Nuclear transfer and intracytoplasmic sperm injection have both been shown to be associated with imprinting defects (87–91). Current clinical reports regarding the safety of assisted reproductive technologies have shown increases in monozygotic twinning (92,93) but De Rycke *et al.* find no significant increase in the number of children born with defects/abnormalities (94). However they suggest that studies to date have only monitored patients for two years and had relatively small sample sizes. Recently two imprinting disorders, BWS and Angelman syndrome, have been reported in association with assisted reproductive technologies (89–91). Additionally, it is possible that the disruption of the epigenetic state of the germline may not be immediately apparent in the offspring of the IVF pregnancy (94), but that problems in resetting the imprints in the germline may occur in children in the subsequent generation (i.e. children of parents who used assisted reproductive technology) (94). Careful study and follow-up of these cases is warranted considering the body of evidence that is developing regarding the fragility of the preimplantation embryo.

CLONING AND IMPRINTING

Maintenance of imprints is also an important issue in the potential development of therapeutic strategies involving cloning. A recent review by Sapienza (95) and paper by Onyango *et al.* (85) investigate the implications for using embryonic stem (ES) cells versus embryonic germ (EG) cells in relation to the resetting of imprints in the germline and preimplantation embryo. The variability of methylation and expression of imprinted genes found in mouse ES cells has raised the issue that the use of the human equivalent of these cells for therapeutic cloning may be problematic. EG cells originate before meiosis whereas ES cells are derived postmeiotically. EG cells are derived from primordial germ cells taken from the developing gonadal ridges of human fetuses, whereas ES cells are derived from the inner cell mass of blastocyst-stage preimplantation embryos. Although ES cells are only a few cell divisions removed from being EG cells themselves, mouse ES cells show great variability in their methylation (96) and expression of imprinted genes (88,97). Furthermore, it is commonly observed that many embryos derived from ES cell are stillborn and exhibit the 'large offspring syndrome' that is reminiscent of BWS (98). Genomic imprinting was discovered because it was realized that the paternal and maternal contributions to the embryo were not equal. Therefore, the utility of ES cells and other somatically derived cell nuclei for therapeutic cloning and nuclear transfer must be questioned until a more complete understanding of the effects on such therapies of epigenetic variation.

PERSPECTIVES

BWS provides a model system to study genomic imprinting, a process that has a profound impact on coordinated gene expression in development and cancer. Future profiling techniques, such as epigenotype microarrays and genome-wide chromatin analysis will permit a more accurate definition of the involvement of imprinted domains in normal development. The concepts that are developed from the study of dysregulation of imprinted domains in BWS are likely to be generally applicable and to increase our understanding of the role of epigenetics and chromatin structure in dysregulation of gene expression in congenital malformations and cancer development.

REFERENCES

1. Ping, A.J., Reeve, A.E., Law, D.J., Young, M.R., Boehnke, M. and Feinberg, A.P. (1989) Genetic linkage of Beckwith–Wiedemann syndrome to 11p15. *Am. J. Hum Genet.*, **44**, 720–723.
2. Wu, N.F. and Kushnick, T. (1974) The Beckwith–Wiedemann syndrome. The exomphalos–macroglossia–gigantism syndrome. *Clin. Pediatr. (Phil.)*, **13**, 452–457.
3. Henry, I., Bonaiti-Pellie, C., Chehensse, V., Beldjord, C., Schwartz, C., Utermann, G. and Junien, C. (1991) Uniparental paternal disomy in a genetic cancer-predisposing syndrome. *Nature*, **351**, 665–667.
4. Moutou, C., Junien, C., Henry, I. and Bonaiti-Pellie, C. (1992) Beckwith–Wiedemann syndrome: a demonstration of the mechanisms responsible for the excess of transmitting females. *J. Med. Genet.*, **29**, 217–220.
5. Weksberg, R. and Shuman, C. (2000) Beckwith–Wiedemann Syndrome and hemihyperplasia. In Cassidy, S. and Allanson, J. (eds), *Management of Genetic Syndromes*. Wiley, New York, pp. 49–70.
6. Pfeifer, K. (2000) Mechanisms of genomic imprinting. *Am. J. Hum. Genet.*, **67**, 777–787.

7. Reik, W., Dean, W. and Walter, J. (2001) Epigenetic reprogramming in mammalian development. *Science*, **293**, 1089–1093.
8. Tilghman, S.M. (1999) The sins of the fathers and mothers: genomic imprinting in mammalian development. *Cell*, **96**, 185–193.
9. Tycko, B. and Efstratiadis, A. (2002) Genomic imprinting: piece of cake. *Nature*, **417**, 913–914.
10. Constancia, M., Pickard, B., Kelsey, G. and Reik, W. (1998) Imprinting mechanisms. *Genome Res.*, **8**, 881–900.
11. Nicholls, R.D. (2000) The impact of genomic imprinting for neurobehavioral and developmental disorders. *J. Clin. Invest.*, **105**, 413–418.
12. Buiting, K., Saitoh, S., Gross, S., Dittrich, B., Schwartz, S., Nicholls, R.D. and Horsthemke, B. (1995) Inherited microdeletions in the Angelman and Prader–Willi syndromes define an imprinting centre on human chromosome 15. *Nat. Genet.*, **9**, 395–400.
13. Morison, I.M. and Reeve, A.E. (1998) A catalogue of imprinted genes and parent-of-origin effects in humans and animals. *Hum. Mol. Genet.*, **7**, 1599–1609.
14. Lee, M.P., DeBaun, M.R., Mitsuya, K., Galonek, H.L., Brandenburg, S., Oshimura, M. and Feinberg, A.P. (1999) Loss of imprinting of a paternally expressed transcript, with antisense orientation to KVLQT1, occurs frequently in Beckwith–Wiedemann syndrome and is independent of insulin-like growth factor II imprinting. *Proc. Natl Acad. Sci. USA*, **96**, 5203–5208.
15. Dao, D., Walsh, C.P., Yuan, L., Gorelov, D., Feng, L., Hensle, T., Nisen, P., Yamashiro, D.J., Bestor, T.H. and Tycko, B. (1999) Multipoint analysis of human chromosome 11p15/mouse distal chromosome 7: inclusion of H19/IGF2 in the minimal WT2 region, gene specificity of H19 silencing in Wilms' tumorigenesis and methylation hyper-dependence of H19 imprinting. *Hum. Mol. Genet.*, **8**, 1337–1352.
16. Paulsen, M., Davies, K.R., Bowden, L.M., Villar, A.J., Franck, O., Fuermann, M., Dean, W.L., Moore, T.F., Rodrigues, N. and Davies, K.E. *et al.* (1998) Syntenic organization of the mouse distal chromosome 7 imprinting cluster and the Beckwith–Wiedemann syndrome region in chromosome 11p15.5. *Hum. Mol. Genet.*, **7**, 1149–1159.
17. Xin, Z., Soejima, H., Higashimoto, K., Yatsuki, H., Zhu, X., Satoh, Y., Masaki, Z., Kaneko, Y., Jinno, Y., Fukuzawa, R., Hata, J. and Mukai, T. (2000) A novel imprinted gene, KCNQ1DN, within the WT2 critical region of human chromosome 11p15.5 and its reduced expression in Wilms' tumors. *J. Biochem. (Tokyo)*, **128**, 847–853.
18. Onyango, P., Miller, W., Lehoczky, J., Leung, C.T., Birren, B., Wheelan, S., Dewar, K. and Feinberg, A.P. (2000) Sequence and comparative analysis of the mouse 1-megabase region orthologous to the human 11p15 imprinted domain. *Genome Res.*, **10**, 1697–1710.
19. Joyce, J.A., Lam, W.K., Catchpoole, D.J., Jenks, P., Reik, W., Maher, E.R. and Schofield, P.N. (1997) Imprinting of IGF2 and H19: lack of reciprocity in sporadic Beckwith–Wiedemann syndrome. *Hum. Mol. Genet.*, **6**, 1543–1548.
20. Sun, F.L., Dean, W.L., Kelsey, G., Allen, N.D. and Reik, W. (1997) Transactivation of Igf2 in a mouse model of Beckwith–Wiedemann syndrome. *Nature*, **389**, 809–815.
21. Reik, W., Bowden, L., Constancia, M., Dean, W., Feil, R., Forne, T., Kelsey, G., Maher, E., Moore, T., Sun, F.L. and Walter, J. (1996) Regulation of Igf2 imprinting in development and disease. *Int. J. Dev. Biol.*, **Suppl. 1**, 53S–54S.
22. Brown, K.W., Villar, A.J., Bickmore, W., Clayton-Smith, J., Catchpoole, D., Maher, E.R. and Reik, W. (1996) Imprinting mutation in the Beckwith–Wiedemann syndrome leads to biallelic IGF2 expression through an H19-independent pathway. *Hum. Mol. Genet.*, **5**, 2027–2032.
23. Morison, I.M. and Reeve, A.E. (1998) Insulin-like growth factor 2 and overgrowth: molecular biology and clinical implications. *Mol. Med. Today*, **4**, 110–115.
24. Morison, I.M., Becroft, D.M., Taniguchi, T., Woods, C.G. and Reeve, A.E. (1996) Somatic overgrowth associated with overexpression of insulin-like growth factor II. *Nat. Med.*, **2**, 311–316.
25. Eggenschwiler, J., Ludwig, T., Fisher, P., Leighton, P.A., Tilghman, S.M. and Efstratiadis, A. (1997) Mouse mutant embryos overexpressing IGF-II exhibit phenotypic features of the Beckwith–Wiedemann and Simpson–Golabi–Behmel syndromes. *Genes Dev.*, **11**, 3128–3142.
26. Li, M., Squire, J.A. and Weksberg, R. (1998) Overgrowth syndromes and genomic imprinting: from mouse to man. *Clin. Genet.*, **53**, 165–170.
27. Weksberg, R., Shen, D.R., Fei, Y.L., Song, Q.L. and Squire, J. (1993) Disruption of insulin-like growth factor 2 imprinting in Beckwith–Wiedemann syndrome. *Nat. Genet.*, **5**, 143–150.
28. Tycko, B. (2000) Epigenetic gene silencing in cancer. *J. Clin. Invest.*, **105**, 401–407.
29. Schofield, P.N., Joyce, J.A., Lam, W.K., Grandjean, V., Ferguson-Smith, A., Reik, W. and Maher, E.R. (2001) Genomic imprinting and cancer; new paradigms in the genetics of neoplasia. *Toxicol. Lett.*, **120**, 151–160.
30. Ohlsson, R., Nystrom, A., Pfeifer-Ohlsson, S., Tohonen, V., Hedborg, F., Schofield, P., Flam, F. and Ekstrom, T.J. (1993) IGF2 is parentally imprinted during human embryogenesis and in the Beckwith–Wiedemann syndrome. *Nat. Genet.*, **4**, 94–97.
31. Weksberg, R., Teshima, I., Williams, B.R., Greenberg, C.R., Pueschel, S.M., Chernos, J.E., Fowlow, S.B., Hoyme, E., Anderson, I.J. and Whiteman, D.A. *et al.* (1993) Molecular characterization of cytogenetic alterations associated with the Beckwith–Wiedemann syndrome (BWS) phenotype refines the localization and suggests the gene for BWS is imprinted. *Hum. Mol. Genet.*, **2**, 549–556.
32. Reik, W., Brown, K.W., Slatter, R.E., Sartori, P., Elliott, M. and Maher, E.R. (1994) Allelic methylation of H19 and IGF2 in the Beckwith–Wiedemann syndrome. *Hum. Mol. Genet.*, **3**, 1297–1301.
33. Li, M., Squire, J.A. and Weksberg, R. (1998) Molecular genetics of Wiedemann–Beckwith syndrome. *Am. J. Med. Genet.*, **79**, 253–259.
34. Leighton, P.A., Saam, J.R., Ingram, R.S., Stewart, C.L. and Tilghman, S.M. (1995) An enhancer deletion affects both H19 and Igf2 expression. *Genes Dev.*, **9**, 2079–2089.
35. Webber, A.L., Ingram, R.S., Levorse, J.M. and Tilghman, S.M. (1998) Location of enhancers is essential for the imprinting of H19 and Igf2 genes. *Nature*, **391**, 711–715.
36. Kanduri, C., Holmgren, C., Pilartz, M., Franklin, G., Kanduri, M., Liu, L., Ginjala, V., Ulleras, E., Mattsson, R. and Ohlsson, R. (2000) The 5′ flank of mouse H19 in an unusual chromatin conformation unidirectionally blocks enhancer-promoter communication. *Curr. Biol.*, **10**, 449–457.
37. Kanduri, C., Pant, V., Loukinov, D., Pugacheva, E., Qi, C.F., Wolffe, A., Ohlsson, R. and Lobanenkov, V.V. (2000) Functional association of CTCF with the insulator upstream of the H19 gene is parent of origin-specific and methylation-sensitive. *Curr. Biol.*, **10**, 853–856.
38. Szabo, P., Tang, S.H., Rentsendorj, A., Pfeifer, G.P. and Mann, J.R. (2000) Maternal-specific footprints at putative CTCF sites in the H19 imprinting control region give evidence for insulator function. *Curr. Biol.*, **10**, 607–610.
39. Chung, J.H., Bell, A.C. and Felsenfeld, G. (1997) Characterization of the chicken beta-globin insulator. *Proc. Natl Acad. Sci. USA*, **94**, 575–580.
40. Bell, A.C. and Felsenfeld, G. (2000) Methylation of a CTCF-dependent boundary controls imprinted expression of the Igf2 gene. *Nature*, **405**, 482–485.
41. Bell, A.C., West, A.G. and Felsenfeld, G. (2001) Insulators and boundaries: versatile regulatory elements in the eukaryotic genome. *Science*, **291**, 447–450.
42. Hark, A.T., Schoenherr, C.J., Katz, D.J., Ingram, R.S., Levorse, J.M. and Tilghman, S.M. (2000) CTCF mediates methylation-sensitive enhancer-blocking activity at the H19/Igf2 locus. *Nature*, **405**, 486–489.
43. Bell, A.C., West, A.G. and Felsenfeld, G. (1999) The protein CTCF is required for the enhancer blocking activity of vertebrate insulators. *Cell*, **98**, 387–396.
44. Weksberg, R., Nishikawa, J., Caluseriu, O., Fei, Y.L., Shuman, C., Wei, C., Steele, L., Cameron, J., Smith, A., Ambus, I., Li, M., Ray, P.N., Sadowski, P. and Squire, J. (2001) Tumor development in the Beckwith–Wiedemann syndrome is associated with a variety of constitutional molecular 11p15 alterations including imprinting defects of KCNQ1OT1. *Hum. Mol. Genet.*, **10**, 2989–3000.
45. Matsuoka, S., Edwards, M.C., Bai, C., Parker, S., Zhang, P., Baldini, A., Harper, J.W. and Elledge, S.J. (1995) p57KIP2, a structurally distinct member of the p21CIP1 Cdk inhibitor family, is a candidate tumor suppressor gene. *Genes Dev.*, **9**, 650–662.
46. Kikuchi, T., Toyota, M., Itoh, F., Suzuki, H., Obata, T., Yamamoto, H., Kakiuchi, H., Kusano, M., Issa, J.P., Tokino, T. and Imai, K. (2002) Inactivation of p57KIP2 by regional promoter hypermethylation and histone deacetylation in human tumors. *Oncogene*, **21**, 2741–2749.
47. Li, Y., Nagai, H., Ohno, T., Yuge, M., Hatano, S., Ito, E., Mori, N., Saito, H. and Kinoshita, T. (2002) Aberrant DNA methylation of p57(KIP2) gene in the promoter region in lymphoid malignancies of B-cell phenotype. *Blood*, **100**, 2572–2577.

48. Shin, J.Y., Kim, H.S., Lee, K.S., Kim, J., Park, J.B., Won, M.H., Chae, S.W., Choi, Y.H., Choi, K.C., Park, Y.E. and Lee, J.Y. (2000) Mutation and expression of the p27KIP1 and p57KIP2 genes in human gastric cancer. *Exp. Mol. Med.*, **32**, 79–83.
49. Hartmann, W., Waha, A., Koch, A., Goodyer, C.G., Albrecht, S., von Schweinitz, D. and Pietsch, T. (2000) p57(KIP2) is not mutated in hepatoblastoma but shows increased transcriptional activity in a comparative analysis of the three imprinted genes p57(KIP2), IGF2, and H19. *Am. J. Pathol.*, **157**, 1393–1403.
50. Lai, S., Goepfert, H., Gillenwater, A.M., Luna, M.A. and El-Naggar, A.K. (2000) Loss of imprinting and genetic alterations of the cyclin-dependent kinase inhibitor p57KIP2 gene in head and neck squamous cell carcinoma. *Clin. Cancer Res.*, **6**, 3172–3176.
51. Lam, W.W., Hatada, I., Ohishi, S., Mukai, T., Joyce, J.A., Cole, T.R., Donnai, D., Reik, W., Schofield, P.N. and Maher, E.R. (1999) Analysis of germline CDKN1C (p57KIP2) mutations in familial and sporadic Beckwith–Wiedemann syndrome (BWS) provides a novel genotype–phenotype correlation. *J. Med. Genet.*, **36**, 518–523.
52. Hatada, I., Nabetani, A., Morisaki, H., Xin, Z., Ohishi, S., Tonoki, H., Niikawa, N., Inoue, M., Komoto, Y. and Okada, A. *et al.* (1997) New p57KIP2 mutations in Beckwith–Wiedemann syndrome. *Hum. Genet.*, **100**, 681–683.
53. O'Keefe, D., Dao, D., Zhao, L., Sanderson, R., Warburton, D., Weiss, L., Anyane-Yeboa, K. and Tycko, B. (1997) Coding mutations in p57KIP2 are present in some cases of Beckwith–Wiedemann syndrome but are rare or absent in Wilms tumors. *Am. J. Hum. Genet.*, **61**, 295–303.
54. Muller, S., van den Boom, D., Zirkel, D., Koster, H., Berthold, F., Schwab, M., Westphal, M. and Zumkeller, W. (2000) Retention of imprinting of the human apoptosis-related gene TSSC3 in human brain tumors. *Hum. Mol. Genet.*, **9**, 757–763.
55. Frank, D., Fortino, W., Clark, L., Musalo, R., Wang, W., Saxena, A., Li, C.M., Reik, W., Ludwig, T. and Tycko, B. (2002) Placental overgrowth in mice lacking the imprinted gene Ipl. *Proc. Natl Acad. Sci. USA*, **99**, 7490–7495.
56. Reece, M., Prawitt, D., Landers, J., Kast, C., Gros, P., Housman, D., Zabel, B.U. and Pelletier, J. (1998) Functional characterization of ORCTL2—an organic cation transporter expressed in the renal proximal tubules. *FEBS Lett.*, **433**, 245–250.
57. Dao, D., Frank, D., Qian, N., O'Keefe, D., Vosatka, R.J., Walsh, C.P. and Tycko, B. (1998) IMPT1, an imprinted gene similar to polyspecific transporter and multi-drug resistance genes. *Hum. Mol. Genet.*, **7**, 597–608.
58. Schwienbacher, C., Sabbioni, S., Campi, M., Veronese, A., Bernardi, G., Menegatti, A., Hatada, I., Mukai, T., Ohashi, H., Barbanti-Brodano, G., Croce, C.M. and Negrini, M. (1998) Transcriptional map of 170-kb region at chromosome 11p15.5: identification and mutational analysis of the BWR1A gene reveals the presence of mutations in tumor samples. *Proc. Natl Acad. Sci. USA*, **95**, 3873–3878.
59. Mitsuya, K., Meguro, M., Lee, M.P., Katoh, M., Schulz, T.C., Kugoh, H., Yoshida, M.A., Niikawa, N., Feinberg, A.P. and Oshimura, M. (1999) LIT1, an imprinted antisense RNA in the human KvLQT1 locus identified by screening for differentially expressed transcripts using monochromosomal hybrids. *Hum. Mol. Genet.*, **8**, 1209–1217.
60. Smilinich, N.J., Day, C.D., Fitzpatrick, G.V., Caldwell, G.M., Lossie, A.C., Cooper, P.R., Smallwood, A.C., Joyce, J.A., Schofield, P.N. and Reik, W. *et al.* (1999) A maternally methylated CpG island in KvLQT1 is associated with an antisense paternal transcript and loss of imprinting in Beckwith–Wiedemann syndrome. *Proc. Natl Acad. Sci. USA*, **96**, 8064–8069.
61. Horike, S., Mitsuya, K., Meguro, M., Kotobuki, N., Kashiwagi, A., Notsu, T., Schulz, T.C., Shirayoshi, Y. and Oshimura, M. (2000) Targeted disruption of the human LIT1 locus defines a putative imprinting control element playing an essential role in Beckwith–Wiedemann syndrome. *Hum. Mol. Genet.*, **9**, 2075–2083.
62. Fitzpatrick, G.V., Soloway, P.D. and Higgins, M.J. (2002) Regional loss of imprinting and growth deficiency in mice with a targeted deletion of KvDMR1. *Nat. Genet.*, **32**, 426–431.
63. Kanduri, C., Fitzpatrick, G., Mukhopadhyay, R., Kanduri, M., Lobanenkov, V., Higgins, M. and Ohlsson, R. (2002) A differentially methylated imprinting control region within the Kcnq1 locus harbours a methylation-sensitive chromatin insulator. *J. Biol. Chem.*, **277**, 18106–18110.
64. Hatada, I., Ohashi, H., Fukushima, Y., Kaneko, Y., Inoue, M., Komoto, Y., Okada, A., Ohishi, S., Nabetani, A. and Morisaki, H. *et al.* (1996) An imprinted gene p57KIP2 is mutated in Beckwith-Wiedemann syndrome. [See comments.] *Nat. Genet.*, **14**, 171–173.
65. Li, M., Squire, J., Shuman, C., Fei, Y.L., Atkin, J., Pauli, R., Smith, A., Nishikawa, J., Chitayat, D. and Weksberg, R. (2001) Imprinting status of 11p15 genes in Beckwith–Wiedemann syndrome patients with CDKN1C mutations. *Genomics*, **74**, 370–376.
66. Yan, Y., Frisen, J., Lee, M.H., Massague, J. and Barbacid, M. (1997) Ablation of the CDK inhibitor p57Kip2 results in increased apoptosis and delayed differentiation during mouse development. *Genes Dev.*, **11**, 973–983.
67. Zhang, P., Liegeois, N.J., Wong, C., Finegold, M., Hou, H., Thompson, J.C., Silverman, A., Harper, J.W., DePinho, R.A. and Elledge, S.J. (1997) Altered cell differentiation and proliferation in mice lacking p57KIP2 indicates a role in Beckwith–Wiedemann syndrome. *Nature*, **387**, 151–158.
68. Caspary, T., Cleary, M.A., Perlman, E.J., Zhang, P., Elledge, S.J. and Tilghman, S.M. (1999) Oppositely imprinted genes p57(Kip2) and igf2 interact in a mouse model for Beckwith–Wiedemann syndrome. *Genes Dev.*, **13**, 3115–3124.
69. Weksberg, R., Shen, D.R., Fei, Y.L., Song, Q.L. and Squire, J. (1993) Disruption of insulin-like growth factor 2 imprinting in Beckwith–Wiedemann syndrome. *Nat. Genet.*, **5**, 143–150.
70. John, R.M., Ainscough, J.F., Barton, S.C. and Surani, M.A. (2001) Distant *cis*-elements regulate imprinted expression of the mouse $p57^{Kip2}$(*Cdkn1c*) gene: implications for the human disorder, Beckwith–Wiedemann syndrome. *Hum. Mol. Genet.*, **10**, 1601–1609.
71. Brown, K.W., Villar, A.J., Bickmore, W., Clayton-Smith, J., Catchpoole, D., Maher, E.R. and Reik, W. (1996) Imprinting mutation in the Beckwith–Wiedemann syndrome leads to biallelic IGF2 expression through an H19-independent pathway. *Hum. Mol. Genet.*, **5**, 2027–2032.
72. Squire, J.A., Li, M., Perlikowski, S., Fei, Y.L., Bayani, J., Zhang, Z.M. and Weksberg, R. (2000) Alterations of H19 imprinting and IGF2 replication timing are infrequent in Beckwith–Wiedemann syndrome. *Genomics*, **65**, 234–242.
73. Cleary, M.A., van Raamsdonk, C.D., Levorse, J., Zheng, B., Bradley, A. and Tilghman, S.M. (2001) Disruption of an imprinted gene cluster by a targeted chromosomal translocation in mice. *Nat. Genet.*, **29**, 78–82.
74. DeBaun, M.R., Niemitz, E.L., McNeil, D.E., Brandenburg, S.A., Lee, M.P. and Feinberg, A.P. (2002) Epigenetic alterations of H19 and LIT1 distinguish patients with Beckwith–Wiedemann syndrome with cancer and birth defects. *Am. J. Hum. Genet.*, **70**, 604–611.
75. Bliek, J., Maas, S.M., Ruijter, J.M., Hennekam, R.C., Alders, M., Westerveld, A. and Mannens, M.M. (2001) 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.*, **10**, 467–476.
76. Engel, J.R., Smallwood, A., Harper, A., Higgins, M.J., Oshimura, M., Reik, W., Schofield, P.N. and Maher, E.R. (2000) Epigenotype–phenotype correlations in beckwith–wiedemann syndrome. *J. Med. Genet.*, **37**, 921–926.
77. Feinberg, A.P. (1993) Genomic imprinting and gene activation in cancer. *Nat. Genet.*, **4**, 110–113.
78. Issa, J.P. and Baylin, S.B. (1996) Epigenetics and human disease. *Nat. Med.*, **2**, 281–282.
79. Ogawa, O., Becroft, D.M., Morison, I.M., Eccles, M.R., Skeen, J.E., Mauger, D.C. and Reeve, A.E. (1993) Constitutional relaxation of insulin-like growth factor II gene imprinting associated with Wilms' tumour and gigantism. *Nat. Genet.*, **5**, 408–412.
80. Okutsu, T., Kuroiwa, Y., Kagitani, F., Kai, M., Aisaka, K., Tsutsumi, O., Kaneko, Y., Yokomori, K., Surani, M.A., Kohda, T., Kaneko-Ishino, T. and Ishino, F. (2000) Expression and imprinting status of human PEG8/IGF2AS, a paternally expressed antisense transcript from the IGF2 locus, in Wilms' tumors. *J. Biochem. (Tokyo)*, **127**, 475–483.
81. Wu, H.K., Squire, J.A., Catzavelos, C.G. and Weksberg, R. (1997) Relaxation of imprinting of human insulin-like growth factor II gene, IGF2, in sporadic breast carcinomas. *Biochem. Biophys. Res. Commun.*, **235**, 123–129.
82. Wu, H.K., Weksberg, R., Minden, M.D. and Squire, J.A. (1997) Loss of imprinting of human insulin-like growth factor II gene, IGF2, in acute myeloid leukemia. *Biochem. Biophys. Res. Commun.*, **231**, 466–472.
83. Weksberg, R., Shuman, C., Caluseriu, O., Smith, A.C., Fei, Y.L., Nishikawa, J., Stockley, T.L., Best, L., Chitayat, D. and Olney, A. *et al.* (2002) Discordant KCNQ1OT1 imprinting in sets of monozygotic twins discordant for Beckwith–Wiedemann syndrome. *Hum. Mol. Genet.*, **11**, 1317–1325.

84. Santos, F., Hendrich, B., Reik, W. and Dean, W. (2002) Dynamic reprogramming of DNA methylation in the early mouse embryo. *Dev. Biol.*, **241**, 172–182.
85. Onyango, P., Jiang, S., Uejima, H., Shamblott, M.J., Gearhart, J.D., Cui, H. and Feinberg, A.P. (2002) Monoallelic expression and methylation of imprinted genes in human and mouse embryonic germ cell lineages. *Proc. Natl Acad. Sci. USA*, **11**, 11.
86. Howell, C.Y., Bestor, T.H., Ding, F., Latham, K.E., Mertineit, C., Trasler, J.M. and Chaillet, J.R. (2001) Genomic Imprinting Disrupted by a Maternal Effect Mutation in the Dnmt1 Gene. *Cell*, **104**, 829–838.
87. Kierszenbaum, A.L. (2002) Genomic imprinting and epigenetic reprogramming: unearthing the garden of forking paths. *Mol. Reprod. Dev.*, **63**, 269–272.
88. Humpherys, D., Eggan, K., Akutsu, H., Hochedlinger, K., Rideout, W.M. III, Biniszkiewicz, D., Yanagimachi, R. and Jaenisch, R. (2001) Epigenetic instability in ES cells and cloned mice. *Science*, **293**, 95–97.
89. Cox, G.F., Burger, J., Lip, V., Mau, U.A., Sperling, K., Wu, B.L. and Horsthemke, B. (2002) Intracytoplasmic sperm injection may increase the risk of imprinting defects. *Am. J. Hum. Genet.*, **71**, 162–164.
90. DeBaun, M.R., Niemitz, E.L. and Feinberg, A.P. (2003) Association of *in vitro* fertilization with Beckwith–Wiedemann syndrome and epigenetic alterations of LIT1 and H19. *Am. J. Hum. Genet.*, **72**, 156–160.
91. Maher, E.R., Brueton, L.A., Bowdin, S.C., Luharia, A., Cooper, W., Cole, T.R., Macdonald, F., Sampson, J.R., Barratt, C.L., Reik, W. and Hawkins, M.M. (2003) Beckwith–Wiedemann syndrome and assisted reproduction technology (ART). *J. Med. Genet.*, **40**, 62–64.
92. Wenstrom, K.D., Syrop, C.H., Hammitt, D.G. and Van Voorhis, B.J. (1993) Increased risk of monochorionic twinning associated with assisted reproduction. *Fertil. Steril.*, **60**, 510–514.
93. Sills, E.S., Tucker, M.J. and Palermo, G.D. (2000) Assisted reproductive technologies and monozygous twins: implications for future study and clinical practice. *Twin Res.*, **3**, 217–223.
94. De Rycke, M., Liebaers, I. and Van Steirteghem, A. (2002) Epigenetic risks related to assisted reproductive technologies: risk analysis and epigenetic inheritance. *Hum. Reprod.*, **17**, 2487–2494.
95. Sapienza, C. (2002) Imprinted gene expression, transplantation medicine, and the "other" human embryonic stem cell. *Proc. Natl Acad. Sci. USA*, **99**, 10243–10245.
96. Olek, A. and Walter, J. (1997) The pre-implantation ontogeny of the H19 methylation imprint. *Nat. Genet.*, **17**, 275–276.
97. Dean, W., Bowden, L., Aitchison, A., Klose, J., Moore, T., Meneses, J.J., Reik, W. and Feil, R. (1998) Altered imprinted gene methylation and expression in completely ES cell-derived mouse fetuses: association with aberrant phenotypes. *Development*, **125**, 2273–2282.
98. Young, L.E., Sinclair, K.D. and Wilmut, I. (1998) Large offspring syndrome in cattle and sheep. *Rev. Reprod.*, **3**, 155–163.