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ARTICLE

Beckwith–Wiedemann Syndrome

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Beckwith–Wiedemann syndrome (BWS) is a clinically heterogeneous overgrowth syndrome associated with an increased risk for embryonal tumor development. BWS provides an ideal model system to study epigenetic mechanisms. This condition is caused by a variety of genetic or epigenetic alterations within two domains of imprinted growth regulatory genes on human chromosome 11p15. Molecular studies of BWS have provided important data with respect to epigenotype/genotype–phenotype correlations; for example, alterations of Domain 1 are associated with the highest risk for tumor development, specifically Wilms' tumor. Further, the elucidation of the molecular basis for monozygotic twinning in BWS defined a critical period for imprint maintenance during pre-implantation embryonic development. In the future, such molecular studies in BWS will permit enhanced medical management and targeted genetic counseling. © 2005 Wiley-Liss, Inc.

KEY WORDS: overgrowth; genomic imprinting; embryonal tumors; chromosome 11p15; imprinted domains; epigenotype; monozygotic twinning

INTRODUCTION

Beckwith–Wiedemann syndrome (BWS) represents a complex disorder both phenotypically and genetically and provides unique opportunities to explore a number of intriguing biological phenomena. Such phenomena include genomic imprinting, monozygotic twins with discordant phenotypes, and genetic contributions to embryonal tumor development. The timing of monozygotic twinning and imprint re-establishment during pre-implantation development appears to be a critical period for the incorporation of epigenetic errors in the plastic embryonic genome. Such epigenetic errors determine a range of phenotypes associated with BWS including the predisposition to embryonal tumor development.

BWS has been documented in a variety of ethnic populations with an incidence of 1/13,700 and is equally represented in males and females [Thorburn et al., 1970; Pettenati et al., 1986]. However, as molecular testing continues to expand the phenotypic spectrum, positive molecular test results in "atypical" cases of BWS, will likely increase the reported incidence. In fact, the phenotypic spectrum of this disorder now appears to include at least a proportion of cases of isolated hemihyperplasia. The syndromic designation of BWS was coined following the first descriptions of this syndrome by Beckwith in 1963 and Wiedemann in 1964. However, artistic depictions suggestive of BWS have been found dating back to the beginning of the Common Era [Beckwith, 1998a].

CLINICAL EVALUATION

Initially, the diagnosis of BWS was defined by the presence of macrosomia (pre-natal and/or post-natal gigantism), macroglossia and abdominal wall defect (omphalocele, umbilical hernia, diastasis recti) [Beckwith, 1963; Wiedemann, 1964]. However, the clinical features of BWS are variable and it is generally accepted that the diagnosis can be established if at least three diagnostic findings are present. Such findings may include those listed above as well as hemihyperplasia, embryonal tumors, adrenocortical cytomegaly, ear anomalies (anterior linear earlobe creases, posterior helical pits), visceromegaly, renal abnormalities, neonatal hypoglycemia, cleft palate, and a positive family history [Pettenati et al., 1986; Elliott et al., 1994a,b; Weng et al., 1995;

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Engstrom et al., 1998]. When there are fewer than three of the above sentinel findings, the following findings may support the diagnosis: polyhydramnios and pre-maturity, enlarged placenta, cardiomegaly or structural cardiac anomalies, nevus flammeus or other hemangiomata, advanced bone age, characteristic facies with midfacial hypoplasia, and monozygotic twining (usually female and discordant). Because of the associated risk for embryonal tumor development, consideration should be given to offering tumor surveillance to individuals when the clinical diagnosis appears equivocal.

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Most individuals with BWS do well both physically and developmentally but approximately 20% die in the perinatal period of complications of pre-maturity, macroglossia, or, rarely, cardiomyopathy [Pettenati et al., 1986; Weng et al., 1995]. Additionally, a proportion of children with BWS will face significant medical management issues and these are addressed in detail elsewhere [Chitayat et al., 1990b; Breslow et al., 1991; Goldman et al., 2003; Weksberg and Shuman, 2004].

The phenotypic variability in BWS reflects its genetic heterogeneity (Fig. 1). BWS is a complex, multigenic disorder caused by alterations in growth regulatory genes on chromosome 11p15 (Fig. 2) [Li et al., 1997, 1998]. As a result, perhaps the easiest approach to understanding the complex etiology involves grouping individuals with BWS according to the clinical assessment and family history. These data along with karyotype and laboratory data are important first steps in categorizing BWS subgroups.

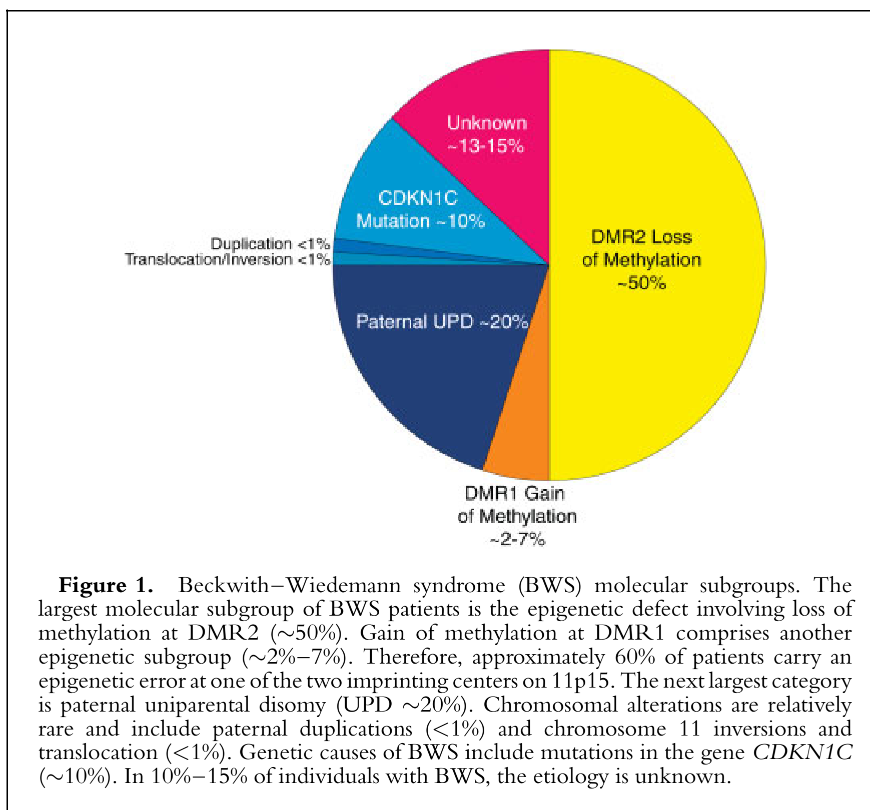

Figure 1. Beckwith–Wiedemann syndrome (BWS) molecular subgroups. The largest molecular subgroup of BWS patients is the epigenetic defect involving loss of methylation at DMR2 (~50%). Gain of methylation at DMR1 comprises another epigenetic subgroup (~2%–7%). Therefore, approximately 60% of patients carry an epigenetic error at one of the two imprinting centers on 11p15. The next largest category is paternal uniparental disomy (UPD ~20%). Chromosomal alterations are relatively rare and include paternal duplications (<1%) and chromosome 11 inversions and translocation (<1%). Genetic causes of BWS include mutations in the gene *CDKN1C* (~10%). In 10%–15% of individuals with BWS, the etiology is unknown.

A large proportion of BWS cases, about 85%, is sporadic and karyotypically normal. Very few individuals are reported with chromosome abnormalities of 11p15. Approximately 10%–15% of cases of BWS are part of autosomal dominant pedigrees demonstrating preferential maternal transmission. Therefore, a detailed family history is an important part of the initial evaluation. Because the phenotype may be variable even within a family and as the facial appearance typically normalizes later in childhood, pedigree review should survey parental birth weights, history of abdominal wall defect, increased tongue size or tongue surgery, and other features of BWS. In adults, the most helpful physical features include prominence of the jaw, enlarged tongue, ear creases and pits, and evidence of repaired omphalocele. Abdominal ultrasound may help to evaluate abnormalities of kidneys and other abdominal organs. Adult heights are usually normal, and other features may be quite subtle or even surgically altered; hence, early childhood photographs are useful adjuncts to family assessment and estimation of recurrence risk.

MOLECULAR/ CYTOGENETIC BASIS OF BWS

In order to understand the molecular basis of BWS one must invoke three concepts: genomic imprinting, somatic mosaicism, and multiple genes. Most genes are normally expressed equally from the paternally and maternally derived alleles. However, genes which are subject to genomic imprinting are expressed predominantly or exclusively from either the maternal or paternal allele in a parent-of-origin specific manner. This parent-of-origin specific imprinting is heritable. During the formation of the germ cell, imprints are erased and re-established based on

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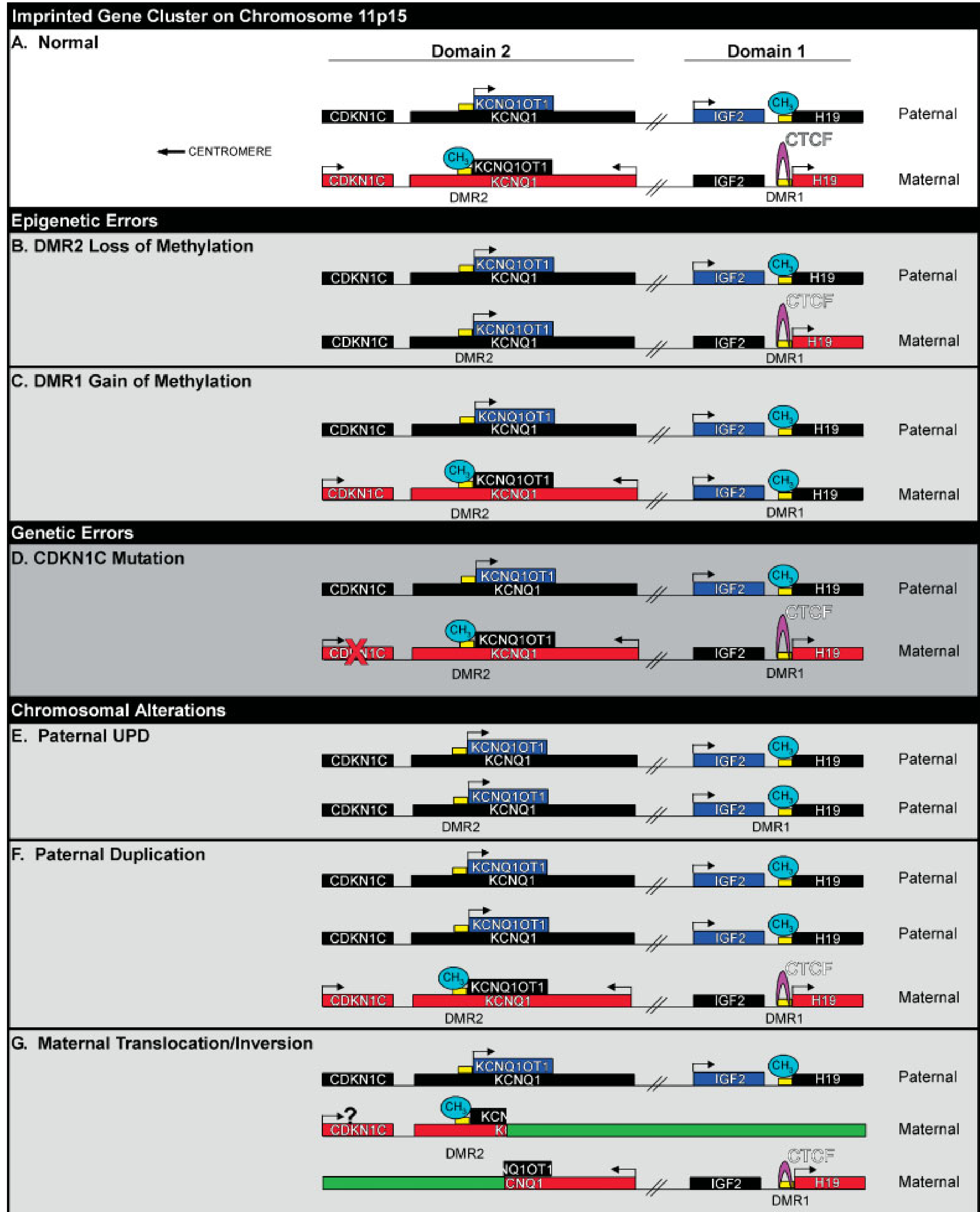

Figure 2.

the sex of the transmitting parent [Barlow, 1994].

The molecular basis of genomic imprinting is epigenetic. That is, heritable changes in gene expression occur without a change in DNA sequence [Wolffe and Matzke, 1999]. These epigenetic mechanisms may include modifications such as DNA methylation, histone protein modification, and chromatin conformation.

Imprinted genes cluster in distinct regions on chromosomes, which are referred to as imprinted domains. These domains are characterized by unique organizational and regulatory systems. An imprinting center is believed to control expression of closely linked imprinted genes [Nicholls, 2000]. Imprinting centers in these domains are characterized by the presence of differential methylation on the paternal and maternal chromosomes, which result in differential *cis*-regulation and transcription of imprinted genes. Therefore, the homologous maternal and paternal genomic regions have different epigenotypes (Fig. 2).

To date there are approximately 40 known imprinted genes in humans, but data from animals suggest that there are likely as many as 100 such genes. In humans, deregulation of imprinting is associated with disease. Such deregulation on chromosome 15 is associated with Prader Willi and Angelman syndromes [Nicholls, 2000] and on chromosome 11 with BWS.

BWS and Genomic Imprinting

The observations of uniparental disomy (UPD), preferential maternal transmission of BWS in autosomal dominant pedigrees, and parent-of-origin effects in chromosome abnormalities associated with BWS together provide convincing evidence that BWS arises from alterations of imprinted genes on 11p15. The genes and imprinted domains of chromosome 11p15 relevant to the Beckwith–Wiedemann are shown in Figure 2. This region spans approximately 1 megabase and includes two imprinted domains, each associated with an imprinting center.

Chromosome 11p15: Imprinted domain 1. Domain 1 contains the two genes insulin-like growth factor 2 (*IGF2*) and *H19* (Fig. 2A). This domain is located on the distal end (telomeric) of the imprinted cluster on 11p15.

IGF2. *IGF2* is a paternally expressed (maternally imprinted) embryonic growth factor. Disruption of *IGF2* imprinting (biallelic expression) has been observed in some patients with BWS [Weksberg et al., 1993] as well as in multiple tumors, including Wilms' tumor [Ogawa et al., 1993; Rainier et al., 1993]. Biallelic IGF2 expression has been found in normal cells from the colons of individuals who develop colon cancer [Niemitz et al., 2004].

H19. *H19* is a maternally expressed (paternally imprinted) gene encoding a biologically active non-translated mRNA that may function as a tumor suppressor [Hao et al., 1993]. The H19 promoter and the associated imprinting center DMR1, several kilobases upstream, are differentially methylated on the two parental chromosomes. Normally, the paternal allele is methylated and the maternal allele is unmethylated (Fig. 2A).

The *H19* and *IGF2* genes are normally coordinately regulated by DMR1 so that on the maternal chromosome only *H19* is expressed whereas on the paternal chromosome only *IGF2* is expressed (Fig. 2A). This expression pattern occurs because DMR1 has binding sites for the insulator protein CTCF, which can bind to unmethylated DNA on the maternal chromosome and inhibit the interaction of the maternal *IGF2* allele with mesodermal and endodermal enhancers downstream of *H19* [Hark and Tilghman, 1998; Hark et al., 1999].

Gain of methylation at the maternal *H19* and DMR1 is associated with loss of *H19* expression and biallelic *IGF2* expression [Joyce et al., 1997]. In 2%–7% of cases of BWS [Bliek et al., 2001; Weksberg et al., 2001] (Fig. 2B) this alteration has been reported and is referred to as *H19*-dependent IGF2 biallelic expression. Gain of methylation of *H19* is almost always seen in sporadic cases without a positive family history. However, there are recent reports of three families that carry heritable DNA sequence abnormalities in DMR1 that can disrupt imprint regulation in Domain 1. These cases exhibit the clinical features of BWS [Sparago et al., 2004; Prawitt et al., 2005].

In many cases of BWS (as well as in isolated Wilms' tumors) biallelic IGF2 expression is accompanied by monoallelic H19 expression. This is referred to as *H19*-independent biallelic expression. The significance of this finding in BWS is not completely understood. These cases show normal methylation and expression of H19 from the maternal allele with biallelic IGF2 expression [Weksberg et al., 2001].

Chromosome 11p15: Imprinted domain 2. Domain 2 is centromeric to Do-

Figure 2. **A**: Imprinted gene cluster on chromosome 11p15 illustrating selected genes. Red boxes represent maternally expressed alleles and blue boxes represent paternally expressed alleles. Arrows represent the direction of transcription. Black boxes denote imprinted alleles that are not expressed. Yellow boxes denote the location of differentially methylated imprinting centers 1 and 2 (DMR1 and DMR2). Light blue circles with CH_3 represent DNA methylation. Two diagonal lines represent an interval of genetic distance not shown. Insulator protein CTCF is shown in purple. **B**: Loss of methylation at DMR2 of BWS patients results in two copies of the "paternal" epigenotype for Domain 2. **C**: Gain of methylation at DMR1 results in H19-dependent IGF2 biallelic expression with loss of H19 expression, i.e., two copies of the paternal epigenotype for Domain 1. **D**: Shows mutations in CDKN1C. **E**: Shows paternal UPD. Patients have two copies of the paternal epigenotype for Domains 1 and 2. **F**: Rare paternal duplications (<1%) carry two copies of the paternal epigenotype and one copy of the maternal epigenotype. **G**: Translocations/inversions (<1%) of maternal origin seen in BWS. The epigenotypes are not yet well characterized.

main 1. Although Domain 2 contains a number of imprinted genes which include: *KCNQ1*, *KCNQ1OT1*, *CDKNIC*, *PHLDA*, and *SLC22A18*, we will limit our discussion here to those genes implicated in BWS and growth regulation.

KCNQ1. *KCNQ1* (previously known as *KvLQT1*) encodes a subunit of a voltage-gated potassium channel. Mutations in this gene have been implicated in several cardiac arrhythmia syndromes (familial atrial fibrillation, Jervell and Lange-Nielsen, and long-QT syndrome 1). *KCNQ1* is maternally expressed in most tissues, with the notable exception of the heart [Lee et al., 1997a].

KCNQ1OT1. *KCNQ1OT1* is a non-coding RNA with antisense transcription to *KCNQ1*. The promoter for *KCNQ1OT1* is located in intron 10 of *KCNQ1*. The 5′ end of this imprinted transcript overlaps with the differentially methylated imprinting center for Domain 2, DMR2 or *KvDMR* (as it was originally called for "*KvLQT1 DMR*") [Lee et al., 1999; Smilinch et al., 1999]. Normally, the maternal allele of DMR2 is methylated and *KCNQ1OT1* is silenced whereas the paternal allele is unmethylated allowing transcription of the KCNQ1OT1 transcript. DMR2 regulates in *cis* the expression of a number of imprinted genes including *CDKN1C* so that preferential expression occurs from the maternal chromosome (Fig. 2C).

Loss of maternal methylation of DMR2 is seen in 50%–60% of patients with sporadic BWS [Lee et al., 1999; Smilinch et al., 1999; Gaston et al., 2000; Bliek et al., 2001; Weksberg et al., 2001]. Deletion of the orthologous sequence in mice results in loss of imprint for several genes neighboring KCNQ1 indicating that this DMR is critical for maintaining imprinted gene expression in Domain 2 [Fitzpatrick et al., 2002]. In human tissue, loss of methylation at DMR2 has been shown to be associated with reduction in CDKN1C (see below) expression and is likely involved in the etiology of BWS.

CDKN1C. The *CDKN1C* gene (that encodes the protein known as $p57^{KIP2}$) is a member of the cyclin-dependent kinase inhibitor family acting to negatively regulate cell proliferation. It is both a tumor suppressor gene and a potential negative regulator of fetal growth. Both these functions and the imprinted expression of this gene suggested it as a candidate for a maternally expressed growth inhibitory gene in BWS.

Mutations in *CDKN1C* have been reported in 5%–10% of sporadic BWS cases (Fig. 2D) [Hatada et al., 1996, 1997; Lee et al., 1997b; Lam et al., 1999; Bliek et al., 2001; Gaston et al., 2001; Li et al., 2001]. Such mutations are found in approximately 40% of BWS cases with a positive family history [O'Keefe et al., 1997; Lam et al., 1999]. However, *CDKN1C* mutations have not been found in all cases of BWS with dominant transmission.

PHLDA2 (also known as *IPL*, *TSSC3*, *BWRIC*) and *SLC22A18* (also known as *ORCTL2*, *TSSC5, BWRIA*, *IMPT1*) are two other imprinted genes in Domain 2 of the 11p15 imprinted region [Qian et al., 1997; Dao et al., 1998]. Both genes show preferential maternal expression in the fetus and are located centromeric to *CDKN1C*. While neither gene has been directly implicated in BWS, both are hypothesized to have negative growth regulatory functions and are thought to be regulated by DMR2 [Fitzpatrick et al., 2002].

11p15 UPD

Approximately, 20% of patients with BWS have paternal UPD, with two paternally derived copies of chromosome 11p15 and no maternal contribution for that region (Fig. 2E) [Henry et al., 1991]. Although the region of UPD varies, UPD for chromosome band 11p15 is always present involving both Domains 1 and 2. The vast majority of patients with UPD exhibit somatic mosaicism. This implies that UPD arises post-zygotically as a result of a somatic recombination. Therefore, it may be found only in some tissues, e.g., in fibroblasts or renal tissue but not in lymphocytes. Since most somatic tissues are not available for testing, the quoted frequency of UPD in BWS almost certainly is an underestimate of the actual frequency.

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This hypothesis is supported by several observations including the risk of embryonal tumor development, the spectrum of tumors, and certain clinical features (birth weight and renal findings) suggesting that at least a proportion of patients with hemihyperplasia represent "mild" BWS [Hoyme et al., 1998]. This is supported by recent findings of 11p15 UPD in cases of isolated hemihyperplasia with or without embryonal tumors [Grundy et al., 1991; Shuman et al., 2002]. Further, another clinical presentation, persistent hyperinsulinemic hypoglycemia, has been reported with somatic mosaicism for 11p15 limited to pancreas [De Lonlay et al., 1997].

11p15 Chromosome Rearrangements

Parent-of-origin-specific chromosomal rearrangements involving 11p15 are associated with the BWS phenotype. Translocations and inversions typically show maternal inheritance [Sait et al., 1994] (Fig. 2G), whereas duplications are typically paternally inherited [Brown et al., 1992; Slavotinek et al., 1997] (Fig. 2F). Patients with 11p15 translocations or inversions exhibit typical features of BWS. These chromosomal alterations can arise de novo or can be vertically transmitted. In contrast, patients with 11p15 duplications may

have atypical clinical features as well as a significant risk of developmental delay [Waziri et al., 1983; Slavotinek et al., 1997]. Duplications of chromosome 11p15 can arise de novo or can result from a balanced paternally derived translocation.

In some individuals, the molecular etiology for BWS remains unidentified. Many of these individuals may have UPD for 11p15, which is undetected due to tissue mosaicism. Alternatively, there may be a mutation or epimutation in genes inside or even outside the 11p15 imprinted cluster that affect establishment of imprinting or tissue-specific expression [Algar et al., 1999]. Additionally, there may be unrecognized mutations in other 11p15 genes.

Phenotype–Genotype/ Epigenotype Correlations

There are positive and negative correlations associated with specific molecular alterations seen in BWS. A highly positive correlation exists between 11p15 UPD and the presence of hemihyperplasia. This most likely reflects the somatic mosaicism associated with 11p15 UPD. For Domain 2, there is a positive correlation for DMR2 alterations and omphalocele as well as for monozygotic twinning (see below) and a positive correlation for *CDKN1C* and omphalocele as well as cleft palate [Lam et al., 1999; Bliek et al., 2001; Gaston et al., 2001; Li et al., 2001; Weksberg et al., 2001; Bliek et al., 2004]. For both of these molecular subtypes in Domain 2 there is a notable absence of Wilms' tumors.

Recent data suggest that the different molecular subgroups of BWS carry distinct tumor risks and susceptibilities to specific tumor profiles. It appears that individuals with 11p15 UPD and *H19* hypermethylation carry the highest tumor risk and preferentially develop Wilms' tumors, whereas BWS cases with loss of methylation at DMR2 have a lower tumor risk and are susceptible to non-Wilms' tumors [Bliek et al., 2001; Weksberg et al., 2001]. These data should not be incorporated into clinical management protocols until they are replicated in larger groups of patients. Even though there are differences in the rates and types of tumors seen in Beckwith–Wiedemann individuals with alterations in Domain 1 versus Domain 2, all BWS patients have a tumor risk which is increased over that in the general population.

PRE-IMPLANTATION EMBRYO—A CRITICAL PERIOD FOR EPIGENETIC ERRORS

Two avenues of investigation related to BWS have suggested that prior to implantation, the human embryo is at risk for epigenetic errors. These investigations include our studies on monozygotic twins discordant for BWS. Also, a number of studies have raised the question that offspring of pregnancies conceived by assisted reproductive technologies are at increased risk of imprinting errors.

Monozygotic Twins Discordant for BWS

There have been multiple reports of monozygotic twins with BWS [Berry et al., 1980; Bose et al., 1985; Litz et al., 1988; Olney et al., 1988; Chien et al., 1990; Clayton-Smith et al., 1992; Franceschini et al., 1993; Orstavik et al., 1995; Leonard et al., 1996]. We had the opportunity to investigate a cohort of monozygotic twins discordant for BWS. The study demonstrated first that the incidence of monozygotic twinning in BWS is dramatically increased and that the majority of this increase is for female rather than male monozygotic twins. Further, in skin fibroblasts from ten monozygotic twin pairs discordant for BWS, only the affected twin showed altered maternal methylation at DMR2, as well as biallelic expression of the antisense transcript of *KCNQ1OT1*. In contrast, hematopoietic cells from both the affected and unaffected twins in these pairs showed similar imprinting abnormalities, likely due to the sharing of blood circulation which is a common feature of monozygotic twinning [Weksberg et al., 2002].

We have postulated that the significant female preponderance in monozygotic twins discordant for BWS and an imprinting defect on chromosome 11 could be related to a variety of sex related factors. One such factor could be the lag in early development of female embryos as compared to male embryos [Hall and Lopez-Rangel, 1996]. This may be secondary to the X-inactivation process, making female monozygotic twin embryos more susceptible to certain developmental errors [Lubinsky and Hall, 1991]. In this regard we suggested that discordance in monozygotic twins for BWS could result from a failure of maintenance methylation during a single cell cycle at or just prior to the twinning event. Thus, the resulting hemimethylated daughter duplex would be converted in the next S phase to a fully methylated and an unmethylated sister chromatid, which would then segregate to different blastomeres and separate in the twinning event. Depending on the timing of twinning it is possible that failure of maintenance methylation could result in mosaicism or complete discordance for imprinting defects in the monozygotic twins.

There are animal models which support such a hypothesis, specifically the genetic studies of the *Dnmt1* gene in mice [Howell et al., 2001]. $Dnmt1_0$ is a specialized oocyte-specific form of the major maintenance DNA methyltransferase required specifically for maintenance methylation of imprinted single copy sequences during the fourth S phase in embryo development. In the absence of $Dnmt1_0$, one-half of the normally imprinted alleles are demethylated and loss of the imprinting phenotype, consistent with mosaic reactivation of normally silenced imprinted alleles, is observed. We suggest that BWS arises when maintenance methylation of DMR2 fails to occur due to the abnormal expression of the human ortholog of $Dnmt1_0$.

In summary, this finding of an increased incidence of monozygotic twins discordant for BWS and failure of DMR2 imprint maintenance directs

our attention to the pre-implantation phase of embryonic development as a critical time period for imprint maintenance at DMR2. Since 50% of BWS individuals carry an epigenetic alteration at *KCNQ1OT1*, which is in most cases not inherited, epigenetic errors in pre-implantation embryonic development would be a credible etiology for epigenetic alterations in singleton BWS cases as well.

Assisted Reproductive Technologies and Imprinting Errors

An independent line of evidence, which implicates the pre-implantation phase of embryonic development as a critical time for imprint maintenance is the finding of an increase in BWS cases, with loss of imprinting at DMR2 in offspring of mothers undergoing assisted reproductive technology (ART). In fact, reports of Angelman syndrome and ART have also been reported indicating that epigenetic errors in early development are not confined to BWS. Three papers [DeBaun et al., 2003; Gicquel et al., 2003; Maher et al., 2003] have reported data suggesting that ART may favor imprinting alterations at the centromeric imprinted 11p15 locus DMR2 and thus may increase the incidence of BWS. These data, although retrospective, highlight the need for follow-up of children born after ART. No specific ART procedure has been shown to increase the risk of BWS to date [Chang et al., 2005]. The procedures of ART that may influence imprinting include the stimulation protocol, the biological technique, the stage of maturation of the gametes, the culture media, and the timing of embryo transfer. Further, it will be important to carefully evaluate whether the underlying issues of infertility rather than the ART procedures in fact pre-dispose to imprinting defects in the pre-implantation phase of embryonic development. Future larger prospective studies will be needed to clarify whether there is indeed a significant increase in the risk of imprinting errors following ART and if so, whether this finding is associated with ART procedures or with the underlying infertility of the parents.

Three papers have reported data suggesting that ART may favor imprinting alterations at the centromeric imprinted 11p15 locus DMR2 and thus may increase the incidence of BWS.

DIAGNOSTIC TESTING

At this time, diagnostic testing is most useful for confirming the diagnosis of BWS and for defining recurrence risks rather than for phenotype/genotype correlations and medical management issues (Table I). One exception to this would involve the finding of a chromosome abnormality such as a duplication of 11p15, because this has

TABLE I. BWS: Genetic, Cytogenetic and Molecular Groups

BWS subgroup	Frequency of BWS cases in this group (%)[1]	Etiology	Inherited/sporadic	Recurrence risk to parents of a child with BWS
Paternal UPD	20	Post-zygotic somatic recombination	Sporadic	Low
DMR2	50–60	Usually epimutation, rarely deletion resulting in epimutation	Usually sporadic	Low, rarely inherited
DMR1	2–7	Rarely deletion resulting in epimutation	Usually sporadic	Low, rarely inherited
11p15 chromosome translocation/inversion	<1	Translocation	Inherited or sporadic	May be as high as 50% if maternal translocation[2]
11p15 chromosome duplication	<1	Duplication	Inherited or sporadic	May be as high as 50% if father is the carrier
CDKN1C mutations	5–10 in sporadic cases 30–50 in AD pedigrees	Mutation	Sporadic or inherited	May be as high as 50% (preferential maternal transmission)[2]

[1]Overall, 85% of BWS cases are sporadic and 15% are associated with vertical transmission. To date 15% of patients with BWS do not have a detected molecular or genetic defect within these subgroups.
[2]Specific figure not known.
UPD, uniparental disomy; AD, autosomal dominant.

a significant association with developmental delay [Slavotinek et al., 1997].

At this time, diagnostic testing is most useful for confirming the diagnosis of BWS and for defining recurrence risks rather than for phenotype/genotype correlations and medical management issues. One exception to this would involve the finding of a chromosome abnormality such as a duplication of 11p15, because this has a significant association with developmental delay.

Therefore, chromosomal analysis including high-resolution banding for chromosome 11 should be undertaken for all children with BWS. Recurrence risks for chromosome abnormalities associated with BWS will depend on the status of the parental karyotypes.

UPD can be assessed by short tandem repeat (STR) analysis of multiple 11p15 loci or by methylation studies of imprinted regions on chromosome 11p15. Because all cases of UPD associated with BWS reported to date involve somatic mosaicism, failure to detect UPD in one tissue (usually lymphocytes) is not conclusive. One should consider obtaining another tissue (such as skin from the overgrown region), especially in the event of surgery. The presence of mosaicism for 11p15 UPD would confer a low recurrence risk for future sibs, as this results from a post-zygotic event.

Loss of methylation at DMR2 is detected by methylation analysis of the 5′ region of this gene and is offered through a variety of service laboratories. Gain of methylation at DMR1 or *H19* can also be detected by methylation analysis. These primary epigenetic defects are usually associated with very low recurrence risks (Table I). However, for both DMR1 and DMR2 there have been rare reports of familial transmission of the methylation defect. In each of these cases, the epigenetic change has been associated with a deletion of the primary DNA sequence [Niemitz et al., 2004; Sparago et al., 2004].

CDKN1C testing has very recently become available as a clinical diagnostic test. If a mutation in *CDKN1C* is found in a child with BWS, the parents should be offered testing because this mutation can be associated with recurrence risks up to 50% depending on the sex of the transmitting parent. Although mutations in *CDKN1C* are usually maternally transmitted, both parents should be tested because there have been two cases of paternal transmission of a *CDKN1C* mutation associated with BWS in the child [Lee et al., 1997b; Li et al., 2001]. If no mutation were found in either parent, prenatal testing for recurrence of a *CDKN1C* mutation remains an option in view of the theoretical possibility of gonadal mosaicism. There are no published cases of such a situation in BWS.

Currently, IGF2 expression studies remain research tools and should not be considered part of the routine diagnostic work-up for individuals with BWS.

DIFFERENTIAL DIAGNOSIS

There are a number of overgrowth syndromes that should be considered in the differential diagnosis of children presenting with macrosomia or other features of BWS. The possibility of maternal diabetes mellitus during pregnancy should be considered and investigated. In addition, the presence of features not commonly associated with BWS might suggest other diagnoses. Some children with hypotonia appear to have enlarged tongues. Especially when developmental delay is present, other diagnoses should be seriously considered. Several syndromes with phenotypes overlapping that of BWS are discussed below. Some cases involving overgrowth do not fit into any of these defined syndromes, but likely represent, other new overgrowth syndromes yet to be defined.

Simpson–Golabi–Behmel syndrome shares the following features with BWS: macrosomia, visceromegaly, macroglossia, and renal cysts. In addition, individuals with Simpson–Golabi–Behmel syndrome can have distinctive and coarse facial features, cleft lip, a high frequency of cardiac defects [Lin et al., 1999], supernumerary nipples, polydactyly, and other skeletal anomalies. Simpson–Golabi–Behmel syndrome has an increased risk of neonatal mortality, and an increased risk for developing embryonal tumors, including Wilms' tumor and hepatoblastoma [Yong, 2000, personal communication]. The actual risk figure for tumor development is not known. Simpson–Golabi–Behmel syndrome is caused by mutations in an X-linked gene, *GPC3*, encoding an extra-cellular proteoglycan (glypican-3) believed to function in growth regulation [Weksberg et al., 1996; Neri et al., 1998].

Sotos syndrome is characterized by pre- and post-natal overgrowth, macrocephaly, variable mental retardation, distinctive facial features including a prominent forehead with receding hairline, downslanting palpebral fissures, pointed chin, and advanced bone age [Cole and Hughes, 1994]. Deletions and point mutations of the *NSD1* gene account for >60% of cases of Sotos syndrome [Kurotaki et al., 2002]. Recently, Baujat et al. [2004] identified two cases with features of Sotos syndrome and 11p15 UPD. As well there have been two cases reported of *NSD1* mutations in individuals with features of BWS and mental retardation [Baujat et al., 2004].

Perlman syndrome is defined by macrosomia, increased risk of neonatal mortality, mental retardation, nephroblastomatosis, and a high incidence of bilateral Wilms' tumor, occurring usually in the first year of life. The characteristic facial appearance includes a round face, upsweep of anterior scalp hair, depressed nasal bridge, and micrognathia. At present, the molecular basis of Perlman syndrome is unknown, but it likely represents a distinct genetic entity

given its autosomal recessive inheritance [Greenberg et al., 1986; Grundy et al., 1992].

Costello syndrome can present in the neonatal period, in the guise of "overgrowth," due to the presence of edema and cardiac defects. These patients can easily be distinguished from BWS patients over time by a number of findings, including their distinctive facial coarsening and failure to thrive [Johnson et al., 1998; van Eeghen et al., 1999].

Hemihyperplasia may be a feature of a number of syndromes other than BWS, including neurofibromatosis type 1, Klippel–Trenaunay syndrome, Proteus syndrome, McCune–Albright syndrome, epidermal nevus syndrome, triploid/diploid mixoploidy, Maffucci syndrome, and osteochrondomatosis or Ollier disease [Hoyme et al., 1998].

CLINICAL MANAGEMENT

There are a number of medical management issues for children with BWS which can be followed and managed according to standard pediatric protocols, e.g., neonatal hypoglycemia, abdominal wall defects and renal dyplasia. This section will address the clinical management of issues specific to BWS including macroglossia and neoplasia.

Macroglossia is typically present at birth and involves increased length and thickness of the tongue. Depending upon the degree of severity, macroglossia can lead to complications involving feeding and respiration in infancy. Later, macroglossia can impede speech articulation and lead to malocclusion as the growth of the mandible is guided, at least in part, by the size of the tongue. As the facial structures grow in childhood, mild to moderate macroglossia can usually be accommodated. However, it is recommended that longitudinal assessments can be undertaken for children with moderate to significant macroglossia and ideally, these should be carried out by a multidisciplinary craniofacial team involving plastic surgeons, orthodontists, and speech pathologists. Tongue resection may alleviate cosmetic concerns and speech difficulties and may be undertaken in order to minimize the need for jaw reduction surgery in adolescence. However, in our experience, it appears that for some children with BWS, jaw reduction surgery may still be required even after tongue resection [Zuker, 1999].

Children with BWS have an increased risk for embryonal tumor development, primarily within the first 5–8 years of age [Sotelo-Avila et al., 1980; Wiedemann, 1983; Pettenati et al., 1986]. The most common tumors include Wilms' tumor and hepatoblastoma, but others including rhabdomyosarcoma, adrenocortical carcinoma, and neuroblastoma have been reported [Chitayat et al., 1990a; Bliek et al., 2001; Gaston et al., 2001; Smith et al., 2001; Weksberg et al., 2001].

The overall risk for tumor development in children with BWS is approximately 7.5% [Wiedemann, 1983] but it appears that a number of factors may influence this risk figure, including the presence of hemihyperplasia [Wiedemann, 1983], nephromegaly [DeBaun et al., 1998], and nephrogenic rests or nephroblastomatosis [Coppes et al., 1999]. In addition, the specific

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molecular etiology for BWS may also influence the risk for tumor development as noted previously. Even though there are differences in the rates and types of tumors seen in Beckwith–Wiedemann individuals with alterations in Domain 1 versus Domain 2, all BWS patients have a tumor risk which is increased over that in the general population. Regardless of the molecular findings, tumor surveillance is recommended for all children with a diagnosis or suspected diagnosis of BWS and this screening should not at this time be adjusted according to molecular testing results.

Tumor surveillance currently recommended includes quarterly evaluation with abdominal ultrasound to the age of 8 years [Craft et al., 1995; Beckwith, 1998b; DeBaun et al., 1998; Borer et al., 1999; Choyke et al., 1999] as well as serum alpha fetoprotein (AFP) to the age of 5 years. AFP levels may be higher in children with BWS in

Tumor surveillance currently recommended includes quarterly evaluation with abdominal ultrasound to the age of 8 years as well as serum AFP to the age of 5 years.

infancy [Everman et al., 2000] and in such cases, monthly AFP assay should be undertaken along with a baseline liver function test. It is important to establish that the level of AFP is decreasing rather than increasing. In the event of rising AFP levels or any ultrasound finding suspicious for neoplasm, a referral to a pediatric oncologist should be undertaken [Vaughan et al., 1995]. Lastly, a baseline MRI imaging of the abdomen has been recommended at the time of diagnosis [Clericuzio et al., 1992; Gylys-Morin et al., 1993; Beckwith, 1998b].

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