Renal Abnormalities in Beckwith-Wiedemann Syndrome Are Associated with 11p15.5 Uniparental Disomy

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Abstract. Beckwith-Wiedemann syndrome (BWS) is a somatic overgrowth syndrome characterized by a variable incidence of congenital anomalies, including hemihyperplasia and renal malformations. BWS is associated with disruption of genomic imprinting and/or mutations in one or more genes encoded on 11p15.5, including CDKN1C (p57KIP2). It was hypothesized that genotypic and epigenotypic abnormalities of the 11p15.5 region affecting CDKN1C were associated with renal abnormalities. Medical records for 159 individuals with BWS were reviewed. All underwent at least one abdominal ultrasonographic evaluation. Testing for paternal uniparental disomy (UPD) at 11p15.5, CDKN1C mutations, and imprinting defects at KvDMR1 was performed for 96, 32, and 47 patients, respectively. Of the 159 patients, 67 (42%) exhibited renal abnormalities, mainly nephromegaly (25%), collecting system abnormalities (11%), and renal cysts (10.5%). The frequency of renal lesions among patients who were tested for genetic abnormalities did not differ from that among patients who were not tested. Paternal UPD was demonstrated in 22 of 96 cases (23%), CDKN1C mutations in eight of 32 cases (25%), and KvDMR1 imprinting defects in 21 of 47 cases (45%). The 22 UPD-positive patients exhibited a significantly higher incidence of renal abnormalities (P = 0.0026). Surprisingly, the eight patients with CDKN1C mutations exhibited no significant increase in the incidence of renal lesions (P = 0.29). Imprinting defects at KvDMR1, which might downregulate CDKN1C, were also not associated with a significant difference in the incidence of renal disease. Whereas UPD at 11p15.5 in BWS was associated with a higher incidence of renal abnormalities, mutations at CDKN1C and KvDMR1 imprinting defects were not, suggesting that imprinted genes on 11p15.5 other than CDKN1C are critical for renal development.

Beckwith-Wiedemann syndrome (BWS) is a congenital disorder characterized by somatic overgrowth, macroGLOSSIA, anterior abdominal wall defects, and macromosia. Additional but variable features include a typical facial appearance, ear lobe creases or pits, facial nevus flammeus, hemihyperplasia, neonatal hypoglycemia, organomegaly, genitourinary abnormalities, and a predisposition to develop embryonal tumors (1,2). Renal abnormalities have been observed in up to 59% (range, 25 to 59%) of patients with BWS (2–4). The most common renal abnormalities are nephromegaly (19 to 59%), simple cysts (13 to 19%), hydrenephrosis (12 to 14%), and medullary cysts (5 to 13%). Other, less common findings include nephrocalcinosis (with or without nephrolithiasis) (4%), increased cortical and pyramidal echogenicity (4%), a double collecting system (4%), and caliceal diverticula (1.3%). The molecular mechanisms causing these renal abnormalities are undefined.

BWS is associated with disruption of genomic imprinting of one or more genes in the 11p15.5 region. Genomic imprinting is a process that results in parent-of-origin-specific gene expression. Approximately 20% of sporadic BWS cases demonstrate mosaicism for paternal uniparental disomy (UPD), with two paternally derived copies of chromosome 11p15.5 and no maternal contribution for that region (5,6) (Figure 1). In approximately 50% of BWS cases, there is an epigenetic alteration at KvDMR1, which is a differentially methylated region at the 5'-end of an antisense transcript (KCNQ1OT1) that is not translated. In normal individuals, the KCNQ1OT1 transcript is imprinted with monoalectic expression from the paternally derived allele. The epigenetic alteration in 50% of BWS cases involves loss of maternal methylation at KvDMR1 and biallelic expression of the antisense transcript KCNQ1OT1. KvDMR1/KCNQ1OT1 has been proposed as an imprinting control center for the proximal cluster of imprinted genes in the 11p15.5 region. These genes include CDKN1C (also known as

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biallelic expression of \( \text{IGF2} \) and \( \text{H19} \) (8,9). Rarely, a primary methylation change at the presence of normal \( \text{H19} \) CH\(_3\). Active transcription of an allele of an imprinted gene is represented by a white box with an arrow, whereas the silenced allele is indicated in black. The double hatch-marks represent several hundred kilobases of 11p15.5 that are not represented.

**Figure 1.** Map of imprinted genes on chromosome 11p15.5. Maternal (Mat) and paternal (Pat) contributions are indicated. The \( \text{KvDMR1} \) region in intron 10 of \( \text{KCNQ1} \) is represented by a gray box within the body of the \( \text{KCNQ1} \) gene, and its allelic methylation is represented as \( \text{CH}_3 \). Active transcription of an allele of an imprinted gene is represented by a white box with an arrow, whereas the silenced allele is indicated in black. The double hatch-marks represent several hundred kilobases of 11p15.5 that are not represented.

\( p^{57\text{KIP2}} \), \( \text{KCNQ1} \) (\( \text{KVLQ} \text{T1} \)), \( \text{SLC22A1} \), and \( \text{TSSC3} \). \( \text{CDKN1C} \), a maternally expressed gene encoding a cyclin-dependent kinase inhibitor, is a negative regulator of fetal growth. Mutations in this gene are detected in approximately 40% of familial BWS cases and approximately 5% of nonfamilial cases (5,7). A second, more telomeric, imprinted gene cluster on chromosome 11p15.5 includes insulin-like growth factor 2 (\( \text{IGF2} \)), which is a paternally expressed fetal growth factor, and \( \text{H19} \), which is an untranslated RNA sequence. In 30% of BWS cases, biallelic expression of \( \text{IGF2} \) occurs in the presence of normal \( \text{H19} \) expression and promoter methylation (8,9). Rarely, a primary methylation change at the \( \text{H19} \) promoter, affecting \( \text{H19} \) expression, occurs. In these cases, loss of maternal monoallelic expression of \( \text{H19} \) is associated with biallelic expression of \( \text{IGF2} \).

Investigation of the genetic features of BWS has begun to provide insight into the molecular mechanisms that control the BWS phenotype. Positive correlations exist between UPD at 11p15.5 and hemihyperplasia (5) and tumors (10); between mutations in \( \text{CDKN1C} \) and omphalocele (7) and cleft palate (11). Studies in genetic murine models demonstrated that \( \text{IGF2} \) and \( \text{CDKN1C} \) control growth and morphogenesis during kidney development (12,13). Although the renal phenotype in experimental murine models paralleling BWS gain-of-function \( \text{IGF2} \) mutations demonstrated striking nephromegaly and general overgrowth (14), loss-of-function \( \text{CDKN1C} \) mutations produced cystic renal disease, as observed in BWS cases. Therefore, we hypothesized that \( \text{CDKN1C} \) is the gene responsible for the dysplastic/cystic renal disease component of BWS. We predicted that the highest rate of renal abnormalities would be observed in cases with \( \text{CDKN1C} \) downregulation, including (1) \( \text{CDKN1C} \) mutations, (2) loss of \( \text{KvDMR1} \) methylation (which might downregulate \( \text{CDKN1C} \) expression), and (3) 11p15 UPD (which results in a decrease in \( \text{CDKN1C} \) expression and an increase in \( \text{IGF2} \) expression) (Figure 3). Therefore, we analyzed the renal phenotypes in these molecular subgroups of BWS (\( n = 159 \)), including 67 cases with renal abnormalities. Surprisingly, we observed that renal abnormalities were associated with paternal 11p15.5 UPD in BWS but not with \( \text{CDKN1C} \) mutations or \( \text{KvDMR1} \) epigenetic lesions, suggesting that genes in the 11p15.5 region other than \( \text{CDKN1C} \) are responsible for the renal anomalies in BWS.

**Materials and Methods**

**Patients and Clinical Procedures**

Medical records for 159 individuals with diagnoses of BWS were reviewed. Of these, 72 were male and 87 were female. The median age at the time of the last clinical review was 6.5 yr (range, 13 to 39 mo). Seventy-six patients were monitored at the Hospital for Sick Children (HSC) (Toronto, Ontario, Canada), and 83 were referred from other centers in North America. The clinical diagnosis of BWS was based on the presence of at least three of the following features (or at least two of these features plus one of neonatal hypoglycemia, abdominal organomegaly, or renal malformation): macrosomia, macroglossia, hemihyperplasia, ear creases/pits, abdominal wall defects (omphalocele or umbilical hernia), or embryonal tumors.

All patients monitored at HSC were offered abdominal computed tomographic or magnetic resonance imaging assessments at the age of 6 mo or at the time of diagnosis (if patients presented at >6 mo of age). All patients underwent abdominal ultrasonography every 3 mo until 8 yr of age. If patients with BWS presented at >8 yr of age, they underwent at least one ultrasonographic evaluation. Further imaging investigations, including renal scanning, voiding cystourethrogram, and excretory urography, were performed if clinically indicated. For patients referred from institutions other than HSC, data from at least one ultrasonographic evaluation were available.

DNA or RNA was obtained for analyses of chromosome 11 UPD, \( \text{CDKN1C} \) mutations, and \( \text{KvDMR1} \) methylation status. UPD testing results are reported for the 96 cases (48 from HSC and 48 from other centers) for which a recently developed, highly informative, sensitive test was applied (15). \( \text{CDKN1C} \) testing was performed for patients with a family history of BWS and/or clinical features suggesting a \( \text{CDKN1C} \) mutation (e.g., omphalocele or cleft palate) (16). Testing for \( \text{KvDMR1} \) methylation status, with a relatively recently developed test, was performed for recently recruited patients. Of the 96 patients tested for UPD, 21 were tested for \( \text{CDKN1C} \) mutations and 34 were tested for \( \text{KvDMR1} \) methylation status. All three tests were performed for 17 patients.

A subset of the patients described here were described elsewhere (15) in an analysis of tumor predisposition. These studies were approved by the Research Ethics Board of the HSC.

**Cell Cultures**

Lymphoblast lines were maintained in RPMI 1640 medium supplemented with 15% fetal calf serum. Fibroblast strains were maintained for <10 passages in a-minimal essential medium supplemented with 10% fetal calf serum.

**Chromosome 11 UPD Analysis**

Chromosome 11 UPD analysis was performed as described previously (15). Briefly, genomic DNA was extracted from either peripheral blood, cultured lymphoblasts, or skin fibroblasts from the probands and their parents. Quantitative multiplex-PCR, using highly polymorphic short tandem repeat (STR) markers, was performed with
three markers within (TH, D11S2362, and D11S1997) and two markers distal to (D11S1998 and D11S1974) the BWS critical region at 11p15.5, to detect somatic cell rearrangements giving rise to paternal UPD of the chromosome 11p15.5 region. The percentage of paternal UPD of alleles at 11p15.5 in the probands was determined from informative alleles for at least two DNA markers within the BWS critical region that exhibited dosage increases of >20%, as determined with the following calculation: (peak area of paternal allele – peak area of maternal allele)/(peak area of paternal allele + peak area of maternal allele). Allele dosages were measured because mosaicism for 11p15.5 UPD exists among these patients.

Southern Blot Analysis of KvDMR1
For analysis of KvDMR1 methylation, genomic DNA was digested with EcoRI and NolI. Digestion products were subjected to electrophoresis on 0.8% agarose gels and were then transferred to GeneScreen Plus membranes (NEN, Boston, MA). Blots were hybridized by using the [32P]dCTP-labeled, DMR probe (a kind gift from M. Higgins Roswell, Park Cancer Institute, Buffalo, NY) (17). The methylation index was determined by dividing the OD of the 4.2-kb band by the combined OD values of the 4.2- and 2.7-kb bands (17).

CDKN1C Mutation Screening
CDKN1C mutation screening was performed as described by Li et al. (16).

Statistical Analyses
Genotype-phenotype correlations between 11p15.5 UPD, CDKN1C mutations, KvDMR1 methylation, and renal abnormalities were assessed by using the χ² test. P values of <0.05 were considered statistically significant.

Results
Renal Abnormalities among Patients with BWS
Nonmalignant Lesions. Nonmalignant renal abnormalities occur with an incidence of up to 59% among patients with BWS. Nephromegaly accounts for approximately one-half of these lesions (2–4). Of our 159 patients with BWS, 67 (42%) exhibited renal lesions (Table 1). Nephromegaly was the most common finding (25%), followed by abnormalities of the collecting system (renal calyces, pelvis, and ureters) (11%). Hydronephrosis was present in 10 of 17 patients with collecting system abnormalities. For six of those patients, the cause was not defined, despite imaging of the kidney and urinary tract. For another three, the hydronephrosis was mild and the cause was not investigated. Yet another patient exhibited moderate hydronephrosis and hydroureter secondary to vesicoureteral reflux. Interestingly, severe vesicoureteral reflux (grade 4 to 5) was detected for one patient for whom hydronephrosis was not detected by ultrasonography. Four patients exhibited caliceal diverticula, as detected by ultrasonography and confirmed by computed tomography. Three patients exhibited a double collecting system. Simple cortical cysts were detected for five (3%) and multiple medullary cysts for 12 (7.5%) of our patients. Nephrocalcinosis and/or nephrolithiasis was observed for six patients (4%) in this study. Increased cortical echogenicity was observed for two patients (1%). Taken together, our results demonstrated nonmalignant renal abnormalities in 42% of our cases. The most common abnormality was nephromegaly (25%), followed by collecting system abnormalities (11%).

Malignant Lesions and Hemihyperplasia. Estimates of the risk of tumor development among patients with BWS vary widely (4 to 21%) (1,18,19). In this series, tumors were diagnosed for 22 of 159 patients (14%), including hepatoblastomas in seven cases, adrenal carcinoma in one, rhabdomyosarcoma in one, and Wilms’ tumor in 13 (8% of all patients). The risk of Wilms’ tumor in BWS is significantly increased among patients with hemihyperplasia and/or nephromegaly (1,18,20).

Previous studies demonstrated that hemihyperplasia occurs in approximately 25% of BWS cases (2). In this study, hemihyperplasia was detected for 53 of 159 patients (33%) and was more frequent among patients with tumors (11 of 22 patients), compared with those without tumors (43 of 137 patients) (P = 0.02). Therefore, patients in this series demonstrated a 14% incidence of tumor formation and a positive association between hemihyperplasia and tumor formation, consistent with previous reports.

Genetic Abnormalities among Patients with BWS
Several regional or single-gene alterations have been demonstrated among patients with BWS (6). These abnormalities encompass regions of 11p15.5 that encode genes including IGF2 and CDKN1C. Because loss-of-function CDKN1C mutations in mice affect renal morphogenesis (12,13), we hypothesized that genotypic and epigenotypic abnormalities of the 11p15.5 region affecting CDKN1C might be associated with renal abnormalities. To investigate this possibility, we first identified imprinting abnormalities and CDKN1C mutations among patients for whom biologic samples were available. To determine whether the subsets of patients for whom genetic testing was performed were representative of the total population, we compared phenotypes between the groups. Our results demonstrated that the incidences of renal lesions, hemihyperplasia, and tumors were similar among patients who were tested for UPD and those who were not (tested for UPD versus not tested: renal lesions, P = 0.402; hemihyperplasia, P =

Table 1. Renal abnormalities among patients with Beckwith-Wiedemann syndrome

<table>
<thead>
<tr>
<th>Renal Abnormality</th>
<th>No. of Patients</th>
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<tbody>
<tr>
<td>Nephromegaly</td>
<td>39 (25%)</td>
</tr>
<tr>
<td>Collecting system abnormalities</td>
<td>17 (11%)</td>
</tr>
<tr>
<td>Wilms’ tumor</td>
<td>13 (8%)</td>
</tr>
<tr>
<td>Medullary cysts</td>
<td>12 (7.5%)</td>
</tr>
<tr>
<td>Simple cortical cysts</td>
<td>5 (3%)</td>
</tr>
<tr>
<td>Nephrocalcinosis or nephrolithiasis</td>
<td>6 (4%)</td>
</tr>
<tr>
<td>Increased cortical echogenicity</td>
<td>2 (1%)</td>
</tr>
<tr>
<td>Total</td>
<td>67 (42.5%)</td>
</tr>
</tbody>
</table>

a. Hydronephrosis and/or hydroureter, 10; vesicoureteral reflux, 1; double collecting system, 3; caliceal diverticula, 4.

b. Nineteen patients exhibited two renal abnormalities and four exhibited three.
0.302; tumors, \( P = 0.735 \)). Similarly, we could not detect differences in these abnormalities between individuals who were tested and those who were not tested for mutations in \( CDKN1C \) (tested for \( CDKN1C \) versus not tested: renal lesions, \( P = 0.847 \); hemihyperplasia, \( P = 0.485 \); tumors, \( P = 0.805 \)) or methylation at \( KvDMR1/KCNQ1OT1 \) (tested for methylation at \( KvDMR1/KCNQ1OT1 \) versus not tested: renal lesions, \( P = 0.260 \); hemihyperplasia, \( P = 0.902 \); tumors, \( P = 0.450 \)). These data demonstrated that the subsets of patients with BWS who were tested for 11p15 UPD, methylation at \( KvDMR1 \), or \( CDKN1C \) mutations were representative of our BWS population.

**Imprinting Abnormalities**

We tested for imprinting abnormalities by determining the methylation status of \( KvDMR1 \) and the presence of paternal 11p15 UPD. Whereas only the paternal allele of \( KCNQ1OT1 \) is expressed in normal individuals, in association with methylation of the maternal \( KvDMR1 \) allele (Figure 1), there is biallelic expression of \( KCNQ1OT1 \) in approximately 50% of patients with BWS, in association with the loss of maternal methylation at \( KvDMR1 \). We tested 47 patients for methylation at the \( KvDMR1 \) locus and observed loss of maternal methylation in 21 of 47 non-UPD cases (45%), consistent with previous reports (Figure 2B). Paternal UPD at 11p15.5 is observed in approximately 20% of BWS cases (6). We tested 96 patients for 11p15.5 UPD, using quantitative multiplex-PCR of highly polymorphic STR markers within the BWS critical region. We detected UPD in 22 of 96 cases (23%), consistent with previous reports (Figure 2C).

**CDKN1C Mutations**

\( CDKN1C \) (p57KIP2), which encodes a cyclin-dependent kinase inhibitor, is maternally expressed in normal individuals. Because mutations in \( CDKN1C \) occur in both familial and nonfamilial BWS cases and homozygous mutational inactivation of murine \( CDKN1C \) causes renal dysplasia, we tested for mutations by sequencing the entire coding region (Figure 2A). Individuals with a positive family history were first screened for \( CDKN1C \) mutations. Mutations were detected for eight of 32 patients. Of these eight patients, three were derived from a single family and carried the same mutation; therefore, the rate of mutation in our familial cases was 20%, consistent with previously published data (16).

**Genotype-Phenotype Correlations**

Characterization of the clinical features and genetic/epigenetic abnormalities of a representative subset of patients with BWS provided the basis for determining whether downregulation of \( CDKN1C \), via mutation or imprinting defects (such as 11p15.5 UPD or loss of methylation at \( KvDMR1 \)), was positively associated with the occurrence of renal abnormalities (Table 2). Of interest, 11p15 UPD-positive patients demonstrated a significantly higher incidence of renal abnormalities \((P = 0.0026)\). Patients with BWS and mosaicism for 11p15 UPD also demonstrated significantly higher incidences of hemihyperplasia and tumors, compared with 11p15 UPD-negative cases, as previously reported (5,10). To determine the contributions of hemihyperplasia and tumors to the new renal association demonstrated by our data, we performed a subgroup analysis of patients with renal lesions but without hemihyperplasia or tumors. For these patients, a positive statistical correlation was demonstrated between 11p15 UPD and renal lesions \((P = 0.017 \text{ and } P = 0.032, \text{ respectively})\). This positive correlation persisted when we controlled for the presence of nephromegaly \((P = 0.003)\) or both nephromegaly and Wilms’ tumor \((P = 0.003)\). Surprisingly, we could observe no statistically significant correlations between \( CDKN1C \) mutations and renal lesions or between hemihyperplasia and tumors, although there was a tendency toward lower incidences of these abnormalities among patients with \( CDKN1C \) mutations (Table 2). Similarly, we could not demonstrate significant correlations between the incidences of renal lesions, hemihyperplasia, or tumors (mostly Wilms’ tumors) and abnormal imprinting at the \( KvDMR1 \) locus \((P = 0.181, P = 0.60, \text{ and } P = 0.99, \text{ respectively})\). Our results confirm previously reported results suggesting positive correlations between UPD and hemihyperplasia and tumor formation. More importantly, our data provide the first demonstration that renal lesions in BWS are positively associated with paternal UPD at 11p15.5 but not with \( CDKN1C \) mutations or imprinting defects at \( KvDMR1 \).

**Discussion**

This study represents, to our knowledge, the first analysis of correlations between renal abnormalities and molecular defects among patients with BWS. On the basis of data obtained with mice (12), we hypothesized that downregulation of \( CDKN1C \) would be associated with renal malformations among patients with BWS. Consistent with our hypothesis, we expected that renal malformations would be positively associated with \( CDKN1C \) mutations in BWS, as well as with two imprinting defects that decrease \( CDKN1C \) activity, i.e., \((1)\) paternal UPD, in which \( CDKN1C \) activity is reduced because of the presence of two paternally derived \( CDKN1C \) alleles, and \((2)\) \( KvDMR1 \) imprinting defects, which might cause downregulation of \( CDKN1C \). Therefore, we investigated genotype-phenotype correlations for renal abnormalities among patients with BWS and \( CDKN1C \) mutations, 11p15.5 UPD, and \( KvDMR1 \) loss of imprinting. Of the 159 patients in this study, 67 (42%) exhibited a variety of renal abnormalities (Table 1). The 11p15 UPD-positive patients exhibited a significantly higher incidence of renal abnormalities, in contrast to patients with \( CDKN1C \) mutations or \( KvDMR1 \) loss of imprinting (Table 2). Our results demonstrated a novel association between 11p15.5 UPD and renal lesions among patients with BWS.

In BWS, paternal 11p15.5 UPD is observed in approximately 20% of cases and abnormal \( KvDMR1 \) methylation in approximately 50% of cases (5–7). Our findings of 23% of cases with paternal 11p15.5 UPD and 45% with abnormal \( KvDMR1 \) methylation are consistent with these results. Five percent of sporadic cases and 40% of familial cases have been reported to involve \( CDKN1C \) germline mutations (5–7). We observed \( CDKN1C \) mutations for eight of 32 patients (25%) by
Figure 2. (A) CDKN1C mutation analysis. (Left) Normal sequence for nucleotides 426 to 438 in exon 2 of CDKN1C. (Right) Heterozygous C→T mutation at nucleotide 432 (numbered relative to the published cDNA sequence; Genbank Accession No. 4557440), resulting in a single amino acid substitution of a glutamine to leucine at residue 58 of the 316-amino acid protein. (B) DNA methylation analysis of KvDMR1. Southern blots of DNA samples digested with NotI and EcoRI hybridized with a [32P]dCTP-labeled DMR probe are presented. The upper band (4.2 kb) is of maternal origin and the lower band (2.7 kb) of paternal origin. Blots indicate a control subject with a methylation index of 0.50 (left), a patient with Beckwith-Wiedemann syndrome (BWS) with a methylation index of 0.24 (center), and a patient with BWS with a methylation index of 0 (right). LOM, loss of methylation. (C) Quantitative multiplex-short tandem repeat analysis to detect paternal chromosome 11 UPD. In a child with BWS and paternal UPD11, an increase in dosage of the paternally derived allele, compared with the maternally derived allele, is observed at DNA markers within the 11p15.5 region [D11S1997 (blue), D11S2362 (green), and TH (black)]. This is in contrast to the normal dosage of two DNA markers on the q arm of chromosome 11 [D11S1974 (green) and D11S1998 (black)].
Table 2. Genotype (UPD and CDKN1C)-phenotype (renal lesions, hemihyperplasia, and tumor) analysis\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>No. of Patients</th>
<th>(P) Value</th>
<th>No. of Patients</th>
<th>(P) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UPD-Positive</td>
<td>UPD-Negative</td>
<td>CDKN1C-Positive</td>
<td>CDKN1C-Negative</td>
</tr>
<tr>
<td>Renal lesions</td>
<td>16/22</td>
<td>27/74</td>
<td>0.0026</td>
<td>2/8</td>
</tr>
<tr>
<td>Hemihyperplasia</td>
<td>15/22</td>
<td>20/74</td>
<td>0.0004</td>
<td>0/8</td>
</tr>
<tr>
<td>Tumors</td>
<td>6/22</td>
<td>8/74</td>
<td>0.054</td>
<td>0/8</td>
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</table>

\(a\) UPD, uniparental disomy.

who were tested. Three of the patients were from a single family, and five were from different families. The relatively high incidence is explained by the fact that a group of individuals with positive family histories was included in the study. Although not all of our patients underwent molecular testing, we determined that the incidences of renal lesions, hemihyperplasia, and tumors were similar for patients who were tested for UPD, CDKN1C mutations, and KvDMR1 methylation and those who were not tested.

Abnormalities of the renal collecting system that are associated with BWS include hydronephrosis and/or hydrourerter, vesicoureteral reflux, double collecting system, and caliceal diverticula. These abnormalities have been observed in approximately 14% of BWS cases (2,3). Consistent with those reports, 17 of the patients (11%) in our study demonstrated such lesions. The most prevalent abnormality was hydronephrosis. Although determination of the underlying cause was limited by the retrospective nature of our study, imaging results for the patients with the most severe grades of hydronephrosis demonstrated the absence of any fixed obstructing lesion. Therefore, it is likely that, in most cases, dilation of the collecting system was secondary to nonobstructive abnormalities, perhaps including abnormalities of the pelvic and ureteric smooth muscle layers. Less common abnormalities of the collecting system included caliceal diverticula and vesicoureteral reflux. The similarity of the prevalence of those abnormalities to that observed in the general population (3) (2.5 and 1.2%, respectively) suggested that the presence of those lesions was coincidental and not secondary to BWS.

Cysts represented the most prevalent renal abnormality among our patients. The majority of the cysts were located in the medulla, rather than the cortex. This is in contrast to findings for patients without BWS, for whom isolated cysts are rare and are located in the cortex (21). Medullary cysts are derived from dilated collecting ducts and loops of Henle and are formed during development of the renal collecting system and medulla (22). Therefore, these cysts may represent a more severe phenotype in a continuum of collecting system abnormalities, with mild pelvic dilation representing a mild phenotype. The relatively high prevalences of nephrocalcinosis and nephrolithiasis in this study (4%) and other studies (3,4) might be secondary to the congenital abnormalities of the collecting system among the patients. Two of the six affected patients in our study exhibited medullary cysts associated with nephrocalcinosis, two exhibited hydronephrosis (which might predispose them to the development of renal stones), and two exhibited isolated nephrocalcinosis. Laboratory data (urinary calcium, creatinine, oxalate, amino acid, uric acid, and citrate levels) were available for four patients. Three of the patients demonstrated hypercalciuria, and results were normal for the fourth. Taken together, these abnormalities highlight the pathogenic role of collecting system maldevelopment in BWS and suggest the need for future studies aimed at defining the natural history of these abnormalities.

Our demonstration of a novel genotype-phenotype correlation between paternal 11p15.5 UPD and renal lesions suggests that this genomic region consists of genes that control renal morphogenesis. Disruption of the function of these genes might cause abnormal patterning of the collecting system in the cortex and medulla, leading to the development of renal cysts and collecting system abnormalities. The 11p15.5 region associated with BWS spans 1 Mb containing several imprinted genes, including IGF2, H19, CDKN1C, and KCNQ1/KCNQ1OT1 (Figure 1). Both CDKN1C and IGF2 were demonstrated to participate in the control of renal morphogenesis in animal studies; however, overexpression of IGF2 produces nephromegaly in mice but does not produce renal medullary dysplasia, as observed for some patients with BWS (14). In contrast, p57\(^{Kip2}\)-H19 mice do exhibit such renal medullary dysplasia (12). Mutations in H19 cause enhanced expression of IGF2, and mice with mutations of both p57\(^{Kip2}\) and H19 exhibit elevated serum IGF-2 levels and more severe renal dysplasia, compared with p57\(^{Kip2}\)-H19 mice (13). That is, increased IGF2 expression exacerbates the p57\(^{Kip2}\)-H19 renal phenotype in mice but is not a primary cause of that phenotype. Our results demonstrating that CDKN1C mutations and imprinting defects at KvDMR1 were not correlated with renal abnormalities strongly suggest that defects in imprinted genes in the 11p15 region other than CDKN1C produce renal abnormalities among human subjects with BWS. Whereas both primary CDKN1C mutations and 11p15.5 UPD significantly reduced CDKN1C expression and imprinting defects at KvDMR1 were not correlated with renal abnormalities strongly suggest that defects in imprinted genes in the 11p15 region other than CDKN1C produce renal abnormalities among human subjects with BWS. Whereas both primary CDKN1C mutations and 11p15.5 UPD significantly reduced CDKN1C expression, an increase in renal malformations was noted only with 11p15.5 UPD (Figure 3, B versus C). Similarly to primary CDKN1C mutations, loss of methylation at KvDMR1 was not associated with renal malformations (Figure 3D). Therefore, imprinted genes or regulatory elements other than CDKN1C or KvDMR1 must account for renal dysplasia among human subjects with BWS. Future experiments directed at determining the role of imprinted genes in this region hold the promise of providing new insights into the genetic control of renal development and, more generally, the relationship between the control of growth and morphogenesis.
Figure 3. Model of genomic imprinting on 11p15.5, including the imprinted genes CDKN1C and KCNQ1, containing the differentially methylated region KvDMR1 (gray box with CH3), a putative imprinting center. (A) Normal expression. Expression of CDKN1C (arrow) is preferentially from the maternal allele (95%), with slight expression from the paternal allele (5%) (23). (B) Paternal uniparental disomy (UPD). Patients with paternal UPD (two paternal copies for a segment of chromosome 11) demonstrate 10% of the normal expression of CDKN1C. However, 11p15.5 UPD extends to other imprinted genes and regulatory regions in band 11p15.5. (C) Mutation in the maternal CDKN1C allele. CDKN1C expression might be reduced to as little as 5%, the contribution from the imprinted paternal allele. (D) Imprinting defects in KvDMR1. Loss of methylation (LOM) has been postulated to downregulate expression of CDKN1C (24), as illustrated by the question mark.

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