Aberrant genomic imprinting in chromosome 11p15-associated congenital growth disorders: consequences for DNA-diagnostics
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Chapter 3

Epigenotyping as a tool for the prediction of tumor risk and tumor type in patients with Beckwith-Wiedemann syndrome (BWS)

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ABSTRACT

Objectives: Patients with Beckwith-Wiedemann syndrome (BWS) have a risk of 7.5% to 10% of developing childhood tumors, 60% of which are Wilms' tumors. Aberrant methylation of two distinct clusters of imprinted genes on chromosome 11p15 is detected in 70% of BWS cases. Our aim was to determine associations between the imprinting status of both imprinting clusters (BWSIC1/2) and the tumor incidence and type.

Study design: Methylation patterns of \textit{H19} and \textit{KCNQ1OT1} were collected in 114 patients with BWS with a clinical diagnosis. The patients were followed until 5 years of age, and tumor incidence and type were registered.

Results: A lower risk of developing childhood tumors was found among patients with a methylation defect limited to BWSIC2 compared with other patients with BWS. No Wilms' tumors were found in this group, whereas in patients with a methylation defect limited to BWSIC1 Wilms' tumor was the most common tumor.

Conclusions: In addition to clinical factors indicative for a high tumor risk (hemihypertrophy, nephromegaly), methylation patterns discriminate between patients with BWS with a high and low tumor risk. It also is possible to predict whether they are at risk of developing a Wilms' tumor. Epigenotyping of patients is important to select the type of screening protocol to be proposed to these patients.
INTRODUCTION

Beckwith-Wiedemann syndrome (BWS) is a congenital overgrowth condition, characterized by multiple features that are variably present. Children are often diagnosed by an increased birth weight in combination with the presence of a large tongue (macroglossia) and abdominal wall defects. Organomegaly and limb asymmetry, hemihypertrophy, also are often observed. Patients with BWS have an increased risk of about 7.5% to 10% of developing childhood neoplasms. Wilms' tumor is the most common tumor found in patients with BWS (60% of all tumors), but other solid childhood tumors also are found.

All children with BWS are subjected to a strict and intense screening protocol to detect cancer in an early stage. In concordance with published screenings protocols, the Emma Children's Hospital in Amsterdam developed a Dutch screening program. It consists of ultrasonography examination of the abdomen every 3 months until 4 years of age, followed by screening every 4 months until 5 years of age, and then screening every 6 months until 8 years of age. At the same time intervals, fetoprotein, elevated in hepatoblastoma, is measured until 4 years of age. Screening at these short intervals increased the number of stage I and II Wilms' tumors detected, hence favoring the outcome of children with BWS with malignant tumors.

The genes involved in BWS are located on chromosome 11p15 and are subject to genomic imprinting. Within this region there are two imprinting domains, each controlled by its own imprinting center; BWS imprinting center 1 (BWSIC1, telomeric domain) and BWS imprinting center 2 (BWSIC2, centromeric domain). Imprinting defects affecting either one or both imprinting centers have been described in BWS. For diagnostic purposes, methylation patterns are established for the genes $H19$ in BWSIC1 and $KCNQ1OT1$ in BWSIC2. We regard the methylation of $H19$ as representative for the imprinting status of BWSIC1, although in some cases, imprinting of $H19$ and IGF2 is uncoupled. In addition, there are cases of $KCNQ1OT1$ and IGF2 loss of imprinting without $H19$ loss of imprinting. This study deals only with the correlation between aberrant methylation at BWSIC1/2 and tumor type and frequency.

Based on methylation defects, patients with BWS can be divided into four groups. Group I patients (20%-25%) display aberrant methylation in both regions because of uniparental disomy (UPD). Group II patients (about 10%) have a methylation defect in BWSIC1, which results in hypermethylation of the maternal $H19$ allele. Group III patients (40%-50%) have a methylation defect in BWSIC2, which results in hypomethylation of the maternal allele of $KCNQ1OT1$. Group IV patients (about 30%) have a normal methylation pattern of both regions. In a small minority of patients (<5%) mutations in a cell-cycle regulator, CDKN1C, are found; these are included in group IV.
Clinical features associated with a high tumor risk are hemihypertrophy and nephromegaly. In addition, methylation patterns might be useful in the further delineation of tumor risk in patients with BWS. The aim of this study is to investigate possible associations between the imprinting status of both imprinting clusters and the tumor incidence type. We expand our data with data already published by others to enlarge the number of patients in the methylation groups.76.

**SUBJECTS AND METHODS**

Patients were referred to either the Hôpital Trousseau in Paris (66 patients) or to the Academic Medical Centre in Amsterdam (48 patients) by pediatricians and geneticists. The patient group published in our previous study19 was included in the current study. Clinical examination of all patients was performed at least until 5 years of age, and only patients meeting the criteria for BWS described in literature were included in this study. Patients <5 years of age were not included in our study because the 5-year follow-up period had not been completed. However, children with BWS who presented with a tumor before 5 years of age were included in the studies.

Methylation indices of both H19 (BWSIC1) and KCNQ1OT1 (BWSIC2) were measured in blood lymphocytes as described before.19,20

**RESULTS**

The inclusion of patients with BWS <5 years of age who developed a tumor creates a bias in the patient population. Therefore, all tumor frequencies in this study are overestimated. The group consists of too many children with tumors (25% in our study vs 10% in the literature). However, this does not influence the distribution of the tumor type/risk within the various genetic groups.

Among group I patients, the tumor incidence is 39% (9/23) (Table 1). We found 12 tumors in these 9 patients; 5 patients developed a Wilms' tumor. A tumor frequency of 23% is observed among group IV patients (7/30). We found 9 tumors in these 7 patients; 5 patients developed a Wilms' tumor. The patient in this group who developed a neuroblastoma carries a CDKN1C mutation.

The 16 group II patients display a significantly (P = .0013) higher risk of developing tumors (10/16, 63%). The only tumor type found in this group is the Wilms' tumor (10/10).

In contrast, among group III patients a significantly lower tumor risk (P = .0002) was found. Of 45 patients, only 2 patients developed a tumor (4%). One patient developed a hepatoblastoma, and one patient developed a thyroid carcinoma (at 14 years of age). A thyroid carcinoma is not a classic BWS-associated tumor; this is the only case found in a patient with BWS. It is therefore possible that this tumor developed independently from the BWS phenotype. No Wilms' tumors were found in group III.
Table 1. Tumor risk versus methylation defect: results of this study

* Ten tumors in 7 patients. One patient developed a neuroblastoma and Wilms' tumor, a second patient hepatoblastoma and leukemia, and a third patient a mammary adenoma (14 years of age) and bilateral pheochromocytomas (19 years of age). † Three patients carried a CDKN1C mutation, and one of them developed a neuroblastoma. ‡ Six tumors in 4 patients, bilateral pheochromocytoma described by E. L. T. van den Akker et al. Eur J Pediatr 2002;161:157-60. § Tumor frequencies were compared with the pooled tumor risk (28/114) with the log likelihood ratio test.
Table 2. Tumor risk versus methylation defect: combined results from this study and data from the literature\textsuperscript{21, 22.}

<table>
<thead>
<tr>
<th>Methylation pattern</th>
<th>Tumor type</th>
<th>Fraction of sample (%)</th>
<th>Tumor frequency (2%) (16/81)</th>
<th>Tumor frequency (18%) (16/92)</th>
<th>Tumor frequency (20%) (16/81)</th>
<th>Total tumor frequency in sample (16%) (63/287)</th>
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</thead>
<tbody>
<tr>
<td>Group I (UPD, H191)</td>
<td>WT, Wilms' tumor</td>
<td>23/114 (20)</td>
<td>5/12</td>
<td>2/2</td>
<td>1/3</td>
<td>20/56 (36)</td>
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<td></td>
<td></td>
<td>9/23</td>
<td>4/10</td>
<td>6/21</td>
<td>15/29 (52)</td>
<td>50/180 (86)</td>
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<td>Group II (H191)</td>
<td></td>
<td>16/114 (14)</td>
<td>10/92 (11)</td>
<td>4/10</td>
<td>15/29 (52)</td>
<td>60/270 (111)</td>
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<td>10/16</td>
<td>10/92 (11)</td>
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<td>Group III (KCNQ4/OT1)</td>
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<td>Group IV (Normal)</td>
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WT, Wilms' tumor.* Tumor frequencies were compared with the pooled tumor risk (65/380) with the log likelihood ratio test.† Wilms’ tumor frequencies were compared with the pooled Wilms' tumor frequency (30 WT/19 non-WT) with the log likelihood ratio test.
**DISCUSSION**

Methylation data for BWSIC1 and BWSIC2 were taken from two additional studies\(^{21}\) and \(^{22}\). (Table 2), resulting in a total of 287 patients. These studies confirm our findings. The overall tumor frequency in this expanded group is 16%. Most likely, the patient population bias observed in our study also is present in the other studies. The results of a third study\(^{18}\) also confirm our findings. Engel et al found no association between BWSIC2 demethylation and tumors. Tumor frequencies in the BWSIC1-affected patients were equal to this study. However, we could not include these data in our statistical analyses because not all patients were screened for both methylation defects.

Among patients with a methylation defect in BWSIC2 (group III), a tumor frequency of 6% was observed, which is significantly lower than the other patients with BWS (\(P < .0001\)). None of the tumors found in this group are Wilms' tumors, which is different from the other groups (\(P = .0003\)). It must be noted that 5 of the 8 group III patients with a tumor are found in the study of Weksberg et al. However, as in our study, none of the tumors are Wilms' tumors. DeBaun et al report a group III patient with a tumor, however, the type is unknown.\(^{21}\)

A significantly higher tumor frequency of 52% is found in patients with a methylation defect in BWSIC1 (\(P = .0003\)). In this group exclusively, Wilms' tumors are found (11/11), which is significantly higher than in the other groups (\(P = .0010\)).

The observation that no Wilms' tumors are found among group III patients whereas it is the only tumor found among group II patients indicates that at least one gene involved in the aetiology of Wilms' tumor is distinct from the genes involved in other tumors associated with BWS. Demethylation of \(KCNQ1OT1\) is associated with tumor types that are found in patients with BWS in a much lower frequency than Wilms' tumors.

Based on methylation patterns, it is possible to discriminate between patients with BWS with a high and low tumor risk. It also is possible to predict whether they are at risk of developing a Wilms' tumor. Epigenotyping of patients is important to select the type of screening protocol to be proposed to these patients.

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**REFERENCES**


Tumour risk BWS