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Phenotype, Cancer Risks and Surveillance in of Beckwith-Wiedemann Syndrome Depending on Molecular Genetic Subgroups

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Abbreviations: BWS = Beckwith-Wiedemann syndrome, IC1 = imprinting center 1, IC2 = imprinting center 2, pUPD = paternal UniParental Disomy, LOM = loss of methylation, GOM = gain of methylation, AFP = alpha-fetoprotein

Abstract

Patients with Beckwith-Wiedemann syndrome (BWS) have an increased risk to develop cancer as a child, especially Wilms tumor and hepatoblastoma. The risk varies depending on the cause of BWS.

We obtained clinical and molecular data in our cohort of children with BWS, including tumor occurrences, and correlated phenotype and genotype. We obtained similar data from larger cohorts reported in literature.

Phenotype, genotype and tumor occurrence were available in 229 own patients. Minor differences in phenotype existed depending on genotype/epigenotype, similar to earlier studies. By adding patients from the literature we obtained data on genotype and tumor occurrence of in total 1971 BWS patients. Tumor risks were the highest in the IC1 (*H19/IGF2*:IG-DMR) hypermethylation subgroup (28%) and pUPD subgroup (16%) and were lower in the *KCNQ1OT1*:TSS-DMR (IC2) subgroup (2.6%), CDKN1C (6.9%) subgroup, and the group in whom no molecular defect was detectable (6.7%). Wilms tumors (median age 24 months) were frequent in the IC1 (24%) and pUPD (7.9%) subgroups. Hepatoblastoma occurred mostly in the pUPD (3.5%) and IC2 (0.7%) subgroups, never in the IC1 and CDKN1C subgroups, and always <30 months of age. In the CDKN1C subgroup 2.8% of patients developed neuroblastoma.

We conclude tumor risks in BWS differ markedly depending on molecular background. We propose a differentiated surveillance protocol, based on tumor risks in the various molecular subgroups causing BWS.

INTRODUCTION

Beckwith-Wiedemann syndrome (BWS) is an overgrowth disorder characterized by perinatal overgrowth, macroglossia, exomphalos, hemihyperplasia and postnatal hypoglycemia, and associated with an increased risk to develop embryonic tumors.[1,2] The prevalence at birth is estimated to be 1/12,000.[3] Two sets of similar but not identical diagnostic criteria are mainly used in clinical practice (Table I).[4,5]

Familial transmission has been reported as occurring in ~15 percent of BWS patients if all patients were grouped together.[6] BWS exhibits etiologic molecular heterogeneity due to a variety of alterations in growth regulating genes located at chromosome 11p15. This chromosome region harbors two independently regulated clusters of imprinted genes (Fig. 1). One cluster contains the reciprocally imprinted genes *IGF2* and *H19* and is under control of *H19/IGF2*:IG-DMR (IC1), upstream of the *H19* promoter.[7] This imprinting center is differentially methylated, methylation being present only at the paternal allele. The second cluster contains (among others) the maternally expressed *CDKN1C* gene and the paternally expressed *KCNQ1OT1* (*LIT1*) gene and is under control of *KCNQ1OT1*:TSS-DMR (IC2), located upstream of the KCNQ1OT1 promoter. This region is methylated on the maternal allele only. The majority of BWS patients (80%) show an aberrant imprinting in either one, or both imprinted clusters (choufani 2013).[8] Aberrant methylation of both ICs is typically explained by a paternal UniParental Disomy (pUPD) of the 11p15 region (20% of BWS cases). Mutations in *CDKN1C* are found in approximately 5-10% of (mostly familial) cases. Infrequently paternal trisomy of 11p15 or a maternal balanced translocation involving the area causes BWS. Approximately 10-15% of cases remain without molecular confirmation of the syndrome despite carrying all clinical characteristics of the syndrome.[9]

BWS patients have an increased incidence of embryonic tumors, especially Wilms tumors, but also hepatoblastoma, neuroblastomas, adrenal carcinomas and rhabdomyosarcomas can occur.[5,10-16] This risk depends on the epigenetic defect of BWS: patients with a molecular abnormality involving the telomeric domain (pUPD) and H19 gain of methylation [GOM]) tend to have a higher risk patients with an abnormality involving the centromeric domain (*CDKN1C* mutations and loss of KCNQ1OT1 methylation [LOM]) tend to develop tumors infrequently.[14,16,17] Several protocols have been suggested for tumor surveillance, consisting typically of abdominal ultrasound and screening of alfa-fetoprotein levels at various ages and intervals.[16, 18-25] All protocols have been based on relatively small groups of patients, and with a limited number of exceptions subtype of BWS were not taken into account for the surveillance protocol.[14-16,26,27]

Here we report on studies in a large cohort of BWS individuals, summarize their phenotype, add data from similar studies in literature in order to correlate the phenotype with the various genetic subgroups in BWS. We determined the relative tumor risks for each of these subgroups, and propose a tumor surveillance system.

METHODS

Patient selection

The Academic Medical Center in Amsterdam started to offer cytogenetic and molecular diagnostics tests for BWS in the early nineties. Since 2000 it functions as the national center of referral for individuals with BWS. Any patient who fulfilled the diagnostic criteria described by DeBaun and/or Elliott (Table I) was allowed to enter the study, irrespective whether the clinical diagnosis BWS could be confirmed molecularly or not.[4,5]
Clinical data of all included patients were obtained either directly by examining patients, or through questionnaires on clinical manifestations forwarded to physicians who submitted samples of patients suspected for having BWS. In 2005 a dedicated outpatient clinic was opened specifically for individuals with BWS. A single clinical geneticist (SMM) evaluated all individuals referred to this clinic, and extensive initial and follow-up data were collected. For the present study the Dutch Childhood Oncology Group pediatric was consulted in June 2015, in order to evaluate whether, since the last clinical contact, a tumor had developed in any of our patients. In all patients with a tumor the major characteristics of that tumor were obtained.

The Medical Ethics Committee of our institution approved this study (#99.15.210). Informed consent was obtained from all participating patients and/or their parents/legal representatives.

Molecular analysis

Studies were performed at the Molecular diagnostics Laboratory of the Academic Medical Centre in Amsterdam. DNA was extracted from peripheral blood lymphocytes and methylation levels of KCNQ1OT1 and H19 were determined by either southern blot methylation sensitive high resolution melting analysis (HRMA)[28] or methylation multiplex ligation-dependent probe amplification (MLPA).[29] In case of loss of imprinting of both KCNQ1OT1 and H19, variable number of tandem repeats (VNTR) studies were performed to confirm the presence of pUPD, as described before.[28] Mutation analysis of *CDKN1C* was not performed routinely. Study participants were classified in 4 genetic subgroups: hypermethylation of *H19/IGF2*:IG-DMR (further indicated in the manuscript as IC1); hypomethylation of *KCNQ1OT1*:TSS-DMR (further indicated in the manuscript as IC2); pUPD; and clinical diagnosis fulfilling the diagnostic criteria of either DeBaun and/or Elliot and without detectable genetic abnormality.

Literature study

A literature search was performed in Pubmed and EMBASE with MESH terms: (neoplasms OR cancer* OR tumor OR tumors OR tumour* OR Wilms OR hepatoblastoma) AND (Beckwith-Wiedemann syndrome OR Beckwith-Wiedemann) AND (genetics OR genetic* OR phenotyp* OR genotyp* OR epigenotyp*). The reference lists of all publications were hand-searched for other potentially useful publications. Case reports were excluded: we included only studies with series of patients of whom phenotype, genotype and tumor characteristics were described. Only tumors that are considered to be malignant and thus listed in the International Classification of Childhood Cancer (ICC3) have been included.[30] We have carefully avoided using patient data more than once as in several publications data of earlier publications were incorporated. If needed this has been checked specifically by contacting the authors of the original publications.

Statistical analysis

The Statistical Package for the Social Sciences (IBM SPSS version 22.0, USA) was used to analyze the data. Descriptive statistics were generated to describe the total sample of patients and the subsamples of genetic subgroups. Differences between the genetic subgroups were tested with ANOVA for parametric data, and Chi-squared tests statistics for non-parametric data. Fisher's exact test was used when appropriate. Two-tailed *P*-values <0.05 were considered to indicate statistical significance.

RESULTS

Characteristics of own study group

In total 244 patients were included in this study. Five patients had a chromosome abnormality and were excluded as the other chromosome imbalances prohibited analysis of the phenotype due to only a 11p15 imbalance. All patients but three were at least five years of age when last data were gathered (mean 15.2 years, median 13.5 years). The distribution over the four genetic subgroups is provided in Table II, in which also the frequencies of manifestations of BWS in the genetic subgroups and in the total patient group are available. The various abnormal morphological characteristics are available in Supplemental Table S-I.

Tumor frequencies in own study group and in literature cohorts

We were able to obtain reliable data on both genotype and tumor occurrence in 229 BWS patients of the present cohort (Table III). The literature search yielded seven studies in which cohorts of patients with BWS were described including the various genetic subgroups and tumors (Weksberg et al., 2001; Gaston et al., 2001; DeBaun et al., 2002; Bliek et al., 2004; Brioude et al., 2013; Ibrahim et al., 2014; Mussa et al., 2015). [5,12,13,16,17,27,31]

In three cohorts not all BWS patients were screened for CDKN1C mutations[5,13], and in two other studies (Brioude et al., 2013; Mussa et al., 2015) BWS patients in whom no molecular defect could be detected were not included.[16,27] We decided to include these five studies in the overview to increase the number of useful data even though this means that the numbers of individuals and tumors in the genetic subgroups ‘CDKN1C’ and ‘no detectable molecular cause’ are minimum estimates. In total data on 1971 BWS patients were available. The highest tumor risk was present in the genetic subgroup IC1hypermethylation (28%), the lowest tumor risk was in the subgroup with IC2 hypomethylation (2.6%). These risks are at the age patients were described, which varied among the various publications. The exact nature of tumors occurring per genetic subgroup is listed in Table III. The risk for specific tumors in the subgroups (IC2, IC1, pUPD, no defect, CDKN1C) were for Wilms tumor 0.2%, 24%, 7.9%, 4.1%, and 1.4%, for hepatoblastoma 0.7%, 0%, 3.5%, 0.3%, and 0%, and for neuroblastoma 0.4%, 0%, 1.4%, 0.6% and 2.8%, respectively. For all other specific tumor types the risk per molecular subgroup was well below 1% (Table III). In addition we studied the age at which BWS patients developed a tumor. If available both mean and median age is provided, and the highest age at which a tumor was detected per genetic subgroup (Table IV).

DISCUSSION

BWS is often diagnosed by combining clinical and molecular findings. Two generally accepted sets of diagnostic criteria are those described by Elliott[4] and DeBaun[5] (Table II). The Elliott criteria are usually stricter than the DeBaun criteria, and also in our series all patients fulfilled the DeBaun criteria while 43.8% of patients fulfilled the Elliott criteria. In the series of patients published by Ibrahim and co-workers (Ibrahim) the sensitivity of the DeBaun criteria were calculated to be higher than the Elliott criteria (83.5% versus 43.5%), and specificity were 83.5% (Elliot criteria) versus 62.3% (DeBaun criteria).[30] Whether in the present cohort the specificity of either set of criteria is higher than the other remains uncertain as we have no information on how frequently samples of patients fulfilling either set of criteria are indeed submitted for molecular analysis. We found in the present study that more than half of patients (varying from 50% to 58.4%) in whom a molecular diagnosis of BWS could be made did not fulfill the Elliott criteria. As reported previously[13, 31] there is also a subgroup of patients with a clinical diagnosis of BWS but no detectable molecular abnormality.[13,31] In this group the percentage of patient that did not fulfill the Elliott criteria (56.4%) was similar to that in the other patient groups (with a detectable molecular cause). We conclude that the sets of diagnostic criteria are both useful, but for neither of the sets do we know with certainty that sensitivity and specificity are truly high. BWS was and still is a clinical diagnosis, in which a molecular confirmation is not always possible, and further studies of the Elliott and DeBaun criteria and other sets of criteria are needed.

Clinical features of BWS

The phenotype of the present cohort is, in general, comparable to that in patients described in other cohorts.[16,27] As can be expected the patients in the group ‘clinical diagnosis’ show the signs that are very characteristic for BWS somewhat more frequently than the patients with a molecular abnormality, as the former patients were diagnosed based on the phenotype only (Table II). Remarkable differences between the various groups are the lower frequency of a high birthweight in the IC2 hypomethylation subgroup, the high frequency of asymmetrical overgrowth in the pUPD subgroup (as described before) and explained by mosaicism for the pUPD), and the relatively low frequency of ear creases, ear pits and facial naevus flameus in the IC1 hypermethylation subgroup. [5,12,14,16,31,32] Also, as reported previously, a low frequency of omphalocele in this latter subgroup was found, and less frequently an enlargement of the internal organs (especially kidney and spleen) in the IC2 hypomethylation subgroup.[32,33] Though not all data was collected by evaluating the cohort personally, the vast majority of cases were seen and examined and so the data in Table II is likely to be very reliable.

Neoplasia

In the present cohort we have found the highest risk to develop cancer in the IC1 hypermethylation subgroup, and to a lesser extend in the pUPD group. In the IC2 hypomethylation subgroup two children with a Wilms tumor were found, which has not been reported before. Niemitz et al. have described two patients with a Wilms tumor and hypomethylation of *KCNQ1OT1* in normal kidney tissue and LOH in the tumor, but unfortunately details regarding methylation results in lymphocytes were not provided.[34]

We provide a complete literature overview evaluating almost 2000 BWS patients, which allows reliable conclusions (Table III). Earlier careful meta-analyses of the literature are available, but in much smaller numbers.[15,27] We realize there is likely still a publication bias in data reported in literature, and in reality frequencies may be somewhat lower.

We evaluated the nature of the tumors in our own patients and patients reported in literature. Wilms tumors and hepatoblastoma are only rarely present in the IC2 hypomethylation subgroup, and Wilms tumors are also very unusual in the CDKN1C group. In all other molecular groups Wilms tumors are frequently occurring. In the IC2 hypomethylation subgroup the variability in tumor types is remarkably large. In the IC1 group no hepatoblastoma has ever been described. In the CDKN1C group reported by Gaston et al.[12] and Brioude et al.[16] individuals developed neuroblastoma at age 6m and 10m, respectively. The median age at which BWS individuals develop cancer in the present cohort has been 24 months for Wilms tumors and 12 months for hepatoblastoma. There is a tendency for Wilms tumors to develop at an earlier age in the IC2 subgroup compared to the IC1 and pUPD groups. Results are compared to literature data in Table IV.

Cancer risks in BWS have been reported correlated with the presence of hemihyperplasia, nephromegaly, nephrogenic rests and nephroblastomatosis.[1,33,35] Mussa et al.[32] found hemihyperplasia and enlarged kidneys in all patients with a Wilms tumor, and similarly DeBaun[10] reported that all patients with a Wilms tumor had enlarged kidneys if evaluated repeatedly. In this publication before molecular subgroups could be made, the nephromegaly was typically bilateral, and the cancer had always arisen in the largest kidney.[10] Gaston et al. found a (statistically insignificant) higher frequency of hemihyperplasia in patients with tumors but this was not subdivided according to molecular subtype.[12] We evaluated this in our cohort according to different molecular genetic subgroups: Wilms tumors were more frequently found in each of the genetic subgroups except for the IC2 subgroup where there is no difference (Supplemental Table S-2). However, for none of the subgroups was this difference statistically significant. In the pUPD group there was a statistically significant increase of hemihyperplasia in the group who developed a Wilms tumor, and this was also found in the group in whom no molecular defect could be detected causing BWS. In the latter group there was also a significantly more frequent occurrence of an enlarged spleen ($p=0.016$). Otherwise in none of the subgroups a marked difference was found for the occurrence of Wilms tumors and the presence of hemihyperplasia, enlarged livers or spleens, or combinations of these. We realize the various subgroups are small and these conclusions should be used with caution. We refrained from performing similar comparisons with hepatoblastoma due to the very small numbers.

Screening: General considerations

Screening individuals for cancer is aimed at improving the outcomes for those who have an increased genetic risk to develop tumors.[24] The outcomes can improve by detecting tumors earlier, at a less advanced stage than they would have at detection without screening. Less advanced tumors generally need less extensive surgery and less intensive chemo- and radiotherapy, and are associated with a better survival.[24] A prerequisite is that the screening schedule is as such that indeed the tumor is detected at a less advanced stage, so the velocity of the growth of the tumor, the sensitivity and specificity of the screenings procedure, the interval between the screening moments, the treatment schedules of the various stages of the tumor, and the effectiveness of these treatment schedules need to be carefully determined.[36] Screening has significant consequences for the emotional wellbeing of patients and their families. It can be positive for them knowing they are being controlled but it can also create recurrent anxieties around each screening moment. Screening can lead to false negative and false positive results. The latter may need additional evaluations and infrequently surgical procedures, with obvious and significant impact on the wellbeing of patients and their families.[19,20]

The threshold level above which the risk to develop cancer is sufficiently high to provide surveillance is a subjective decision. The UK Wilms Tumor Surveillance Working Group suggested that surveillance should be offered to children who are at a greater risk than 5% risk of Wilms tumor.[37] Other studies did not mention specifically a threshold Wilms tumor risk for inclusion in surveillance, though in practice a 5% threshold for a general tumor risk was used. We have followed these authors and use the threshold level of 5% risk for all tumors together and, admittedly somewhat arbitrarily, added a 2% risk as threshold to screen for specific tumors.

Screening has financial implications. In most countries these are limited for the patients and their families themselves, but these may be significant for society. A cost-effectiveness evaluation should be part of general evaluations of screening procedures.[38] The total of the above influences on screening should be used to weigh the potential benefits and disadvantages of any screening schedule, and to establish protocols that address adequately the needs of the population under screening. An overview of the earlier reported recommendations in BWS in which the various molecular pathogeneses have been taken into account, is provided in Table V.

Background for Wilms tumor screening

Wilms tumors are embryonal kidney tumors that are almost invariably present before 10 years of age.[19] The median age of identification of Wilms tumors in our cohort is 18 months and of all studies together it is 24 months. Exceptionally Wilms tumors have been reported in BWS patients over 5 years of age, including at 10 years,[32] 12 years,[12,16] and 13 years in a patient with a cytogenetically visible deletion of 11p13.[39] Long-term survival in Wilms tumors is >90% for localized tumors and >70% for advanced tumors.[40] Advanced stage Wilms tumors need more intensive chemotherapy and radiotherapy.[41] Detection of Wilms tumor at an earlier stage reduced treatment-related morbidity in some studies [22,42] but not in others.[43,44] No results of reliable studies are available that show that early detection has a significant impact on the overall survival of BWS individuals. Craft et al. reported lack of a difference in outcome or stage distribution of the tumor between screened and unscreened population screening.[44]

False positive results of screening have been reported such as cysts, nephrogenic rests or foci of renal dysplasia.[19,42] The doubling time estimated for growth rate in Wilms tumors is 11-40 days.[45-47] This rapid tumor growth indicates only an interval of three and four months between screening moments is appropriate. McNeil et al. concluded that ultrasound screening of the abdomen at least until the age of 7 years is a cost-effective method to screen BWS patients if one considers costs of the screening and costs of treating a low stage tumor versus a late stage tumor.[38]

Background for hepatoblastoma screening

Hepatoblastomas are malignant liver cancers that consist of fetal liver cells, more mature liver cells and bile duct cells.[48] Ninety percent of hepatoblastomas occur before the age of four years, at a mean age of 22 months and median age of 16 months, and only exceptionally at an older age.[49] In BWS all hepatoblastoma occurred <30months of age (Table IV).[50] We have been unable to find a reliable description of an exception. In children with BWS it was shown that hepatoblastoma was diagnosed at a significantly younger age (median age 6 months) compared to children with hepatoblastoma without BWS (median age 16 months), and also the stage at diagnosis tended to be lower.[50] All patients are treated with chemotherapy and a surgical resection is attempted after tumor shrinkage.[24] After complete resection patients have an event-free survival of > 90%.[51] Patient with tumors that are initially non-resectable have an event-free survival rate of <70%, and those with metastases have an event-free survival rate of 20-30%.[51,52] Thus, early detection by effective screening could lower tumor advancement and treatment-related morbidity.[50,53] In over 96% of patients with hepatoblastoma serum alpha-fetoprotein (AFP) levels are elevated. Since AFP levels tend to be elevated in BWS individuals anyway, this urges for careful interpretation of screening results to avoid false positive results.[22,40,54] AFP levels can be elevated when abdominal ultrasounds do not allow visualization of a tumor, [40,55] and especially a rise of AFP levels after a few weeks is a strong indicator for further evaluations.[40] Rojas et al. reported on a small series of patients with hepatoblastoma who were screened for recurrences.[56] They found that AFP was elevated 1-11months before the tumor was detected by the surveillance imaging, and also reported false positive results. A similar study showed AFP to be elevated until two months before imaging showed an abnormality, and these authors reported on false negative results.[57] The half-life of AFP is 5-6 days.[58] Hepatoblastoma can grow very rapidly, doubling time has been reported as low as a few weeks.[22]

Authors of several early publications have concluded the usefulness of AFP screening should be doubted due to interpretation difficulties,[59] uncertainty whether it allows discovery of hepatoblastoma at such an earlier age that this changes prognosis, the relatively low occurrence of hepatoblastoma in BWS individuals, and the need for very frequent sampling for AFP for a potentially useful surveillance.[16,22,59]

Background for neuroblastoma screening

Neuroblastoma is a common pediatric cancer arising from the developing sympathetic nervous system, and can follow a highly variable course, from spontaneous regression to aggressive metastatic tumors. Neuroblastoma are usually diagnosed between 0 and 4 years of age (median 19 months). [60] Less than 5% occur at 10 years of age or above.[61] A neuroblastoma can be classified as low risk, intermediate risk and high risk depending on age, stage, histopathology, DNA index (ploidy) and MYCN amplification.[62] Depending on the stage, treatment consists of surgery combined with chemotherapy, radiotherapy, and more recently immunotherapy. Survival of low and intermediate risk is excellent (90%) but for high risk neuroblastoma this is only 40-50%.[63] Homovanillic acid and vanillylmandelic acid (HVA and VMA) are good biomarkers to detect neuroblastoma.[64] Population screening resulted in increased detection of tumors but these were tumors with favorable biology and pathology.[65,66] For conditions with a high risk for neuroblastoma such as in the NPARM group of *PHOX2B* mutations, ultrasound of the abdomen and urinary VMA and HVA every three months until the age of two years has been recommended and subsequent screening was depending on the risk on developing tumor.[61]

Screening proposal

The earlier suggested surveillance protocols for individuals with BWS in which molecular subgroups were taken into account, are summarized in Table V. They differ in screening methods, frequency and duration. We add to these an amended surveillance protocol based on:

a. The marked differences of occurrence of tumors in the various molecular genetic subgroups which indicate that the molecular background needs to be taken into account.

b. Screening is indicated in BWS patients with a IC1 hypermethylation, pUPD, and no detectable molecular abnormality, but not in BWS patients with a IC2 hypomethylation as in the latter patients the risk to develop a tumor is 2.6%. Raising the awareness of physicians in charge of BWS individuals with a IC2 epimutation that there is only a small increased chance of developing a tumor is indicated.

c. The number of reported BWS patients with a CDKN1C mutation is too low to determine the risk for tumor development in general, and for separate risks for Wilms tumors, hepatoblastoma, and neuroblastoma with certainty. We suggest to offer screening to the families, with a full explanation of the benefits and drawbacks. If a family decides for tumor screening we suggest to offer a complete screening. For Wilms tumor and hepatoblastoma the screening can therefore be the same screening as for BWS patients with a pUPD.

d. The presence or absence of hemihyperplasia, enlarged liver and/or spleen and/or kidney does not alter the screening protocol.

e. BWS patients with a IC1 hypermethylation should only be screened for Wilms tumors as hepatoblastoma does not occur; patients with pUPD or no detectable molecular abnormality should be screened for both Wilms tumors and hepatoblastoma.

f. Based on doubling time for growth rate screening for Wilms tumors should be performed every 3 months. Based on median and mean age of occurrence of Wilms tumors, screening is indicated from birth until age five years. The frequency of all type of tumors after age 4 years is well below 5% for each study individually and for each molecular genetic subgroup, and we do not advocate screening for this age group.

g. Presence of Wilms tumors is screened by renal sonographies although local circumstances may make MRI screening more useful.

h. Based on doubling time for growth rate screening for hepatoblastoma should be performed every 3 months. Based on the median and mean age of occurrence of hepatoblastoma screening is indicated between 0 and 36 months of age. As screening for Wilms tumors by imaging is indicated until 48 months, in practice abdominal imaging including both kidneys and liver will be performed simultaneous until that age in patients with pUPD, CDKN1C and those with no detectable molecular abnormality.

i. Existence of hepatoblastoma is screened by liver sonographies although local circumstances may make MRI screening more useful. We do not advocate AFP screening as there is insufficient proof this screening changes morbidity or mortality of BWS patients who develop a hepatoblastoma, while the burden of repeated blood sampling in young children and consequences for the emotional well-being for the families is considerable. We do not advocate abdominal palpation by parents because we concur with others that this may exacerbate parental anxiety and affect parent-child relationship, especially if a mass is not detected during “ parental surveillance”.[40]

j. The presence of neuroblastoma can be screened by urinary excretion of VMA and HVA and abdominal ultrasound every three months until the age of two years. Due to the relatively low risk and early age of patients in whom neuroblastoma have been found we do not advocate screening in older children.

k. We realize that the presently suggested surveillance protocol may need adaptation if markedly more BWS patients are reported in sufficient detail. Especially the screening for BWS patients with a CDKN1C mutation may need adaptation if such data would be available. Two additional studies describing larger series of patients with CDKN1C have been reported, including the occurrence of tumors, increasing the number of CDKN1C patients to 93, while the number of patients with cancer remained 6 (6.4%).[67,68] This may indicate that if a sufficiently large number of BWS patients with a *CDKN1C* mutation are reported, the tumor risk may be below 5% and surveillance may not be indicated. We also realize no screening protocol will detect every tumor and occasionally a tumor will develop in a BWS child in whom surveillance is discontinued; this is an inescapable characteristic of screening if the screening procedure has disadvantages as well, which is invariably the case.[40]

CONCLUSIONS

We show that tumor risk may vary considerably in genetic subgroups of BWS as some subgroups have a high risk of developing a Wilms tumor or hepatoblastoma while others have a low risk. Current screening protocols usually do not take this into account. We therefore propose a new screening protocol that is based on our own experience and an overview of literature, and offers a state-of-the-art of 2015. We realize that several important issues are still insufficiently studied, such as the burden of screening for BWS children and their families, and the influence this has on their wellbeing. Also the proof that in each molecular subgroup morbidity and mortality is changed sufficiently to counterbalance disadvantages is almost completely lacking. Until such studies are available we hope the present overview and surveillance protocol will be of benefit to the BWS children and their families.

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LEGENDS

Figure 1. Schematic representation of the imprinting control regions (ICR) on chromosome 11p15. BWSIC1 contains the reciprocally imprinted embryonic growth factor IGF2 (expressed from paternal allele) and the noncoding RNA H19 (expressed from maternal allele). Disturbance of the IC1 methylation results in overexpression of IGF2. BWSIC 2 contains the cell-cycle inhibitor CDKN1C and the noncoding RNA KCNQ1OT1 (expressed from the paternal allele). Mutations in CDKN1C (expressed from the maternal allele) and disturbance of methylation at IC2 results in reduced expression of CDKN1C. Both IC1 and IC2 can be disturbed in BWS, resulting in embryonic overgrowth. Note that the sizes and distances are not drawn to scale.

Figure 2. Suggested surveillance in patients with Beckwith-Wiedemann syndrome depending on molecular subgroup.

Table I. Two major sets of diagnostic criteria used for Beckwith – Wiedemann Syndrome.

Table II. Phenotype in 244 patients with Beckwith-Wiedemann syndrome comparing overall phenotype to those in the various genetic subgroups.

Table III. Overview of cohorts of individuals with Beckwith-Wiedemann syndrome and frequencies of tumors in genetic subgroups.

Table IV. Overview of age at detection of tumors in cohorts of individuals with Beckwith-Wiedemann syndrome.

Table V. Overview of suggested screening protocols taking molecular subgroups into account.

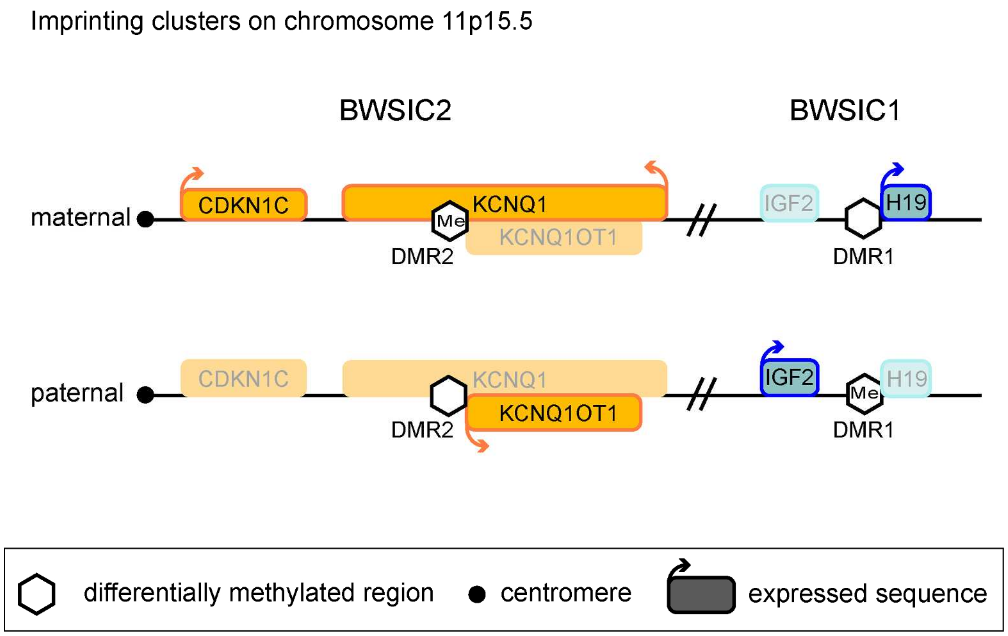

Figure 1. Schematic representation of the imprinting control regions (ICR) on chromosome 11p15. BWSIC1 contains the reciprocally imprinted embryonic growth factor IGF2 (expressed from paternal allele) and the noncoding RNA H19 (expressed from maternal allele). Disturbance of the IC1 methylation results in overexpression of IGF2. BWSIC 2 contains the cell-cycle inhibitor CDKN1C and the noncoding RNA KCNQ1OT1 (expressed from the paternal allele). Mutations in CDKN1C (expressed from the maternal allele) and disturbance of methylation at IC2 results in reduced expression of CDKN1C. Both IC1 and IC2 can be disturbed in BWS, resulting in embryonic overgrowth. Note that the sizes and distances are not drawn to scale.

190x118mm (300 x 300 DPI)

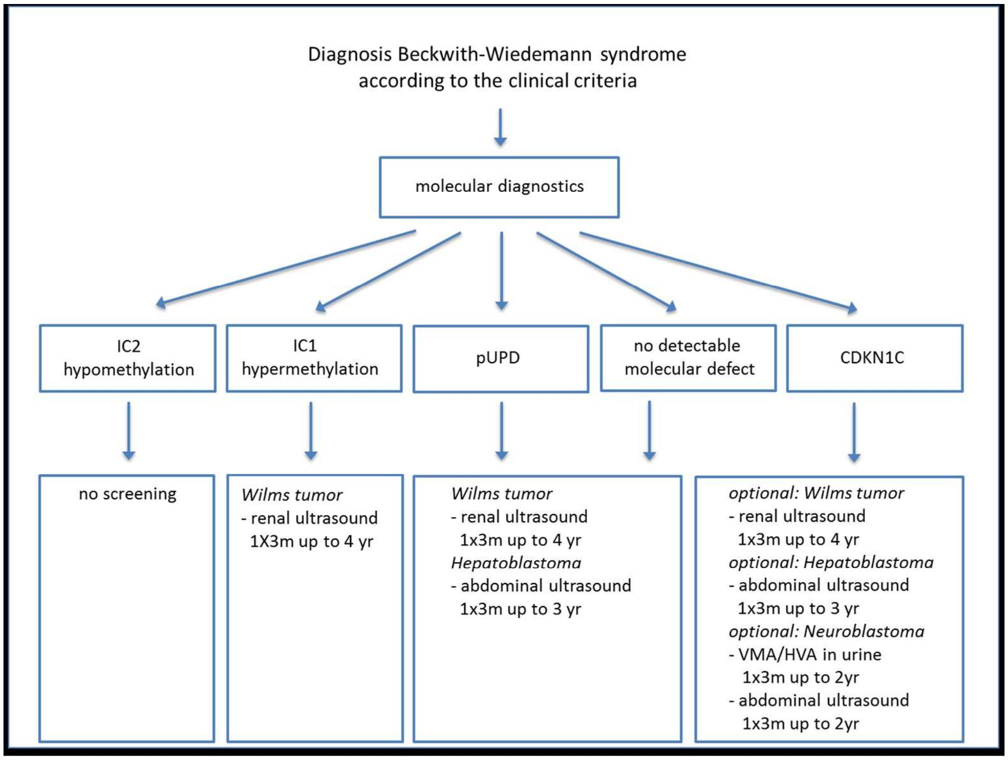

Figure 2. Suggested surveillance in patients with Beckwith-Wiedemann syndrome depending on molecular subgroup.
256x193mm (150 x 150 DPI)

Table I. Two major sets of diagnostic criteria used for Beckwith – Wiedemann Syndrome.

	DeBaun et al. (2002)	Elliot et al. (1994)
Beckwith-Wiedemann syndrome present if:	a. clinical diagnosis by physician, and b. at least 2 criteria present	a. at least 3 major features present, or b. 2 major + 3 or 4 minor criteria present
Birth weight > 90th centile	criterion	Major
Macroglossia	criterion	Major
Abdominal wall defect	criterion	Major
Postnatal hypoglycemia	criterion	Minor
Ear creases or ear pits	criterion	Minor
Hemihyperplasia	-	Minor
Nephromegaly	-	Minor

Table II. Phenotype in 244 patients with Beckwith-Wiedemann syndrome comparing overall phenotype to those in the various genetic subgroups

	Total (%)	IC2 LOM (%)	IC1 GOM (%)	pUPD (%)	clinical diagnosis (%)	p-value
Number	244 (100%)	125 (51.2%)	20 (8.2%)	44 (18%)	55 (22.5%)	
Gender (M:F)	112:132	57:68	10:10	21:23	24:31	
Criteria Elliott[1]	107/244 (43.8%)	52/125 (41.6%)	10/20 (50%)	21/44 (47.7%)	24/55 (43.6%)	0.477
Growth						
Birth weight>90th centile	167/172 (97%)	44/85 (51.8%)	11/15 (73.3%)	28/32 (87.5%)	29/40 (72.5%)	0.002
Hemihyperplasia	103/223 (46.2%)	38/ 115 (33%)	11/19 (57.9%)	36/42 (85.7%)	18/47 (38.3%)	<0.001
Facial						
Macroglossia	198/240 (82.5%)	106/123 (86.2%)	17/20 (85%)	34/43 (79.1%)	41/54 (75.9%)	0.361
Ear creases	76/229 (33.2%)	40/ 119 (33.6%)	2 /18 (11.1%)	13/40 (32.5%)	21/52 (40.4%)	0.158
Ear pits	47/222 (21.2%)	28/114 (24.6%)	1/ 19 (5.3%)	11/ 39 (28.2%)	7/50 (14%)	0.095
Facial naevus flammeus	100/226 (44.2%)	63/ 118 (53.4%)	3/20 (15%)	14/39 (35.9%)	20/49 (40.8%)	0.007
Other dysmorphic signs[2]	44/152 (28.9%)	15/ 76 (19.7%)	5/14 (35.7%)	9/32 (28.1%)	15/30 (50%)	0.019
Abdomen						
Abdominal wall defect						
Omphalocele	52/235 (22.1%)	39/122 (32%)	0/20 (0%)	5/39 (12.8%)	8/54 (14.8%)	0.001
Umbilical hernia	100/223 (44.8%)	50/114 (43.9%)	8/20 (40%)	16/38 (42.1%)	26/51 (51%)	0.771
Diastasis recti	100/199 (50.2%)	20/103 (19.4%)	6/18 (33.3%)	8/34 (23.5%)	12/44 (27.3%)	0.516
Nephromegaly	56/210 (26.7%)	14/106 (13.2%)	8/20 (40%)	17/38 (44.7%)	17/46 (37%)	0.000
Hepatomegaly	44/208 (21.2%)	19/109 (17.4%)	4/20 (20%)	7/34 (20.6%)	14/45 (31.3%)	0.308
Splenomegaly	21/204 (10.3%)	8/104 (7.7%)	3/20 (15%)	4/34 (11.8%)	6/46 (13%)	0.637
Other						
Cardiac anomaly[3]	22/215 (10.2%)	17/109 (15.6%)	1/ 20 (5%)	2/39 (5.1%)	2/47 (4.3%)	0.074
Hypoglycemia	89/147 (60.5%)	44/ 70 (62.9%)	6/ 13 (46.2%)	20/30 (66.7%)	19/34 (55.9%)	0.559
Developmental delay	18/179 (10%)	7/87 (8%)	1/19 (5.3%)	2/34 (5.9%)	8/39 (20.8%)	0.148

[1] All patients fulfilled the diagnostic criteria of DeBaun et al (2002)

[2] A full list of all other signs is available as Supplemental material Table S-I.

[3] VSD (n=5), ASD (n=4), persistent ductus arteriosus (n=2), open foramen ovale (n=2), pulmonic stenosis (n=1), cardiomyopathy with thickened ventricle septum (n=1), septum hypertrophy (n=1) valvular aorta stenosis (n=1)

LOM = loss of methylation; GOM = gain of methylation

Table III. Overview of cohorts of individuals with Beckwith-Wiedemann syndrome and frequencies of tumors in genetic subgroups.

Study	N	Tumors per subgroup		Tumor type
Weksberg et al., 2001	125	IC2	5/35[1]	2 hepatoblastoma, 2 rhabdomyosarcoma, 1 gonadoblastoma
		IC1	1/3	1 Wilms
		UPD	6/21	5 Wilms, 1 hepatoblastoma
		No defect	4/17[2]	4 Wilms
		CDKN1C	0/5	none
Gaston et al., 2001	97[3]	IC2	1/45	1 thyroid carcinoma (11yr)
		IC1	5/11	4 Wilms, 1 ganglioneuroma
		UPD	4/11[4]	2 Wilms, 1 Wilms + neuroblastoma, 1 mamma adenoma (14yr)+ pheochromocytoma (19yr)
		No defect	1/24	1 Wilms
		CDKN1C	1/2	1 neuroblastoma
DeBaun et al., 2002	92	IC2	1/39	not specified
		IC1	4/10	
		UPD	5/12	
		No defect	6/31	
		CDKN1C	not studied	
Bliek et al., 2004[5]	66	IC2	2/27	1 thyroid carcinoma (14yr), 1 hepatoblastoma
		IC1	6/9	6 Wilms
		UPD	7/13[6]	4 Wilms, 1 adrenal carcinoma, 1 neuroblastoma, 1 hepatoblastoma, 1 pheochromocytoma, 1 leukemia, 1 mammary adenoma
		No defect	3/17	2 Wilms, 1 neuroblastoma
		CDKN1C	not studied	
Brioude et al., 2013[7]	407	IC2	8/257	2 neuroblastoma, 2 hepatoblastoma, 1 sarcoma, 1 rhabdomyosarcoma, 1 thyroid carcinoma, 1 melanoma
		IC1	8/35	8 Wilms tumor
		UPD	14/81[8]	10 Wilms tumor, 2 adrenocortical carcinoma, 2 hepatoblastoma, 1 rhabdomyosarcoma, 1 neuroblastoma, 1 acute lymphoid leukemia
		No defect:	not studied	
		CDKN1C	3/34	1 neuroblastoma, 1 ganglioneuroma, 1 acute lymphoid leukemia
Ibrahim et al.,2014[7,9,10]	637	IC2	2/288	1 hepatoblastoma, 1 rhabdomyosarcoma
		IC1	3/28	3 Wilms
		UPD	4/99	1 Wilms, 3 hepatoblastoma
		No defect	5/201	3 Wilms, 1 adrenocortical carcinoma, 1 neuroblastoma
		CDKN1C	1/21[11]	1 Wilms
Mussa et al., 2015[7,12]	318	IC2	4/190	2 neuroblastoma, 1 rhabdomyosarcoma, 1 germinoma
		IC1	8/31	7 Wilms, 1 pancratoblastoma
		UPD	13/87	3 Wilms, 5 hepatoblastoma, 2 neuroblastoma, 1 pancreatoblastoma, 1 adrenal carcinoma, 1 hemangioteloma
		No defect	not studied	
		CDKN1C	0/10	none
Present study[13]	229	IC2	3/114	2 Wilms, 1 hepatoblastoma
		IC1	6/19	6 Wilms
		UPD	6/44	3 Wilms,1 hepatoblastoma, 1 myopepithelial cell carcinoma (13 yr), 1 pheochromocytoma
		No defect	4/52[14]	3 Wilms, 1 Wilms + 1 hepatoblastoma+ 1 rhabdomyosarcoma
		CDKN1C	not studied	
Pooled data	**1971**	**IC2**	**26/995 (2.6%)**	2 Wilms, 7 hepatoblastoma, 3 thyroid ca, 5 rhabdomyosarcoma, 1 sarcoma, 4 neuroblastoma, 1 melanoma, 1 gonadoblastoma, 1 germinoma, 1 not specified
		IC1	**41/146 (28%)**	35 Wilms, 1 ganglioneuroma, 4 not specified, 1 pancratoblastoma
		UPD	**59/368 (16%)**[15]	29 Wilms, 13 hepatoblastoma, 1 pancreatoblastoma, 1 hemangiotheloma, 4 adrenocortical carcinoma, 2 mammary adenoma, 1 rhabdomyosarcoma, 1 myoepithelial cell carcinoma, 5 neuroblastoma, 3 pheochromocytoma, 2 ALL, 5 not specified
		No defect	**23/342 (6.7%)**[16]	14 Wilms, 1 hepatoblastoma, 1 rhabdomyosarcoma, 2 neuroblastoma, 1adrenocortical carcinoma, 6 not specified
		CDKN1C	**5/72 (6,9%)**	1 Wilms, 2 neuroblastoma,1 ganglioneuroma, 1 acute lymphatic leukemia
		All	**155/1923 (8%)**	

[1] only 59 of 125 patients evaluated for IC2

[2] only 67 have been completely evaluated

[4] 6 tumors in 4 patients

[3] used other diagnostic criteria than the DeBaun or Elliott criteria
[5] only patients from France included (Dutch patients included in present study)
[6] 10 tumors in 7 patients
[7] only patients with a genetic defect included
[8] 17 tumors in 14 patients
[9] includes patients reported by Engel et al., 2000
[10] adapted figures as patients with isolated hemihypertrophy were excluded and additional data is included
[11] not all patients have been tested for CDN1C (personal communication)
[12] includes patients reported by Mussa et al., 2012
[13] series include Bliek et al., 2001 and Dutch patients in Bliek et al., 2004
[14] 6 tumors in 4 patients
[15] 67 tumors in 59 patients
[16] 25 tumors in 23 patients

Table IV. Age at detection of Wilms tumors and other tumors in cohorts of individuals with Beckwith-Wiedemann syndrome.

Study	**Mean Age**			**Median Age**			**Other data**
	All tumors	*Wilms tumor*	*Hepato-blastoma*	*All tumors*	*Wilms tumor*	*Hepato-blastoma*	
Green et al. 1993					26m		eldest age Wilms 7.9 yr
DeBaun and Tucker 1998	14m						5 hepatoblastoma, 6 Wilms, 2 neuroblastoma
Gaston et al. 2001 - IC2 - IC1 - pUPD - no defect	58m 132m 25m 79m 12m	34.5m - 25m 55m 12m (n=1)		18m	24m		1 Wilms 12 yr, 1 thyroid carcinoma 11 yr, 1 mamma adenoma 14yr. 1 pheochromocytoma 19yr Neuroblastoma at 4m, 10m
Clericuzio et al. 2003			6 m			5 m	
Mussa et al. 2012							1 Wilms 10 yr (bilateral)
Brioude et al. 2013 - IC2 - IC1 - pUPD	24m 35m 24m 16m	21m - 24m 17m	3m 1.5m - 5m	21m 28.5 24m 16m	22,5m - 24m 18,5m	3m 1.5m - 5m	1 Wilms 12 yr, 1 ALL at 120m, 1 sarcoma at 74m 1 thyroid carcinoma at 75m 3 neuroblastoma at <1 m, 4m, 6m
Trobaugh-Lotrarario et al. 2014			8m			6m	eldest age hepatoblastoma 30m
Ibrahim et al. 2014 - IC2 - IC1 - pUPD - no defect	24m - 37.5m 25.5m 32m	33m - 37.5m 24m (n=1) 32m	8m - - 8m -	24m - 37.5m 6m 36m	36m - 37.5m 24m (n=1) 36m	6m - - 6m -	
Present study - IC2 - IC1 - pUPD - no defect	28m 39m 31m 27.5m 41m	30m 11m 31m 34m 41m	11.5m 14m (n=1) - 9m (n=1) -	18m 14m 33m 57m 41m	18m 14m 33m 30m 41m	12m	eldest age Wilms 5.5 yr eldest age hepatoblastoma 30m
All studies - IC2 - IC1 - pUPD - no defects	28m 38m 28m 29m 32m	28m 11m 25m 29m 32m	7m 6m - 7m -	14m 13m 24m 12m 30m	24m 11m 24m 20m 30m	6m 2m - 6m -	6 Wilms >5yr all hepatoblastoma <30m all neuroblastoma <12m

Table V. Overview of suggested screening protocols taking molecular subgroups into account.

Publication	Abdominal ultrasound		AFP		Other
	Frequency	*Duration*	*Frequency*	*Duration*	
Rump et al., 2005					
IC2	indicated for hepatoblastoma	n.m.[1]			
IC1	indicated for Wilms	n.m.			
pUPD	indicated for Wilms	n.m.			
CDKN1C	n.m.				
Santiago et al., 2008					
IC2	once at age 3m	once	-		physical exam 1 x 1m for 0-1yr and 1 x 3m for 2-5yr
IC1	1 x 6m	0-6 yr	1 x 3m	0-4 yr	physical exam 1 x 3m for 0-6yr
pUPD	1 x 6m	0-6 yr	1 x 3m	0-4 yr	physical exam 1 x 3m for 0-6yr
CDKN1C	once at age 3m	once	-		physical exam 1 x 1m for 0-1yr and 1 x 3m for 2-5yr
Brioude et al., 2013					
IC2	once at diagnosis; if hemihyperplasia or organomegaly[2] 1 x 3m	0-6 yr			physical exam 1 x 1m for 0-2yr and 1 x 3-6m for 2-6yr
IC1	1 x 3m	0-6 yr			physical exam 1 x 1m for 0-1yr, 1 x 3m for 1-6yr, 1 x yr after 6yr
pUPD	1 x 3m	0-6 yr			physical exam 1 x 1m for 0-1yr, 1 x 3m for 1-6yr, 1 x yr after 6yr
CDKN1C	1 x 3m	0-6 yr			physical exam 1 x 1m for 0-1yr, 1 x 3m for 1-6yr, 1 x yr after 6yr
Mussa et al., 2015					
IC2	"Questionable"		"Questionable"		
IC1	1 x 3-6m	0-3 yr	-		
pUPD	n.m.		indicated	n.m.	no indication on frequency provided
CDKN1C	n.m.		-		
Cooper et al., 2005					
IC2	Indicated for hepatoblastoma	n.m.	-		
IC1	indicated for Wilms	n.m.	-		
pUPD	indicated for Wilms	n.m.	-		
CDKN1C	n.m.		-		
Present proposal			not indicated		physical exams by parent(s) not indicated
IC2	not indicated				
IC1	indicated for Wilms 1 x 3m	0-4yr			
pUPD	indicated for Wilms and hepatoblastoma 1 x 3m	0-4yr[3]			
CDKN1C	facultative for Wilms and hepatoblastoma 1 x 3m	0-4yr[3]			facultative: urinary VMA/HVA excretion 1 x 3m for 0-2yr
	facultative for neuroblastoma 1 x 3m	0-2yr			
no detectable defect	indicated for Wilms and hepatoblastoma 1 x 3m	0-4yr[3]			

[1] n.m. = not mentioned

[2] enlarged liver or spleen or kidney

[3] For hepatoblastoma indicated till 36m but in practice it will be performed till 48m together with Wilms screening

SUPPLEMENTAL MATERIALS

Phenotype, Cancer Risks and Surveillance in Beckwith-Wiedemann Syndrome Depending on Molecular Genetic Subgroups

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Table S-I. Unusual morphological signs in 244 individuals with Beckwith-Wiedemann syndrome.

Sign	Number of individuals showing sign
Trigonocephaly	1
Plagiocephaly	1
Dolichocephaly	2
Wide anterior fontanel	1
Low frontal hairline	2
Prominent forehead	3
Wide eyebrows	1
Wide palpebral fissures	1
Blepharophimosis	1
Strabismus	3
Periorbital fullness	2
Downward slanted palpebral fissures	2
Upward slanted palpebral fissures	3
Epicanthi	6
Hyperetelorism	3
Wide nasal bridge	4
Depressed nasal bridge	4
Short nose	3
Broad nasal tip	2
Upturned nasal tip	1
Wide nares	1
Flat face	1
Flat malae	1
Full cheeks	1
Smooth philtrum	2
Long philtrum	1
Thin vermillions	1
Full lips	1
Highly arched palate	1
Low set ears	1
Posteriorly rotated ears	1
Prominent ears	1
Deep flexion creases hands	1

Single flexion crease hand	1
Genua valga	1
Partial syndactyly toes 2+3	2
Hypermobile joints	1
Pectus carinatum	1

SUPPLEMENTAL MATERIALS

Phenotype, Cancer Risks and Surveillance in Beckwith-Wiedemann Syndrome Depending on Molecular Genetic Subgroups

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Supplemental Table S-2. Correlations between hemihyperplasia and enlarged visceral organs and the presence of Wilms tumors for the various molecular genetic subgroups of Beckwith-Wiedemann syndrome.

Molecular subgroup	**Physical sign**	**Frequency Wilms tumor**	**Fisher's exact test**
IC2	hemihyperplasia	1/33	
	no hemihyperplasia	1/72	
	all	2/105	0.532
	splenomegaly	0/7	
	no splenomegaly	2/88	
	all	2/95	1
	hepatomegaly	0/18	
	no hepatomegaly	2/82	
	all	2/100	1
	hepato- or splenomegaly	0/29	
	no hepato- or splenomegaly	2/85	
	all	2/114	1
	hemihyperplasia + hepato- or splenomegaly	0/8	
	no hemihyperplasia + hepato- or splenomegaly	2/106	
	all	2/114	1
	nephromegaly	0/14	
	no nephromegaly	2/84	
	all	2/98	1
IC1	hemihyperplasia	3/11	
	no hemihyperplasia	3/7	

	all	6/18	0.672
	splenomegaly	1/3	
	no splenomegaly	5/16	
	all	6/19	1
	hepatomegaly	1/4	
	no hepatomegaly	5/15	
	all	6/19	1
	hepato- or splenomegaly	3/8	
	no hepato- or splenomegaly	3/11	
	all	6/19	1
	hemihyperplasia + hepato- or splenomegaly	1/4	
	no hemihyperplasia + hepato- or splenomegaly	5/15	
	all	6/19	1
	nephromegaly	3/7	
	no nephromegaly	3/12	
	all	6/19	0.617
pUPD	hemihyperplasia	0/36	
	no hemihyperplasia	2/6	
	all	2/42	0.017*
	splenomegaly	1/4	
	no splenomegaly	1/30	
	all	2/34	0.225
	hepatomegaly	1/7	
	no hepatomegaly	1/27	
	all	2/34	0.374
	hepato- or splenomegaly	2/18	
	no hepato- or splenomegaly	1/26	
	all	3/44	0.558
	hemihyperplasia + hepato- or splenomegaly	0/15	
	no hemihyperplasia + hepato-		

	or splenomegaly	3/29	
	all	3/44	0.540
	nephromegaly	2/17	
	no nephromegaly	1/21	
	all	3/38	0.577
No defect	hemihyperplasia	4/17	
	no hemihyperplasia	0/28	
	all	4.45	0.016*
	splenomegaly	2/6	
	no splenomegaly	1/38	
	all	3/44	0.045
	hepatomegaly	2/13	
	no hepatomegaly	1/29	
	all	3/42	0.222
	hepato- or splenomegaly	3/19	
	no hepato- or splenomegaly	1/33	
	all	4/52	0.132
	hemihyperplasia + hepato- or splenomegaly	3/6	
	no hemihyperplasia + hepato- or splenomegaly	1/46	
	all	4/52	0.003*
	nephromegaly	3/16	
	no nephromegaly	1/28	
	all	4/44	0.129