ORIGINAL ARTICLE

AMERICAN JOURNAL OF medical genetics PART A

Phenotype, Cancer Risk, and Surveillance in Beckwith–Wiedemann Syndrome Depending on Molecular Genetic Subgroups

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Manuscript Received: 27 January 2016; Manuscript Accepted: 29 May 2016

Patients with Beckwith–Wiedemann syndrome (BWS) have an increased risk to develop cancer in childhood, 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 the literature. Phenotype, genotype and tumor occurrence were available in 229 of our 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 1,971 BWS patients. Tumor risks were 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 before 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.

Key words: Wiedemann–Beckwith syndrome; Wilms tumor; hepatoblastoma; neuroblastoma; genotype–phenotype correlation

How to Cite this Article:
Maas SM, Vansenne F, Kadouch DJM, Ibrahim A, Bliek J, Hopman S, Mannens MM, Merks JHM, Maher ER, Hennekam RC. 2016. Phenotype, cancer risk, and surveillance in Beckwith–Wiedemann syndrome depending on molecular genetic subgroups.
Am J Med Genet Part A 170A:2248–2260.

INTRODUCTION

Beckwith–Wiedemann syndrome (BWS) is an overgrowth disorder characterized by perinatal overgrowth, macroglossia, exomphalos,

Conflict of interest: The authors have no potential conflicts of interest to disclose.

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Article first published online in Wiley Online Library (wileyonlinelibrary.com): 15 July 2016
DOI 10.1002/ajmg.a.37801

hemihyperplasia, and postnatal hypoglycemia, and associated with an increased risk to develop embryonic tumors. [Cohen, 2005; Weksberg et al., 2005]. The prevalence at birth is estimated to be 1/12,000 [Hennekam et al., 2010]. Two sets of similar, but not identical, diagnostic criteria are mainly used in clinical practice (Table I) [Elliott et al., 1994; DeBaun et al., 2002].

Familial transmission has been reported in about 15% of all BWS patients grouped together [Weksberg et al., 2010]. Our own experience is that familial occurrence is considerably lower and that this figure needs re-evaluation. 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 [Azzi et al., 2014]. This imprinting center is differentially methylated, methylation being present only at the paternal allele. The second cluster contains (among others) the maternally expressed *CDKN1C* and the paternally expressed *KCNQ1OT1* (*LIT1*) 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 et al., 2013]. Aberrant methylation of both ICs is typically explained by a mosaic paternal uniparental disomy (pUPD) of 11p15 (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 [Scott et al., 2006a]. Approximately 10–15% of patients remain without molecular confirmation of the syndrome despite carrying all clinical characteristics of the syndrome [Bliek et al., 2001]. Whether more in depth analysis of these cases will demonstrate abnormalities in one genes already known to be involved in BWS, whether an unusual pattern of mosaicism or disturbance of methylation is present, or whether other genetic or epigenetic mechanisms play a role in pathogenesis remains unknown.

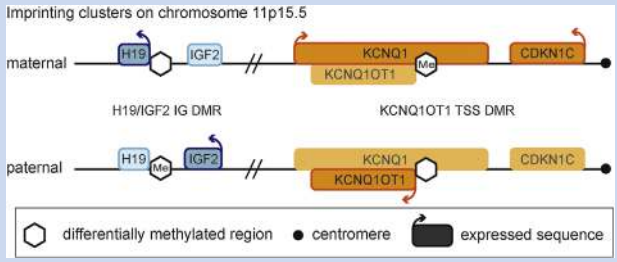

FIG. 1. Schematic representation of the imprinting control regions (ICR) on chromosome 11p15. BWSIC1 contains the reciprocally imprinted embryonic growth factor IGF2 (expressed from paternal allele) and the noncoding RNA H19 (expressed from maternal allele). Disturbance of the IC1 methylation results 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. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmga].

TABLE I. Two Major Sets of Diagnostic Criteria Used for Beckwith–Wiedemann Syndrome

	DeBaun et al. [2002]	Elliott et al. [1994]
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

BWS patients have an increased incidence of embryonic tumors, especially Wilms tumors, but also of hepatoblastomas, neuroblastomas, adrenal carcinomas, and rhabdomyosarcomas [DeBaun et al., 1998; Engel et al., 2000; Gaston et al., 2001; DeBaun et al., 2002; Bliek et al., 2004; Cooper et al., 2005; Rump et al., 2005; Brioude et al., 2013]. 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 and patients with an abnormality involving the centromeric domain (*CDKN1C* mutations and loss of KCNQ1OT1 methylation [LOM]) tend to have a lower risk [Weksberg et al., 2001; Cooper et al., 2005; Brioude et al., 2013]. Several protocols have been suggested for tumor surveillance, consisting typically of abdominal ultrasound and screening of alpha-fetoprotein levels at various ages and intervals [Shah, 1983; Beckwith, 1998a; Choyke et al., 2003; Scott et al., 2006b; Zarate et al., 2009; Choufani et al., 2010; Teplick et al., 2011; Brioude et al., 2013; Eggermann et al., 2014]. 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 [Cooper et al., 2005; Rump et al., 2005; Santiago et al., 2008; Brioude et al., 2013; Mussa et al., 2016].

Here, we report on studies in a large cohort of BWS individuals, summarize their phenotype, add data from similar studies in the literature in order to correlate the phenotype with the various genetic subgroups. We determined the relative tumor risks for each subgroup 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 1990s. 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 [Elliott et al., 1994; DeBaun et al., 2002].

Clinical data of all included patients were obtained either directly by examining patients (41%), or through questionnaires on clinical manifestations forwarded to physicians who submitted samples of patients suspected for having BWS (59%). In 2005, a dedicated outpatient clinic 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 patient. 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 studies were performed on a diagnostic basis and are funded by health insurances in the Netherlands. 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) [Alders et al., 2009] or methylation multiplex ligation-dependent probe amplification (MLPA) [Schouten et al., 2002]. 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 [Alders et al., 2009]. Mutation analysis of *CDKN1C* was not performed routinely. Study participants were classified in four 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 tumor* 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 malignant and, thus, listed in the International Classification of Childhood Cancer (ICC3) were included [Steliarova-Foucher et al., 2005]. 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. This is excluding five patients who had a chromosome abnormality and were excluded as the other chromosome imbalances prohibited analysis of the phenotype due to only a 11p15 imbalance. All but three patients were at least 5 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 the frequencies of manifestations of BWS in the genetic subgroups and in the total patient group are listed. The various abnormal morphological characteristics are available in Supplemental Table SI.

Tumor Frequencies in Our 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 [Gaston et al., 2001; Weksberg et al., 2001; DeBaun et al., 2002; Bliek et al., 2004; Brioude et al., 2013; Ibrahim et al., 2014; Mussa et al., 2016].

In three cohorts not all BWS patients were screened for *CDKN1C* mutations [DeBaun et al., 2002; Bliek et al., 2004], and in two other studies [Brioude et al., 2013; Mussa and Ferrero, 2015] BWS patients in whom no molecular defect could be detected were not included [Brioude et al., 2013; Mussa et al., 2016]. 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 1,971 BWS patients were available. The highest tumor risk was present in the genetic subgroup

TABLE II. Phenotype in 244 Patients With Beckwith–Wiedemann Syndrome Comparing Overall Phenotype to Those in the Various Genetic Subgroups[a]

	Total (%)	IC2 LOM (%)	IC1 GOM (%)	pUPD (%)	Clinical diagnosis (%)	*P*-value[b]
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[c]	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	112/172 (65.1)	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[d]	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	46/199 (23.1)	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[e]	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[f]	18/179 (10)	7/87 (8)	1/19 (5.3)	2/34 (5.9)	8/39 (20.8)	0.148

[a]Totals not always add up to 244 as a feature may not always be recorded in all patient. LOM, loss of methylation; GOM, gain of methylation.
[b]*P* values: *P* values refer to the frequency of each variable between the four subgroups, so all genetic subgroups are compared at the same time.
[c]All patients fulfilled the diagnostic criteria of DeBaun et al. [2002].
[d]A full list of all other signs is available as Supplemental material Table SI.
[e]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).
[f]Definition according to American Psychiatric Association. 2013. Diagnostic and statistical manual of mental disorders: DSM-5. Washington, D.C.: American Psychiatric Association.

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). If all patients were subdivided in year groups, the percentage developing a Wilms tumor never exceeded the limit for screening (of 2%), not even if all BWS individuals were grouped together irrespective the molecular subgroup.

DISCUSSION

BWS is often diagnosed by combining clinical and molecular findings. Two generally accepted sets of diagnostic criteria are described by Elliott et al. [1994] and DeBaun et al. [2002] (Table I). The Elliott criteria are usually stricter than the DeBaun criteria, and in our series all patients fulfilled the DeBaun criteria, while 43.8% fulfilled the Elliott criteria. In the series of patients published by [Ibrahim et al., 2014], the sensitivity of the DeBaun criteria were calculated to be higher than the Elliott criteria (83.5% vs. 43.5%), and specificity were 83.5% (Elliot criteria) versus 62.3% (DeBaun criteria). Whether in the present cohort the specificity of either set of criteria is higher 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, there is a subgroup of patients with a clinical diagnosis of BWS, but no detectable molecular abnormality [Bliek et al., 2004; Ibrahim et al., 2014] 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 set do we know with certainty that sensitivity and specificity are truly high,

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[a]	Two hepatoblastoma, two rhabdomyosarcoma, one gonadoblastoma
		IC1	1/3	One Wilms
		UPD	6/21	Five Wilms, one hepatoblastoma
		N.D.*	4/17[b]	Four Wilms
		CDKN1C	0/5	None
Gaston et al. [2001]	97[c]	IC2	1/45	One thyroid carcinoma (11 yr)
		IC1	5/11	Four Wilms, one ganglioneuroma
		UPD	4/11[d]	Two Wilms, one Wilms + neuroblastoma, one mamma adenoma (14 yr) + pheochromocytoma (19 yr)
		N.D.*	1/24	One Wilms
		CDKN1C	1/2	One neuroblastoma
DeBaun et al. [2002]	92	IC2	1/39	Not specified
		IC1	4/10	
		UPD	5/12	
		N.D.*	6/31	
		CDKN1C	N.S.**	
Bliek et al. [2004][e]	66	IC2	2/27	One thyroid carcinoma (14 yr), one hepatoblastoma
		IC1	6/9	Six Wilms
		UPD	7/13[f]	Four Wilms, one adrenal carcinoma, one neuroblastoma, one hepatoblastoma, one pheochromocytoma, one leukemia, one mammary adenoma
		N.D.*	3/17	Two Wilms, one neuroblastoma
		CDKN1C	N.S.**	
Brioude et al. [2013][g]	407	IC2	8/257	Two neuroblastoma, two hepatoblastoma, one sarcoma, one rhabdomyosarcoma, one thyroid carcinoma, one melanoma
		IC1	8/35	Eight Wilms tumor
		UPD	14/81[h]	Ten Wilms tumor, two adrenocortical carcinoma, two hepatoblastoma, one rhabdomyosarcoma, one neuroblastoma, one acute lymphoid leukemia
		N.D.*	N.S.**	
		CDKN1C	3/34	One neuroblastoma, one ganglioneuroma, one acute lymphoid leukemia
Ibrahim et al. [2014][g,i,j]	637	IC2	2/288	One hepatoblastoma, one rhabdomyosarcoma
		IC1	3/28	Three Wilms
		UPD	4/99	One Wilms, three hepatoblastoma
		N.D.*	5/201	Three Wilms, one adrenocortical carcinoma, one neuroblastoma
		CDKN1C	1/21[k]	One Wilms
Mussa and Ferrero [2015][g,l]	318	IC2	4/190	Two neuroblastoma, one rhabdomyosarcoma, one germinoma
		IC1	8/31	Seven Wilms, one pancratoblastoma
		UPD	13/87	Three Wilms, five hepatoblastoma, two neuroblastoma, one pancreatoblastoma, one adrenal carcinoma, one hemangioteloma
		N.D.*	N.S.**	
		CDKN1C	0/10	None
Present study[m]	229	IC2	3/114	Two Wilms, one hepatoblastoma
		IC1	6/19	Six Wilms
		UPD	6/44	Three Wilms, one hepatoblastoma, one myopepithelial cell carcinoma (13 yr), one pheochromocytoma
		N.D.*	4/52[n]	Three Wilms, one Wilms + one hepatoblastoma + one rhabdomyosarcoma
		CDKN1C	N.S.**	
Pooled data	1971	IC2	26/995 (2.6%)	Two Wilms, seven hepatoblastoma, three thyroid ca, five rhabdomyosarcoma, one sarcoma, four neuroblastoma, one melanoma, one gonadoblastoma, one germinoma, one not specified
		IC1	41/146 (28%)	Thirty-five Wilms, one ganglioneuroma, four not specified, one pancratoblastoma
		UPD	59/368 (16%)[o]	Twenty-nine Wilms, 13 hepatoblastoma, one pancreatoblastoma, one hemangiotheloma, four adrenocortical carcinoma, two mammary adenoma, one rhabdomyosarcoma, one myoepithelial cell carcinoma, five neuroblastoma, three pheochromocytoma, two ALL, five not specified
		N.D.*	23/342 (6.7%)[p]	Fourteen Wilms, one hepatoblastoma, one rhabdomyosarcoma, Two neuroblastoma, one adrenocortical carcinoma, six not specified
		CDKN1C	5/72 (6,9%)	One Wilms, two neuroblastoma, one ganglioneuroma, one acute lymphatic leukemia
		All	155/1923 (8%)	

[a]Only 59 of 125 patients evaluated for IC2.
[b]Only 67 have been completely evaluated.
[c]Used other diagnostic criteria than the DeBaun or Elliott criteria.
[d]Six tumors in four patients.
[e]Only patients from France included (Dutch patients included in present study).
[f]Ten tumors in seven patients.
[g]Only patients with a genetic defect included.
[h]Seventeen tumors in 14 patients.
[i]Includes patients reported by Engel et al. [2000].
[j]Adapted figures as patients with isolated hemihypertrophy were excluded and additional data is included.
[k]Not all patients have been tested for CDN1 C (personal communication).
[l]Includes patients reported by Mussa et al. [2012].
[m]Series include Bliek et al. [2001] and Dutch patients in Bliek et al. [2004].
[n]Six tumors in four patients.
[o]Sixty-seven tumors in 59 patients.
[p]Twenty-five tumors in 23 patients.
*N.D. = no defect.
**N.S. = not studied.

TABLE IV. Age at Detection of Wilms Tumors and Other Tumors in Cohorts of Individuals With Beckwith–Wiedemann Syndrome

	Mean age			Median age			
Study	All tumors	Wilms tumor	Hepato-blastoma	All tumors	Wilms tumor	Hepato-blastoma	Other data
Green et al. [1993]					26 m		Eldest age Wilms 7.9 yr
DeBaun and Tucker [1998]	14 m						Five hepatoblastoma, six Wilms, two neuroblastoma
Gaston et al. [2001]	58 m	34.5 m		18 m	24 m		One Wilms 12 yr, one thyroid carcinoma
IC2	132 m	–					Eleven years, one mamma adenoma 14 yr
IC1	25 m	25 m					One pheochromocytoma 19 yr
pUPD	79 m	55 m					Neuroblastoma 4 and 10 m
No defect	12 m	12 m (n = 1)					
Clericuzio et al. [2003]			6 m			5 m	
Mussa et al. [2012]							One Wilms 10 yr (bilateral)
Brioude et al. [2013]	24 m	21 m	3 m	21 m	22.5 m	3 m	One Wilms 12 yr, one ALL 120 m
IC2	35 m	–	1.5 m	28.5 m	–	1.5 m	One sarcoma 74 m
IC1	24 m	24 m	–	24 m	24 m	–	One thyroid carcinoma 75 m
pUPD	16 m	17 m	5 m	16 m	18.5 m	5 m	Three neuroblastoma <1, 4, and 6 m
Trobaugh-Lotrario et al. [2014]			8 m			6 m	Eldest age hepatoblastoma 30 m
Ibrahim et al. [2014]	24 m	33 m	8 m	24 m	36 m	6 m	
IC2	–	–	–	–	–	–	
IC1	37.5 m	37.5 m	–	37.5 m	37.5m	–	
pUPD	25.5 m	24 m (n = 1)	8 m	6 m	24 m (n = 1)	6 m	
No defect	32 m	32 m	–	36 m	36 m	–	
Present study	28 m	30 m	11.5 m	18 m	18 m	12 m	Eldest age Wilms 5.5 yr
IC2	39 m	11 m	14 m (n = 1)	14 m	14 m		Eldest age hepatoblastoma 30 m
IC1	31 m	31 m	–	33 m	33 m		
pUPD	27.5 m	34 m	9 m (n = 1)	57 m	30 m		
No defect	41 m	41 m	–	41 m	41 m		
All studies	28 m	28 m	7 m	14 m	24 m	6 m	Six Wilms >5 yr
IC2	38 m	11 m	6 m	13 m	11 m	2 m	All hepatoblastoma <30 m
IC1	28 m	25 m	–	24 m	24 m	–	All neuroblastoma <12 m
pUPD	29 m	29 m	7 m	12 m	20 m	6 m	
No defects	32 m	32 m	–	30 m	30 m	–	

although we expect this is the case. BWS still is a clinical diagnosis, in which molecular confirmation is not always possible, and further studies of the Elliott and DeBaun criteria and other sets of criteria, including studies set up specifically to test for sensitivity and specificity, are needed.

Clinical Features of BWS

The phenotype of the present cohort is, in general, comparable to that in other cohorts [Brioude et al., 2013; Mussa et al., 2016]. As can be expected, the patients in the group "clinical diagnosis" show the signs that are very characteristic for BWS 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 nevus flammeus in the IC1 hypermethylation subgroup [Gaston et al., 2001; DeBaun et al., 2002; Cooper et al., 2005; Mussa et al., 2012; Brioude et al., 2013; Ibrahim et al., 2014]. 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 [Goldman et al., 2002; Mussa et al., 2012].

Though not all data were collected by evaluating the cohort personally, the vast majority of cases were seen and examined and so the data in Table II is likely 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. [2005] 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.

We provide a complete literature overview evaluating almost 2,000 BWS patients, which allows reliable conclusions (Table III). Earlier careful meta-analyses of the literature are available, but in much smaller cohorts [Rump et al., 2005; Mussa et al., 2016]. We realize there is likely still a publication bias, and in reality frequencies may be somewhat lower.

We evaluated the nature of the tumors in our own patients and patients reported in the literature. Wilms tumors and hepatoblastoma are only rarely present in the IC2 hypomethylation subgroup, and Wilms tumors are very unusual in the CDKN1C group. In all other molecular groups Wilms tumors occur frequently. In the IC2 hypomethylation subgroup, the variability in tumor types is remarkably large. In the IC1 group no hepatoblastoma has been described, although we cannot exclude with complete certainty that the four patients reported to have an unspecified tumor did not have a hepatoblastoma [DeBaun et al., 2002]. In the CDKN1C group individuals developed neuroblastoma at age 6 and 10 months, respectively [Gaston et al., 2001; Brioude et al., 2013].

The median age at which BWS individuals develop cancer in the present cohort was 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 the literature data in Table IV.

Cancer risks in BWS have been correlated with the presence of hemihyperplasia, nephromegaly, nephrogenic rests, and nephroblastomatosis [Beckwith, 1998b; Goldman et al., 2002; Cohen, 2005]. Mussa et al. [2012] found hemihyperplasia and enlarged kidneys in all patients with a Wilms tumor, and similarly DeBaun et al. [1998] reported that all patients with a Wilms tumor had enlarged kidneys if evaluated repeatedly. In the latter publication, before molecular subgroups could be distinguished, the nephromegaly was typically bilateral, and the cancer had always arisen in the largest kidney [DeBaun et al., 1998]. Gaston et al. [2001] found a (statistically insignificant) higher frequency of hemihyperplasia in patients with tumors, but this was not subdivided according to molecular subtype. We evaluated the associations of physical signs with cancer risk 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 SII). However, for no subgroup 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. Indeed, this may indicate that this group has a very low mosaicism for pUPD which escaped detection. In the latter group there was significantly more frequently an enlarged spleen ($P = 0.016$). Otherwise in no subgroup a marked difference was found for the occurrence of Wilms tumors and the presence of hemihyperplasia, enlarged liver or spleen, 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 [Teplick et al., 2011]. The outcomes can improve by detecting tumors earlier, at a less advanced stage. Less advanced tumors generally need less extensive surgery and less intensive chemo- and radiotherapy, and are associated with a better survival [Teplick et al., 2011]. A prerequisite is that the screening schedule is such that, indeed, the tumor is detected at a less advanced stage, so the velocity of the tumor growth, the sensitivity, and specificity of the screenings procedure, the interval between the screenings, the treatment schedules of the various stages of the tumor, and the effectiveness of these treatment schedules need to be carefully determined [Lapunzina, 2005].

Screening has significant consequences for the emotional well-being of patients and their families. It can be positive for parents to know their child is being monitored, but on the other hand it can also create anxieties around each screening episode, as has been described in surveillance of adults after having had cancer [Thompson et al., 2010]. 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 [Beckwith, 1998a; Choyke et al., 2003].

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 [Scott et al., 2006b]. Other studies did not specifically mention 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, if the total risk to develop a tumor is between 2% and 5%. This has been checked in the present study for each tumor type detected in a patient and it was determined whether the threshold was reached, taking the various genetic subgroups into account. Only Wilms tumor, hepatoblastoma, and neuroblastoma as individual tumor had a higher frequency than the 2% threshold.

Screening has financial implications. In most countries these are limited for the patients and the families, but may be significant for society. A cost-effectiveness evaluation should be part of general evaluations of screening procedures [McNeil et al., 2001].

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 adequately address the needs of the population. 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 [Beckwith, 1998b]. 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 [Mussa et al., 2012], 12 years [Gaston et al., 2001; Brioude et al., 2013], and 13 years in a patient with a

TABLE V. Overview of Suggested Screening Protocols Taking Molecular Subgroups Into Account

	Abdominal ultrasound		AFP		
Publication	**Frequency**	**Duration**	**Frequency**	**Duration**	**Other**
Rump et al. [2005]					
IC2	Indicated for hepatoblastoma	n.m.			
IC1	Indicated for Wilms	n.m.			
pUPD	Indicated for Wilms	n.m.			
CDKN1C	n.m.				
Santiago et al. [2008]					
IC2	Once at age 3 m	Once	–		Physical exam 1 × 1 m for 0–1 yr and 1 × 3 m for 2–5 yr
IC1	1 × 6 m	0–6 yr	1 × 3 m	0–4 yr	Physical exam 1 × 3 m for 0–6 yr
pUPD	1 × 6 m	0–6 yr	1 × 3 m	0–4 yr	Physical exam 1 × 3 m for 0–6 yr
CDKN1C	Once at age 3 m	Once	–		Physical exam 1 × 1 m for 0–1 yr and 1 × 3 m for 2–5 yr
Brioude et al. [2013]					
IC2	Once at diagnosis; if hemihyperplasia or organomegaly[a] 1 × 3 m	0–6 yr			Physical exam 1 × 1 m for 0–2 yr and 1 × 3–6 m for 2–6 yr
IC1	1 × 3 m	0–6 yr			Physical exam 1 × 1 m for 0–1 yr, 1 × 3 m for 1–6 yr, 1 × yr after 6 yr
pUPD	1 × 3 m	0–6 yr			Physical exam 1 × 1 m for 0–1yr, 1 × 3 m for 1–6 yr, 1× yr after 6 yr
CDKN1C	1 × 3 m	0–6 yr			Physical exam 1 × 1 m for 0–1yr, 1 × 3 m for 1–6 yr, 1× yr after 6 yr
Mussa and Ferrero [2015]					
IC2	"Questionable"		"Questionable"		
IC1	1 × 3-6 m	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 × 3 m	0–4 yr			
pUPD	Indicated for Wilms and hepatoblastoma 1 × 3 m	0–4 yr[b]			
CDKN1C	Facultative for Wilms and hepatoblastoma 1 × 3 m	0–4 yr[b]			Facultative: urinary VMA/HVA excretion 1 × 3 m for 0–2 yr
	Facultative for neuroblastoma 1 × 3 m	0–2 yr			
No detectable defect	Indicated for Wilms and hepatoblastoma 1 × 3 m	0–4 yr[b]			

n.m., not mentioned.
[a]Enlarged liver or spleen or kidney.
[b]For hepatoblastoma indicated till 36 m but in practice it will be performed till 48 m together with Wilms screening.

cytogenetically visible deletion of 11p13 [Seshachalam et al., 2011]. Long-term survival in Wilms tumors is >90% for localized tumors and >70% for advanced tumors [Tan and Amor, 2006]. Advanced stage Wilms tumors need more intensive chemotherapy and radiotherapy [Pritchard-Jones, 2002]. Detection of Wilms tumor at an earlier stage reduced treatment-related morbidity in isolated Wilms tumor and in BWS related Wilms tumor in some studies [Pritchard-Jones, 2002; Kaste et al., 2008; Zarate et al., 2009], but not in other BWS related Wilms tumor studies [Shackney et al., 1978; Craft et al., 1995]. No results of reliable studies are available showing that early detection has a significant impact on the overall survival. Craft et al. [1995] reported lack of a difference in outcome or stage distribution of the tumor between screened and unscreened populations.

False positive screening results have been reported such as cysts, nephrogenic rests, or foci of renal dysplasia [Beckwith, 1998a; Choyke et al., 1999]. The doubling time estimated for growth rate in Wilms tumors is 11–40 days [Shackney et al., 1978; Craft, 1999; Zoubeck et al., 1999]. This rapid tumor growth indicates only an interval of 3 and 4 months between screening is appropriate. McNeil et al. [2001] concluded that ultrasound screening of the abdomen at least until the age 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.

Background for Hepatoblastoma Screening

Hepatoblastomas are malignant liver cancers that consist of fetal liver cells, more mature liver cells and bile duct cells [Roebuck and Perilongo, 2006]. Ninety percent of hepatoblastomas occur before age 4 years, at a mean age of 22 months and median age of 16 months, and only exceptionally at an older age [Surveillance Epidemiology, and End Results (SEER) Program, 2001; Tannuri et al., 2015]. In BWS all hepatoblastoma occurred before 30 months (Table IV) [Tannuri et al., 2015]. We have been unable to find a reliable description of an exception. In children with BWS hepatoblastoma was diagnosed at a significantly younger age (median age 6 months) compared to children with hepatoblastoma without BWS (median age 16 months), and the stage at diagnosis tended to be lower [Trobaugh-Lotrario et al., 2014]. Depending on stage and location, patients are treated surgically or by chemotherapy [Teplick et al., 2011]. After complete resection patients have an event-free survival of >90% [Katzenstein et al., 2002; Perilongo et al., 2004]. 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% [Katzenstein et al., 2002; Perilongo et al., 2004]. Thus, early detection by effective screening could lower tumor advancement and treatment-related morbidity [Rao et al., 2008; Trobaugh-Lotrario et al., 2014]. In over 96% of patients with hepatoblastoma serum alpha-fetoprotein (AFP) levels are elevated and especially a rising trend is indicative for a hepatoblastoma. Since AFP levels tend to be elevated in BWS individuals anyway, this urges for careful interpretation of screening results to avoid false positive results [Everman et al., 2000; Tan and Amor, 2006; Zarate et al., 2009]. AFP levels can be elevated when abdominal ultrasounds do not allow visualization of a tumor [Clericuzio et al., 2003; Tan and Amor, 2006] and especially a rise of AFP levels after a few weeks is a strong indicator for further evaluations [Tan and Amor, 2006]. Rojas et al. [2014] reported on a small series of patients with hepatoblastoma who were screened for recurrences. They found that AFP was elevated 1–11 months before the tumor was detected by the surveillance imaging, and also reported false positive results. A similar study showed AFP to be elevated until 2 months before imaging showed an abnormality, and these authors reported on false negative results [Semeraro et al., 2013]. The half-life of AFP is 5–6 days [Murray and Nicholson, 2011]. Hepatoblastoma can grow very rapidly, doubling time has been reported as low as a few weeks [Zarate et al., 2009].

Authors of several early publications concluded the usefulness of AFP screening should be doubted due to interpretation difficulties [Mussa and Ferrero, 2015], uncertainty whether it allows earlier discovery of hepatoblastoma such 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 [Zarate et al., 2009; Brioude et al., 2013; Mussa and Ferrero, 2015]. We conclude that there are important doubts about AFP-screening and that the use of AFP in surveillance should be balanced against the burden of repeated blood sampling in younger children and anxieties around sampling for families.

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. Neuroblastomas are usually diagnosed between 0 and 4 years of age (median 19 months) [London et al., 2005]. Less than 5% occur at 10 years or above [Irwin and Park, 2015]. A neuroblastoma can be classified as low risk, intermediate risk, and high risk depending on age, stage, histopathology, DNA index (ploidy), and MYCN amplification [Monclair et al., 2009]. Depending on the stage, treatment consists of observation, 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% [Davenport et al., 2012]. Homovanillic acid and vanillylmandelic acid (HVA and VMA) are good biomarkers to detect neuroblastoma [Strenger et al., 2007]. Population screening resulted in increased detection of tumors but these were tumors with favorable biology and pathology [Schilling et al., 2002; Hiyama et al., 2008]. 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 3 months until age 2 years has been recommended and subsequent screening was depending on the risk of developing tumor [Irwin and Park, 2015].

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 (Fig. 2), and duration. We add to these an amended surveillance protocol based on the following points:

(1) 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.
(2) 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 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 slightly increased risk of developing a tumor is indicated.
(3) 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 complete screening. For Wilms tumor

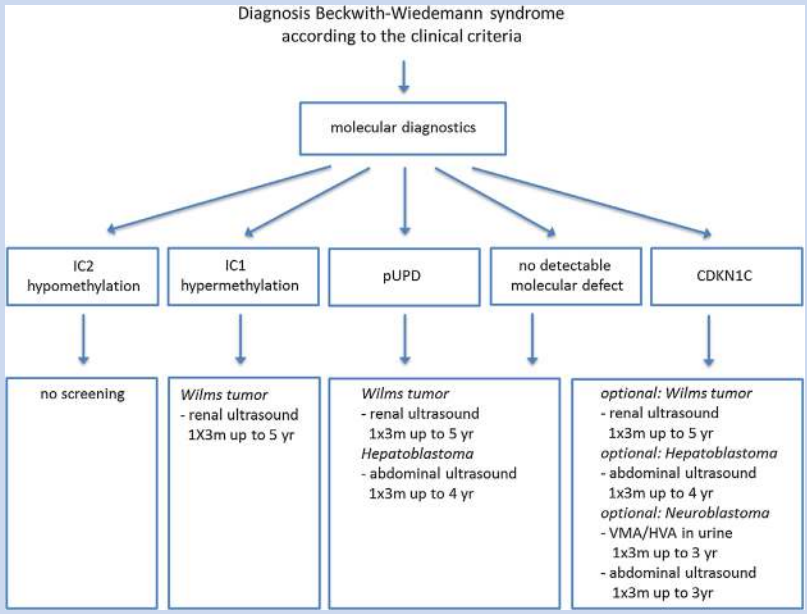

FIG. 2. Suggested surveillance in patients with Beckwith–Wiedemann syndrome depending on molecular subgroup. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/journal/ajmga].

and hepatoblastoma the screening can, therefore, be the same screening as for BWS patients with a pUPD.

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

(5) BWS patients with 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.

(6) 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 5 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.

(7) Presence of Wilms tumors is screened by renal sonographies although local circumstances may make MRI screening more useful. Renal sonographies will be the first technique of choice for financial reasons, but there is increasing use of total body MRIs for various purposes in several countries. Organs can be reliably visualized in a very short MRI time, which may makes it very feasible to use in young children, and we recognize the reliability of MRIs for this goal.

(8) 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, abdominal imaging including both kidneys and liver should be performed simultaneously in patients with pUPD, CDKN1C and those with no detectable molecular abnormality.

(9) Existence of hepatoblastoma is screened by liver sonographies although local circumstances may make MRI screening an alternative (see point 7). 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 the parent-child relationship, especially if a mass is not detected during "parental surveillance" [Seshachalam et al., 2011].

(10) Neuroblastoma can be screened for by urinary excretion of VMA and HVA and by abdominal ultrasound every 3 months until the age 2 years and should be discussed with parents as an option. 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.

(11) 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 become 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 six (6.4%) [Romanelli et al., 2010; Brioude et al., 2015]. 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 [Seshachalam et al., 2011].

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 a literature overview, and offers a state-of-the-art of 2016. 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 benefit the BWS children and their families. The present study is performed in the Netherlands and the UK, where molecular diagnostics are available to everyone and in typical circumstances without additional costs. In addition, management is generally based primarily on available evidence rather than medicolegal concerns. We recognize that in some countries the above recommendations may be modified to reflect local medical and non-medical practices.

ACKNOWLEDGMENTS

We thank all patient and their parents for their generous participation in the study. We are grateful to the many clinicians who have helped recruit patients to the study and provided clinical data. We thank Hester Groot, Jan Lieverst, and Ardine Reedijk for providing information from the Dutch Childhood Oncology Group pediatric cancer registry. We also thank Lot Schutte for helping with the SPSS database.

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