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# Investigation of (epi)genotype causes and follow-up manifestations in the patients with classical and atypical phenotype of Beckwith-Wiedemann spectrum

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## Abstract

Beckwith-Wiedemann syndrome (BWS) is a genomic imprinting disorder, characterized by macroglossia, abdominal wall defects, lateralized overgrowth, and predisposition to embryonal tumors. It is caused by the defect of imprinted genes on chromosome 11p15.5, regulated by imprinting control (IC) domains, IC1, and IC2. Rarely, *CDKN1C* and chromosomal changes can be detected. The aim of this study is to retrospectively evaluate 55 patients with BWS using the new diagnostic criteria developed by the BWS consensus, and to investigate (epi)genetic changes and follow-up findings in classic and atypical phenotypes. Loss of methylation in IC2 region (IC2-LoM), 11p15.5 paternal uniparental disomy (pUPD11), and methylation gain in IC1 region (IC1-GoM) are detected in 31, eight, and five patients, respectively. Eleven patients have had no molecular defects. Thirty-five patients are classified as classical and 20 as atypical phenotype. Patients with classical phenotype are more frequent in the IC2-LoM (25/31), while patients with atypical phenotype are common in the pUPD11 group (5/8). Malignant tumors have developed in six patients (10.9%); three of these patients have IC1-GoM, two pUPD11, one IC2-LoM genotype, and four an atypical phenotype. We observed that the face was round in the infantile period and elongated as the child grew-up, developing prognathism and becoming asymmetrical if hemi-macroglossia was present in the classical phenotype. These findings were mild in the atypical phenotype. These results support the importance of using the new diagnostic criteria to facilitate the diagnosis of patients with atypical phenotype who have higher tumors risk. This study also provides important information about facial gestalt.

**KEYWORDS**
atypical phenotype, Beckwith-Wiedemann spectrum, classical phenotype, embryonal tumors, facial gestalt

## 1 | INTRODUCTION

Beckwith-Wiedemann Syndrome (BWS) (OMIM #130650) is the most common overgrowth syndrome caused by epigenetic and/or genetic alterations within the chromosome 11p15.5 region. The reported prevalence is 1/10,340 live births (Mussa et al., 2013). The frequency of BWS is more common in births with assisted reproductive technologies compared to the general population (Mussa et al., 2017).

  

The clinical findings of BWS are divided into two groups; the more characteristic ones being cardinal features (macroglossia, omphalocele, lateralized overgrowth, multifocal Wilms tumor-WT- or nephroblastomatosis, hyperinsulinism, and pathology findings) and the ones supporting the clinical diagnosis as suggestive features (birth weight greater>2 standard deviation scores-SDS-, facial nevus simplex, polyhydramnios, placentamegaly, ear crease/pit, transient hypoglycemia, organomegaly, umbilical hernia, diastasis recti, and typical embryonal tumors) (Brioude et al., 2018).

Epigenetic and genetic defects cause BWS by changing the expression of imprinted genes localized in 11p15.5, which play a role in fetal and postnatal growth and cell proliferation (Mussa, et al., 2016a). Imprinted genes in the 11p15.5 region are classified into two domains as Impriting Center 1 (IC1) and Imprinting Center 2 (IC2). The IC1 telomeric domain controls *H19* and *IGF2* expression, while IC2 centromeric domain regulates *KCNQ1OT1*, *CDKN1C*, and *KCNQ1* expressions (Mussa, et al., 2016c). *H19* in the IC1 domain has only maternal expression and acts as a tumor suppressor. *IGF2* has paternal expression and encode IGF2, which is a fetal growth factor. The KCNQ1OT1 paternal-expressed protein is a non-coding transcription factor that regulates the expression of *CDKN1C* and *KCNQ1* genes, which are maternally expressed. The *CDKN1C* encodes p57, a negative regulator of cell proliferation. *KCNQ1* also encodes a voltage-gated potassium channel (Mussa, et al., 2016a; Mussa, et al., 2016b; Mussa, et al., 2016c).

Approximately 50% of patients with BWS have IC2 hypomethylation (loss of methylation- LoM) on the maternal allele, 20% have paternal uniparental disomy (pUPD11) in 11p15.5, 5–10% of IC1 hypermethylation (gain of methylation-GoM) on the maternal allele, and 5% heterozygous mutation on the maternal *CDKN1C* allele (Eggermann, et al., 2014; Wang et al., 2020). Defects involving pUPD11 can be found beyond the 11p15 region and affect all chromosomes (genome-wide paternal uniparental isodisomy, GWpUPD). Up to 9.3–10% of patients with pUPD11 may have GWpUPD (Duffy et al. 2019a; Brioude et al., 2018). More rarely, in 3–6% of patients, duplications, deletions, or chromosome translocations/ inversions can occur, affecting the 11p15 region (Brioude et al., 2018). However, a genetic/epigenetic defect may not be detected in approximately 20% of clinically diagnosed cases (Brioude et al., 2018; Cooper et al., 2005).

Embryonal solid tumor (WT, hepatoblastoma, neuroblastoma, rhabdomyosarcoma, and adrenal carcinoma) risk is approximately 8–10% in the first decade of life (Mussa, et al., 2016b).

The tumor risk is known to correlate with the molecular subgroup; IC1-GoM (28%) and pUPD11 patients (16%) have a higher tumor risk than *CDKN1C* mutations (6.9%) and IC2-LoM (2.6%) patients (Mussa, et al., 2016b; MacFarland, et al., 2018).

Recently, the BWS consensus has developed a new clinical diagnostic scoring system (Brioude et al., 2018). With the consensus suggestions, this syndrome is expanded as the Beckwith-Wiedemann spectrum (BWSp) covering classical BWS without a molecular diagnosis and BWS-related phenotypes with an 11p15.5 molecular anomaly. BWSp is divided into three clinical subtypes as classical, atypical form, and isolated lateralized overgrowth (ILO). BWS consensus underlined that previous scoring system have had mostly captured the classical types in patients with IC2-LoM and pUPD11 cases with higher tumor risk have been skipped since they had more atypical phenotypes.

The aim of this study is to retrospectively reevaluate patients using newly developed diagnostic criteria by the BWS consensus and investigate (epi)genetic changes and follow-up symptoms in classical and atypical phenotypes.

## 2 | METHODS

### 2.1 | Patients

A total of 55 patients (30 boys, 25 girls) between 2 days and 7 years of age (median 6 months) were included in this study (Suppl Table 1). Written consent was obtained from the parents for using their clinical and laboratory data. The clinical diagnosis of BWS was made according to the previous diagnostic criteria (3 major or 2 major and 1 minor criteria were required for clinical diagnosis) designed by Weksberg et al. (2010). Genetic test was recommended for patients who fulfilled these clinical diagnosis criteria. The patients without genetic testing were not included in the cohort. The patients whose molecular tests were found to be normal were named as "genetic test negative." It was planned to perform whole exome sequencing for differential diagnosis of other unknown overgrowth syndromes in these patients.

Fifty-two patients were followed for 3 to 21 years. Abdominal ultrasound was performed every three months until the age of 7 and 11 month, once a year after the age of 8, in terms of a tumor risk. Alpha-fetoprotein measurements were also made every three months until 4 years of age. Their growth was recorded during the follow-up period. Height (cm) and weight (grams and kg) were measured by the pediatrician at 3, 6, 12, 18, 24, and 30 months, yearly between 3 and 13 years, and then at 15 and 18 years of age. The frequency of height and weight measurements varied between a total of 10 and 31 for boys and girls in different age groups. No statistical analysis was performed between groups. The patients were followed up by the same pediatric geneticist specialist who worked in the same single center since three decades.

All patients were re-evaluated, scored, and classified retrospectively according to the new diagnostic criteria developed by the BWS consensus by both medical charts and physical examination (Brioude et al., 2018).

The BWS consensus divided the features of BWS patients into two groups as cardinal and suggestive:

Cardinal features: macroglossia, omphalocele, lateralized overgrowth, multifocal, and/or bilateral WT or nephroblastomatosis, hyperinsulinism, and pathology findings (adrenal cortex cytomegaly, placental mesenchymal dysplasia, or pancreatic adenomatosis).

Suggestive features: birth weight greater>2 standard deviation scores (SDS), facial nevus simplex, polyhydramnios and/or placentomegaly, ear crease/pit, transient hypoglycemia, nephromegaly

and/or hepatomegaly, umbilical hernia and/or diastasis recti, typical BWSp tumors (neuroblastoma, rhabdomyosarcoma, unilateral WT, hepatoblastoma, adrenocortical carcinoma, or pheochromocytoma). Transient hypoglycemia was diagnosed when it lasted less than one week, without necessitating any medication. Hyperinsulinemia was diagnosed when hypoglycemia lasted more than one week requiring an escalated treatment (Brioude et al., 2018).

For each cardinal feature two points and for each suggestive feature one point was given.

A patient with four or more points (2 of which must come from a cardinal feature), was considered as clinical BWS. A testing was demanded in patients with a score $\geq 2$.

The BWS consensus also classified the patients as classical, atypical, and ILO subgroups (Brioude et al., 2018). Duffy et al. (2019a) designated the following criteria for selecting patients suitable for these subgroups:

-Patients with two or more cardinal features except hyperinsulinism and lateralized overgrowth and more than six clinical scores were classified as **Classical BWSp**;**-Atypical BWSp** included (1) patients with a clinical score of <6 and at least one cardinal feature or (2) patients with a clinical score $\geq 6$ with hyperinsulinism and lateralized overgrowth as two cardinal features or (3) patients diagnosed with BWS after presenting with hyperinsulinism or (4) patients diagnosed with BWS after presenting with the tumor (except for a single cardinal feature LO);

-For the **ILO group,** it was sought whether patients had a clinical score of <4 and LO, as the only cardinal feature.

Since the patients presented in this study were evaluated molecularly according to the old scoring system, the "ILO" patients who had not been tested could not be included in this cohort. Written informed consent was obtained from the patients or the parents.

## 2.2 | Genetic studies

The methylation level of the chromosome 11p15.5 region and the presence of UPD were detected by Methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA), Salsa ME-030-C3 BWS/RSS containing methylation sensitive probes for H19/IGF2: IG-DMR (IC1) and KCNQ1OT1/TSS-DMR (IC2) regions (Scott et al. 2008). An adaptation to normal MLPA, MS-MLPA simultaneously detected changes in copy numbers and methylation. After hybridization of the MLPA probes, DNA was digested with a methylene-sensitive enzyme. Comparison of peak heights of undigested and digested DNA showed the methylation level of a particular probe. This kit contained four MS-MLPA probes targeting the H19 gene and four MS-MLPA probes targeting the KCNQ1OT1 locus. All MS-MLPA probes at each locus were expected to give similar results.

The interpretation of the MLPA test was done as described by Eggerman et al. (2016) and Priolo et al. (2008). It had been recommended to use the mean or median methylation status of these probes to determine the methylation status of each locus. In our study, the mean methylation indices for IC2 were mean ± SD: 0.53 ± 0.049 (range 0.41–0.64) in 30 normal samples, while the mean ± SD for IC1 was 0.53 ± 0.046 (range 0.41–0.62). The value between 0 and 0.484 in the IC2 region was accepted as methylation loss (LoM), between 0.576 and 1 in the IC1 region as methylation gain (GoM). The presence of both GoM at IC1 and LoM at IC2 in a patient showed paternal uniparental disomy of 11p15.5.

In 31 patients with IC2 hypomethylation, the methylation index range for IC2 was between 0.00 and 0.25 (mean ± SD: 0.12 ± 0.06), while the methylation index for IC1 in five patients with IC1 hypermethylation was between 0.74 and 0.84 (mean ± SD: 0.79 ± 0.045). In six patients with paternal 11p15.5 UPD, the methylation index for the IC1 region was 0.73–0.91 (mean ± SD: 0.82 ± 0.05), and for the IC2 region the range was 0.23–0.35 (mean ± SD: 0.28 ± 0.04). Since methylation indices in two patients with paternal 11p15.5 UPD were between normal and threshold values, the range was 0.61–0.69 (0.66 ± 0.035) for IC1 and 0.28–0.47 (mean ± SD: 0.38 ± 0.07) for IC2, they were accepted as borderline UPD (Russo, et al., 2016).

Mutations of *CDKN1C* gene were investigated by sanger sequencing in the patients who had normal methylation pattern in 11p15.5 region. SNP array analysis was performed to detect the extent of a deletion or duplication and also to detect the possibility of low-level mosaicism that is below the limit of detection of methylation testing, as recommended by BWS consensus in patients with no methylation disorder in 11p15.5 and *CDKN1C* mutation (Brioude 2018). Unfortunately, GWpUPD could not be performed because of it high cost and need for SNP-array analysis together with patients and parents. Mosaicism occurs in most patients of BWSp and different tissues may have different ratios of affected cells. Methylation analysis in different tissues could not be performed in this study. The limitations of this study were not performing methylation in different tissues and GWpUPD analysis with SNP array.

# 3 | RESULTS

## 3.1 | Initial clinical manifestations and molecular results

The diagnosis age was between 2 days and 7 years of age (median 6 months); nine in the neonatal period, 33 in infantile period (29 days–12 months), eight between 13 months and 3 years, and four between 4 and 7 years of age (suppl Table 1).

The initial clinical findings of the patients according to molecular subtype were summarized in Table 1. Thirty-one patients (56.4%) had methylation loss in IC2, eight patients (14.5%) had pUPD11 (two of them had borderline methylation defects), and five patients (9.1%) had methylation gain in IC1. Both methylation level of the chromosome 11p15.5 region and *CDKN1C* gene sequencing and SNP-array analysis were normal in the remaining 11 patients.

The most frequent findings in our cohort were macroglossia (92.7%), postnatal macrosomia (58.2%), abdominal wall defect (49%),

**TABLE 1** Distribution of clinical characterization of all BWS patients according to molecular subtype

| Clinical features | Overall patients *n*: 55 (100%) | ICR2-LoM *n* = 31 (56.4%) | pUPD11 *n* = 8 (14.5%) | ICR1-GoM *n* = 5 (9.1%) | Genetic tests *N* *n* = 11 (20%) |
|---|---|---|---|---|---|
| Macroglossia | 51/55 (92.7%) | 31/31 (100%) | 6/8 (75%) | 4/5 (80%) | 10/11 (90%) |
| Prenatal macrosomia | 21/55 (41.8%) | 11/31 (35.5%) | 4/8 (50%) | 3/5 (60%) | 4/11 (36.4%) |
| Postnatal macrosomia | 32/55 (58.2%) | 17/31 (54.8%) | 5/8 (62.5) | 4/5 (80%) | 6/11 (54.5%) |
| Abdominal wall defect | 27/55 (49.1%) | 16/31 (51.6%) | 4/8 (50%) | 2/5 (40%) | 5/11 (45.4%) |
| Omphalocele | 8/55 (14.5%) | 6/31 (19.3%) | — | — | 2/11 (18.2%) |
| Umbilical hernia | 13/55 (23.6%) | 6/31 (19.3%) | 4/8 (50%) | 1/5 (20%) | 2/11 (18.3%) |
| Diastasis recti | 6/55 (10.9%) | 4/31 (12.9%) | — | 1/5 (20%) | 1/11 (9.1%) |
| Lateralized overgrowth | 26/55 (47.3%) | 14/31 (45.2%) | 7/8 (87.5%) | 2/5 (40%) | 3/11 (27.3%) |
| Transient hypoglycemia | 18/55 (38.2%) | 10/31 (32.2%) | 3/8 (37.5%) | 2/5 (40%) | 3/11 (27.3%) |
| Hyperinsulinism | 3/55 (5.4%) | 1/31 (3.2%) | 2/8 (25%) | — | — |
| Ear pit/creases | 27/55 (49%) | 18/31 (58%) | 4/8 (50%) | 2/5 (40%) | 3/11 (27.3%) |
| Organomegaly | 19/55 (34.5%) | 7/31 (22.6%) | 4/8 (50%) | 5/5 (100%) | 3/11 (27.3%) |
| Facial nevus simplex | 7/55 (12.7%) | 6/31 (19.3%) | — | — | 1/11 (9.1%) |
| Malignant Tumor | 6/55 (10.7%) | 1/31 (3.2%) | 2/8 (25%) | 3/5 (60%) | — |
| Wilms tumor | 4/55 (7.3%) | — | 1/8 (12.5%) | 3/5 (60%) | — |
| Rabdomyosarcoma | 1/55 (1.8%) | 1/31 (3.2%) | — | — | — |
| Adrenocortical carcinoma | 1/55 (1.8%) | — | 1/8 (12.5%) | — | — |
| Benign tumor (angiomyolipoma) | 1/55 (1.8%) | — | — | 1/5 (1.8%) | — |

Abbreviations: *n*, patient number; IC1-GoM, Imprinting Center 1 gain of methylation; ICR2-LoM, Imprinting Center 2 loss of methylation; pUPD11, Paternal uniparental disomy of chromosome 11; N, Normal.

ear pits/crease (49%), lateralized overgrowth (47.3%), prenatal macrosomia (41.8%), organomegaly (34.5%), transient hypoglycemia (32.7%), and hyperinsulinism (5.4%). Of the 27 patients with abdominal wall defects, 13 had umbilical hernias, eight had omphalocele, and six had diastasis recti. While 18 patients had transient hypoglycemia, three patients had hyperinsulinemia. Seven patients (12.7%) had nevus simplex on the face.

We categorized molecular subtypes and clinical findings of the patients retrospectively according to classical and atypical phenotype in Tables 2 and 3. Thirty-five (63.6%) patients were classified as classical, while 20 patients (36.4%) were atypical phenotype. Patients with atypical phenotype were mostly found in the pUPD11 subgroup (62.5%), followed by IC1-GoM (40%), and less frequently in the IC2-LoM subgroup (16%). However, classical phenotype was also mostly found in patients with IC2-LoM (83.9%) and IC1-GoM (60%) subgroups.

**TABLE 2** Comparison of molecular subtype the patients with classic or atypical phenotype

| Molecular subtypes | Classical BWS | Atypical BWS | Total |
|---|---|---|---|
| IC2-LOM | 25/31 (83.9%) | 6/31 (16.1%) | 31 (100%) |
| pUPD11 | 3/8 (37.5%) | 5/8 (62.5%) | 8 (100%) |
| IC1-GOM | 3/5 (60%) | 2/5 (40%) | 5 (100%) |
| Genetic test normal | 4/11 (36.4%) | 7/11 (63.6%) | 11 (100%) |
| Overall patients | 35/55 (63.6%) | 20/55 (36.4%) | 55 (100%) |

Abbreviations: IC1-GOM, Imprinting center 1 gain of methylation; ICR2-LOM, Imprinting center 2 loss of methylation; pUPD11, Paternal uniparental disomy of chromosome 11.

In the classical phenotype, most frequently detected findings were macroglossia (100%), abdominal wall defect (68.5%), ear pits/folds (62.8%), prenatal macrosomia (42.8%), organomegaly (42.8%), lateralized overgrowth (40%), and transient hypoglycemia (31.4%). In patients with atypical phenotype, the most common findings were macroglossia (80%), postnatal macrosomia (65%), lateralized overgrowth (60%), and transient hypoglycemia (35%). Three patients with hyperinsulinism had an atypical phenotype.

The photographs of patients with different molecular subtypes of BWS and classical or atypical phenotype are shown in Figure 1. In patients with classical phenotype, coarse and round face, prominent cheek, micrognathia, and severe macrosomia were noticeable in IC1-GoM subgroup (Figure 1(a)), while IC2-LoM subgroup showed round face, severe macroglossia, facial nevus simplex, micrognathia (Figure 1(b)), umbilical hernia, (Figure 1(c)) and ear creases (Figure 1(d), (e)). The patient with the pUPD genotype had moderate macroglossia,

**TABLE 3** Comparison of clinical features in the patients with classic and atypical phenotype

| Clinical features | Classic BWS *n*: 35 | Atypical BWS *n*: 20 | Total patients *n*: 55 |
|---|---|---|---|
| Macroglossia | 35/35 (100%) | 16/20 (80%) | 51/55 (92.7%) |
| Prenatal macrosomia | 15/35 (42.8%) | 6/20 (30%) | 21/55 (41.8%) |
| Postnatal macrosomia | 19/35 (54.3%) | 13/20 (65%) | 32/55 (58.2%) |
| Abdominal wall defect | 24/35 (68.5%) | 3/20 (15%) | 27/55 (49.1%) |
| Omphalocele | 8/35 (22.8%) | – | 8/55 (14.5%) |
| Umbilical hernia | 10/35 (28.6%) | 3/20 (15%) | 13/55 (23.6%) |
| Diastasis recti | 6/35 (17.1%) | – | 6/55 (10.9%) |
| Lateralized overgrowth | 14/35 (40%) | 12/20 (60%) | 26/55 (47.3%) |
| Transient hypoglycemia | 11/35 (31.4%) | 7/20 (35%) | 18/55 (38.2%) |
| Hyperinsulinism | – | 3/20 (15%) | 3/55 (5.4%) |
| Ear pit/creases | 22/35 (62.8%) | 5/20 (25%) | 27/55 (49%) |
| Organomegaly | 15/35 (42.8%) | 4/20 (20%) | 19/55 (34.5%) |
| Facial nevus simplex | 6/35 (17.1%) | 1/20 (5%) | 7/55 (12.5%) |
| Malignant tumor | 2/35 (5.7%) | 4/20 (20%) | 6/55 (10.7%) |
| IC1-GOM | 1/35 (2.8%) | 2/20 (10%) | 3/55 (5.4%) |
| pUPD11 | – | 2/20 (10%) | 2/55 (3.6%) |
| IC2-LOM | 1/35 (2.8%) | – | 1/55 (1.8%) |

Abbreviations: n, patient number; IC1-GOM, Imprinting center 1 gain of methylation; ICR2-LOM, Imprinting center 2 loss of methylation; pUPD11, Paternal uniparental disomy of chromosome 11.

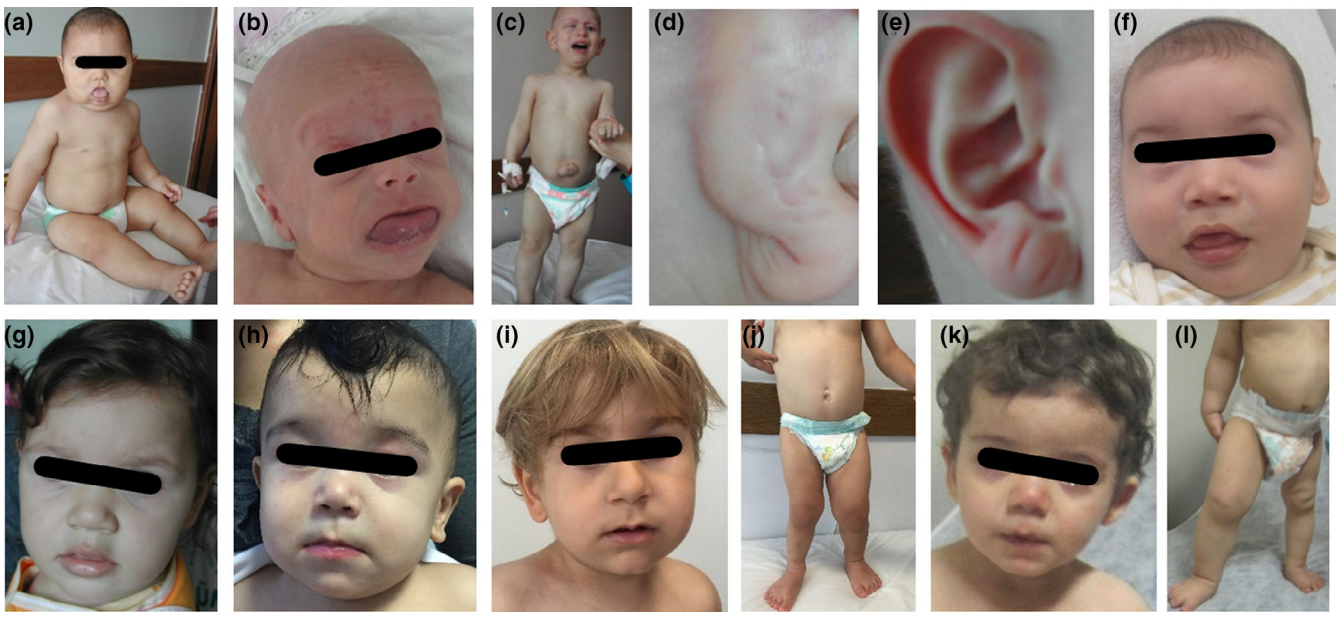


**FIGURE 1** Photographs of patients with BWS with classical and atypical phenotypes according to each molecular subtype. IC2: imprinting center 2 loss of methylation; IC1: imprinting center 1 gain of methylation; pUPD11: paternal uniparental disomy of chromosome 11. The patients with classical phenotype (Top row) with IC1 (a), IC2 (b)–(e) and pUPD11 (f) subtype. Note IC1 patients have round and coarse face, micrognathia, prominent cheek, and macrosomia, while IC2 patients have round face, severe macroglossia, naevus flammeus at the glabella, typical ear creases, and umbilical hernia. The patients with atypical phenotype (bottom row) with IC1 (g), IC2 (h), pUPD11 (i)–(l) subtype. Note coarse face in IC1 (g), round face with prominent cheeks in IC2 (h), close to normal face in pUPD11 (i),(k), and lateralized overgrowth in pUPD11 patients (j),(l) [Color figure can be viewed at wileyonlinelibrary.com]

round face, and prominent cheeks (Figure 1(f)). In patients with atypical phenotype, while the IC1 genotype had the coarse face (Figure 1(g)), the patient with IC2 genotype had a round face and prominent cheeks (Figure 1(h)). The patients with pUPD genotype had a face close to normal (Figure 1(i),(k)) whereas prominent lateralized overgrowth was a common finding (Figure 1(j),(l)).

Three patients died during operation (one patient due to a tumor at 11 months, one patient during a tongue reduction at 2 years, and another one for an intervention of ompholocele at 5 months of age). Fifty-two patients were followed for 3 to 21 years.

### 3.2 | Follow-up features

While 51 patients (92.7%) had macroglossia, only six of them had respiratory distress and nutritional difficulties during infancy, and three (6.5%) required tongue reduction operation. A patient with IC2-LoM and classical phenotype died when he was 2 years old while undergoing tongue reduction surgery. Second patient with an atypical phenotype and a negative molecular diagnosis had undergone four times tongue reduction operations between the ages of 2 and 6 years. The other patient with pUPD11 had an


**FIGURE 2** Facial photographs of the patients with classical phenotype at different ages. IC2: imprinting center 2 loss of methylation; IC1: imprinting center 1 gain of methylation; pUPD11: paternal uniparental disomy of chromosome 11. Top row: first patient (IC1) at the ages of 8 months and 4.5 years (a),(b), the second patient (IC1) at the ages of 7 months and 12 years (c),(d), third patient (IC2) at the ages of 6 months and 6 years (e),(f). Second row: The first patient (IC2) at the age of 1 and 10 years (g,h), second patient (IC2) at the ages of 18 months and 10 years (i),(j), third patient (IC2) at the ages of 4 and 13 years (k),(l). Third row: The first patient (IC2) at the ages of 4.5 months and 4.5 and 14 years (m)-(o), second patient (IC2) at the age of 7 and 17 years (p),(r), third patient (IC2) the age of at 23 years (s). Bottom row: First patient (pUPD11) at four and 18 months of age (t),(u), second patient (pUPD11) at 22 years (ü), fourth patient (IC2) at 18 years of age (v), last patient (clinical BWS, no molecular diagnosis) at the age of 15 and 21 years (y),(z). As the child grows, it is observed that the round face gets longer and prognathism and triangular face develops. The face becomes asymmetrical depending on the degree and the unilaterality of macroglossia [Color figure can be viewed at wileyonlinelibrary.com]

atypical phenotype, and had undergone this surgery at the age of 1 year.

We evaluated the facial photographs of the patients with classical phenotype at different ages. While two patients with IC1 genotype had micrognathia (Figure 2(a) (c)) at the age of 8 and 7 months, a normal chin (Figure 2(b)) and moderate prognathism (Figure 2(d)) were detected at the age of 4.5 and 12 year, respectively (Figure 2 (b), (d)). The photographs of eight patients with the IC2 phenotype were presented in Figure 2(e)-(s). Similar to the IC1 phenotype, we noticed that the round face got longer as child grew and prognathism developed gradually from late childhood to adulthood as the result of severe macroglossia. While round face with prominent cheeks was seen in the 4-month and 18-month photographs of the patient with the pUPD11 genotype (Figure 2(t),(u)), severe prognathism was also observed in another 22-year-old patient with pUPD11 (Figure 2u). The photographs of the last patient with negative molecular diagnosis of BWSp (Figure 2(y),(z)) showed severe hemimacroglossia, prognathism, and asymmetric face at the age of 15 and 21 years.

The frequency of prenatal and postnatal overgrowth was found to be 41.8% and 58.2%, respectively, in this study and prenatal macrosomia was mostly associated with IC1-GoM patients (60%). We analyzed the mean height and weight SDS according to Turkish standards for 31 patients with macrosomia (Table 4). We observed that the mean height SDS increased toward the age of 3 and decreased after 6. The mean height SDS at 3 and 7 years of ages were found to be +2.6 and + 1.5, respectively, in male patients, while they were + 1.9 and + 0.7 for girls. Nine of ten patients reached the final height with normal percentile; the mean final height of five male patients was + 0.71 SDS, while five female patients were + 0.25 SDS. Only the final height of one male patient was over +2 SDS.

Six patients (10.7%) developed malignant tumors: four WT (at the ages of 7 months, 12 months, 18 months and 3 years), one orbital rhabdomyosarcoma (at 12 months of age), and one adrenocortical carcinoma (at 8 months of age). While only one of the 31 patients (3.2%) with IC2-LoM developed rabdomyosarcoma, three of five patients (60%) with IC1-GoM had WT (two of them bilaterally). Two of eight patients (25%) with pUPD11 also developed WT and adrenocortical carcinoma. One patient with the IC-GoM presented a benign tumor (angiomyolipoma) at 3 years of age (Table 1). Tumor developed in 20% of patients with atypical phenotype and in 5.7% of patients with classical phenotype (Table 3). The first admissions of patients with adrenocortical carcinoma with an atypical phenotype and rhabdomyosarcoma in the eye with classical phenotype were tumor complaints; others were detected during the screening. Only seven patients (the youngest at the age of 4) were under the age of 7 in the last examination, still with the risk of tumors in this study.

## 4 | DISCUSSION

### 4.1 | Initial findings and (Epi)genotype–phenotype correlations

Since there is a strong correlation between phenotype and epigenotype in BWS, it is very important to identify the disease-

**TABLE 4** The mean height and weight SDS according to age and gender of 31 patients with macrosomia

| | Female | | Male | |
|---|---|---|---|---|
| Age | Height (+SDS (*n*)) | Weight (+SDS (*n*)) | Height (+SDS (*n*)) | Weight (+SDS (*n*)) |
| 3 mo | 0.7 (7) | 1.56 (7) | 0.8 (8) | 1.15 (8) |
| 6 mo | 0.6 (10) | 1.4 (10) | 0.7 (12) | 1.6 (12) |
| 12 mo | 0.7 (12) | 1.5 (12) | 0.8 (14) | 1.7 (14) |
| 18 mo | 1.1 (12) | 1.7 (12) | 0.9 (15) | 2.2 (15) |
| 24 mo | 1.3 (13) | 1.1 (13) | 2.2 (15) | 2.0 (15) |
| 3 yrs | 1.9 (14) | 1.5 (14) | 2.6 (16) | 1.9 (16) |
| 4 yrs | 1.4 (13) | 1.1 (13) | 1.5 (17) | 1.6 (17) |
| 5 yrs | 1.2 (14) | 1.1 (14) | 2.1 (17) | 1.9 (17) |
| 6 yrs | 1.2 (13) | 0.9 (13) | 1.5 (17) | 1.6 (17) |
| 7 yrs | 0.7 (12) | 0.9 (12) | 1.5 (15) | 1.6 (15) |
| 8 yrs | 0.9 (10) | 1.1 (10) | 1.6 (13) | 1.8 (13) |
| 9 yrs | 0.8 (8) | 0.8 (8) | 1.4 (10) | 2.0 (10) |
| 10 yrs | 1.3 (7) | 0.6 (7) | 1.6 (8) | 1.3 (8) |
| 11 yrs | 0.9 (7) | 0.6 (7) | 1.4 (8) | 1.2 (8) |
| 12 yrs | 0.6 (6) | 0.5 (6) | 1.2 (7) | 0.7 (7) |
| 13 yrs | 0.31 (6) | 0.1 (6) | 2.1 (6) | 0.9 (6) |
| 15 yrs | 0.33 (5) | 0.01 (5) | 1.8 (6) | 0.5 (6) |
| 18 yrs + | 0.25 (5) | 0.05 (5) | 0.71 (5) | 0.3 (5) |

Abbreviations: *n*, patient number; mo, month; SDS, Standard deviation score; yrs, years.

causing molecular defect. The distribution of clinical findings also differ according to the molecular subgroup. While 56.4% of patients in our study had IC2-LoM, 14.5% of patients also had pUPD11, and 9.1% of patients had methylation gain in IC1-GoM. These findings were consistent with the three largest published studies, which had included 407, 244, 318, and 407 patients with BWS (Brioude, et al., 2013; Mussa, et al., 2016b; Maas, et al., 2016, Duffy et al.,2019a). There was no patient with *CDKN1C* gene mutation nor chromosomal abnormalities in 11p15.5 region in our study. The studies investigating the prevalence of molecular subgroups of BWS according to ethnic groups revealed that Asian cohort had a significantly lower frequency of IC1-GoM (6.1%, vs. 8.6) and a higher frequency of chromosome abnormality (6.1% vs. 1.7%) and *CDKN1C* gene mutation (7.1% vs. 3.2%) than in North American and European patients (Duffy, et al., 2019b; Sasaki, et al., 2007; Luk, 2017). Although our sample number was not large, the results were similar to the European/North American cohorts. Genetic/epigenetic test was normal in 11 patients (20%) in the present study similar to previous reports (Choufani, et al., 2010; Brioude et al., 2018, Duffy et al., 2019a).

When the frequency of clinical features for BWS patients in four molecular subgroups was evaluated (Table 1), we found that IC1-GoM, IC2-LoM, pUPD11, and no genetic defect subgroups showed similar distribution to those reported in the literature (Brioude, et al., 2013; Mussa, et al., 2016c; Maas, et al., 2016, Duffy et al., 2019a). Macroglossia was the most common finding (92.7%) in all molecular subtypes with BWS; its frequency was 100% in the IC2-LOM and 75% in pUPD11 in the present study, which was consistent with other reported cohorts (Mussa, et al., 2016c; Brioude, et al., 2013; Maas, et al., 2016). Abdominal wall defects, including omphalocele, umbilical hernia, and rectus diastasis was found in almost 60% of patients with BWS and omphalocele was common in patients with IC2-LoM subgroup (Broidue, et al., 2013; Duffy, et al., 2019a). In the present study, the frequency of abdominal wall defects was 49%; all of the genetically diagnosed patients with omphalocele were in the IC2 subgroup (Table 1). The patients with IC1-GoM were associated with a higher frequency of organomegaly in our study similar to the literature (Broidue, et al., 2013). Lateralized overgrowth was detected in 47.3% of patients while the highest rate was in the pUPD11 subgroup with 87.5% in our cohort similar to reported studies (Mussa, et al., 2016c; Brioude, et al., 2013; Maas, et al., 2016; Ibrahim, et al., 2014).

It had been reported that approximately 30–50% of cases with BWS had a history of neonatal hypoglycemia and only 5% of cases with severe hyperinsulinism needed an aggressive treatment. Hyperinsulinism was common in patients with pUPD11 and IC1-GoM subgroups (Adachi et al., 2013; Kalish et al., 2016; Munns & Batch, 2001). In our study, the rate of hypoglycemia was 38.2% and the majority of patients were in the pUPD11 group (Table 1). Three patients (5.4%) had hyperinsulinism requiring treatment, two of them were pUPD11 and one was IC2 subtype.

Since methylation indices in two patients with paternal 11p15.5 UPD were between normal and threshold values, these two patients were accepted as borderline UPD (low rate mosaicism). Their clinical findings were not different from other patients with pUPD11. It is not known whether there is a relationship between the severity of the phenotype and the level of mosaicism (Mussa, et al., 2016a; Kalish, et al., 2017).

## 4.2 | Follow-up clinical findings

The most important problems in the follow-up of patients with BWS are tumor risk and macroglossia (Mussa et al., 2016d; Brioude, et al., 2018). Macroglossia is the most common feature of the BWS spectrum. Orthodontic anomalies, speech disorders, breathing, drooling, feeding, and swallowing difficulties, which may persist into adulthood, have been reported in patients with macroglossia (Brioude, et al., 2018). The relative size of the macroglossia decreases as the child grows up; however, it is known that 40% of cases require tongue reduction surgery (Kadouch, et al., 2012). In our study, six patients with macroglossia had severe respiratory distress and nutritional difficulties during infancy, and three of these patients had tongue reduction operations. In addition, the presence and the degree and the unilaterality of macroglossia contributes to the shaping of the face and the development of prognathism.

In patients affected by BWS, rapid growth had been observed beginning in the last half of the intrauterine period and continuing to early childhood. The growth rate was found to decrease toward 7–8 years of age (Weng, et al., 1995; Pappas, et al. 2015; Brioude, et al., 2018; Duffy, et al., 2019a; Mussa, et al., 2016d). In patients with macrosomia presented here, the height increase became prominent toward 3 years of age and in most of the cases height returned to normal after 10 years of age. Nine of 10 patients who reached the final height were within normal limits. Similar to earlier studies, the frequency of prenatal and postnatal macrosomia were found to be 41.8% and 58.2%, respectively, in this study and prenatal macrosomia mostly associated with in the patients who had IC1-GoM (Brioude, et al., 2013, Maas, et al., 2016; Mussa, et al., 2016e).

Embryonal tumors occur in ~8–10% of children with BWS; the most common types are WT (52%), hepatoblastoma (14%), neuroblastoma (10%), rhabdomyosarcoma (5%), and adrenal carcinoma (3%) (Maas, et al., 2016). Six of the 55 patients (10.9%) in the present study developed malignant tumors; four (66.5%) WT, one orbital rhabdomyosarcoma, and one adrenocortical carcinoma. Although there are some differences in the mean age of diagnosis among tumor types, the overall cancer risk is highest in the first two years of life, and clinical experience shows that the risk of cancer decreases gradually before puberty (Brioude et al., 2018). In six of our patients, the malignant tumor occurred at 7–18 months of age, benign tumor (angiomyolipoma) was seen in one patient at the age of three. Each molecular subgroup of BWS has a different tumor type and incidence. IC1-GoM is the type with the highest malignant and benign tumor incidence (28%), followed by pUPD11 (16%), *CDKN1C* gene mutation (6.9%), and the lowest IC2-LoM (2.6%) (MacFarland, et al., 2018). A

strong relationship has been reported between IC1-GoM and WT development in BWS (Maas, et al., 2016; Mussa, et al., 2016b and 2016c). While three patients with WT were in the IC1-GoM group in our study, the patient with one WT was in the pUPD11 group. One patient with pUPD11 also had adrenocortical carcinoma. The patient with orbital rhabdomyosarcoma was in the IC2-LoM subgroup. It has been reported that although the tumor incidence is lowest in IC2-LoM (2.6%), rhabdomyosarcoma is mostly associated with this subgroup patients (Maas, et al., 2016).

Mussa et al. (2016c) evaluated 318 BWS patients and reported a correlation between malignant neoplasm and lateralized overgrowth and organomegaly. Between our six patients who developed tumor, four had lateralized overgrowth, and five had organomegaly.

### 4.3 | Clinical classification

BWS consensus emphasized that the disease should be considered a spectrum rather than a syndrome and proposed to classify the patients as classical, atypical form, and ILO (Brioude et al., 2018). Authors underlined that previous scoring system mostly captured the classical types in patients with IC2-LoM and that pUPD11 cases with higher tumor risk had been skipped since they had more atypical phenotypes. Duffy et al. (2019a) defined criteria to select patients suitable for these subgroups and evaluated their cohort according to these criteria. They evaluated 322 patients according to the BWS spectrum, for the first time, it revealed that 64.3% of the patients were in the classical, 17.1% in the atypical, and 18.6% in the ILO phenotype. In the classical group patients, 46.9% were IC2-LoM, 18.4% were pUPD11, 7.2% were IC1-GoM subgroups, and 2.4% were *CDKN1C* mutation. In the atypical group, the rates of pUPD11, IC2-LoM, IC1-GoM, and *CDKN1C* mutation were 12.7%, 21.8%, 38.2%, and 3.6%, respectively. The present study was in accordance with this first study; 63.6% patients were classified as classical phenotype who were mostly in IC2-LoM subgroup and the patients with atypical phenotype were mostly in pUPD11, followed by IC1-GoM (Table 2).

In the study of Duffy et al., (2019a) tumor development rate was 1% in patients with classical phenotype, while it was 11.3% in atypical phenotypes and 19.6% in ILO phenotype. While four of the patients presented here who developed malignant tumors were atypical phenotype, two of them were in classical phenotype, tumor development rate was 5.7% in classical phenotype, and 20% in atypical phenotype (Table 3). In our cohort, there was no patient with ILO phenotype. It was possible that our cohort might have excluded ILO patients since testing was requested according to the old clinical scoring. The new scoring is important as it draws attention to atypical and LA cases with higher tumor risk and covers a larger group of patients. Our results supported the importance of evaluating the patients according to the criteria that could facilitate diagnosis of the patient group with atypical phenotype, who had an increased cancer risk.

For the first time, in this study, the distribution of cardinal and suggestive findings and the change in BWS-specific facial features from infantile period to adulthood in atypical and classical phenotype were evaluated.

When we evaluated the distribution of cardinal and suggestive findings of BWS into atypical and classical phenotypes (Table 3), it was observed that typical BWS findings such as macroglossia, abdominal wall defect, ear pit/creases, organomegaly, prenatal macrosomia, and facial nevus simplex were more common in the patients with classical phenotype compared to atypical patients. In patients with atypical phenotype, the most common findings were postnatal macrosomia, lateralized overgrowth, hyperinsulinism, and hypoglycemia. Two of three patients with refractory hyperinsulinism had an atypical phenotype and all were accompanied by lateralized overgrowth. Kalish et al. (2016) evaluated 28 children with hyperinsulinism and BWS and observed that 26 were in the pUPD11 with lateralized overgrowth. Lateralized overgrowth was more common in patients with atypical BWS and most of them were pUPD subgroup in our cohort.

Hunter, et al., (1994) described the facial findings of 13 children with BWS who had between 19 months and 15 years of age. They stated that during this early period the face was round to oval with prominent cheeks, and emphasized that the typical facial appearance improved as the age got older. However, Gazzin, et al. (2019) evaluated adult BWS patients, and showed prognathism in their photographs. In the patients presented here with classical phenotype, while coarse face with prominent cheeks and micrognathia were noticeable in IC1 subgroup, IC2 subgroup showed a round face, prominent cheeks, micrognathia, severe macroglossia, facial naevus simplex, and ear creases. In the pUPD11 subgroup, these findings were moderate. We observed that as the child grew up, after the age of 6–7 years, the face gradually became longer and moderate or severe prognathism developed consistent with macroglossia degree in classical phenotype. The face became also asymmetrical in the presence of hemimacroglossia. However, in patients with atypical phenotype, while those with IC1 subgroup had mild coarse face, the face appearance was close to normal in IC2 and pUPD11 subgroups.

## 5 | CONCLUSION

The patients with BWSp were classified according to clinical phenotype; 63.6% were classical and 36.4% were atypical phenotype. The patients with atypical phenotype were mostly in pUPD11 and IC1-GoM group, whereas classical phenotype was associated with IC2 group. While typical BWS findings such as macroglossia, abdominal wall defect, ear pit/creases, organomegaly, prenatal macrosomia, and facial nevus simplex were more common in the patients with classical phenotype, postnatal macrosomia, lateralized overgrowth, and hypoglycemia were more common in patients with atypical phenotype. In classical phenotype, as the child grew up, the round face got longer; moderate and severe prognathism developed depending on the degree of macroglossia. However, in the atypical phenotype, these BWS specific facial features were either very mild or absent. Malignant tumor developed before the age of three in 10.9% of patients,

and the majority of patients developing tumors had atypical phenotype. This study is the second study classifying BWS patients as classical and atypical phenotype. It is also the first one investigating the distribution of cardinal and suggestive findings and the change in BWS-specific facial features from infantile period to adulthood in both phenotypes. We speculate that the new classification may help us to better diagnose the patients with atypical phenotype who have a higher tumor risk in BWSp.

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## CONFLICT OF INTEREST

The authors declare that they have no competing interests.

## AUTHOR CONTRIBUTION

Beyhan Tüysüz, Mehmet Vural, and Nilay Güneş provided clinical data and designed the study, wrote, and revised the manuscript. Filiz Geyik and Gözde Yeşil performed MLPA and sanger analysis, TC oncological clinical data. All authors read and approved the final manuscript.

## ETHICS STATEMENT

The data presented in this study were retrieved from the routine clinical care facilities of Cerrahpasa School of Medicine, Istanbul, Turkey.

## DATA AVAILABILITY STATEMENT

Authors confirm that all relevant data are included in the article and/or its supplementary information files.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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