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Epigenotype, genotype, and phenotype analysis of patients in Taiwan with Beckwith–Wiedemann syndrome

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ABSTRACT

Background: Beckwith–Wiedemann syndrome (BWS) is a congenital overgrowth disorder predisposing to tumorigenesis that results from abnormal expression or function of imprinted genes of chromosome 11p15.5.
Methods: Forty-seven patients in Taiwan with clinical suspicion of BWS were referred for diagnostic testing based on methylation profiling of *H19*-associated imprinting center (IC) 1 and *KCNQ1OT1*-associated IC2 using high-resolution melting analysis, multiplex ligation-dependent probe amplification, or high-resolution quantitative methylation profiling.
Results: Twenty-eight patients received a clinical diagnosis of BWS (the presence of 3 major features or 2 major features and at least 1 minor feature), 18 had suspected BWS (the presence of at least 1 major feature), and 1 had isolated Wilms' tumor. Nineteen patients were identified with IC2 hypomethylation (including 1 with isolated Wilms' tumor), 1 with IC1 hypermethylation, 2 with paternal uniparental disomy, and 1 with *CDKN1C* mutation. Several clinical features were found to be statistically different ($P < 0.05$) between the 2 groups—clinical diagnosis of BWS (n = 28) or suspected BWS (n = 18)—including macroglossia, pre- or postnatal gigantism, abdominal wall defect, ear creases, facial nevus flammeus, BWS score, and the molecular diagnosis rate. Molecular lesion was detected in 81% of patients with the presence of three major features, compared with 33% and 28% of those with two or one major feature, respectively. The mean BWS score was 5.6 for 19 subjects with "IC2 hypomethylation", compared with 3.8 for 2 subjects with pUPD. The BWS score of one subject with *CDKN1C* mutation and one with IC1 hypermethylation was 6 and 7, respectively.
Conclusions: The BWS score was positively correlated with the molecular diagnosis rate ($P < 0.01$). The BWS database of epigenotype, genotype, and phenotype is expected to promote better genetic counseling and medical care of these patients.

1. Introduction

Beckwith-Wiedemann syndrome (BWS; MIM #130650) is a congenital overgrowth disorder predisposing to tumorigenesis caused by abnormal expression or function of imprinted genes of the chromosome 11p15.5 imprinting gene cluster. The estimated prevalence of BWS in North West of Italy is about 1 in 10,000 live birth [1]. It is characterized by macrosomia, macroglossia, omphalocele or umbilical hernia, intra-abdominal visceral organomegaly, ear creases or

Abbreviations: BWS, Beckwith–Wiedemann syndrome; IC, imprinting center; pUPD, paternal uniparental disomy; UPD, uniparental disomy; ART, assisted reproductive technology; HRM, high-resolution melting; MS-MLPA, methylation-specific multiplex ligation-dependent probe amplification.

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pits, renal abnormalities, facial nevus flammeus, neonatal hypoglycemia, hemihypertrophy, cardiac anomalies, neoplasia, polydactyly, cleft palate, and a 7.5% reported risk of developing embryonal Wilms' tumor, hepatoblastoma, neuroblastoma, or adrenocortical carcinoma [1–13].

Several advances have helped define the molecular defect of this disorder since the first clinical report of BWS in the 1960s [14,15]. The chromosome 11p15.5 imprinting region harbors two imprinting domains, *IGF2*/*H19* and *CDKN1C*/*KCNQ1*/*KCNQ1OT1*. *IGF2* is expressed from the paternal allele, and *H19* is expressed from the maternal allele. *KCNQ1OT1* is expressed from the paternal allele, whereas *CDKN1C* and *KCNQ1* are both expressed from the maternal allele. The *H19*-associated imprinting center (IC) 1 is methylated on the paternal allele and unmethylated on the maternal allele. The *KCNQ1OT1*-associated IC2 is methylated on the maternal allele and unmethylated on the paternal allele. In patients with BWS, hypomethylation at IC2 occurs in 50%–60%; hypermethylation at IC1 occurs in 5%–10%; *CDKN1C* mutations occur in 5%–10% (in 5% of sporadic cases and in 40% of familial BWS cases); and paternal uniparental disomy (pUPD) 11p15.5 occurs in 10%–20% [2–8,10,11].

Although consensus diagnostic criteria for BWS have not been defined, the presence of three major features (macroglossia, prenatal or postnatal overgrowth, and abdominal wall defects) or two major features and one minor feature (e.g., ear creases or pits, facial nevus flammeus, hemihypertrophy, neonatal hypoglycemia, midface hypoplasia, cardiomegaly, renal abnormality, or polyhydramnios) is required for the clinical diagnosis of BWS [16].

Phenotype and genotype/epigenotype correlations in European and North American BWS patients have been reported in the literature. For example, hemihypertrophy is strongly associated with uniparental disomy (UPD). Omphalocele occurs more frequently in patients with IC2 hypomethylation or *CDKN1C* point mutations, whereas macrosomia, macroglossia, and an increased risk of embryonic tumors are more commonly associated with IC1 hypermethylation [6,17–19]. At present, there are only limited reports of the molecular basis and clinical features of BWS in Asia [20,21]. The aim of this study was to characterize the epigenotype, genotype, and phenotype of Taiwanese patients with BWS.

2. Patients and methods

2.1. Patient selection

A retrospective chart review was carried out for 47 patients with clinical suspicion of BWS (23 males and 24 females; age range, 2 days to 20 years) diagnosed from January 2007 through July 2015 in Mackay Memorial Hospital, Taipei, Taiwan. Among these 47 patients, 28 were given a clinical diagnosis of BWS (the presence of 3 major features or 2 major features and at least 1 minor feature), 18 with suspected BWS (the presence of at least one major feature), and 1 with isolated Wilms' tumor. All information was obtained from the medical records. The chart review was performed by a single author (HYL) to ensure consistent extraction of information. Written informed consent was obtained from a parent for children and from patients over 18 years. The study was approved by the ethics committee of Mackay Memorial Hospital, Taipei, Taiwan.

2.2. Clinical assessments

Clinical manifestations were recorded based on the diagnostic criteria proposed by Zarate et al. [16], including major features (macroglossia, prenatal or postnatal overgrowth, and abdominal wall defects) and minor features (ear creases or pits, renal abnormalities, facial nevus flammeus, neonatal hypoglycemia, hemihypertrophy, congenital cardiac malformations, neoplasia, polydactyly, cleft palate, and intra-abdominal visceral organomegaly) (Fig. 1). Ibrahim et al. [4] developed a novel weighted scoring system to categorize patients presenting with the most common features of BWS. We calculated a total diagnostic score for each patient according to the BWS molecular abnormality outcome score (maximum = 8), giving a differentially weighted score based on the presence of the following features: macroglossia (2.5), exomphalos (1.5), organomegaly (1), macrosomia (1), facial nevus flammeus (1), hemihypertrophy (0.5), and hypoglycemia (0.5). Other data abstracted from the records included sex, history of conception by assisted reproductive technology (ART), gestational age at birth, and parental age, as well as the patient's length, weight, head circumference, and chest circumference at birth.

2.3. Molecular studies

All DNA was extracted from peripheral blood using the Chemagic DNA Blood Kit (Chemagen, Baesweiler, Germany), and the MethylCode Bisulfite Conversion Kit (Invitrogen, Carlsbad, CA) was used to treat the DNA (1 mg) with bisulfite according to the manufacturer's instructions [22]. All diagnostic examinations were performed by methylation profiling of *H19*-associated IC1 and *KCNQ1OT1*-associated IC2 using high-resolution melting (HRM) analysis, methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA), or high-resolution quantitative methylation profiling with a methylation-specific polymerase chain reaction assay (EpiTYPER and MassARRAY; Agena Bioscience, San Diego, CA, USA) [8,23–26]. The detailed procedures were described previously [8]. If pUPD was identified by MS-MLPA or MassARRAY, microsatellite analysis was performed to confirm these cases [20]. For the remaining cases with negative results, *CDKN1C* mutation was detected as previously described [27].

2.4. Data and statistical analysis

We compared the clinical features and BWS score between patients with clinical diagnosis (n = 28) versus those with suspected BWS (n = 18) using Student's *t*-test for continuous variables and Fisher's exact test for categorical variables. Two-tailed *P*-values were computed. The relationship between molecular diagnosis rate and BWS score of these 46 patients was evaluated using Pearson's correlation coefficient (r), and testing for statistical significance ($P < 0.05$) was performed using Fisher's r-z transformations. All statistical analyses were performed with SPSS version 11.5 (SPSS Inc., Chicago, Illinois, USA). Statistical significance was set at $P < 0.05$.

3. Results

Among these 47 patients, 19 were identified with IC2 hypomethylation (including 1 with isolated Wilms' tumor), 1 with IC1 hypermethylation, 2 with pUPD, and 1 with *CDKN1C* mutation (Tables 1, S1–S4). Three patients (patients No. 9, 19, and 35) were conceived by ART. Both patients No. 9 and 19 were identified with IC2 hypomethylation. Their BWS score were 5 and 7, respectively. Patient No. 35 had normal molecular study results and a BWS score of 1. Among 28 patients with a clinical diagnosis of BWS, abdominal wall defect was the most common manifestation (93%), followed by macroglossia (89%) and pre- or postnatal overgrowth (75%). Ear creases or pits and facial nevus flammeus were the two most common minor features (71%), followed by intra-abdominal visceral organomegaly (61%), renal abnormalities (46%), and hemihypertrophy (36%). Several clinical features were found to be statistically different ($P < 0.05$) between the two groups [i.e., "clinical diagnosis of BWS" (n = 28) and "suspected BWS" (n = 18)], including macroglossia (89% vs. 50%), pre- or postnatal overgrowth (75% vs. 39%), abdominal wall defect (93% vs. 17%), ear creases or pits (71% vs. 39%), facial nevus flammeus (71% vs. 17%), BWS score (6.0 ± 1.1 vs. 2.6 ± 0.9; maximum = 8), and the molecular diagnosis rate (61% vs. 28%). No other clinical features differed significantly between the two groups (Tables 2, S5 and Fig. 1). Molecular lesion was

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