UNDER-DIAGNOSED BECKWITH-WIEDEMANN SYNDROME AMONG EARLY-ONSET OBESE CHILDREN

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THE AUTHORS DECLARE NO CONFLICT OF INTEREST

Introduction:

- Beckwith-Wiedemann syndrome (BWS) is a clinical and genetically heterogeneous entity encompassing overgrowth with a widely variable clinical phenotype that attenuates with age. Although there is no complete consensus, proposed criteria to warrant clinical suspicion for BWS are the presence of at least 3 major or 2 major plus 1 minor features. Obesity can be a feature associated with macrosomy, but it is not considered a criterion.
- Early diagnosis of BWS is crucial due to the increased risk for developing embryonal malignancies (mainly below 5 years of age).
- Several genetic and epigenetic aberrations affecting the imprinted 11p15 locus can cause BWS, all of them leading to the down-regulation of maternally expressed genes and/or the up-regulation of paternally expressed genes. Two of the genes in these regions, *IGF2* and *CDKN1C*, contribute to growth regulation and are directly related to the pathogenesis of BWS. (Figure 1A)

Objective:

- We aimed to determine the presence of underdiagnosed BWS in early-onset "non-syndromic" obese children in our department.

Patients and methods:

- We studied 159 children (95 males/64 females) diagnosed with early-onset (< 5 years) severe (BMI-SDS >+3 SDS) obesity.
- A custom-made methylation-specific multiple-ligand-probe-assay (MS-MLPA), with HhaI as a methylation-sensitive restriction enzyme, was used to analyze blood cell DNA methylation at the 11p15.5 region. The assay contains 11 probes, including one for the imprinting-center-1 (IC1) locus (*H19*) and one for IC2 (*KCNQ1*). Probes located at fully unmethylated loci were included as technical controls, as well as probes without the HhaI recognition site for methylation quantification.
- Hypomethylation at the *KCNQ1* locus was identified in two of the 157 patients. The same MS-MLPA assay was useful to discard unbalanced genomic rearrangements or uniparental disomy at this 11p15.5 region, considering that the methylation level at H19 was within the reference range.
- A decrease of 60% in the methylation level at *KCNQ1* locus was detected in patient 1, while in patient 2 the reduction was approximately 33% (Figure 1B). The different percentage of methylation reduction suggests different degrees of mosaicism for the alteration in the two samples. Both patients with 11p15.5 epimutations had been referred to our Pediatric Endocrinology clinic due to severe childhood obesity. Neither of them fulfilled the minimum criteria for clinical diagnosis of BWS (Table). Kidney ultrasound and plasma alpha-fetoprotein levels (after diagnosis) were normal in both girls.
- Patient 1, with a higher degree of methylation impairment, presented no single criteria for diagnosis of BWS. She had suitable birth anthropometry, height and bone age according to target height and chronological age and her obesity was ameliorating as age progressed (Figure 2).
- Patient 2, with a lesser degree of methylation impairment, had one major [pre- (birth length and weight 53 cm and 4.0 kg, respectively) and post-natal macrosomy (height +2.7 SDS)] and two minor criteria [gestational polyhydramnios and advanced bone age (+ 2 years over chronological age)]. She presented a more severe predominantly abdominal obesity (BMI > +8 SDS) while in adolescence (Figure 3).

Figure 1A

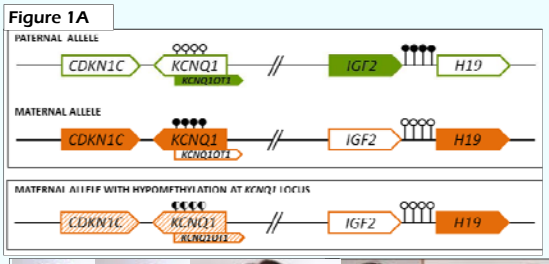

Figure 1B

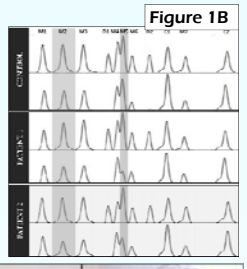

Figure 1A: Schematic representation of the imprinted 11p15.5 region. IC1 (H19-associated imprinting center) is methylated on the paternal allele and unmethylated on the maternal chromosome. IC2 is unmethylated on the paternal chromosome and methylated on the maternal allele, leading to the non-expression of KCNQ1OT1 and the activation of *CDKN1C* and *KCNQ1*. Hypomethylation at IC2, which is found in 50% of BWS as well as in these two patients, is shown in the scheme below. This epigenetic alteration leads to the expression of KCNQ1OT1 on the maternal allele and subsequently reduces expression of *CDKN1C* and *KCNQ1*. **Figure 1B:** MS-MLPA results of both patients. Probes from the 11p15.5 region are shown in grey (H19-M2 and KCNQ1-M5), among other probes used as dosage (C1 and C2) and digestion controls (D1 and D2). In each case, the peak pattern displayed above represents the non-digested products. The patterns below display the methylation status after digestion with a methylation-sensitive restriction enzyme. No methylation alteration was detected with the H19 probe. However, a reduction in the peak of the KCNQ1 probe was detected in both patients, implying a decrease of methylation status at this locus.

Figure 2: Photographs of patient 1 at age 16. **Figure 3:** Photographs of patient 2 at age 14.

		CBWS / IBWS frequencies	Patient 1	Patient 2
Prenatal overgrowth		84% / 58%	No	Yes
Prenatal features	*Birth weight*		- 0.43 SDS	+ 1.94 SDS
	Gestational polyhydramnios	-	No	Yes
	Embryonary tumor	7.5%	No	No
Chronological age at first examination			7.84 years	8.33 years
	Pubertal stage		Tanner I	Tanner I
Postnatal overgowth		84% / 58%	No	Yes
	Height		130.7 cm (+0.91 SDS)	139.8 cm (+2.27 SDS)
	Target height		169.0 cm (+1.35 SDS)	162.1 cm (+0.15 SDS)
	Age at the onset of obesity		4 years	1 year
	BMI		26.10 kg/m^2 (+3.55 SDS)	36.58 kg/m^2 (+8.58 SDS)
	Whole body fat (DXA)		18612 g (42.5%; +3.15 SDS)	37078 g (44.8%; +3.19 SDS)
Advanced bone age		-	No	Yes
	Bone age at first examination (G&P)		7.25 years	10.5 years
Visceromegaly		56% / 42%	No	No
Macroglosia		98% / 83%	No	No
Abdominal wall defect		84% / 39%	No	Diastais rectii
Hemihyperplasia		41% / 25%	No	No
Ear lobe anomalies		53% / 58%	No	No
Facial nevus		30% / 33%	No	No
Kidney ultrasonography			Normal [age 16 y.]	Normal [age 12y.]
Plasma alpha-fetoprotein (N.V.1-15)			1.86 ng/ml [age 16 y.]	1.58 ng/ml [age 12y.]
Additional analytical findings	*Fasting glucose (mg/dl)*		78	89
	Fasting insulin (µU/ml)		16.0	16.3
	HOMA index		3.08	3.58
	HbA1c		5.2%	5.6%
	Dyslipidemia		No	HDL < 35 mg/dl

Table 1. Clinical features of patients (both females of European ancestry), with respect to the clinical criteria of BWS. CBWS: Complete form of BWS; IBWS: Incomplete form of BWS (Gaston V, et al. Eur J Hum Genet 2001; 9: 409-18.)

Conclusions:

- Some overgrowth syndromes, particularly BWS, can present clinically as early-onset obesity, with mild or no prenatal overgrowth and no other features, leading to misdiagnosis and misclassification as "common" obesities.
- Genetic testing for BWS should be considered in early-onset obesity. MS-MLPA is an useful and efficient diagnostic tool.