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RESEARCH ARTICLE

Clinical profile of a patient cohort with Beckwith-Wiedemann syndrome treated at the Hospital Infantil de México Federico Gómez (2007–2012)

Rodrigo Moreno-Salgado,[1*] Constanza García-Delgado,[1] Alicia Cervantes-Peredo,[2,3] Leticia García-Morales,[4] Luis Enrique Martínez-Barrera,[1] Rosenda Peñaloza-Espinosa,[5] Verónica Fabiola Morán-Barroso[1]

ABSTRACT

Background. Beckwith-Wiedemann syndrome (BWS) (OMIM 130650) has an incidence of 1:13,700 newborns. Patients characteristically suffer from overgrowth, macroglossia and abdominal wall defects. BWS has diverse etiologies with several genetic and epigenetic mechanisms related to imprinted gene expression in 11p15 being involved.
Methods. The clinical profile of a cohort of BWS patients treated at the Hospital Infantil de Mexico Federico Gomez during the last 6 years was analyzed. A total of 19 patients with diagnostic criteria for BWS were included.
Results. Among the clinical characteristics identified in this study were preterm birth (33%), *nevus flammeus* (47%), macroglossia (89%), medial facial hypoplasia (68%), hemihyperplasia (36.8%) and abdominal wall defects (68%). No embryonic tumor or cardiopathies were identified. A familiar case was described.
Conclusions. Clinical follow-up of BWS patients should be strict and include the participation of the medical team and the patient's family. In order to offer genetic counseling, molecular diagnosis should ideally be provided due to the heterogeneity of the etiology of BWS.

Key words: Beckwith-Wiedemann syndrome, epigenetics, overgrowth.

INTRODUCTION

Beckwith-Wiedemann syndrome (BWS) (OMIM 130650) is one of the most important syndromes in the pediatric age, particularly because of its association with tumor development. It was described in the 1960s by Beckwith and Wiedemann who highlighted its clinical characteristics in various patients who shared the triad of symptoms: abdominal wall defects (particularly omphalocele), macroglossia and overgrowth (Figure 1). For this reason it was initially known as EMG syndrome: *exomphalos, macroglossia, gigantism*.[1,2]

BWS has a frequency of 1:13,700 newborns;[3] however, recent studies have considered a higher frequency (1:12,000). It is very probable that the syndrome may be even more frequent due to variability of phenotypic expression. Many patients may be underdiagnosed, including patients with diagnosis of "isolated" hemihyperplasia or Wilms tumor.[1,4] At present there is a consensus for diagnosis, taking into consideration the clinical characteristics and classifying them according to major and minor clinical findings (Table 1).[5] Approximately 85% of the cases present sporadically. The remainder correspond to a familial presentation.[6] BWS affects a similar proportion of

1 Departamento de Genética, Hospital Infantil de México Federico Gómez
2 Servicio de Genética, Hospital General de México Dr. Eduardo Liceaga
3 Facultad de Medicina, Universidad Nacional Autónoma de México
4 Departamento de Endocrinología, Hospital Infantil de México Federico Gómez
5 Sistemas Biológicos Universidad Autónoma Metropolitana-Xochimilco
* Becario del Instituto Carlos Slim, Mexico, D.F., Mexico

México D.F., México

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males and females, although a slightly higher proportion has been observed in males (53%).[7]

The etiology of BWS is as complex as it is interesting. It is caused by genetic and epigenetic alterations that modify the expression of various genes in the p15.5 region of chromosome 11. The activity of this region is regulated by the epigenetic mechanism of genomic imprinting. Epigenetics describes the hereditary and reversible changes in the genetic expression that do not imply alterations in the DNA sequence, i.e., not due to mutations but to mechanisms that regulate gene expression, modifying the chromatin structure.[8,9]

One of the best characterized epigenetic processes is genomic imprinting, a mechanism by which the male or female germ line confers specific marks (or imprints) to certain regions of the chromosome during the formation and maturation of gametes. These marks, once established, are mitotically inherited and are maintained throughout development and growth of an organism except in primordial germinal cells in which they are erased to give place to a new imprint, depending on the gender of the organism.[9] One of the characteristics of the imprinted genes is their monoallelic expression. In the majority of the 25,000 human genes, both alleles, inherited from the father as well as the mother are expressed. When there is imprinting, only the gene from the ovum or the spermatocyte is expressed, whereas the other remains silenced.

DNA methylation is a key epigenetic mechanism in genomic imprinting. Establishment of differentially methylated regions rich in cytosine-phosphate-guanine dinucleotide (CpG) generates conformational changes in the chromatin that do or do not allow interaction with transcription factors to promote gene expression or gene silencing.[10]

The 11p15.5 region has at least 12 imprinted genes. It is divided into two domains regulated by a control center of the imprint (CI) with a region with a different methylation pattern in the paternal and maternal chromosomes. Domain 1 (telomeric/distal) contains two imprinted genes: *IGF2* (*insulin-like growth factor 2*) expressed in the paternal allele and *H19*, an imprinted, maternally expressed transcript transcribed in a noncoding RNA. These genes are regulated by CI1.[1,3] Domain 2 (centromeric/proxi-

Table 1. Clinical findings in Beckwith-Wiedemann syndrome

Significant
• Positive family history for BWS
• Macrosomy
• Macroglossia
• Omphalocele or umbilical hernia
• Creases in the posterior border of the helix
• Visceromegaly (liver, spleen, kidneys, adrenal glands and pancreas)
• Hemihiperplasia
• Tumor of embryonic origin (Wilms tumor, hepatoblastoma and rhabdomyosarcoma)
• Cytomegaly of adrenal fetal cortex (pathognomonic)
• Renal alterations (including structural, nephromegaly and nephrocalcinosis)
• Cleft palate (rare)
• Placental mesenchymal dysplasia
• Cardiomegaly
• Cardiomyopathy (rare)
Less significant
• Neonatal hypoglycemia
• *Nevus flammeus*
• Medial facial hypoplasia, infraorbital creases
• Cardiopathy or structural cardiac defect
• Rectus diastasis
• Advanced bone age
• Perinatal
— Prematurity
— Polyhydramnios

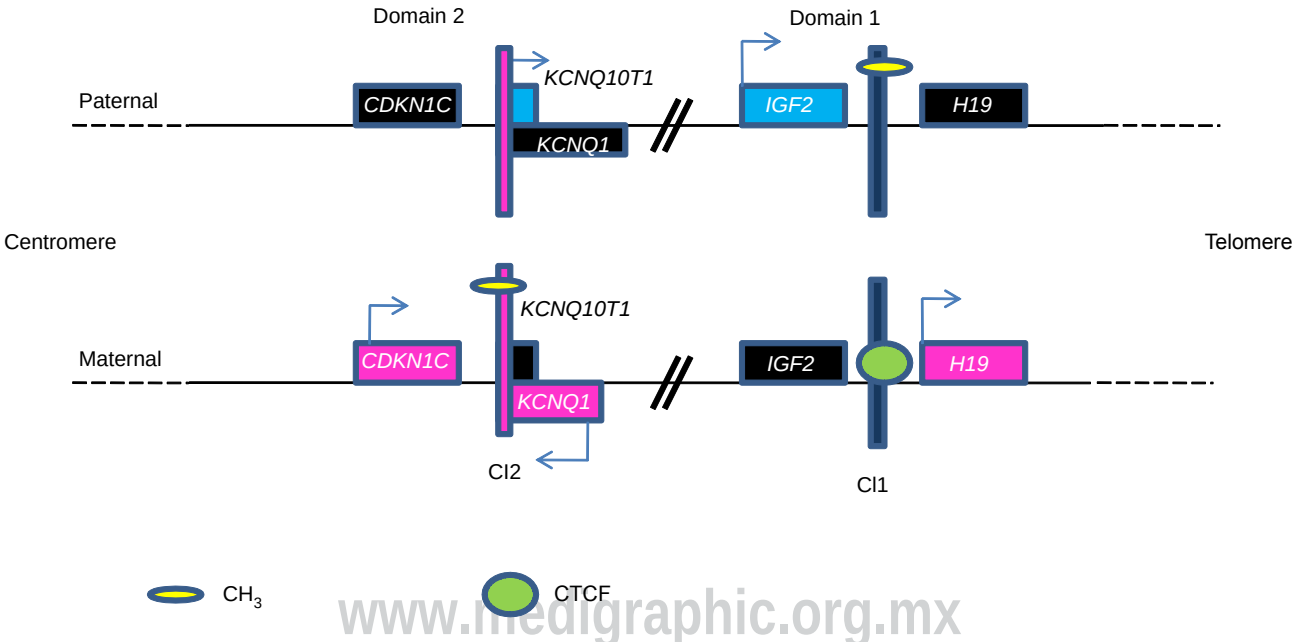
Figure 1.

Patients with Beckwith-Wiedemann Syndrome. **(A)** Retroauricular pits. **(B)** Macroglossia. **(C)** Hemihyperplasia.

mal) contains various imprinted genes, among them the *CDKN1C* gene (*cyclin-dependent kinase inhibitor 1C*), which is a negative regulator of the cell cycle expressed in the maternal allele. CI2 is located in a CpG island in intron 10 of the *KCNQ1*gene *(potassium voltage-gated channel, KQT-like subfamily, member 1*) with expression in the maternal allele.[1] In the paternal allele, *KCNQ1OT1* or *LIT1* (*KCNQ1 opposite strand/antisense transcript 1*) is expressed, which is an antisense transcript of *KCNQ1*. Figure 2 shows a section of the 11p15.5 region, the main genes that have maternal or paternal expression and the methylation state of both CI are noted.[1,11,12]

An alteration in the genomic imprint leads to activation of an allele that should have been silenced or the silencing of an allele that should have been active. This may cause diseases and anomalies in growth and development.[9,12,13] Changes in imprinting that cause BWS are varied and include cytogenetic changes such as microduplications of the paternal allele of domain 1 (corresponding to 1% of the BWS cases, in particular those associated with mental retardation), microdeletions of domain 2 in the maternal allele and balanced rearrangements in the maternal chromosome. These alterations lead to overgrowth due to gain of function of growth-promoting genes such as *IGF2* or to loss of function of negative growth regulators such as *CDKN1C*. Certain hereditary mutations in the maternal allele of the *CDKN1C* gene result in 5 to 10% of the sporadic cases of BWS and are found in ~40–50% of familial cases. In up to 80% of the patients with BWS there are epigenetic alterations of the methylation pattern. Hypomethylation of the maternal allele in domain 2 is found in 50% of the affected individuals[6,14] and hypermethylation of the maternal CI1 in 5%. Other alterations in methylation are generated by microdeletions or microduplications in the CI.[2,3,5,6,15-17]

Another event that could alter the imprinting and lead to biallelic expression of genes or to its lack of expression is uniparental disomy (UPD). In this situation, both ho-



CI1, control of imprint 1; CI2, control of imprint 2; CH_3, methylated region; CTCF, transcription factor of C. Genes not expressed: black. Genes with expression of the paternal allele: blue. Genes with expression of the maternal allele: pink. Arrows indicate the direction of the transcription of the gene.

Figure 2. Part of the 11p15 region is shown. In the paternal allele, CI1 is methylated, which allows the expression of *IGF2*. CI2 unmethylated with expression of the transcript *KCNQ1OT1.* The maternal CI1 allele interacts with the CTCF factor allowing the expression of the transcript *H19*. CI2 is methylated, allowing the expression of *KCNQ1*.

mologous chromosomes (belonging to the same chromosome pair) or chromosomal segments are inherited from a single progenitor.[10] In 20% of patients with BWS, UPD of the 11p15 region is demonstrated.[4]

Alterations of the CI1 or CI2 regions have significant clinical implications in BWS patients as, for example, it has been reported that cases of hemihyperplasia are associated with alterations in CI1[13] as well as the presence of Wilms tumor, whereas omphalocele is more frequent with changes in CI2.[1] Also, as reported by some authors, a history of assisted reproduction techniques in patients with BWS suggests a greater incidence of this syndrome with these techniques when compared with the general population; however, further studies in these cases are necessary.[18]

Considering the molecular alterations in the 11p15.5 region and its relation with different diseases as well as the significance of molecular diagnosis in the prognosis and genetic counseling of patients, it was proposed to carry out the following protocol "Implications of the methylation pattern in the 11p15.5 region as etiological mechanism of the isolated hemihyperplasia" (HIM/2012/007). As part of the controls of this protocol, the 11p15.5 region was to be studied at the molecular level in five patients with BWS. For this, it was necessary to review the clinical records and analyze the characteristics in patients with diagnosis of BWS and treated at the HIMFG during the previous 6 years.

SUBJECTS AND METHODS

Using the electronic clinical records of the HIMFG, identification was made of patients with diagnosis of "Syndromes of congenital malformations with excessive early growth", in accordance with the Internal Classification of Diseases (ICD10), category Q873, which includes those patients with diagnosis of BWS. The review period was from January 2007 to December 2012. Patients who did not meet diagnostic criteria for BWS were eliminated from the study. The clinical characteristics of the patients with BWS were classified according to the international criteria. A comparison was made of the population being studied with respect to what has been published in the literature. For the investigation protocol (HIM/2010/007) five patients were invited to participate. Clinical photographs were taken after obtaining informed consent. Molecular findings of these patients will be reported later in the context of the results of the protocol.

RESULTS

From the review of the general clinical file records of the HIMFG, a total of 29 patients were identified who were registered as having "Congenital malformation syndromes with excessive early growth." Five cases were excluded because they did not meet the clinical characteristics for the diagnosis of BWS and those who presented another clinical syndrome; four patients with Sotos Syndrome (OMIM 117550) and one with Klippel-Trenaunay-Weber Syndrome (OMIM 149000). This review included a total of 19 cases that met the clinical characteristics of BWS (Figure 1), of which 58% were males and 42% were females with ages ranging from 5 months to 27 years (average age 9.6 years; mean 9 years) (Table 2). Ten patients had karyotypes with GTG banding technique in peripheral blood and were reported as normal. We identified a family with two affected individuals (first cousins).

DISCUSSION

In the cohort of 19 patients diagnosed with BWS and treated at the HIMFG during a 6-year period, there was a predominance of male patients (58 vs. 42%). This is consistent with reports by Cohen.[7] Only one of the cases (5.26%) corresponded to a twin pregnancy, apparently discordant monozygotic, with one of the twins having BWS. A higher incidence has been reported of females in monozygotic twins that tend to be discordant.[3,7,14] According to international recommendations, the apparently healthy twin will be followed-up.

A familial case was identified with two individuals who were first cousins (patients 4 and 5, Table 2). One (patient 4) had a cleft palate. Both mothers were apparently healthy. Due to the maternal inheritance and the association with cleft palate, it is important to consider searching for mutations in the *CDKN1C* gene in this family.

In our cohort of patients we found prematurity (33 vs. 27% reported in the literature) (Table 3), *nevus flammeus* (47 vs. 54%), macroglossia (89 vs. 97%), midface hypoplasia (68 vs. 85%) and hemihyperplasia (36.8 vs. 14%) (Table 2).[7] Although abdominal wall defect is a classic characteristic for diagnosis of BWS, it was

found less frequently (68 vs. 80% reported in the literature) (Table 4).[7]

Interestingly, in this group of patients there was no reported incidence of tumors. In particular, no patients with Wilms tumors were identified. All cases had multidisciplinary follow-up carried out including abdominal ultrasonography and alpha-fetoprotein levels. The presence of tumors has been varied in different series, ranging from 4 to 25%.[1,5,7] Failure to identify, up to this time, may indicate that this complication could be related to size of the sample and age of the patients. An increased incidence of tumors has been found in patients between 8 and 10 years of age. In our series, seven patients were <8 years of age and will be followed up.

No patient had cardiac disease or structural cardiac defects compared with other studies that reported frequencies of 6.6-20%.[3,7] Although two patients have yet to undergo cardiac evaluation, they do not exhibit any clinically suspicious data. However, we must take into consideration that although the cohort corresponds to 6 years of experi-

Table 2. Clinical characteristics of the cohort of patients studied

Case # and gender	*Age*	*Facial nevus flammeus*	*Infraorbital creases*	*Folds in helix*	*Medial facial hypoplasia*	*Macroglossia*	*Cleft palate*	*Umbilical hernia*	*Rectus diastasis*	*Omphalocele*	*Hemihyperplasia*
% reported by Cohen[7]		54%		63%	85%	97%		80%			14%
1 M	1 year										
	5 months	+	+	+	+	+	-	-	-	-	+
2 M	4 years										
	2 months	+	+	+	+	+	-	-	-	-	-
3 M	15 years	+	+	+	+	+	-	-	-	+	-
4 F*	9 years	+	+	+	+	+	+	-	-	-	-
5 F	19 years										
	9 months	-	+	+	+	+	-	-	-	+	-
6 F*	11 years	+	+	+	+	+	-	-	+	-	-
7 M	8 years										
	2 months	-	+	+	+	+	-	-	-	+	-
8 M	5 years										
	3 months	+	+	+	+	+	-	+	-	-	-
9 M	4 years										
	5 months	+	-	+	+	+	-	-	-	-	-
10 M	18 years										
	9 months	-	-	+	+	+	-	+	-	-	-
11 F	15 years	+	+	+	+	+	-	+	-	-	-
12 M	14 years										
	4 months	-	-	+	-	+	-	-	-	+	+
13 F	11 years										
	9 months	-	-	-	-	-	-	-	-	+	+
14 M	14 years										
	1 month	-	-	+	+	-	-	+	-	-	+
15 M	27 years	-	-	-	-	+	-	-	-	-	-
16 F	2 years	-	-	+	-	+	-	-	-	+	+
17 M	3 years										
	2 months	-	-	+	-	+	-	+	-	-	+
18 F	1 year	-	-	-	-	+	-	+	-	-	+
19 F	5 months	+	+	+	+	+	-	-	-	-	-
Total		9	10	16	13	17	1	6	1	6	7
%		47.3%	52.6%	84.2%	68.4%	89%	5.2%	31.5%	5.2%	31.5%	37%
										68.4% abdominal alterations	

M, male; F, female.*First cousins according to maternal lineage.

Table 3. Perinatal history

	Macrosomy	*Birth weight (g)*	*Polyhydramnios*	*Prematurity*	*Neonatal HG*
1	+	4800	+	-	+
2	-	3200	+	-	-
3	+	5000	+	+	+
4	-	3400	-	-	-
5	-	3800	+	-	+
6	+	4300	-	-	-
7	+	3950	-	-	+
8	-	3300	-	+	-
9	-	2675	-	+	NA
10	+	4850	NA	-	NA
11	-	2200	-	+	-
12	+	4300	-	-	+
13	-	3200	-	+	+
14	NA	NA	-	NA	+
15	+	4650	-	-	+
16	-	3475	-	-	+
17	NA	NA	NA	-	-
18	+	5300	+	-	+
19	-	2485	-	+	+
	8/17 47%	Average 3758	5/17 29.4%	5/18 33.3%	11/17 61.1%

NA, Not available; HG, hypoglycemia.

Table 4. Visceral alterations

	Intraabdominal visceromegaly	*Cytomegaly of adrenal cortex*	*Cardiomegaly*	*Cardiomyopathy*	*Structural cardiac anomalies*	*Renal anomalies*	*Advanced bone age*	*Neoplasms*
1	+	-	-	-	-	+	-	-
2	+	-	-	-	-	-	+	-
3	+	-	-	-	-	-	+	-
4	-	-	-	-	-	-	+	-
5	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	+	-
8	-	-	-	-	-	-	+	-
9	-	-	-	-	-	-	-	-
10	+	-	-	-	-	+	-	-
11	+	-	-	-	-	-	NA	-
12	-	-	-	-	-	+	-	-
13	+	-	-	-	-	-	-	-
14	+	+	-	-	-	+	+	-
15	+	-	-	-	-	+	-	-
16	+	-	-	-	-	+	NA	-
17	-	-	NA	NA	NA	-	NA	-
18	+	-	NA	NA	NA	-	NA	-
19	-	-	-	-	-	-	-	-
F	10/19	1/19	0/17	0/17	0/17	7/19	6/15	0/19
P	52.6%	5.3%	0%	0%	0%	36.8%	40%	0%

NA, not available; F, frequency; P, percentage.

ence, it included only 19 patients. This may imply that the cohort is small to include all the previously reported characteristics of BWS.

Preliminarily, there were 19 patients identified in the HIMFG with BWS during the last 6 years. There was a slight prevalence of male patients. The majority of the cases were sporadic and only one familial case was identified with two patients. The characteristics found correspond with major and minor clinical findings for establishing diagnosis of BWS. It is noteworthy that in this cohort there have been no neoplasms or cardiac diseases described.

Patients with BWS require multidisciplinary management that should include, among others, medical geneticists, endocrinologists, pediatricians and surgeons, in addition to being monitored for neoplasms because of the elevated lifetime frequency in these patients, even though no neoplasm was currently present in these patients. For this reason, strict surveillance should be carried out with family and institutional involvement.

In order to be able to provide comprehensive genetic counseling, ideally there should be a molecular diagnosis available, due to the heterogeneity in the etiology of BWS.

Correspondence: Dra. Verónica Fabiola Morán Barroso
Departamento de Genética
Hospital Infantil de México Federico Gómez
Mexico, D.F., Mexico
E-mail: veronicafabiolamoranbarroso@yahoo.com.mx;
vfmoran@himfg.edu.mx

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