Adrenal cytomegaly with elevated serum androgen levels in a patient with Beckwith-Wiedemann syndrome

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Abstract. Beckwith–Wiedemann syndrome (BWS) is infrequently associated with adrenocortical carcinoma (ACC) or non-hormone-producing adrenal cytomegaly, but we recently, encountered a single case of adrenal cytomegaly in a patient with BWS, which was difficult to distinguish from androgen-producing adrenocortical carcinoma (ACC). Here, we describe the case of a 4-month-old female who presented with clitoromegaly, hemihypertrophy, and an adrenal mass identified during the prenatal period. The mass was located in detected at the left suprarenal region and detected at 20 weeks of gestational age. At birth, she also presented with clitoromegaly and elevated serum levels of 17 α-hydroxyprogesterone, dehydroepiandrosterone, and testosterone at birth and experienced hyper-insulinemic hypoglycemia, which improved following diazoxide therapy. We initially suspected androgen-producing ACC with metastasis and the left adrenal mass was resected accordingly when the patient reached 4 months of age. However, histological examination revealed adrenal cytomegaly. Genetic analysis revealed paternal uniparental disomy, and the patient was finally diagnosed as having BWS. Resection of the left adrenal gland restored the serum androgen levels to normal physiological levels without any recurrence. While it is reasonably well known that BWS is sometimes accompanied by virilization due to androgen-producing ACC, our findings are among the first to suggest that adrenal cytomegaly can also increase androgen hormone production. Thus, we propose that adrenal cytomegaly should be considered one of the differential diagnoses when accompanied with hyperandrogenism in BWS patients.

Key words: Beckwith-Wiedemann syndrome, Adrenocortical carcinoma, Adrenal cytomegaly, Clitoromegaly

BECKWITH–WIEDEMANN SYNDROME (BWS) is a genetic overgrowth syndrome associated with the dysregulation of the genes on chromosome 11p15.5, a region closely associated with adrenal hyperplasia and growth regulation [1]. A small proportion (7.5%) of patients are affected by embryonic tumors, such as Wilms tumors, neuroblastomas, hepatoblastomas, and adrenocortical carcinomas (ACC) [2]. ACC is rare during childhood and frequently metastasizes with poor prognosis [3]. Moreover, ACC often produces hormones, such as cortisol in 60% of adults and androgens in 90% of children [4]. In contrast, adrenal cytomegaly is not a malignant tumor but a degenerative dysfunction of the adrenal gland [5]. Adrenal cytomegaly is histologically characterized by numerous, large cells with hyperchromatic nuclei in the adrenal cortex, which can be detected in the adrenal glands of 3% of newborns and 6.5% of premature infants [6]. In addition, adrenal cytomegaly is closely associated with several specific syndromes, including congenital adrenal hypoplasia, erythroblastosis fetalis, and BWS [7]. Notably, unlike ACC, adrenal cytomegaly has not previously been associated with hormone production [8]. Both adrenal cytomegaly and ACC are sometimes accompanied with BWS, and it may be difficult to distinguish them from pathological findings alone, but the prognosis is completely different, so it is important to distinguish between them.

Here, we describe the case of a young patient with BWS presenting with clitoromegaly, hyper-insulinemic hypoglycemia, and hemihypertrophy along with an adrenal mass identified during the prenatal period. We initially suspected that the clitoromegaly was the result of an androgen-producing ACC with metastasis and performed histological evaluation of the resected adrenal gland to confirm this hypothesis.
Case Report

An older (38 years old) woman with a history of two pregnancies and one live birth presented for her 20 weeks ultrasound; her female fetus had developed a suprarenal mass (2 × 2 cm in size) on her left side. Thereby, this case was referred to the Nihon University Itabashi Hospital for detailed analysis of the mass. The infant was born at 36 weeks and 5 days via normal vaginal delivery, with a length of 45.2 cm (–0.78 SD), a body weight of 2,368 g (–0.61 SD), and an enlarged placenta (weight 772 g; normal weight is 500–600 g). The amniotic fluid volume was normal but periodic blood examination for preterm birth revealed that the infant had hyper-insulinemic hypoglycemia (plasma glucose <1 mg/dL, insulin 4.4 μU/mL). Despite this, there were no clinical symptoms of hypoglycemia such as irritability, tremors, or convulsions. This hypoglycemia was resolved following the initiation of diazoxide therapy at a dose of 7 mg/kg/day. Although the left suprarenal mass was initially suspected of being a neuroblastoma, this diagnosis was excluded following detailed examination (Table 1). The infant was then discharged from our department at 44 days after birth in a good condition, although a genetic diagnosis for her hyper-insulinemic hypoglycemia had not been determined.

At the age of 1 month, the patient presented with several classical symptoms of BWS including macroglossia, high arched palate, omphalocele, ear creases, and facial naevus flammeus, and was marked as being likely to have BWS. We also considered 21-hydroxylase deficiency because of the presence of clitoromegaly (labia fusion and common urogenital sinus were absent) (Fig. 1), and an elevated serum 17-hydroxyprogesterone (17-OHP) level (9.9 ng/mL, normal values <2 ng/mL) during her neonatal screening. However, other common manifestations of 21-hydroxylase deficiency, such as skin pigmentation and electrolyte imbalance, were absent.

![Fig. 1 Clitoromegaly](image)

The cross section of clitoral size is 7 mm (Prader 1), there is no common urogenital sinus, pigmentation, labia heals.

<table>
<thead>
<tr>
<th>Table 1 Detailed examination</th>
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<tr>
<td>Blood count</td>
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</tr>
<tr>
<td>WBC 13,200/μL</td>
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<tr>
<td>RBC 5.81 × 10⁶/μL</td>
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<td>Hb 22.4 g/dL</td>
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<td>Ht 40%</td>
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<td>Plt 17/μL</td>
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<tr>
<td>AST 28 U/L</td>
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<tr>
<td>ALT 4 U/L</td>
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<tr>
<td>LDH 487 U/L</td>
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<tr>
<td>ALP 417 U/L</td>
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<tr>
<td>BUN 11 mg/dL</td>
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<tr>
<td>Cr 0.8 mg/dL</td>
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<tr>
<td>Na 137 mEq/L</td>
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<tr>
<td>K 3.5 mEq/L</td>
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<tr>
<td>Cl 103 mEq/L</td>
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<tr>
<td>glucose &lt;1 mg/dL</td>
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<td>IRI 4.4 μU/mL</td>
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IRI, immunoreactive insulin; 17-OHP, 17α-hydroxyprogesterone; CEA, carcinoembryonic antigen; AFP, alpha-fetoprotein; NSE, neuron specific enolase; sIL-2R, soluble interleukin-2 receptor; HVA, homovanillic acid; VMA, vanillylmandelic acid.
Moreover, no physical signs of Cushing syndrome were present and serum levels of cortisol and ACTH were 4.0–9.3 μg/dL and 15.4–81.6 pg/mL, respectively. In addition, patient length and body weight at birth were −2.0 SDS and height and body weight at 23 months of age were +1.0 SDS; thus, although the patient exhibited some increased growth, she was not obese. Based on these findings, both congenital adrenal hyperplasia and Cushing syndrome were excluded from the differential diagnosis.

Left hemihypertrophy, a typical characteristic of BWS, was first observed in the patient at 4 months of age, when we resected the left adrenal mass to confirm its definitive diagnosis and the risk of metastasis in the case of a malignant carcinoma. Laparotomy findings revealed that the left adrenal gland was swollen albeit isolated and non-invasive to other organs. No other metastatic findings were observed. Total left adrenalectomy was then performed without rupture during surgery and subsequent histopathological examination revealed adrenal hyperplasia and cytomegaly, with no signs of a malignant carcinoma. Serum levels of dehydroepiandrosterone (DHEA), 17-OHP, and testosterone rapidly normalized, returning to normal range within just 30 minutes, following the removal of the left adrenal gland. Adrenocortical hormone supplement therapy (hydrocortisone 10 mg/m$^{2}$) was then initiated, and no hypoglycemic events were recorded following the discontinuation of the diazoxide therapy at 8 months and hydrocortisone at 12 months of age. In addition, serum levels of 17-OHP, DHEA, and testosterone remained within normal range even at 13 months of age while those of cortisol and ACTH remained unchanged when compared to the values recorded when prior to resection of the left adrenal gland. There were also no signs of malignant carcinoma (Fig. 2).

We then proceeded to confirm the genetic diagnosis of BWS using, methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) using patient blood-derived DNA and the Salsa MLPA kit with a BWS/RSSME030 BWS/RSS probe mix (MRC-Holland). The results confirmed the hypermethylation of ICR1–DMR (average of four $H19$ sites: 0.56 (>0.55)) and hypomethylation of ICR2–DMR (average of four $KCNQ1OT$ sites: 0.40 (<0.45)) (Fig. 3). These findings suggested the presence of uniparental disomy (UPD) but were not conclusive. Subsequent analysis of the resected adrenal mass revealed clear hypermethylation of ICR1–DMR (average of four $H19$ sites: 0.96 (>0.55)) and hypomethylation of ICR2–DMR (average of four $KCNQ1OT$ sites: 0.27 (<0.45)), confirming the presence of paternal UPD (pUPD) (Fig. 4).

**Histopathological findings**

The diagnosis of ACC using histopathological findings alone is difficult; therefore, most clinicians use a predefined scoring system to help distinguish ACC from other tumors. The Weiss score is often used to differentiate ACC from adrenocortical adenoma in adults, while the Wienke criteria are more accurate in predicting clinical outcomes in younger children [9]. The Wienke criteria specifically consider various parameters including the weight and size of the tumor(s) and their histopathological findings from perioperative evaluations in addition to the histopathological findings following resection [10].

Here, the mass was found to be 6 × 3.5 × 1 cm in size and slightly yellowish in color via macroscopic analysis.

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![Fig. 2 Clinical course](image_url)
The adrenal cortex exhibited a complex morphology with incomplete nodular formation (Fig. 5) and microscopic evaluation revealed that the adrenal gland lacked the typical three-layer cortical zonation. The cortex was also shown to be hypertrophic based on its increased thickness and enlargement of its cells. A mass of large to huge eosinophilic cytomegalic cells occupied the center of the glands deep into the transient zone (Fig. 6).

**Fig. 3** MS-MLPA (peripheral blood DNA)

The mild hypermethylation of ICR1–DMR (average of four *H19* sites) and mild hypomethylation of ICR2–DMR (average of four *KCNQ1OT* sites) indicated the presence of pUPD.

**Fig. 4** MS-MLPA (resected adrenal mass)

The hypermethylation of ICR1–DMR (average of four *H19* sites) and hypomethylation of ICR2–DMR (average of four *KCNQ1OT* sites) are more clearly than Fig. 3 and confirming the presence of pUPD.
the mass appeared to be a remnant of the fetal zone. The cytomegalic cells were two to three times the normal size and exhibited hyperchromatic pleomorphic nuclei and abundant vacuolated eosinophilic cytoplasm. Some enlarged cells contained pseudo-inclusions in their nuclei and rare mitotic figures were also observed. No necrosis or vascular invasion was present (Fig. 7).

Current diagnosis of ACC relies upon a combination of the Weiss or Wienke index and immunohistochemical staining (Ki-67) using an index of cellular proliferation [11]. In this case, the latter revealed a positivity rate of just 1% (where >5% reflects suspected ACC, and >10% indicates an increased possibility of recurrence following resection). Thus, the patient was diagnosed as exhibiting adrenal hyperplasia with adrenal cytomegaly rather than carcinoma. Moreover, when this was combined with the presence of only a single criterion (positive for atypical mitotic figures) from the Wienke index, we suspected that the resection in this case should produce a good clinical outcome.

**Discussion**

This paper describes a case of cystic adrenal cytomegaly presenting with clitoromegaly and elevated serum androgen levels in BWS. Histological findings revealed adrenal gland hyperplasia and hypertrophy with cytomegaly, which may have resulted in the hypersecretion of androgens in our patient. Adrenal cytomegaly is occasionally observed in BWS, and 7.5% of patients with BWS develop some form of carcinoma [2]. However, our patient presented with an adrenal mass with several characteristics associated with ACC including clitoromegaly and elevated androgen expression. Therefore, it was difficult to clinically distinguish between ACC and other adrenal mass diseases including adrenal cytomegaly.

The BWS triad includes macrosomia, macroglossia, and abdominal wall defects. Other features include hemihypertrophy, distinct facial features, ear and infraorbital creases, and hypoglycemia. The incidence of BWS is 1/13,700 births, with 15% of cases being familial and 85% sporadic [12]. In the 7.5% of BWS patients who develop tumors, most tumors occur during the patients’ first 8 years of life [13] with the incidence of childhood
tumors reaching approximately 10% in these patients Wilms tumors are the most frequent (52%), followed by hepatoblastoma (14%), neuroblastoma (10%), rhabdomyosarcoma (5%), and ACC (3%). Nevertheless, although ACC is relatively rare in BWS, patients with pUPD have an increased risk for this type of malignancy. Moreover, the risk of ACC increases in the presence of hemihypertrophy [1]. Therefore, our patient presented with two significant risk factors for ACC: pUPD and hemihypertrophy.

Owing to the presence of clitoromegaly and elevated serum androgen levels at birth, we initially suspected that the patient had ACC. ACC is sometimes accompanied by BWS during childhood and is recorded in 0.72/10 million births and 0.6% of all pediatric tumors. They most frequently occur at <5 years of age or between 40 and 50 years of age [14]. Metastasis is frequent, and these aggressive tumors are associated with a 5-year survival rate of only 16–38% [3]. The most consistent risk factors associated with poor prognosis are advanced stage and incomplete surgical resection, with other risk factors including higher grade, older age, hormonal hypersecretion, and increased tumor size. Recurrence occurs in 70–80% of patients following resection; therefore, surgery is the only standard treatment if the cancer is localized. As ACC often produces functional hormones such as cortisol, in 60% of adults, and androgens, in 90% of children [4], symptoms of Cushing syndrome and virilization are also commonly associated with a diagnosis of ACC. In contrast, nonfunctional ACC (non-hormone-producing) is often recognized by abdominal pain or abdominal distension. Hishiki et al. reported a case of androgen-producing ACC, which was identified based on high serum levels of 17-OHP [15]. Given this, we decided to resect the mass to confirm the histological diagnosis and suppress the progression of the clitoromegaly. However, histopathological examination revealed that this mass presented as an adrenal cytomegaly, a benign tumor, rather than ACC. Adrenal cytomegaly, first described by Kampmeier et al. in 1927 [16], is associated with various syndromes such as Rh incompatibility [7] and trisomy 13 and 18, and is observed at low frequencies in healthy adults [17] and in those with BWS [7].

These data allowed us to formulate a hypothesis describing the mechanism of hyperandrogenism in this case. In general, the permanent cortex, transient cortex, and fetal cortex are derived from adrenal cortical origin at about 7–8 weeks of viviparity. The permanent cortex then secretes glucocorticoids, while the transient cortex secretes DHEA and the androgens, and the fetal cortex secretes DHEA [18]. Usually, the fetal cortex degenerates between 2 weeks and 2 months of age and disappears completely before a normal child turns one, leaving only the permanent and transient cortex tissues. However, Atemman [7] and Wockel et al. [19] reported that fetal adrenal glands with adrenal cytomegaly could be histologically detected in the adrenal cortical gland at birth and at 2 months of age in patients with BWS. In such cases, the fetal cortex may be retained longer than usual following exposure to chronic stress, e.g., prenatal infection and exogenous steroids [7]. Thus, the elevated 17OHP levels in our patient may have resulted from a positive feedback loop in which 17-OHP cross-reacts with the steroids being produced by the enlarged adrenal gland to increase its own expression [17]. This is supported by the fact that the resected adrenal gland was significantly larger than usual (weight 25 g, five times the normal value). This gland was even increased in size compared to standard adrenal glands from BWS patients which typically exceed 16 g. This enlargement is likely the result of the swelling associated with cytomegaly [20].

Despite this, it remained unclear whether the excess androgen production in this patient was the result of adrenal cytomegaly. However, total resection of the adrenal gland resolved this confusion as there was an immediate reduction in the excess androgens, including 17-OHP, DHEA, and testosterone, following its removal. Histological identification of the resected adrenal gland confirmed the initial diagnosis of adrenal cytomegaly and supported our hypothesis that this adrenal cytomegaly is likely the source of the excess androgens. However, we were unable to confirm this functionality as we experienced some technical difficulties when completing the immunohistochemical staining against the hormones themselves.

We do note there may be some argument that surgical resection of the left adrenal gland may not have been appropriate in this patient, as our histological examination revealed a diagnosis of adrenal cytomegaly (degenerative cyst) rather than ACC (malignancy). However, given the age of the patient and the frequency of ACC and other embryonic tumors (7.5%) in BWS patients [12] and the presence of several other high-risk factors, such as large mass (>3 cm), rapid growth, suspected metastasis, and excessive adrenocortical hormones presenting as Cushing signs, virilization, and hypertension [1], we felt that the risk of malignancy warranted aggressive treatment of the condition. We propose that in any case where a patient is suspected of harboring an ACC and exhibits at least one of these high-risk factors, the tumor should be fully resected. Moreover, our results indicated that such total resection may be useful in reducing the over-secretion of the androgens and the progression of clitoromegaly in this patient.

While genital manifestations such as clitoromegaly, hypospadias, and testicular enlargement are historically
rare in patients with BWS [21, 22], the true frequency of these events is largely unknown. Our patient only presented with clitoromegaly, but other genital abnormalities, such as labial fusion and a common urogenital sinus were absent. We suggest that this clitoromegaly was primarily induced by the increased androgen levels in this patient in utero, as it stopped progressing following the resection of the left adrenal gland and the normalization of the serum androgen levels. On the contrary, clitoromegaly may be a symptom of BWS. Therefore, it cannot be concluded whether clitoromegaly is due to elevated androgen or a symptom of BWS.

At the time of writing this manuscript, the patient was 13 months old (9 months post surgery). Her initial hyperinsulinemic hypoglycemia was treated with diazoxide, but this condition gradually improved over time suggesting that the hyperinsulinemic hypoglycemia was likely transient, which is reasonably common in BWS patients. Nevertheless, the most critical concern remains the potential occurrence of carcinomas and thus a several-year follow-up is strongly recommended [23] especially as our patient presents with an increased risk of carcinoma resulting from her hemihypertrophy and pUPD background. Although she remains in good health without any signs of carcinoma, evaluations, including abdominal ultrasonography focusing on the liver, kidneys, and adrenals and measurement of serum DHEA and alphafetoprotein, should be completed every 3–4 months until she reaches 8 years of age, when tumor risks for BWS patients begin to decrease.

Conclusions

Here, we have described the case of a young patient with BWS caused by pUPD with adrenal cytomegaly presenting with clitoromegaly and hemihypertrophy. We were unable to produce a conclusive diagnosis for the adrenal mass based only on its clinical characteristics and imaging. Initial evaluations suggested that this mass may have been an androgen-producing ACC accompanied by BWS, but histological examination was required to confirm the actual diagnosis. Although clinical signs of hemihypertrophy and the presence of pUPD are high-risk factors for ACC, our findings suggest that hyperplasia of the cortical cortex with histologically adrenal cytomegaly should also be considered among the differential diagnoses in patients with hyperandrogenism.

Statement of Ethics

Written informed consent was obtained from the patient for genetic analysis and publication of this case report and any accompanying images.

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Disclosure Statement

None of the authors have any potential conflicts of interest associated with this report.

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