Beckwith-Wiedemann syndrome (BWS) is the most common congenital overgrowth syndrome involving tumor predisposition and congenital malformations [1, 2]. BWS is caused by various epigenetic or genetic alterations that disrupt the imprinted genes in two imprinted domains on chromosome 11p15.5. In domain 1, insulin-like growth factor 2 (\textit{IGF2}) and \textit{H19} are monoallelically expressed, and in domain 2, \textit{CDKN1C}, a growth repressor, and \textit{KCNQ1OT1} are monoallelically expressed. In each domain, an imprinting center, \textit{H19-DMR} or \textit{KvDMR1}, regulates the expression of imprinted genes. In BWS, several mechanisms result in increased expression of \textit{IGF2} and/or decreased expression of \textit{CDKN1C}. \textit{KvDMR1} loss of methylation occurs in 50% of BWS patients, and paternal uniparental disomy (UPD) on chromosome 11p15 is found in 20%.

The clinical findings of BWS are highly variable because of the heterogeneity of the underlying molecular etiology, and milder phenotypes may not be readily identified [1, 2]. Classically, BWS must be considered when exomphalos, macroGLOSSIA, or gigantism is noted; however, recent advances in molecular testing have expanded the diagnostic potential for BWS for patients with no or few clinical features [3].

Congenital hyperinsulinism (HI) comprises various genetic disorders due to inappropriate insulin secretion by pancreatic \( \beta \)-cells [4, 5]. Severe hypoglycemia is the major feature of HI and has a risk of seizures and brain damage if untreated. Mutations in ATP-sensitive potassium (\( K_{ATP} \)) channel genes, \textit{ABCC8} and \textit{KCNJ11}, on chromosome 11p15.1, are the most common causes of HI and account for 40-45% of all cases but, in nearly half of the cases, the genetic etiology remains unknown. HI is usually isolated, but in rare cases may be part of a
genetic syndrome, such as BWS and Sotos syndrome.

We report an infant with HI but without apparent clinical features suggestive of BWS, but diagnosed BWS by molecular testing due to the somatic mosaicism of paternal UPD on chromosome 11p15.

**Clinical Report**

This female patient was the first child of nonconsanguineous parents and had been conceived naturally. Fetal sonography suggested bilateral mild hydronephrosis at the prenatal age of 23 weeks, but the pregnancy was uncomplicated. The patient was delivered by cesarean section at 38 weeks gestation due to breech presentation. Her birth weight was 3,738 g (>90th percentile), height was 52 cm (>90th percentile), and she was physically evaluated as normal.

She developed severe hyperinsulinemic hypoglycemia 1.5 hours after birth and was diagnosed with hyperinsulinemic hypoglycemia (plasma glucose 17 mg/dL and serum insulin 37.3μU/mL with undetectable ketone bodies, normal lactate). The serum GH and cortisol were 9.18 ng/mL and 11 μg/dL, respectively. The glucose infusion rate required to maintain a blood glucose concentration >60 mg/dL was 20 mg/kg/min. She was apparently normal, without macroglossia, exomphalos, hemihypertrophy or ear anomaly. Light brown irregular nevi on the shoulder, back and upper limb were apparent. Renal ultrasonography showed bilateral mild hydronephrosis, as observed on prenatal ultrasound. Her hypoglycemia failed to respond to maximum doses of diazoxide (20 mg/kg/d). Instead of diazoxide, continuous intravenous infusions of octreotide were started at the age of two weeks and the dose was slowly titrated up to 40 µg/kg/d. While continuing medical therapy, the surgical indication was also considered as a case of unresponsive HI. To determine the histopathological form, \(^{18}\text{F}\)-fluoro-L-DOPA ([\(^{18}\text{F}\)]DOPA) positron emission tomography (PET) was performed, as described by Ribeiro et al. [6]. The patient demonstrated uptake in the head and body of the pancreas (Fig. 1a). The standardized uptake of the head, body and tail was 5.5, 4.4 and 3.7, respectively. As the result was a non-single focal form, i.e. multi-focal or diffuse form, it seemed that partial pancreatectomy was impossible.

At the age of one month, a few days after the maximum dose of octreotide, the glucose infusion rate could be decreased gradually. Normoglycemia without glucose infusion could be maintained one week later and the treatment was changed to continuous subcutaneous octreotide injection at the age of two months. The dose of octreotide was reduced in a stepwise manner and was discontinued at the age of 3 months. Subsequently, there were no episodes of hypoglycemia.

At the ages of 2 and 8 months, computed tomography (CT) with contrast demonstrated a mass adjacent to the upper segment of the left kidney (Fig. 1b). The mass measured 38 × 17 mm, with homogeneous density comparable to the spleen, and was not enhanced. Renal ultrasonography demonstrated no blood flow inside the mass. CT and MRI imaging also showed an enlarged mass occupying the anterior mediastinum, totally covering the heart to 20 mm thickness, indicating thymic hyperplasia (Fig. 1c). Tumor markers were

![Fig. 1](image)

(a) Representative patterns of \([^{18}\text{F}]\)DOPA uptake. Maximum intensity projection obtained 30 min after injection. Multifocal or diffuse uptake in the head and body of the pancreas. (b) CT with contrast showed a mass adjacent to the upper segment of the left kidney (arrows). (c) CT with contrast showed an enlarged mass occupying the anterior mediastinum (arrows), indicating thymic hyperplasia.
not elevated and these masses showed gradual regression, therefore, histological evaluation could not be performed. At the age of 8 months, she demonstrated normal growth and neurodevelopmental progress, with no apparent clinical features of BWS (Fig. 2).

Materials and Methods

**K**\textsubscript{\text{ATP}} **genes analysis**

Genomic DNA was extracted from peripheral leukocytes. Mutation analysis of K\textsubscript{ATP} genes, ABCC8 and KCNJ11, was performed by sequencing coding exons and flanking intronic regions including 30-100bp. The PCR products were purified on 1.0% agarose gel and were sequenced directly with ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, USA) using an automated sequencer ABI Prism 310 Genetic Analyzer (Applied Biosystems). Multiple ligation-dependent probe amplification (MLPA) of ABCC8 was performed by using Salsa MLPA Kit (MRC-Holland, Amsterdam, Netherlands).

**Molecular analysis of BWS**

To analyze paternal UPD, genomic DNA was extracted from peripheral blood lymphocytes of the patient and her parents. For quantitative polymorphism analyses, tetrnucleotide repeat markers (D11S1997, HUMTH01, and D11S1984) from 11p15.4-p15.5 were amplified and separated by electrophoresis on an Applied Biosystems 3130 genetic analyzer (Applied Biosystems); data were quantitatively analyzed with GeneMapper software (Applied Biosystems). The peak height ratios of the paternal allele to maternal allele were calculated. The percentage mosaicism of paternal UPD was calculated as: % mosaicism = (\(k - 1\)) / (\(k + 1\)) \times 100, where \(k\) is the ratio of the intensity of the paternal to maternal alleles of the sample [7]. To confirm the range of UPD, we also used another marker D11S2001 on 11p13 region. We also investigated methylation status in KvDMR1 and H19-DMR, mutation analysis of CDKN1C by sequencing as described previously [8].

These studies were approved by ethical committee of Akita University Graduate School of Medicine and written informed consent was obtained from her parents.

Results

We first suspected mutations in the K\textsubscript{ATP} channel genes. We obtained written informed consent for molecular testing from her parents, and genomic DNA was extracted from peripheral blood lymphocytes of the patient for direct sequencing of ABCC8 and KCNJ11, but no mutations were found; however, a rare homozygous single nucleotide polymorphism (SNP) was found in intron 8 of ABCC8 (rs1800850; A>G change, minor allele frequency was 6.7%). Then, the SNP in her parents was directly sequenced. The patient had A/A genotype, her father had G/A genotype, but her mother had G/G genotype, which suggested deletion of her maternal allele or paternal UPD on chromosome 11p15 (Fig. 3a). MLPA of ABCC8 showed that the patient had two copies of all exons, and we concluded that the homozygous SNP might have resulted from paternal UPD. At the age of three months, we started chromosome 11p15 molecular analysis in order to define her diagnosis.

The results of microsatellite marker analysis for markers D11S1997, HUMTH01, D11S1984, D11S2001 are shown in Fig. 3b. The percentage mosaicism was 70.9%, 72.8%, 72.4% and 73.5%, respectively. These results were consistent with a diagnosis of mosaic paternal UPD on chromosome 11p15. Methylation-sensitive Southern blots showed H19-DMR hypermethylation and KvDMR1 hypomethylation, supporting her genetic diagnosis (data not shown). No CDKN1C mutation was detected.
The neonatal hypoglycemia, macrosomia and hydronephrosis observed in our patient fulfill the generally accepted criteria of BWS (i.e. two major findings and one minor finding) [2]; however, we had difficulty in the diagnosis of BWS because macrosomia is commonly involved in HI and, above all, there were no apparent clinical features of BWS. She also showed an extrarenal mass and an enlarged thymus, but whether they are symptoms of BWS is uncertain at present. Balcom et al. reported hyperplasia of the thymus that caused pulmonary hypoplasia in an infant with BWS [9], but there are few reports about an association between the thymus and BWS.

There are no absolute criteria for the clinical diagnosis of BWS and there exist milder phenotypes of BWS which do not fulfill the criteria [1, 2]. Recently, with the development of molecular genetic analysis, epigenetic alterations of chromosome 11p15 have been detected in patients with no or few clinical features of BWS; for example, isolated hemihyperplasia [10], isolated Wilms tumor [11], and isolated cardiac tumor [3].

In BWS, it has been estimated that the incidences

![Fig. 3](a) SNP(rs1800850) of ABCC8. The patient had A/A genotype, her father had G/A genotype, but her mother had G/G genotype. (b) Microsatellite marker analysis for markers D11S1997, HUMTH01, D11S1984 and D11S2001. The percentage mosaicism of paternal UPD was 70.9%, 72.8%, 72.4% and 73.5%, respectively.
of hypoglycemia, macrosomia, and renal abnormalities are 50%, 88%, and 59%, respectively [12]; however, to our knowledge, there have been no other reports of BWS phenotype only with hypoglycemia, macrosomia, and renal abnormalities. Goldman et al. reported that BWS with paternal UPD was associated with a higher incidence of renal abnormalities [13]. The most common findings are nephromegaly, simple cysts, hydronephrosis and medullary cysts [12-14]. The grade of hydronephrosis was reported to be mild to severe with vesiculoureteral reflux (VUR). Our case did not demonstrate VUR and diuretic renography with 99mTc-MAG3 showed a normal washout pattern. Although this information supported the diagnosis, it might be difficult to reach a diagnosis for less characteristic cases in the neonatal period. Given that the genetic etiology is still unknown in nearly half of HI, some HI might be involved in undiagnosed BWS with no apparent clinical features.

The underlying mechanism leading to HI in BWS remains unclear, and the severity, duration, and response to treatment with diazoxide and octreotide are variable [15, 16]. In the majority of BWS patients, hypoglycemia will be asymptomatic and resolve within the first few days of life. Less than 5% of patients will have hypoglycemia beyond the neonatal period and, in rare cases, there will be no response to medical therapy and partial pancreatectomy will be required. Hussain et al. reported histological and functional studies of BWS with paternal UPD using a pancreas obtained at partial pancreatectomy [16]. Histological findings showed marked proliferation of endocrine tissue forming irregular nodules and functional studies suggested a K\textsubscript{ATP} trafficking defect. In their case, as in our case, the clinical features of BWS were not obvious at birth, but developed postnatally.

BWS caused by paternal uniparental disomy is basically a mosaic, that is, originates as a consequence of postzygotic error [17]. The clinical features, therefore, is inherently variable since the features depend on the timing of the error during the postzygotic process. If an error occurred in the earlier stage of development, the clinical features are more evident. Conversely, if the error occurred in the later stage of development and confined to certain somatic organs (e.g., pancreas), the BWS features are less evident. The mosaic ratio of peripheral blood is reasonably high to diagnose BWS, however this does not tell the mosaic ratio in other somatic tissues. Therefore, we consider that diagnosis of UPD11.5 mosaicism is important for differential diagnosis of unknown HI.

Precise genetic analysis of the K\textsubscript{ATP} channel and [18F]DOPA PET scan diagnosis are essential in the management of diazoxide-unresponsive patients [4, 5, 18]. The focal form is due to the combination of a paternally-inherited mutation and paternal isodisomy of the 11p15 region, which is specific to islet cells within the focal region. Recessive mutations are responsible for the diffuse form. However, some previous papers report that dominant mutations also have diffuse histology. Interestingly, [18F]DOPA PET in our patient showed a non-single focal form, i.e. multi-focal or diffuse form. To our knowledge, there have been no reports of [18F]DOPA PET in HI due to BWS. If no mutations are found in known genes and [18F]DOPA PET does not show a typical form, there is a possibility that HI is caused by undiagnosed BWS with no apparent clinical features.

Early diagnosis of BWS is particularly important because patients with BWS have a predisposition to embryonal tumors, most commonly Wilms tumor and hepatoblastoma, and a variety of other malignant and benign tumors [19, 20]. The risk is approximately 7.5% and most of the tumors occur in the first 8–10 years of life; therefore, tumor surveillance is recommended for all children with confirmed or suspected BWS every 3 months to the age of 8 years by abdominal ultrasound and every 3 months to the age of 4 years by alpha fetoprotein assay [3]. In this regard, it is significant to recognize the existence of BWS patients with no or few clinical features, which might be diagnosed only by molecular testing.

In summary, we identified an infant with HI but without apparent clinical features suggestive of BWS, which was diagnosed by molecular testing as being due to somatic mosaicism of paternal UPD on chromosome 11p15. BWS could be very difficult to diagnose on clinical examination and should be taken into consideration also in children presenting with apparently isolated congenital anomalies of the spectrum of the syndrome, such as hyperinsulinism. Many cases without the typical and well-known facial phenotype are emerging, imposing a new clinical paradigm on the approach to this condition.

**Conflicts of Interest**

The authors have no conflicts of interest to declare.
References