Coexistence of paternally-inherited ABCC8 mutation and mosaic paternal uniparental disomy 11p hyperinsulinism

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Abstract

Background: Beckwith–Wiedemann syndrome (BWS) is an overgrowth syndrome with variable clinical phenotype and complex molecular aetiology. It is mainly caused by dysregulation of the chromosome 11p15 imprinted region, which results in overgrowth in multiple tissues, often in a mosaic manner.

Case presentation: A large-for-gestational-age infant without any other somatic features of BWS presented with medically refractory hyperinsulinism (HI) requiring 80% pancreatectomy. Next generation sequencing with congenital HI sequencing panel identified a pathogenic ABCC8:c.1792C > T (p.Arg598Ter) variant of paternal origin, suggestive of focal HI. However, pancreatic histology revealed atypical findings of coalescing nests and trabeculae of adenomatosis scattered with islets with isolated enlarged, hyperchromatic nuclei scattered throughout the pancreas. Methylation analysis, SNP-based chromosomal microarray and short tandem repeat markers analysis revealed mosaic segmental paternal uniparental disomy (UPD) 11p15.5-p15.1 in the pancreatic tissue, but not the peripheral blood, suggestive of BWS/BW-spectrum HI.

Conclusions: This case highlights the importance of integrating the clinical presentation and subsequent clinical course, together with radiological, genetic and histological findings in the definitive diagnosis of this rare yet clinically important entity. In addition, this is the first report that demonstrated the level of paternal inherited c.1792 T pathogenic variant in the pancreatic tissue being directly correlated to the mosaic level of pUPD.

Keywords: Congenital hyperinsulinism, Hyperinsulinism, Beckwith-Wiedemann syndrome, UPD11

Background

Congenital hyperinsulinism (HI) is the most common cause of persistent hypoglycaemia in infants. It is characterized by dysregulated insulin secretion from pancreatic β-cells and is a group of heterogeneous conditions that vary in terms of clinical severity, histopathology and molecular aetiology. Inactivating mutations of the ABCC8 and KCNJ11 genes, which are located on 11p15.1 and encode the SUR1 and Kir6.2 subunits of the pancreatic β-cell ATP-sensitive potassium channel (KATP channel) respectively, are the most common genetic aetiology of HI [1].

There are two major histological subtypes — diffuse and focal HI. The two have distinct molecular aetiology and response to medical treatment. Rarely, some patients have atypical histology that could not be easily classified into either focal or diffuse forms [2]. They have...
enlargement of β-cell nuclei that is distinct from diffuse 
HI in several discrete regions of the pancreas, which 
suggests the possibility of mosaicism [3].

Beckwith–Wiedemann syndrome (BWS) is an over-
growth syndrome with variable clinical phenotype and 
complex molecular aetiology. It is mainly caused by the 
dysregulation of the chromosome 11p15 imprinted re-
gion, which results in overgrowth in multiple tissues, 
often in a mosaic manner [4]. While only a small pro-
portion of HI are associated with BWS, transient HI oc-
curs in up to 50% of BWS neonates, and 5% have 
persistent HI requiring medical and/or surgical manage-
ment [5, 6]. The exact mechanism of HI in patients with 
BWS has remained unclear. In a cohort of children with 
HI and BWS, it was demonstrated that most did not 
have a concomitant K ATP defect, however they did have 
pancreatic lesions significantly larger than those seen in 
cases of focal HI [5]. For the small proportion of BWS 
with a concomitant paternally transmitted K ATP mu-
tation, their HI were remarkably severe and prolonged [5]. 
Somatic features of BWS may not be readily apparent in 
these patients compared to classical BWS [5].

Herein, we report a case of a large-for-gestational-age 
infant with medically refractory HI due to a paternally 
transmitted K ATP mutation, who was subsequently diag-
osed with mosaic BWS related to mosaic segmental 
pUPD (paternal uniparental disomy) 11 based on mo-
olecular testing of the pancreatic lesion.

Case presentation
A female infant was born at 37 weeks of gestation to a non-
sconsanguineous Chinese couple, with a birth weight of 4.3 
kg (>2SD). Antenatal history was unremarkable with no 
gestational diabetes, polyhydramnios nor placentalmegaly. 
She presented with a hypoglycaemic seizure in the first 
hour of life and required a high glucose infusion rate (GIR) 
of 20 mg/kg/min to maintain normoglycaemia. Physical 
examination showed macrosomia but no other dysmorphic 
features (Fig. 1a). Critical samples taken when blood glu-
cose was 2.8 mmol/L on day 2 of life were compatible with 
hyperinsulinaemic hypoglycaemia (insulin = 33.9mIU/L, 
blood beta-hydroxybutyrate < 0.5 mmol/L). She was started 
on the highest dose of diazoxide (15 mg/kg/day) with hy-
drochlorothiazide with no response. Octreotide (15mcg/kg/
day) was therefore added on with partial response, and she 
still required a GIR of 11 mg/kg/min.

18F-Dopa PET scan at 3 months of age showed accen-
tuated 18F-dopa uptake in the pancreatic body com-
pared with a lesser degree of diffuse activity in 
pancreatic head and tail, suggestive of a focal lesion (Fig. 
1b). There was no organomegaly or asymmetric kidneys. 
The first partial pancreatectomy was performed at 5 
months of age. A distal lesion was identified by gross in-
spection intraoperatively, and a distal resection (~ 5% 
pancreatectomy) was performed. Post-operatively, a high 
GIR requirement at 11 mg/kg/min was still required. 
Histology from resected tissue revealed no evidence of 
pancreatic adenomatosis. Therefore, a second operation 
was carried out with real-time frozen section evaluation, 
resulting in an 80% pancreatectomy. Resected pancreatic 
tissue revealed multiple discrete areas of adenomatosis 
interspersed between areas of normal exocrine acini. 
There were areas of coalescing nests and trabeculae (Fig. 
1c) that were negative for p57 staining, suggestive of ad-
enomatous hyperplasia; whilst some areas contained is-
lets with isolated enlarged, hyperchromatic nuclei and 
exocrine acini at the periphery (Fig. 1d). These enlarged 
nuclei were positive for p57 staining. Post-operatively, 
the GIR could be further lowered to 3 mg/kg/min but 
she was unable to be completely weaned off her dextrose 
infusion. She was subsequently restarted on diazoxide 
with no response, and hence changed to octreotide. She 
finally managed to be weaned off from intravenous dext-
rose with reasonable fasting tolerance of 9 h at the age 
of 7 months.

Molecular analysis
Peripheral blood of proband and both parents, and the 
resected pancreatic tissue (at two different areas: Pan-
creas 1A and Pancreas 2A) from the proband were col-
lected for genomic DNA extraction and further 
molecular genetic analysis.

A heterozygous pathogenic ABCC8 NM_000352.4: 
c.1792C > T p.(Arg598Ter) was found in the DNA ex-
tracted from peripheral blood of proband through NGS 
gen panel analysis. This nonsense loss-of-function 
c.1792T variant was previously reported [7]. Sanger 
DNA sequencing confirmed the NGS finding and con-
firmed the variant was inherited from the father. The 
proportion of the c.1792C and c.1792T variant present 
in Pancreas 1A and 2A were of approximately 10%:90 
and 30%:70%, respectively, estimated by peak height for 
the c.1792C and c.1792T in the sequence chromatogram 
(Fig. 2a). Absolute quantitation by digital PCR analysis 
showed that the proportion of c.1792T was approxi-
mately 88.9 and 70.6% in Pancreas 1A and 2A, respect-
ively (Fig. 2b), which was concomitant to the level of 
mosaicism of pUPD11p region carrying the c.1792T 
variant inherited from the father.

DNA methylation analysis for chromosome 11p15 
showed normal methylation pattern at both IC1 (H19-
IGF2 imprinting centre) and ICR2 (KCNQ1OT1/KCNQ1 
imprinting centre) in the peripheral blood leucocytes of 
the proband. However, gain of methylation at IC1 and 
loss of methylation at IC2 were detected in the DNA ex-
tracted from resected pancreas, consistent with a diag-
nosis of Beckwith-Wiedemann Syndrome due to pUPD.
SNP-based chromosomal microarray (CMA) analysis showed copy number neutrality in DNA extracted from the peripheral blood of proband and parents. However, the pancreatic tissue showed a 17.44 Mb region of copy number neutral loss of heterozygosity (LOH) in 11p15.5-p15.1, suggesting segmental UPD 11, with a higher level of mosaicism for Pancreas 1A (~90%) compared to Pancreas 2A (~70%) (Fig. 2c) for the same region. Trio genotyping analysis on Pancreas 1A and parents using UPDtool [8] based on the SNP genotype results from CytoScan 750 k SNP array further revealed that the 17.44 Mb of LOH in Pancreas 1A was paternally inherited and the rest of the chromosome 11 was biparental, confirming mosaic segmental pUPD 11p15.5-p15.1. CMA was suggested to be a sensitive tool to investigate low level of mosaic segmental UPD [4],

Fig. 1 a. Picture of the proband with no somatic features suggestive of BWS. 1b. 18F-Dopa PET scan showed accentuated 18F-dopa uptake in the pancreatic body, with a lesser degree of diffused activity in pancreatic head and tail, suggestive of a focal lesion. 1c-e. Histology of resected pancreatic tissue. c. The pancreas shows preserved acinar architecture with prominent islets of Langerhans (arrowhead), consist of coalescing nests and trabeulae of endocrine cells. d. High power field showing some islets containing isolated, enlarged, hyperchromatic nuclei, which is over 2 times the size of the nuclei in the adjacent islet cells. e. Immunohistochemical stains confirmed the nests and trabeulae of endocrine cells are positive for neuroendocrine marker chromogranin and many of them express insulin by immunohistochemistry.
however that sensitivity varies between array types in the case of the Affymatrix CytoScan 750 k SNP array, mosaicism above 20% can be detected. Therefore, short tandem repeat (STR) markers analysis as a different molecular approach was performed to verify the level of mosaicism. Based on the peak height ratios of the maternal and paternal alleles detected in the DNA extracted from Pancreas 1A and 2A (Table 1), paternal allele from D11S1363 to D11S1923 in the 11p15.5-p15.4 region accounted for 83–90% and 68–76% respectively.
results were consistent with the mosaic level of pUPD in 11p15.5-q15.4 shown in CMA. The rest of the chromosome 11 was biparental in both pancreatic sites.

Discussion
We described an infant with severe HI resulting from a paternally-inherited ABCC8 mutation in conjunction with mosaic segmental pUPD11p15 demonstrated in the pancreatic tissue from the second resection but not in peripheral blood leucocytes, suggestive of BWS/BWS-spectrum HI. With pUPD11p15, the loss of maternal allele resulted in a loss of $H19$ and $CDKN1C$ expression, which usually negatively regulates cell proliferation; whereas the biallelic $IGF-II$ expression promotes cell growth [9]. Therefore, pancreatic adenosomatous hyperplasia and hyperinsulinism were attributed to the combination of the $K_{ATP}$ defect along with the pUPD11 and the imbalance of imprinted genes at 11p15 region. In contrast to the classical histological findings in focal HI related to a paternally-inherited ABCC8 mutation with lesion confines to a small localized area, our patient had multiple foci of adenosomatous hyperplasia throughout the pancreas. Furthermore, the level of mosaicism of UPD cells in the pancreas correlated with the shifted allele frequency of the ABCC8 mutation. To our understanding, this is the first report using the accurate and sensitive assays to demonstrate the direct correlation of the paternally inherited ABCC8 c.1792T level with mosaic level of pUPD.

Other than being macrosomic, our patient had no other somatic features of BWS. The consideration of testing for BWS was triggered by the atypical histological findings. This distinct pancreatic histology had been described in children with Beckwith-Wiedemann Spectrum [5, 10, 11]. In a large series of 28 patients with BWS and persistent HI, their phenotypes were reported to range from isolated, subtle hemihypertrophy or umbilical hernia to frank BWS phenotype with multiple somatic features [6]. Only four of them had concomitant $K_{ATP}$ mutations. Therefore, it was suggested that, even in the absence of somatic features of BWS, testing should be considered in HI cases with large ‘focal’ pancreatic lesions with or without a $K_{ATP}$ mutation [5]. The diagnosis of BWS is important due to their inherent vulnerability to embryonal tumours, affecting up to 8% of BWS patients [4, 12]. Calton et al. reported a similar case of large/multifocal focal HI resulting from a paternally inherited recessive ABCC8 mutation [11]. That patient, like our patient, had no clinical features of BWS. BWS testing was only performed at the age of 20 months when he developed hepatoblastoma. Again, similar to our patient, pUPD11p was identified in the affected tissue (hepatoblastoma tissue and the stored pancreatic tissue), but not in peripheral blood or buccal DNA [11]. This highlights that infants with HI related to mosaic BWS could also develop BWS-associated tumours due to mosaic UPD, and that tumour surveillance is indicated. It has been suggested that the tumour risk could be associated with the level of mosaicism for UPD within specific organs [13]. Since tissues from other organs were not available for testing in our patient, it is unclear whether other organs are affected by pUPD11p. Therefore, tumour surveillance during early childhood is warranted.

Table 1 Analysis of short tandem repeat (STR) marker inheritance and their allelic ratio in pancreas DNA from two different loci (Pancreas 1A and 2A) from the proband

| STR     | Cytoband | Maternal | Paternal | Pancreas 1A | Pancreas 2A | Pancreas 1A mat:pat allelic ratio | Pancreas 2A mat:pat allelic ratio | Interpretation   |
|---------|----------|----------|----------|-------------|-------------|---------------------------------|---------------------------------|------------------|
| D11S1363| 11p15.5  | a, b     | a        | a           | a           | 0.1:0.9                         | 0.24:0.76                        | mos paternal UPD |
| D11S1984| 11p15.5  | a        | a, b     | a           | a           | 0.110.89                        | 0.25:0.75                        | mos paternal UPD |
| CHR11-TH| 11p15.5  | a, b     | c, d     | c           | c           | 0.170.83                        | 0.32:0.68                        | mos paternal UPD |
| D11S1923| 11p15.4  | a, b     | c, d     | b, c        | b, c        | 0.120.88                        | 0.26:0.74                        | mos paternal UPD |
| D11S1338| 11p15.4  | a, b     | a        | a           | a           | nil                             | nil                             | uninformative    |
| D11S904 | 11p14.2  | a, b     | c, d     | a           | a           | 0.11:1                          | 1:1                             | biparental       |
| D11S2632| 11q12    | a, b     | c        | b, c        | b, c        | 0.11:1                          | 1:1                             | biparental       |
| D11S956 | 11q12.1  | a, b     | c, d     | b, c        | b, c        | 0.11:1                          | 1:1                             | biparental       |
| D11S898 | 11q22.1  | a, b     | a, b     | a           | a           | 0.1:1                           | 1:1                             | uninformative    |
| D11S1299| 11q23.3  | a, b     | c        | b, c        | b, c        | 0.11:1                          | 1:1                             | biparental       |
| D11S488 | 11q24.1  | a, b     | c, d     | a           | a           | 0.11:1                          | 1:1                             | biparental       |

mat:pat allelic ratio: maternal to paternal allelic ratio
nil: unable to provide allelic ratio as monoallelic pattern in observed in Pancreas 1A and 2A DNA
biparental: Pancreas 1A and 2A DNA show inheritance of one allele from each parent
mos paternal UPD: the inheritance of alleles in the pancreas 1A and 2A DNA is not unambiguously biparental (due to presence of low level of maternal allele), but is consistent with mosaic UPD of paternal origin
uninformative: unable to delineate inheritance by the STR marker pattern
With the variability of mosaicism between tissues in patients with BWS, the source of DNA for molecular analysis is extremely important. In our patient, absence of mosaicism in the peripheral blood leukocytes would have wrongly concluded as ‘normal study’ if the pancreatic tissues were not sent for further analysis. Therefore, similar to other mosaic conditions, affected tissue should always be sent for further molecular analysis if possible [4].

Conclusions
This case highlights the importance of integrating the clinical presentation and subsequent clinical course, together with radiological, genetic and histological findings in the definitive diagnosis of this rare yet clinically important entity. In managing HI caused by both pUPD11p and K<sub>ATP</sub> mutation, the HI course could be severe, and hypoglycaemia might persist despite extensive pancreatotomy, trial of resuming medical treatment should be considered, allowing better glycaemic control.

Abbreviations
BWS: Beckwith–Wiedemann syndrome; CMA: Chromosomal microarray; GIR: Glucose infusion rate; HI: Hyperinsulinism; LOH: Loss of heterozygosity; UPD: Uniparental disomy

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Authors’ contributions
JYL was the major writer of this manuscript and is the paediatric endocrinologist taking care of the case. KYC was a major contributor in writing the molecular findings of this case report. DDO, JMK and BHC had been involved in revising this case report critically for important intellectual content. All authors read and approved the final manuscript. KYC, KSV and AG analysed and interpreted the molecular analysis. FL performed the histological examination of the pancreas. All authors read and approved the final manuscript.

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Not applicable.

Consent for publication
Written informed consent to write and publish this case report was obtained from the family.

Competing interests
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this case report.

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