Coexistence of Mosaic Uniparental Isodisomy and a KCNJ11 Mutation Presenting as Diffuse Congenital Hyperinsulinism and Hemihypertrophy

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Established Facts

- Rarely, hyperinsulinaemic hypoglycaemia can present as part of Beckwith-Wiedemann-syndrome (BWS), a severe overgrowth disorder characterized by macroglossia, abdominal wall defects, hemihypertrophy, macrosomia, hypoglycaemia and an increased risk of tumors.
- Focal hyperinsulinism where uniparental disomy (UPD) of chromosomes 11p15.5–11p15.1 occurs in a single pancreatic cell. This unmasks a paternally inherited K-ATP channel mutation and provides the cell with a selective growth advantage due to the inappropriate expression of imprinted genes at 11p15.5, leading to clonal expansion.

Novel Insights

- BWS should be considered in patients with newly diagnosed hyperinsulinaemic hyperglycaemia even in the absence of additional clinical features.
- UPD should also be considered in all patients with persistent hyperinsulinism with a paternally inherited, recessively acting K-ATP channel mutation even in the absence of focal pancreatic disease. A mitotic recombination event, which led to the UPD of chromosome 11, may have occurred earlier and most likely prior to the differentiation of the pancreatic precursor cells.

Key Words

Congenital hyperinsulinism · Uniparental isodisomy · KCNJ11 · Beckwith-Wiedemann syndrome · Hypoglycaemia

Abstract

Background: Isolated hyperinsulinaemic hypoglycaemia (HH) commonly results from recessively inherited mutations in the ABCC8 and KCNJ11 genes that are located on chromosome 11p15.1. More rarely, HH can feature in patients with Beckwith-Wiedemann syndrome (BWS), a congenital overgrowth disorder, resulting from defects at a differentially methylated region telomeric to the K-ATP channel genes at chromosome 11p15.5. Subject: We undertook genetic testing in a patient with diazoxide-unresponsive HH diagnosed at birth. Physical examination later revealed hemihypertrophy of the right arm, a feature of BWS. Results: We identified a novel mosaic, paternally-inherited KCNJ11 mutation(s) in the patient. Further analysis confirmed uniparental disomy (UPD) of chromosome 11, which extended across the KCNJ11
gene at 11p15.1 and the BWS locus at 11p15.5. **Conclusion:** These results highlight the importance of considering UPD as a mechanism of disease in patients with HH and a paternally inherited K-ATP channel mutation, especially when additional syndromic features are present.

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**Introduction**

Hyperinsulinaemic hypoglycaemia (HH) is characterized by the unregulated secretion of insulin despite low blood glucose and is the major cause of persistent hypoglycaemia in the newborn and infancy periods [1]. A rapid diagnosis and appropriate treatment is essential in order to prevent brain injury. A range in the clinical severity of disease is observed, with some patients having asymptomatic hypoglycaemia, whilst others present with severe, medically unresponsive disease that requires pancreatectomy.

Heterogeneity is also observed with regard to the under-lying genetic aetiology with mutations in 9 different genes reported [2]. Of these, the commonest genetic etiology is loss-of-function mutation(s) in the ABCC8 and KCNJ11 genes, which encode the sulphonylurea receptor 1 and inward-rectifying potassium (Kir6.2) subunits of the pancreatic ATP-sensitive potassium (K-ATP) channel. Mutations in these two genes account for 45% of all HH cases and are important to identify as the mode of inheritance of mutations provides information that can guide clinical management. Histologically, two forms of the disease exist; diffuse and focal. Focal lesions develop when paternal uniparental disomy (UPD) of chromosome 11p15 occurs in a single cell within the developing pancreas. This results in an imbalance of imprinted genes involved in cell cycle regulation at 11p15.5, leading to an imbalance of imprinted and dysregulation of genes that are important for cell cycle regulation [4]. As UPD is a sporadic event, which may occur during embryogenesis, there is often variability in the tissues that are affected. This can explain the differences in phenotype observed between individuals with BWS.

Recently, a few patients with BWS and persistent HH due to paternal UPD of chromosome 11, which extends from the BWS locus at 11p15.5 to the K-ATP channel genes at 11p15.1, have been reported. In these patients, the persistent hyperinsulinism results from the unmasking of a recessively inherited mutation in ABCC8 or KCNJ11 within the pancreatic tissue [5, 6]. We now report a patient with congenital hyperinsulinism with hemihypertrophy resulting from novel paternally inherited, recessively acting KCNJ11 mutation(s), which have been unmasked by paternal uniparental isodisomy that extends from 11p15.5 to 11p15.1 within the pancreatic tissue.

**Methods**

**Genetics**

Genomic DNA was extracted from peripheral leukocytes and formalin-fixed, paraffin-embedded pancreatic tissue, using standard procedures. The coding regions and intron/exon boundaries of the KCNJ11 and ABCC8 genes were amplified by PCR using DNA extracted from leukocytes. The PCR products were Sanger sequenced, and the traces were compared to published sequences using Mutation Surveyor Software.

Seven microsatellite markers spanning chromosomes 11p15.5–11p15.1 were amplified in DNA extracted from the formalin-fixed, paraffin-embedded pancreatic tissue and leukocyte DNA from the patient and both parents. The resulting data were analysed using Genemarker software (Soft Genetics, State College, Pa., USA).

**Results**

**Clinical Characteristics**

The female patient was macrosomic at birth (3.8 kg at 35 weeks’ gestation) and developed refractory hypoglycaemia immediately after birth. Laboratory investiga-
Isodisomy and a Coexistence of Mosaic Uniparental Isodisomy within the pancreas. The father who is heterozygous for both mutations is clinically unaffected, consistent with the mutation(s) being recessively acting. In our patient, it is likely that the persistent hyperinsulinism was due to a loss of functional K-ATP channels within the pancreatic beta cell as a result of the KCNJ11 mutation(s) [2]. This is further supported by the patient’s lack of response to the drug diazoxide, which works by binding to and opening functional K-ATP channels, thereby preventing insulin secretion. However, functional studies will be required to confirm whether both or just one of the novel mutations is pathogenic.

Whilst the majority of cases with paternally inherited K-ATP channel mutations have a focal pancreatic lesion, our patient had diffuse pancreatic disease. The identification of mosaic UPD within the leukocyte DNA prompted us to consider the possibility of diffuse disease, which was later confirmed by histological analysis. This finding highlights the importance of performing imaging studies in all individuals with paternally inherited K-ATP channel mutations and medically unresponsive disease prior to surgery. The level of mosaicism observed within the pancreatic and leukocyte DNA in our patient was high (90%). This suggests that the mitotic recombination event, which led to UPD of chromosome 11, occurred early during development in our patient and most likely prior to the differentiation of the pancreatic precursor cells, thus resulting in all, or at least the majority of the cells, within the pancreas being affected. This is in contrast to focal hyperinsulinism, where UPD of chromosomes 11p15.5–11p15.1 is not detected in the blood as the

Discussion

We report a patient with congenital HH and hemihypertrophy with two novel, paternally inherited KCNJ11 mutations, which have been unmasked by mosaic paternal uniparental isodisomy within the pancreas. The father who is heterozygous for both mutations is clinically unaffected, consistent with the mutation(s) being recessively acting. In our patient, it is likely that the persistent hyperinsulinism was due to a loss of functional K-ATP channels within the pancreatic beta cell as a result of the KCNJ11 mutation(s) [2]. This is further supported by the patient’s lack of response to the drug diazoxide, which works by binding to and opening functional K-ATP channels, thereby preventing insulin secretion. However, functional studies will be required to confirm whether both or just one of the novel mutations is pathogenic.

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Fig. 1. a Sequence electropherograms showing the p.R221H (c.662G>A; left panel) and p.Q299H (c.879G>C; right panel) KCNJ11 mutation(s). The proband is mosaic for both mutations, whilst the father is heterozygous. Neither mutation was found in the sample from the mother. b Electropherograms demonstrating the results of microsatellite analysis of the informative marker (D11S1984) in the proband (pancreatic tissue) and her parents (leukocytes). Data for the additional 6 informative markers are not shown. The x-axis indicates the product size [base pairs (bp)], and the y-axis the product quantity (arbitrary units). The results illustrate mosaic UPD with a larger peak for the paternal allele (180 bp) compared with the maternal allele (184 bp). c Results of chromosome 11 microsatellite analysis for DNA extracted from the proband (pancreatic tissue) and her parents (leukocytes). The 7 informative markers, which demonstrated paternal UPD, are shown along with their approximate location on chromosome 11. The position of KCNJ11 and the differentially methylated BWS locus are provided.
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