Mosaic paternal uniparental isodisomy and an ABCC8 gene mutation in a patient with permanent neonatal diabetes and hemihypertrophy

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ABSTRACT

Objective: Activating mutations in the KCNJ11 and ABCC8 genes encoding the Kir6.2 and SUR1 subunits of the pancreatic K\textsubscript{ATP} channel are the most common cause of permanent neonatal diabetes. In contrast to KCNJ11 where only dominant heterozygous mutations have been identified, recessively acting ABCC8 mutations have recently been found in some patients with neonatal diabetes. These genes are co-located on chromosome 11p15.1, centromeric to the imprinted Beckwith-Wiedemann Syndrome (BWS) locus at 11p15.5. We investigated a male with hemi-hypertrophy, a condition classically associated with neonatal hyperinsulinaemia and hypoglycaemia, who developed neonatal diabetes aged 5 weeks.

Research Design and Methods: The KCNJ11 and ABCC8 genes and microsatellite markers on chromosome 11 were analysed in DNA samples from the patient and his parents.

Results: A paternally inherited activating mutation (N72S) in the ABCC8 gene was identified in the proband. The mutation was present at 70% in the patient's leukocytes and 50% in buccal cells. Microsatellite analysis demonstrated mosaic segmental paternal uniparental isodisomy (UPD) of 11pter-11p14 in the proband which encompassed the ABCC8 gene and the BWS locus.

Conclusion: We report a patient with neonatal diabetes, hemihypertrophy and relatively high birth weight resulting from telomeric segmental paternal UPD of chromosome 11 which unmasks a recessively acting gain-of-function mutation in the ABCC8 gene and causes deregulation of imprinted genes at the BWS locus on 11p15.5.
Neonatal diabetes is a rare condition of hyperglycaemia usually presenting during infancy or soon thereafter (1). Aetiologies other than autoimmunity are far more prevalent if diabetes is diagnosed before the age of six months (2). Recent developments have highlighted the importance of ion channel mutations (“Channelopathies”) in the aetiology of both permanent and transient forms of this condition (3-5). The pancreatic \(\beta\)-cell ATP-sensitive potassium (K\(_{\text{ATP}}\)) channel is composed of four inward rectifying potassium channel (Kir6.2) subunits, encoded by the gene \(KCNJ11\), and four sulfonylurea receptor (SUR1) subunits encoded by \(ABCC8\). Its role is to control insulin release in response to glucose metabolism within the \(\beta\)-cell, through alterations in adenine nucleotide availability and binding (6). Inactivating mutations in either \(KCNJ11\) or \(ABCC8\) cause persistent hyperinsulinaemic hypoglycaemia of infancy (PHHI) (7; 8). Recently, Gloyn and colleagues described activating mutations in \(KCNJ11\) as the cause of many cases of permanent (9) and a few cases of transient (10) neonatal diabetes: this has been confirmed in a large follow-up series (5; 11). The mutant K\(_{\text{ATP}}\) channels display reduced sensitivity to ATP-induced closure, thus preventing both membrane depolarisation and insulin release in response to glucose. Clinically, it has been demonstrated that many, but not all, children affected by mutations in \(KCNJ11\) can replace insulin therapy with high dose oral sulfonylureas, usually glyburide (known in the UK as glibenclamide) (12).

Activating mutations in the \(ABCC8\) gene have also been linked to neonatal diabetes (3; 4; 13; 14) (5) (15). Mutations may result in either a transient or permanent form of the disease. Like the \(KCNJ11\) mutations, they cause the K\(_{\text{ATP}}\) channel to remain open even when ATP is elevated by glucose metabolism. Clinically, neonatal diabetes due to \(ABCC8\) mutations can also be abrogated through the therapeutic use of sulfonylureas (3; 4).

Hemihypertrophy describes asymmetric overgrowth of part or half of the body. It may be isolated or part of the Beckwith-Wiedemann syndrome (BWS), a condition characterised by macroglossia, abdominal wall defects, neonatal hyperinsulinaemia and increased risk of embryonal tumours (16). Both isolated hemihypertrophy and BWS can be caused by mosaic, paternal uniparental isodisomy of chromosome 11p15 (17; 18).

**SUBJECT AND METHODS**

A male child weighing 3.87Kg at 40 weeks gestation (75\(^{\text{th}}\) percentile) was born to non-consanguineous, Somali parents. There had been two previous uneventful pregnancies. The proband was discharged home on the day of birth but returned to hospital aged 36 days, failing to thrive, severely dehydrated and acidic (pH 6.99 Normal Range 7.3-7.4). The initial blood glucose measured was 50 mmol/l with a bicarbonate of 5 mmol/l (NR 21-34mmol/l) and urinary ketones of > 160 mg/dl. A diagnosis of neonatal diabetes was made, and subsequent to resuscitation the child was stabilised on twice daily subcutaneous insulin. Both parents had normal fasting glucose levels.
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(father 5.2mmol/l aged 36 years; mother 4.6mmol/l at 31 years) and no prior history of diabetes.

The child had left hemihypertrophy (Figure 1) but no macroglossia, anterior abdominal wall defects, ear creases or peri-auricular pits as seen in BWS. Echocardiography of the heart revealed branch pulmonary artery stenosis but no cardiomegaly whilst serial abdominal ultrasounds identified increasing left renal hypertrophy compared to a normal right kidney.

**Investigations.** The young age of the patient at presentation suggested a diagnosis of neonatal diabetes. Blood was collected for DNA analysis of chromosome 6 anomalies causing transient neonatal diabetes (19) and for KCNJ11 mutation analysis (which at this time was the only “channelopathy” described as causing permanent neonatal diabetes). These tests were both normal. Glutamic Acid Decarboxylase (GAD), insulin and islet cell auto-antibodies were all negative. The child’s karyotype was normal: 46 XY. However, microsatellite analysis of chromosome 11 revealed the child to be mosaic for paternal uniparental isodisomy of 11pter-11p14 (Table 1, Figure 2a). As neonatal diabetes is not characteristic of either BWS or isolated hemihypertrophy, this prompted a review of potential candidate genes for diabetes that were encompassed within this region of UPD. The SUR1 gene (ABCC8), which lies within this region, was a logical candidate, as it coassembles with Kir6.2 (KCNJ11) to form the K\textsubscript{ATP} channel, and activating mutations in KCNJ11 are known to cause neonatal diabetes (9-11).

The ABCC8 gene was sequenced as described previously (14). The father was identified as being heterozygous for a missense mutation, N72S (c.215A>G) whilst the proband’s leukocyte DNA showed mosaicism for the N72S mutation (Figure 2b). The N72S mutation was not found in 250 normal chromosomes and the affected residue was conserved across species. Quantification by real-time PCR (TaqMan assay) demonstrated that the N72S mutation was present at approximately 70% in leukocyte DNA and 50% in buccal cells. Briefly, genomic DNA from leukocytes and buccal cells was amplified using a mutation-specific TaqMan approach. Genomic DNA from the father who is heterozygous for the N72S mutation was used in serial dilution to produce standard curves to determine linear range and accuracy of quantification (primer and probe sequences are available on request). Reactions contained 5µl TaqMan Fast universal PCR mastermix without Amperase, 0.5µl Assays-by-Design (Applied Biosystems, Warrington, UK) probe and primer mix (corresponding to 36µl of each primer and 8µl of each probe), 2.5µl water and 2µl of DNA at a concentration of 100ng/µl. Amplification conditions consisted of a single cycle of 95°C for 20 seconds followed by 40 cycles of 95°C for 1 second and 60°C for 20 seconds. The relative level of mutant transcript relative to wild-type transcript was determined by the \( \Delta \Delta CT \) method (20). Each test was carried out in triplicate.

Functional studies in *Xenopus* oocytes (for methods see refs (9; 10)) demonstrated that the N72S mutation results in a reduced
sensitivity to inhibition by MgATP. Half-maximal block of homozygous N72S channels was produced by 23±1 µM ATP (n=10) compared to 15±1 µM (n=6) for wild-type channels. Two-electrode voltage-clamp studies showed a concomitant small increase in the whole-cell \( K_{\text{ATP}} \) resting current (14) (Figure 3). Homozygous N72S channels were substantially blocked by the sulphonylurea tolbutamide \textit{in vitro} (Figure 3).

Identification of an \textit{ABCC8} mutation, together with the patient’s relatively poor control on twice-daily insulin (Glycated Haemoglobin 8.3% NR 4.0-6.0%), the known efficacy of sulfonylureas in neonatal diabetes caused by \( K_{\text{ATP}} \) channel mutations (21), and the tolbutamide block of mutant N72S channels observed in functional studies, prompted a trial of the sulfonylurea glyburide. Over a two-week period, in hospital under intensive monitoring, regular subcutaneous insulin was withdrawn and glyburide was gradually increased from a starting dose of 0.1mg/kg/day to a maximum dose of 1mg/kg/day.

The therapeutic trial was terminated at 2 weeks due to the complete absence of a clinical response, with high blood sugars (up to 40 mmol/l) requiring the repeated administration of short-acting insulin to improve glycaemia and prevent metabolic decompensation. The C-peptide level before initiating glyburide was <94 pmol/l and remained at <94 pmol/l on 1 mg/kg/day glyburide. The child is now over 18 months of age and is treated with a basal bolus insulin regimen of four injections daily (≈ 1unit/kg/day) to maintain growth and health (recent HbA1c 6.6%). Although his neurological development at this stage seems normal, he remains under regular surveillance both for neurocognitive development and for his increased tumour risk.

**DISCUSSION**

Uniparental isodisomy is a rare abnormality that provides insights into the genetic basis of human disease. Its identification in two cases of transient neonatal diabetes (22) led to identification of imprinting anomalies associated with the transient form of this condition. UPD can also unmask genetic disorders with an autosomal recessive pattern of inheritance (23). In this case, the child’s hemihypertrophy and relatively high birth weight (75\textsuperscript{th} centile) did not fit with a diagnosis of permanent neonatal diabetes as, if anything, these features would be expected to co-segregate with hypoglycaemia secondary to hyperinsulinaemia (as seen in BWS). Since UPD can unmask recessive disorders, SUR1 (\textit{ABCC8}) was an obvious candidate, due to its position within the disomic region of interest, its importance in insulin secretion and the fact that focal adenomatous hyperplasia of islet cells with congenital hyperinsulinaemia, has been described in which loss of maternal 11p15 is associated with homozygosity of an \textit{ABCC8} mutation inherited from a heterozygous father (24).

Recent studies have identified activating mutations in \textit{ABCC8} as causing both transient and permanent neonatal diabetes (3-5). Several cases of permanent neonatal diabetes responded to glyburide at doses ranging from 0.22mg/kg/day to 0.59mg/kg/day (3), including one child with a dominant \textit{ABCC8} mutation (15) and at least
four children with recessively inherited ABCC8 mutations (Hattersley, Flanagan & Ellard, unpublished observations). Unfortunately, the child reported here was unresponsive to glyburide at a dose of as much as 1mg/kg/day. The complete absence of any clinical response in our infant prompted our withdrawal of glyburide after a two-week trial although a further trial when the child is older may be warranted given the in vitro response. The lack of a therapeutic response to sulphonyureas in our patient is puzzling, as the mutant N72S channels were blocked by tolbutamide in “in vitro” studies. It is possible that other imprinted genes involved in the paternal UPD might confer a reduced response.

In conclusion, we report a patient with UPD of chromosome 11 who presented with neonatal diabetes as opposed to the classical phenotype of hypoglycaemia due to hyperinsulinaemia. Paternal UPD of chromosome 11pter-11p14, encompassing the KATP channel genes and the Beckwith Wiedemann syndrome locus, unmasked a recessively acting gain-of-function ABCC8 gene mutation thus causing diabetes in addition to features of Beckwith Wiedemann syndrome. As has previously been described, the heterozygous father has no history of glucose intolerance nor evidence of diabetes in adult life as this is a recessively acting mutation (14). The level of mosaicism for the N72S mutation varied between leukocytes (~70% mutation) and buccal cells (~50% mutation). As the child is alive and well, the mutation load within the pancreas remains unknown. Given the evidence that children with UPD11 can have highly variable levels of mosaicism in different organs, we hypothesise that the pancreas must exhibit a high level of paternal UPD cells to present with this phenotype, the somatic recombination causing the paternal UPD occurring very early during embryogenesis (25).

We have recently identified nine other patients with recessively inherited ABCC8 mutations causing neonatal diabetes (14).

ACKNOWLEDGEMENTS
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Table 1 Microsatellite analysis of proband revealing mosaic segmental paternal uniparental disomy of chromosome 11.

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Microsatellite analysis was performed according to standard methods with the primer sets listed in the Table. After 28 cycles of PCR, amplimers were resolved on an ABI 3100 platform, and maternal and paternal allele levels were compared in DNA from blood and buccal samples of the proband. The underlined figures indicate alleles present at unexpectedly high dosage. Microsatellites D11STH and D11S1318 lie within the BWS region, while D11S921 and D11S902 bracket KCNJ11 and ABCC8.
FIGURE LEGENDS

Figure 1  Photograph of proband showing hemi hypertrophy of left leg.

Figure 2  Results of microsatellite analysis and ABCC8 sequencing
(a) Analysis of microsatellite D11S1318 (2.29Mb from pter, ie within the BWS locus). X-axis indicates product size (base pairs); Y-axis indicates product quantity (arbitrary units). Note the smaller peak for the maternal allele in the proband’s blood sample compared to the buccal sample. (b) Pedigree showing mutation status and sequencing electropherograms. The solid symbol indicates the affected proband and the father is shown as an unaffected carrier of the mutation (dot within an unfilled symbol). The mutated base is indicated by an arrow on the electropherograms.

Figure 3: Resting whole-cell $K_{ATP}$ currents are slightly larger for homomeric N72S mutant channels and are blocked by sulphonylureas. Mean steady-state whole-cell $K_{ATP}$ currents for wild-type (WT), homomeric (hom) N72S and heterozygous (het) N72S channels, as indicated evoked by a voltage step from −10 to −30 mV before (white bars, resting condition) and after application of the metabolic inhibitor 3mM azide (grey bars) and in the presence of 3mM azide plus 0.5 mM tolbutamide (black bars). The number of oocytes is given below the bars.
Figure 1
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Figure 2

a) 

![Genetic analysis results for different samples including proband blood, proband mouthbrush, mother, and father showing specific genetic patterns.]

b) 

![Pedigree chart illustrating family relationships with the identification of N72S mosaic and normal (N72S/N) and normal (N/M) genotypes. A specific genetic pattern is highlighted in the N72S mosaic sample.]
Figure 3