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SHORT COMMUNICATION

SLC2A2 mutations can cause neonatal diabetes, suggesting GLUT2 may have a role in human insulin secretion

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Abstract
Aims The gene *SLC2A2* encodes GLUT2, which is found predominantly in pancreas, liver, kidney and intestine. In mice, GLUT2 is the major glucose transporter into pancreatic beta cells, and biallelic *Slc2a2* inactivation causes lethal neonatal diabetes. The role of GLUT2 in human beta cells is controversial, and biallelic *SLC2A2* mutations cause Fanconi–Bickel syndrome (FBS), with diabetes rarely reported. We investigated the potential role of GLUT2 in the neonatal period by testing whether *SLC2A2* mutations can present with neonatal diabetes before the clinical features of FBS appear.
Methods We studied *SLC2A2* in patients with transient neonatal diabetes mellitus (TNDM; $n=25$) or permanent neonatal diabetes mellitus (PNDM; $n=79$) in whom we had excluded the common genetic causes of neonatal diabetes, using a combined approach of sequencing and homozygosity mapping.
Results Of 104 patients, five (5%) were found to have homozygous *SLC2A2* mutations, including four novel mutations (S203R, M376R, c.963+1G>A, F114LfsX16). Four out of five patients with *SLC2A2* mutations presented with isolated diabetes and later developed features of FBS. Four out of five patients had TNDM (16% of our TNDM cohort of unknown aetiology). One patient with PNDM remains on insulin at 28 months.
Conclusions *SLC2A2* mutations are an autosomal recessive cause of neonatal diabetes that should be considered in consanguineous families or those with TNDM, after excluding common causes, even in the absence of features of FBS. The finding that patients with homozygous *SLC2A2* mutations can have neonatal diabetes supports a role for GLUT2 in the human beta cell.

Keywords Fanconi–Bickel syndrome · FBS · GLUT2 · Neonatal diabetes mellitus · Permanent neonatal diabetes mellitus · PNDM · *SLC2A2* · TNDM · Transient neonatal diabetes mellitus

Abbreviations

FBS	Fanconi–Bickel syndrome
PNDM	Permanent neonatal diabetes mellitus
SNP	Single-nucleotide polymorphism
TNDM	Transient neonatal diabetes mellitus

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Introduction

The gene *SLC2A2* encodes GLUT2, a facilitative glucose transporter. The pancreas, liver, kidney and intestine all express GLUT2 [1]. Biallelic inactivation of *Slca2* in mice leads to severe diabetes with failure to thrive, marked hyperglycaemia with relatively low insulin and high glucagon levels, and death usually at 2–3 weeks of age [2]. The mice can be rescued by exogenous insulin, indicating that the insulin-secretory defect is the major cause of lethality [2]. Glucose-stimulated insulin secretion is normalised when *Slc2a2* expression is restored in *Slc2a2* null murine beta cells [3].

The role of GLUT2 in man is controversial. Expression of GLUT2 in human beta cells is much lower than in mice [4–6]. Humans with biallelic inactivating *SLC2A2* mutations do not normally present with diabetes, but do develop Fanconi–Bickel syndrome (FBS) [7, reviewed in 8]. FBS characteristically involves a renal Fanconi syndrome with glycosuria, galactosuria, aminoaciduria, proteinuria and phosphaturia, short stature, rickets, poor growth, hepatomegaly, and glucose and galactose intolerance. Diagnosis normally occurs in late infancy as clinical features of FBS develop. In some cases galactosaemia screening leads to earlier diagnosis [8].

Monogenic neonatal diabetes mellitus typically presents before 6 months, and may be permanent (PNDM) or transient (TNDM). PNDM is usually due to heterozygous mutations in *KCNJ11*, *ABCC8* or *INS*. TNDM is most commonly due to an imprinting disorder at chromosome 6q24, and also to mutations in *KCNJ11* or *ABCC8* [9]. In around one-third of cases the cause has not been identified.

There is one report of a premature (34 weeks' gestation) infant with neonatal diabetes and hypergalactosaemia who was homozygous for a truncating *SLC2A2* mutation [10]. She received intermittent insulin, but it is not clear whether this persisted until her death from hepatic failure with pneumonia at 10 months.

We tested a large series of patients with neonatal diabetes to determine whether *SLC2A2* mutations cause neonatal diabetes, which presents before clinical features of FBS are apparent.

Methods

This study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the local ethics committee, and written informed consent was obtained from each participant or their parent.

Genetic analysis

We studied *SLC2A2* in patients with TNDM (n=25) or PNDM (n=79) in whom we had excluded where appropriate the common genetic causes of neonatal diabetes [TNDM: *KCNJ11*, *ABCC8*, *INS, PLAGL1* (6q24); PNDM: *KCNJ11*, *ABCC8*, *INS*, *EIF2AK3*]. We sequenced the gene for all patients, who either had TNDM or had PNDM and a possible recessive mutation suggested by a homozygous region >3 cM encompassing the *SLC2A2* gene on genome-wide single-nucleotide polymorphism (SNP) analysis using the Affymetrix (Santa Clara, CA, USA) SNP chip 500 K or v6.0.

We amplified the 11 coding exons of the *SLC2A2* gene by PCR (primers available on request). PCR products were sequenced using standard methods on an ABI 3730 (Applied Biosystems, Warrington, UK) and were compared with the published sequence NM_000340.1 using Mutation Surveyor v3.2 (SoftGenetics, State College, PA, USA). Where an *SLC2A2* mutation was identified, siblings and parents were tested when samples were available.

Results

Molecular genetics One PNDM patient had a homozygous *SLC2A2* mutation c.339del (F114LfsX16) and has been reported previously [11]. Among the 25 TNDM patients, four (16%) had homozygous *SLC2A2* mutations, three of which are novel: c.609T>A (S203R), c.1127T>G (M376R), c.963+1G>A (IVS7+1G>A); and one has been reported previously: c.157C>T (p.R53X) [8]. Further details of the mutations are presented in Table 1. All nine parents tested were carriers of the mutations. Patient 2 has a sister homozygous for the same mutation who was not diagnosed with diabetes.

Clinical features Clinical details of the patients with *SLC2A2* mutations are shown in Table 2. Parental consanguinity was reported in four out of five cases. All four patients with birthweight records had intrauterine growth reduction with birthweights at or below the third centile. Diabetes diagnosis was before 6 weeks. Insulin doses are similar to those in other cases of neonatal diabetes [9] and C-peptide values in two patients were low at diagnosis in the presence of marked hyperglycaemia, suggesting the diabetes resulted from relative insulin deficiency.

At the diagnosis of diabetes, only one patient had features suggesting FBS (rickets at 6 weeks). All patients later developed typical clinical features of FBS, which were recognised up to 30 months after diabetes was diagnosed. The older sister of patient 2, who was homozygous for the same mutation, had FBS features but was not diagnosed with neonatal diabetes.

Discussion

There are now six confirmed cases of neonatal diabetes due to *SLC2A2* mutations: the five in this paper, plus that

Table 1 Details of homozygous *SLC2A2* mutations identified

Mutation characteristic	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Mutation (protein)	p.Ser203Arg	p.Met376Arg	p.?	p.Arg53X	p.Phe114LeufsX16
Mutation (DNA)	c.609T>A	c.1127T>G	c.963+1G>A	c.157C>T	c.339del
Exon	5	9	Intron 7 splice donor site	3	3
Novelty	Not previously reported	Not previously reported	Not previously reported	Previously reported [8]	Previously reported [11]
Comments	Conserved across mammals, fish and reptiles In silico modelling indicates site is in transmembrane region	Conserved across mammals, fish and reptiles In silico modelling indicates site is in transmembrane region	Predicted to cause aberrant splicing	Premature termination at codon 53	Premature termination at codon 129
Family member testing	Both parents carriers	Both parents carriers Sister homozygous for mutation	Both parents carriers	Both parents carriers	Mother carrier No paternal sample available

reported by Yoo et al [10]. This result is important as it implies a role for GLUT2 in glucose regulation in humans.

All five *SLC2A2* mutations are highly likely to be pathogenic as they co-segregated and are either null mutations or missense mutations which affect a highly conserved residue within an important part of the protein (see Table 1). In addition all five patients have developed FBS.

The *SLC2A2*-positive cases reported here represent 16% of our undiagnosed TNDM cohort. Although a rare cause of neonatal diabetes, our results suggest that it is worth testing

Table 2 Clinical features of patients with *SLC2A2* mutations

Patient characteristic	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Country of origin	P.R. China	Oman	Iran	Sudan	Saudi Arabia
Consanguinity	None known	First cousins	First cousins	First cousins	First cousins
Birth details					
Sex	Female	Male	Female	Male	Male
Birthweight (kg)	1.85	2.5	2.0	Not known	2.5
Gestation (weeks)	37	40	40	40	39
Centile	0.7	1.2	0.04	Not known	3.1
Features of diabetes					
Age at NDM diagnosis (days)	1	7	30	42	35
Glucose at NDM diagnosis (mmol/l)	21	22	28	24	13
C-peptide at NDM diagnosis (pmol/l)	660	730	NA	NA	NA
Initial insulin treatment (U/kg per day)	0.6 (+ glibenclamide 0.06 mg/kg per day)	1.0	NA	0.5	0.5
Age at remission	12 months	6 months	18 months	10 months	Not remitted at 28 months
Features of FBS that subsequently developed					
Age first feature noted	3 months	3 months	4 years	6 weeks	10 months
Features of FBS	Hepatomegaly, glycosuria, proteinuria, hypophosphataemia, delayed bone age	Hepatomegaly, glycosuria, proteinuria, rickets, raised ALP	Hepatomegaly, glycogen storage disorder on liver biopsy	Skeletal abnormalities, hypophosphataemia, raised ALP, metabolic acidosis	Hepatomegaly, glycosuria, aminoaciduria, acidosis, hypophosphataemia, hypocalcaemia, raised ALP
Family history	Nil of note	Elder sister with similar features but no known NDM (homozygous for same mutation)	Nil of note	Two deceased older siblings with similar features but no known NDM (not genotyped)	Deceased elder sister with similar features and diabetes at 3 years (not genotyped)

ALP, alkaline phosphatase; NA, not available; NDM, neonatal diabetes mellitus; P.R. China, People's Republic of China

when other common causes have been excluded, particularly when the patient is born to related parents and has possible features of FBS, such as persistent glycosuria after resolution of their diabetes.

It is not known why neonatal diabetes is only diagnosed in a minority of patients with *SLC2A2* mutations (4%; six in approximately 154 reported FBS cases). It is unlikely that these mutations are functionally different from those found in patients who develop FBS without neonatal diabetes, as the mutation found in patient 4 was previously known in FBS [8], and in one family an older sister with the same homozygous mutation had FBS but was not known to have diabetes. It is not known if the majority of FBS patients who are not diagnosed with neonatal diabetes have mild resolving diabetes that is undetected or, despite the fluctuating glucose levels expected in FBS patients, do not meet diabetes diagnostic criteria. Most of our patients came to medical attention due to intercurrent illness and the diabetes had remitted before the first features of FBS become apparent. It is an interesting possibility that the renal glycosuria seen in FBS may greatly reduce the blood glucose levels despite there being a defect in insulin secretion.

We consider that the neonatal diabetes found in patients with recessive inactivating *SLC2A2* mutations is likely to reflect impaired insulin secretion given: (1) the response to insulin therapy in doses similar to other subtypes of neonatal diabetes; (2) the relatively low C-peptide levels at glucose values >20 mmol/l measured at diagnosis in two of our patients; (3) the inappropriately low insulin secretion associated with glucose intolerance reported in many FBS patients [8, 12–15]; and (4) the low birthweight seen in all patients, similar to other causes of neonatal diabetes, and consistent with reduced fetal insulin secretion in utero. We could not find data to assess if birthweight was reduced in FBS patients who are not diagnosed with neonatal diabetes. Further studies are needed on this, as a normal birthweight in FBS without neonatal diabetes would argue against fetal insulin secretion being reduced in all patients with *SLC2A2* mutations.

The homozygous recessive inactivating mutations in humans result in less frequent and less severe neonatal diabetes than the biallelic inactivation of *Slc2a2* in mice [2]. This is in keeping with GLUT2 being produced in lower amounts and having a less important role in glucose-stimulated insulin secretion in humans than in rodents [4].

While our finding of neonatal diabetes in a subgroup of patients suggests GLUT2 has a role in human insulin secretion, we cannot define the mechanism from our work. Expression studies suggest GLUT1 is the major human beta cell glucose transporter from fetal through to adult life [4–6]. Ninety per cent of beta cells were positive for GLUT1 from 20 weeks' gestation to adulthood, but only 10–20% were positive for GLUT2 until infants were over 8 months of age [5], and there are lower levels of expression of GLUT2 compared with GLUT1 and GLUT3 in adults [6]. Patients who are haploinsufficient for GLUT1 due to heterozygous *SLC2A1* mutations have epilepsy from impaired glucose transport across the blood–brain barrier [16, 17] and do not have diabetes. This may be because in beta cells 50% of GLUT1 is sufficient, or it may be due to compensation by other glucose transporters. Complete deficiency of GLUT1 due to a homozygous mutation would be expected to be fatal in utero. GLUT1 mutations have not been found to cause monogenic diabetes [18]. Glucose phosphorylation, rather than glucose transport, is normally likely to be the rate-limiting step for insulin secretion [19, 20].

It has been proposed that GLUT2 may have a role as a signalling molecule as well as a simple transporter [21]. GLUT2 confers insulin secretion but GLUT1 does not when expression levels are adjusted to ensure a similar glucose flux [22]. The higher K_m of GLUT2, compared with GLUT1 or GLUT3 [23], could allow glucose sensing by GLUT2 at physiological concentrations. In the absence of functional GLUT2, failure of an as yet unclear signalling cascade may cause the impaired insulin secretion. A common SNP close to *SLC2A2* has been shown to influence fasting glucose [24], but further investigations could not detect a defect in beta cell function using data from fasting and stimulated samples [25]. However, this is a different scenario from having no functional GLUT2, as seen in our patients.

In conclusion, we have shown that *SLC2A2* mutations can cause neonatal diabetes that occurs before overt clinical features of FBS appear. FBS should be considered as a cause of neonatal diabetes once other common origins are excluded, particularly when parents are related or when the diabetes is transient. This finding implies a role for GLUT2 in insulin secretion in humans as well as in rodents.

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Contribution statement SE and ATH conceived and designed the research. FHS analysed and interpreted the data. SEF and JALH designed the genetic analysis, interpreted data. FLSS, AA-S, AMH, MA and AK provided and interpreted clinical data. All authors either drafted the article or revised it for critically important intellectual content and approved the final version of the article.

References

1. Thorens B, Cheng Z-Q, Brown DF, Lodish HF (1990) Liver glucose transporter: a basolateral protein in hepatocytes and intestine and kidney cells. Am J Physiol 259(Cell Physiol 28):C279–C285
2. Guillam M-T, Hümmler E, Schaerer E et al (1997) Early diabetes and abnormal postnatal pancreatic islet development in mice lacking Glut-2. Nat Genet 17:327–330
3. Guillam M-T, Dupraz P, Thorens B (2000) Glucose uptake, utilization, and signaling in GLUT2-null islets. Diabetes 49:1485–1491
4. De Vos A, Heimberg H, Quartier E et al (1995) Human and rat beta cells differ in glucose transporter but not in glucokinase gene expression. J Clin Invest 96:2489–2495
5. Richardson CC, Hussain K, Jones PM et al (2007) Low levels of glucose transporters and K^+ ATP channels in human pancreatic beta cells early in development. Diabetologia 50:1000–1005
6. McCulloch LJ, van de Bunt M, Braun M, Frayn KN, Clark A, Gloyn AL (2011) GLUT2 (*SLC2A2*) is not the principal glucose transporter in human pancreatic beta cells: Implications for understanding genetic association signals at this locus. Mol Genet Metab 104:648
7. Santer R, Schneppenheim R, Dombrowski A, Götze H, Steinmann B, Schaub J (1997) Mutations in *GLUT2*, the gene for the liver-type glucose transporter, in patients with Fanconi-Bickel syndrome. Nat Genet 17:324–326
8. Santer R, Steinmann B, Schaub J (2002) Fanconi-Bickel syndrome—a congenital defect of facilitative glucose transport. Curr Mol Med 2:213–227
9. Murphy R, Ellard S, Hattersley AT (2008) Clinical implications of a molecular genetic classification of monogenic beta-cell diabetes. Nat Clin Pract Endocrinol Metab 4:200–213
10. Yoo H-W, Shin Y-L, Seo E-J, Kim G-H (2002) Identification of a novel mutation in the *GLUT2* gene in a patient with Fanconi-Bickel syndrome presenting with neonatal diabetes mellitus and galactosaemia. Eur J Pediatr 161:351–353
11. Habeb AM, Al-Magamsi MSF, Eid IM et al (2011) Incidence, genetics, and clinical phenotype of permanent neonatal diabetes mellitus in northwest Saudi Arabia. Pediatr Diabetes. doi:10.1111/j.1399-5448.2011.00828.x
12. Manz F, Bickel H, Brodehl J et al (1987) Fanconi-Bickel syndrome. Pediatr Nephrol 1:509–518
13. Garty R, Cooper M, Tabachnik E (1974) The Fanconi syndrome associated with hepatic glycogenosis and abnormal metabolism of galactose. J Pediatr 85:821–823
14. Chesney RW, Kaplan BS, Colle E et al (1980) Abnormalities of carbohydrate metabolism in idiopathic Fanconi syndrome. Pediatr Res 14:209–215
15. Taha D, Al-Harbi N, Al-Sabban E (2008) Hyperglycemia and hypoinsulinemia in patients with Fanconi–Bickel syndrome. J Pediatr Endocrinol Metab 21:581–586
16. De Vivo DC, Trifiletti RR, Jacobson RI, Ronen GM, Behmand RA, Harik SI (1991) Defective glucose transport across the blood–brain barrier as a cause of hypoglycorrhachia, seizures and developmental delay. N Engl J Med 325:703
17. Seidner G, Alvarez MG, Yeh J-I et al (1998) GLUT-1 deficiency syndrome caused by haploinsufficiency of the blood–brain barrier hexose carrier. Nat Genet 18:188–191
18. Baroni MG, Sentinelli F, Massa O (2001) Single-strand conformation polymorphism analysis of the glucose transporter gene *GLUT1* in maturity-onset diabetes of the young. J Mol Med 79:270–274
19. Whitesell RR, Powers AC, Regen DM, Abumrad NA (1991) Transport and metabolism of glucose in an insulin-secreting cell line, beta TC-1. Biochemistry 30:11560–11566
20. MacDonald PE, Joseph JW, Rorsman P (2005) Glucose-sensing mechanisms in pancreatic beta-cells. Phil Trans R Soc B 360:2211
21. Leturque A, Brot-Laroche E, Le Gall M (2009) GLUT2 mutations, translocation, and receptor function in diet sugar managing. Am J Physiol Endocrinol Metab 296:E985–E992
22. Hughes SD, Quaade C, Johnson JH, Ferber S, Newgard CB (1993) Transfection of AtT-20ins cells with GLUT-2 but not GLUT-1 confers glucose-stimulated insulin secretion. J Biol Chem 268:15205–15212
23. Brown GK (2000) Glucose transporters: structure, function and consequences of deficiency. J Inherit Metab Dis 23:237–246
24. Dupuis J, Langenberg C, Prokopenko I et al (2010) New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. Nat Genet 42:105–116
25. Ingelsson E, Langenberg C, Hivert MF et al (2010) Detailed physiologic characterization reveals diverse mechanisms for novel genetic loci regulating glucose and insulin metabolism in humans. Diabetes 59:1266–1275