Clinical and histological heterogeneity of congenital hyperinsulinism due to paternally inherited heterozygous *ABCC8/KCNJ11* mutations

Ved Bhushan Arya^{1,2,*}, Maria Guemes^{1,2,*}, Azizun Nessa¹, Syeda Alam², Pratik Shah^{1,2}, Clare Gilbert², Senthil Senniappan^{1,2}, Sarah E Flanagan³, Sian Ellard³ and Khalid Hussain^{1,2}

¹Developmental Endocrinology Research Group, Clinical and Molecular Genetics Unit, Institute of Child Health, University College London, 30 Guilford Street, London WC1N 1EH, UK, ²London Centre for Paediatric Endocrinology, Great Ormond Street Hospital for Children, London WC1N 3JH, UK and ³Institute of Biomedical and Clinical Science, University of Exeter Medical School, Exeter EX2 5DW, UK

*V B Arya and M Guemes contributed equally to this work

Correspondence should be addressed to K Hussain
Email
Khalid.Hussain@ucl.ac.uk

Abstract

Context: Congenital hyperinsulinism (CHI) has two main histological types: diffuse and focal. Heterozygous paternally inherited *ABCC8/KCNJ11* mutations (depending upon whether recessive or dominant acting and occurrence of somatic maternal allele loss) can give rise to either phenotype. However, the relative proportion of these two phenotypes in a large cohort of CHI patients due to paternally inherited heterozygous *ABCC8/KCNJ11* mutations has not been reported.

Objective: The purpose of this study is to highlight the variable clinical phenotype and to characterise the distribution of diffuse and focal disease in a large cohort of CHI patients due to paternally inherited heterozygous *ABCC8/KCNJ11* mutations.

Design: A retrospective chart review of the CHI patients due to heterozygous paternally inherited *ABCC8/KCNJ11* mutations from 2000 to 2013 was conducted.

Results: Paternally inherited heterozygous *ABCC8/KCNJ11* mutations were identified in 53 CHI patients. Of these, 18 (34%) either responded to diazoxide or resolved spontaneously. Fluorine-18 L-3, 4-dihydroxyphenylalanine positron emission tomography computerised tomography (¹⁸F DOPA–PET CT) scanning in 3/18 children showed diffuse disease. The remaining 35 (66%) diazoxide-unresponsive children either had pancreatic venous sampling (*n* = 8) or ¹⁸F DOPA–PET CT (*n* = 27). Diffuse, indeterminate and focal disease was identified in 13, 1 and 21 patients respectively. Two patients with suspected diffuse disease were identified to have focal disease on histology.

Conclusions: Paternally inherited heterozygous *ABCC8/KCNJ11* mutations can manifest as a wide spectrum of CHI with variable ¹⁸F DOPA–PET CT/histological findings and clinical outcomes. Focal disease was histologically confirmed in 24/53 (45%) of CHI patients with paternally inherited heterozygous *ABCC8/KCNJ11* mutations.

European Journal of Endocrinology
(2014) 171, 685–695

Introduction

Congenital hyperinsulinism (CHI) is one of the main causes of hypoglycaemia and is characterised by inappropriate insulin secretion (1). Mutations in nine different genes (*ABCC8*, *KCNJ11*, *GLUD1*, *GCK*, *HADH*, *SLC16A1*,

UCP2, *HNF4A* and *HNF1A*) have been reported so far as the genetic causes of CHI (1, 2). The pancreatic β-cell ATP-sensitive potassium channel (K_{ATP} channel) regulates glucose-mediated insulin release and is composed of two

subunits: Kir6.2 encoded by *KCNJ11* and SUR1 encoded by *ABCC8* gene (2). Both genes are localised in the 11p15.1 region and mutations in these accounts for the majority of CHI patients (3, 4, 5, 6, 7, 8, 9, 10, 11).

There are two main forms of CHI (focal and diffuse) that are clinically identical but differ in histology, underlying genetic mechanism and management. In diffuse CHI, histologically there is an increase in the β -cell nuclear size throughout the pancreas. Diffuse CHI is genetically heterogeneous and most commonly due to mutations in *ABCC8* or *KCNJ11* genes (3, 12, 13, 14, 15). Diazoxide, an agonist that targets the SUR1 subunit, is usually ineffective in patients with autosomal recessive form of *ABCC8* or *KCNJ11* mutations. The management of the medically unresponsive diffuse CHI patients involves near-total pancreatectomy.

The focal lesions are characterised by nodular hyperplasia of islet-like cell clusters with ductuloinsular complexes and scattered giant β -cell nuclei with normal surrounding tissue (16). The focal CHI results from a paternally inherited heterozygous *ABCC8/KCNJ11* mutation together with a somatic loss of the maternal chromosome in the 11p15 region (most likely caused by paternal isodisomy) (16, 17). The resulting loss of heterozygosity (LOH) renders the β -cells biallelic for the abnormal foci, altering the K_{ATP} channel and resulting in dysregulated insulin secretion within the focal lesion (16, 17, 18). The consequent imbalance in the expression of adjacent imprinted genes implicated in cell proliferation (such as *CDKN1C* and *H19* normally expressed from the maternal allele and *IGF2* paternally expressed) within the 11p15.5 region leads to focal islet cell adenomatous hyperplasia (19, 20, 21, 22). Focal CHI is usually medically unresponsive, although diazoxide-responsive focal CHI has been recently described (23). Targeted surgical removal of the lesion will cure the patient.

Fluorine-18 1-3, 4-dihydroxyphenylalanine positron emission tomography computerised tomography (^{18}F DOPA-PET CT) scan can help to differentiate the focal from diffuse forms of CHI and aids in the clinical management of these patients (24). Before the advent of ^{18}F DOPA-PET CT, pancreatic venous sampling (PVS) and selective pancreatic arterial calcium stimulation with hepatic venous sampling (ASVS) were used to differentiate between the two subtypes (25, 26). A recent systematic review and meta-analysis has found ^{18}F DOPA-PET CT to be far superior to PVS and ASVS in diagnosing and localising focal CHI (27).

Patients with CHI associated with heterozygous paternally inherited *ABCC8/KCNJ11* mutations may or

may not have the second hit of somatic maternal allele loss in the pancreatic β -cell. Accordingly, these patients can either have focal or diffuse disease. Furthermore, diffuse disease can either be diazoxide-responsive or unresponsive depending on the underlying molecular basis of the mutation and other unclear genetic or environmental influences (28). There are isolated case reports of heterozygous paternally inherited *ABCC8/KCNJ11* mutations leading to diffuse CHI in the literature (3, 10, 29, 30). The proportion of patients with paternally inherited heterozygous *ABCC8/KCNJ11* mutations who develop focal CHI has not been reported in any study so far. Herein, we describe the heterogeneous clinical presentation and the histological basis of a relatively large cohort of patients with heterozygous paternally inherited *ABCC8/KCNJ11* mutations. We also report the proportion of focal and diffuse disease in a large cohort of CHI patients with paternally inherited heterozygous *ABCC8/KCNJ11* mutations.

Subjects and methods

The study was approved by the Ethics Committee of Great Ormond Street Children's Hospital and the Institute of Child Health. Informed written consent was obtained from the parents of children enrolled for molecular genetic testing for CHI (*ABCC8/KCNJ11* sequencing).

This is a descriptive study of the clinical characteristics of children with CHI due to paternally inherited heterozygous *ABCC8/KCNJ11* mutations. Clinical presentation, disease course and outcome for consecutive patients with CHI associated with paternally inherited heterozygous *ABCC8/KCNJ11* mutations between the years 2000 and 2013 were retrospectively reviewed.

CHI was diagnosed by a controlled fasting test that demonstrated an inappropriately detectable serum insulin and/or C-peptide concentration and/or inappropriately low β -hydroxybutyrate and non-esterified fatty acids concentrations in the presence of hypoglycaemia (plasma glucose concentration <3.0 mmol/l). The patients with confirmed diagnosis of CHI underwent molecular genetic analysis after informed consent (see below). Diazoxide (5–15 mg/kg per day in three divided doses) was commenced as the first-line medical treatment. The patients were defined as diazoxide unresponsive if age appropriate fasting tolerance could not be achieved by treatment with 15 mg/kg per day diazoxide for a minimum of 5 days. Diazoxide-unresponsive patients associated with paternally inherited heterozygous *ABCC8/KCNJ11* mutation underwent further investigations (PVS or

^{18}F DOPA-PET CT) to differentiate between focal and diffuse disease. PVS was performed and interpreted as described previously by de Lonlay-Debeney *et al.* (26). The detailed protocol used for ^{18}F DOPA-PET CT and analysis of the images has already been described (31).

Diazoxide-unresponsive diffuse disease was managed by octreotide injections (5–30 $\mu\text{g/kg}$ per day in three to four divided doses) $\pm$ near-total pancreatectomy along with carbohydrate-rich feeds. The focal disease was managed with the resection of the focal lesion or partial pancreatectomy. Post-surgery, glycaemic control was periodically assessed by 24-h blood glucose profile and controlled fast to ensure cure of the focal CHI and appropriate control of diffuse CHI.

Genetic testing

Genomic DNA was extracted from peripheral leukocytes using standard procedures. The single-coding exon of the *KCNJ11* gene and the 39 exons of the *ABCC8* gene were amplified using the PCR. Unidirectional sequencing was performed using universal M13 primers and a Big Dye Terminator Cycle Sequencing Kit v3.1 (Applied Biosystems) according to the manufacturer's instructions. The reactions were analysed on an ABI 3730 Capillary Sequencer (Applied Biosystems) and the sequences were compared with the reference sequences (NM_000525 and NM_000352.2) using Mutation Surveyor v3.24 (SoftGenetics, PA, USA). Dosage analyses by multiplex

ligation-dependent probe amplification were done in patients with a heterozygous *ABCC8/KCNJ11* mutation and diffuse disease on ^{18}F DOPA-PET scan/PVS (32).

LOH was investigated by microsatellite analysis of DNA extracted from paraffin-embedded pancreatic tissue and peripheral leukocytes, mainly when there was a discrepancy between the histology and ^{18}F DOPA-PET CT/PVS result. Six markers (D11S2071, D11S1964, D11S419, D11S1397, D11S1888 and D11S4138) spanning chromosome 11p15.1–11p15.5 were amplified by PCR and allele peak heights were compared using GeneMarker v1.85 (SoftGenetics).

Results

Over a period of 14 years (2000–2013), more than 300 children with confirmed biochemical and genetic diagnosis of CHI were managed in our centre. Of these, 53 children had paternally inherited heterozygous *ABCC8/KCNJ11* mutation (*ABCC8*, 42 and *KCNJ11*, 11). Of the 35 different *ABCC8* mutations seen in 42 patients, 19 affected a single amino acid (missense or in-frame deletion/insertion). Of the ten different *KCNJ11* mutations seen in 11 patients, eight affected a single amino acid (missense or in-frame deletion/insertion), one was a frame shift mutation and one was a non-stop codon mutation resulting in extension of the Kir6.2 protein sequence by three amino acids (Fig. 1). As shown in Fig. 1, mutations occurred throughout SUR1 and Kir6.2.

Figure 1

Paternal mutations mapped onto the SUR1 and Kir6.2 protein. All SUR1 intronic splice site mutation mutants have been highlighted in red. All mutants with * are responsive to diazoxide (transmembrane domain (TMD) and nucleotide-binding domain (NBD)). A full colour version of this figure available via <http://dx.doi.org/10.1530/EJE-14-0353>.

Table 1 Clinical and biochemical characteristics, mutational analysis and clinical outcome of 53 patients with paternally inherited *ABCC8/KCNJ11* mutations.

Patient ID	GA (weeks), birth weight (g)	Gender	Age at presentation (weeks)	Blood glucose (mmol/l)	Serum insulin (mU/l)	Mutation protein description (DNA description)	LOH	Dzx Resp	PET CT/PVS	Outcome
ABCC8										
1	40, 3150	Male	52	2.4	4.3	L1431F/N (c.4291C>T/N)		Yes	–	On Dzx at 6.4 years
2	40, 4000	Male	2	2.4	1.9	p.7/N (c.2697+4A>T/N)		Yes	–	Off Dzx at 2 years
3	40, 5010	Female	<1	2.6	8.6	E1507K/N (c.4519G>A/N)		Yes	–	On Dzx at 2.3 years
4	40, 5600	Male	<1	2.0	7.5	A1508P/N (c.4522G>C/N)		Yes	–	On Dzx at 12 years
5	37, 4820	Male	<1	2.0	9.0	A1153T/N (c.3457G>A/N)		Yes	–	On Dzx at 4 years
6	38, 3630	Female	72	3.1	<2	A1153T/N (c.3457G>A/N)		Yes	–	On Dzx at 2 years
8	35, 2820	Male	<1	1.2	12.9	A1185V/N (c.3554C>T/N)		Yes	–	Off Dzx at 8 months
9	36, 4450	Male	<1	2	28.5	p.7/N (c.3992-9G>A/N)		Yes	–	Off Dzx after 4.5 months
10	41, 2780	Female	<1	1	9.3	D1472N/N (c.4414G>A/N)		Yes	–	Off Dzx after 10 months
11	38, 3750	Male	<1	2.6	5.90	V601I/N (c.1801G>A/N)		Yes	–	Off Dzx after 14 months
13	40, 4160	Female	<1	2.4	14.8	V185fs/N (c.554delT/N)		No	Diffuse	Off octreotide at 5 years
14	37, 3090	Male	<1	0.6	12.4	p.7/N (c.3992-9G>A/N)		No	Focal	On octreotide at 9.5 years
15	40, 3600	Male	<1	2.7	6.7	H627fs/N (c.1879delC/N)		No	Diffuse	Off octreotide at 18 months
16	40, 4700	Female	<1	2.0	<2	E1507K/N (c.4519G>A/N)		NA	–	No treatment required
17	40, 4200	Male	<1	1.0	<2	D1031N/N (c.3091G>A/N)		NA	–	No treatment required
18	41, 4850	Male	<1	1.2	10.1	M1V/N (c.1A>G/N)		No	Diffuse (PVS)	Near-total pancreatectomy (95%)
19	38, 2400	Male	<1	2.1	16.3	D1194V; R1437Q/N (c.3581A>T; c.4310G>A/N)	Yes	No	Focal	Hypoglycaemia resolved after removal of focal lesion
20	40, 4580	Female	<1	1.1	103	A1493T/N (c.4477G>A/N)	Yes ^a	No	Diffuse	Near-total pancreatectomy (95%)
21	40, 4600	Male	<1	1.2	22.5	K890fs/N (c.2669_2675del/N)	Yes	No	Focal	Hypoglycaemia resolved after removal of focal lesion
22	40, 4335	Male	<1	2.6	3.4	p.7/N (c.3992-9G>A/N)	Yes	No	Focal	Partial pancreatectomy
23	40, 3030	Male	<1	1.8	15.6	p.7/N (c.3992-9G>A/N)	Yes	No	Focal	Partial pancreatectomy
24	40, 2770	Male	<1	2.2	3.4	p.7/N (c.580-1G>C/N)		No	Indeterminate (PVS)	Partial pancreatectomy – focal lesion on histology
25	41, 4290	Male	2	2.3	4.32	E128K/N (c.382G>A/N)		No	Focal	Hypoglycaemia resolved after removal of focal lesion
27	40, 5095	Male	<1	2.0	10.5	L1171X/N (c.3512delT/N)	Yes	No	Focal	Hypoglycaemia resolved after removal of focal lesion
29	37, 3560	Male	<1	1.6	21.8	p.7/N (c.1629-2A>C/N)		No	Focal	Hypoglycaemia resolved after removal of focal lesion
30	40, 2750	Male	<1	1.5	16.4	G111R/N (c.331G>A/N)	Yes	No	Focal	Hypoglycaemia resolved after removal of focal lesion
31	37, 3340	Male	<1	2.1	15	H627fs/N (c.1879delC/N)	Yes	No	Focal	Hypoglycaemia resolved after removal of focal lesion
32	41, 4950	Male	<1	1.2	11.95	R934X/N (c.2800C>T/N)		No	Focal	Hypoglycaemia resolved after removal of focal lesion
33	40, 3080	Female	12	1.5	4.6	S12X/N (c.35C>A/N)	Yes	No	Focal	Hypoglycaemia resolved after removal of focal lesion
34	39, 3600	Male	<1	2.5	8	R1494W/N (c.4480C>T/N)		No	Diffuse (PVS)	Near-total pancreatectomy (95%)
37	36, 3410	Male	<1	0.9	17.42	R1494W/N (c.4480C>T/N)		No	Focal	Partial pancreatectomy

Table 1 Continued

Patient ID	GA (weeks), birth weight (g)	Gender	Age at presentation (weeks)	Blood glucose (mmol/l)	Serum insulin (mU/l)	Mutation protein description (DNA description)	LOH	Dzx Resp	PET CT/PVS	Outcome
38	39, 4900	Female	<1	1.4	23.61	A113V/N (c.338C>T/N)		No	Diffuse (PVS)	Near-total pancreatectomy (95%)
39	36, 3210	Male	<1	0.6	114	Mosaic Q54X (c.160C>T)		No	Diffuse	Near-total pancreatectomy (95%)
40	41, 4300	Female	<1	2.1	17	Q954X/N (c.2860C>T/N)		No	Diffuse	Near-total pancreatectomy (95%)
41	36, 2730	Male	<1	?	?	p.7/N (c.1333-1013A>G/N)	Yes	No	Focal	Hypoglycaemia resolved after removal of focal lesion
42	37, 4480	Female	<1	1.7	5.6	A1263T/N (c.3787G>A/N)		No	Diffuse (PVS)	Near-total pancreatectomy (95%)
45	39, 3960	Male	<1	2.4	23.6	p.7/N (c.1-? c.1176+1?/N)		No	Focal	Hypoglycaemia resolved after removal of focal lesion
46	34, 3800	Male	<1	0.8	201	D855E/N (c.2565C>A/N)		No	Focal	Hypoglycaemia resolved after removal of focal lesion
47	41, 4780	Male	<1	2.4	15.9	M429X/N (c.1254_1284dup/N)		No	Focal	Hypoglycaemia resolved after removal of focal lesion
48	40, 3800	Female	<1	2.4	5.9	M429X/N (c.1254_1284dup/N)		No	Diffuse	On octreotide at 7 months
49	40, 4100	Male	<1	0.8	9.1	R74W/N (c.220C>T/N)		No	Focal	Hypoglycaemia resolved after removal of focal lesion
51	38, 3730	Female	<1	1.9	21.6	E995X (c.2983G>T)		No	Focal	Hypoglycaemia resolved after removal of focal lesion
<i>KCNJ11</i>										
7	38, 3780	Female	<1	1.7	29.6	M209I/N (c.627G>A/N)		Yes	Diffuse	On Dzx at 2.8 years
12	40, 3450	Male	<1	2	6.7	R54H/N (c.161G>A/N)		Yes	–	Off Dzx after 4 months
26	36, 3880	Male	<1	<1.0	6.1	E292K/N (c.874G>A/N)		No	Diffuse (PVS)	Near-total pancreatectomy (95%)
28	40, 4600	Male	<1	1.3	38.87	R136fs/N (c.405dupG/N)		No	Diffuse (PVS)	Near-total pancreatectomy (95%)
35	38, 4120	Female	<1	2.2	12.0	T294M/N (c.881C>T/N)		No	Diffuse (PVS)	Near-total pancreatectomy (95%)
36	37, 3960	Male	<1	1.9	7.0	G312C/N (c.934G>T/N)		No	Focal	Hypoglycaemia resolved after removal of focal lesion
43	40, 4100	Female	<1	1.0	35	I284del/N		Yes	Diffuse	Off Dzx at 4 years of age
44	40, 4580	Female	32	2.2	7	(c.850_852delATC/N) p.*391Rext*94/N (c.1171T>C/N)		No	Focal	Partial pancreatectomy, currently on Dzx
50	37, 3980	Male	<1	0.5	2.9	T62M/N (c.185C>T/N)		Yes	–	Off Dzx at 1 month
52	40, 5190	Male	<1	2.5	8.4	R177W/N (c.529A>T/N)		Yes	Diffuse	On Dzx at 3 months of age
53	40, 3920	Male	<1	2.1	9.2	E292K/N (c.874G>A/N)		Yes	Diffuse	Off Dzx at 5 months of age

Dzx, diazoxide; PET, positron emission tomography; PVS, pancreatic venous sampling; GA, gestational age; Wt, weight; LOH, loss of heterozygosity; Resp, responsiveness.

*This patient had giant focal lesion and virtually occupied the whole of pancreas.

Clinical and biochemical characteristics

The mean (± 2 s.d.) gestational age and birth weight of our cohort was 38.9 (± 1.8) weeks and 3980 (± 760) g respectively. Of these, 19 infants (36%) were macrosomic (birth weight > 2 s.d.) at birth. The gender distribution was 37 males and 16 females. The majority of patients in our cohort were of Caucasian descent. Only one proband was a product of the consanguineous marriage. There were two pairs of two siblings and the rest of the cohort was unrelated. The median age of presentation with hypoglycaemia was < 1 week (range < 1 –130 weeks). More than 85% (46/53) patients presented with hypoglycaemia within first week of life. The mean ($\pm$ s.d.) serum insulin during hypoglycaemia was 21.1 (± 34.2) mU/l. In three infants in whom serum insulin was undetectable, C-peptide level was inappropriately elevated for the blood glucose measurement. The serum ammonia was within normal range and β -hydroxybutyrate/non-esterified fatty acids were inappropriately low in all children (Table 1).

Clinical course, ^{18}F DOPA–PET CT and PVS findings and final outcome

Diazoxide-responsive group ► In two (4%) patients (Table 1: patients 16 and 17), CHI resolved spontaneously

within few weeks before investigations were repeated in our centre (Fig. 2). Previous functional work has established milder disease phenotype with a mutation seen in one of these two patients (*ABCC8* (E1507K)) (33). The mutation identified in the second patient (*ABCC8* (D1031N)) is not reported in dbSNP and predicted to be disease causing (Table 2).

Sixteen patients (30%) with presumably diffuse CHI responded to diazoxide treatment (Fig. 2). In three patients from diazoxide-responsive group who underwent ^{18}F DOPA–PET CT, diffuse uptake throughout the pancreas was noticed. Nine patients (17%) successfully managed to come off diazoxide treatment after a variable period of time. The remaining seven (13%) patients were on diazoxide for a median duration of 2.8 years (range 3 months–12 years) at the time of writing.

The mutations in this subgroup are predicted to be pathogenic by various mutation prediction programmes (Table 2) (34, 35).

Diazoxide-unresponsive group ► The remaining 35 (66%) patients were unresponsive to maximum doses of diazoxide and were investigated either by using PVS ($n=8$) or ^{18}F DOPA–PET CT scan ($n=27$).

PVS was suggestive of diffuse disease in seven patients and no differentiation between focal and diffuse was

Figure 2

Clinical course, ^{18}F DOPA–PET CT/pancreatic venous sampling findings and outcome.

Table 2 *In silico* pathogenicity prediction of diazoxide-responsive patients' mutations.

	Prediction				Mutation
Mutation	Found in dbSNP	Provean	SIFT	MutationTaster	previously reported
ABCC8					
L1431F (c.4291C>T)	No	Deleterious	Damaging	Disease causing	(38) ^a
p.? (c.2697+4A>T)	No	Not available	Not available	Disease causing	Novel
E1507K (c.4519G>A)	Yes	Deleterious	Damaging	Disease causing	(32)
A1508P (c.4522G>C)	No	Deleterious	Damaging	Disease causing	(38) ^a
A1153T (c.3457G>A)	No	Deleterious	Damaging	Disease causing	Novel
A1185V (c.3554C>T)	No	Neutral	Damaging	Disease causing	Novel
p.? (c.3992-9G>A)	No	Not available	Not available	Disease causing	(5) ^a
D1472N (c.4414G>A)	Yes	Deleterious	Damaging	Disease causing	(24) ^a
V601I (c.1801G>A)	No	Neutral	Tolerated	Disease causing	Novel ^a
V185fs (c.554delT)	Yes	Not available	Not available	Disease causing	Novel
H627fs (c.1879delC)	No	Not available	Not available	Disease causing	(9) ^a
D1031N (c.3091G>A)	No	Neutral	Tolerated	Disease causing	Novel
M1V (c.1A>G)	No	Neutral	Damaging	Disease causing	(6) ^a
A1493T (c.4477G>A)	Yes	Deleterious	Damaging	Disease causing	(14) ^a
R1494W (c.4480C>T)	Yes	Deleterious	Damaging	Disease causing	(25) ^a
KCNJ11					
M209I (c.627G>A)	No	Neutral	Damaging	Disease causing	Novel
R54H (c.161G>A)	No	Deleterious	Damaging	Disease causing	Novel ^a
E292K (c.874G>A)	No	Deleterious	Damaging	Disease causing	Novel
T62M (c.185C>T)	No	Deleterious	Damaging	Disease causing	(4)
R177W (c.529A>T)	No	Deleterious	Damaging	Disease causing	Novel

^aThese patients were included in reference (3).

possible in one patient. The suspected diffuse disease patients were managed with near-total pancreatectomy. Although histology confirmed diffuse disease in six patients, there was one patient who had focal adenomatous hyperplasia which was presumably missed on PVS. The patient with indeterminate PVS underwent partial pancreatectomy and focal lesion was seen on the histology of the resected pancreas.

Of the 27 patients who were investigated with ¹⁸F DOPA-PET scan, focal uptake was seen in 21 and diffuse uptake in six patients (Fig. 3). The focal lesion was successfully managed either with lesionectomy (*n*=16) or partial pancreatectomy (*n*=4). One patient was managed successfully with octreotide injections.

The patients with diffuse tracer pancreatic uptake were either managed with near-total pancreatectomy (*n*=3) or octreotide injections (*n*=3). One patient from this subgroup had atypical histology with pancreatic β-cell enlargement in parts of body and head of the pancreas due to mosaic interstitial paternal uniparental isodisomy for chromosome 11p15.1 (36).

Discussion

This study reports the proportion of focal disease in a large cohort of patients with CHI associated with paternally

inherited heterozygous *ABCC8/KCNJ11* mutations. In addition, it highlights the clinical and histological heterogeneity of CHI associated with paternally inherited heterozygous *ABCC8/KCNJ11* mutation.

Mutations in the *ABCC8/KCNJ11*, which account for the majority of genetically confirmed CHI cases, can either be biallelic or monoallelic (3, 4, 37). Biallelic *ABCC8/KCNJ11* mutations result in diffuse CHI, whereas monoallelic mutations can either be asymptomatic

Figure 3

¹⁸F DOPA-PET CT image of patient 40 showing diffuse tracer uptake throughout the pancreas in a patient with paternally inherited heterozygous *ABCC8* mutation. Full colour version of this figure available via <http://dx.doi.org/10.1530/EJE-14-0353>.

(recessive acting), or lead to focal CHI (if paternally inherited and associated with loss of somatic maternal 11p allele) or diffuse CHI (dominant acting). Diazoxide, a K_{ATP} channel opener, which is the first-line management for CHI, is usually ineffective in biallelic disease and monoallelic focal disease (3, 4). However, recently a case of diazoxide-responsive focal CHI was described (23). It may be possible that as more diazoxide-responsive CHI patients are investigated with ^{18}F DOPA-PET CT scan, more diazoxide-responsive focal lesions are identified. However, currently diazoxide responsiveness in our institution is generally considered equivalent to excluding monoallelic focal disease and this group of patients are not usually considered as suitable candidates for further investigations with ^{18}F DOPA-PET CT. On the other hand, diazoxide-unresponsive subgroup, particularly associated with paternally inherited monoallelic *ABCC8/KCNJ11* mutations or no identified *ABCC8/KCNJ11* mutations, is investigated with ^{18}F DOPA-PET CT to identify focal or diffuse subtype before proceeding with definitive treatment as the treatment for focal (limited pancreatectomy) and diffuse CHI (near-total pancreatectomy) is drastically different (38).

In our cohort of 53 CHI patients associated with paternally inherited monoallelic *ABCC8/KCNJ11* mutations, 18 (33%) patients either responded to diazoxide or resolved spontaneously and are likely to have dominant acting CHI. Mutations in these 18 patients are predicted to be pathogenic by different mutation prediction programmes (Table 2). Functional work has already established some of these mutants to be dominant acting and exerting their effect by a dominant-negative mechanism (33, 39, 40). Although there was no history of hypoglycaemia in the fathers of our cohort, formal evaluation with controlled provocation fasting studies has not been done. Other studies also have reported parents to be asymptomatic carriers of dominant acting mutations (41). In view of severe diazoxide side effects and parental request, three patients from this subgroup underwent ^{18}F DOPA-PET CT imaging to avoid long-term treatment with diazoxide. This was in the hope that these patients could be managed with removal of focal lesion if found to be the underlying cause for CHI. However, as suspected from the phenotype of diazoxide-responsiveness, ^{18}F DOPA-PET CT identified diffuse tracer uptake throughout the pancreas.

Evaluation with ^{18}F DOPA-PET CT in 27 diazoxide-unresponsive patients with paternally inherited heterozygous *ABCC8/KCNJ11* mutations identified focal disease in 21 (78%) patients. Majority of these patients presented within the first week of life. There was no difference in the

age of presentation between those who were diagnosed with diffuse or focal CHI. Apart from one patient who was managed with octreotide therapy, these patients were either managed with resection of the focal lesion or partial pancreatectomy. Follow-up assessment highlighted age-appropriate fasting tolerance and resolution of hypoglycaemia in all except one (19/20; 95%) after the removal of the focal lesion. Similar to our results, Lord *et al.* (42) recently reported that 94% of their cohort of focal CHI after pancreatectomy remained euglycaemic and required no treatment for blood glucose abnormalities. In the cohort reported by Beltrand *et al.* (43), 91.4% (43/47) patients with focal CHI had complete resolution of hypoglycaemia post-surgery. One patient in whom the evidence of CHI persisted despite the removal of a histologically confirmed focal lesion was managed with diazoxide and regular feeds. A repeat ^{18}F DOPA-PET CT revealed diffuse tracer uptake in the remaining pancreas. Molecular basis for CHI in this particular patient is unclear and further studies are in progress.

In six (21%) diazoxide-unresponsive patients investigated with ^{18}F DOPA-PET CT, diffuse tracer uptake throughout the pancreas was noticed (Fig. 3). Three of these patients were managed with near-total pancreatectomy and in one of these patients, histology and microsatellite analysis was suggestive of giant focal lesion (44). Diffuse uptake in this particular patient was due to the size of the focal lesion, which occupied nearly the whole pancreas. Diffuse CHI has been described in the literature with paternally inherited *ABCC8/KCNJ11* mutations. Fernandez-Marmiesse *et al.* (10) reported five patients with paternally inherited K_{ATP} mutations and whose post pancreatectomy pathology result was not consistent with that of focal lesion. Similar findings have been reported by other studies (1, 21, 27, 30).

There are a number of possible mechanisms as to how a heterozygous *ABCC8/KCNJ11* mutation can lead to diffuse disease. For example there could be a post-zygotic second hit within the pancreas or a maternally inherited mutation may reside within a non-coding regulatory/intronic region of the *ABCC8/KCNJ11* gene. Recently, in a patient with focal CHI but no exonic or splice site *ABCC8/KCNJ11* mutation, next-generation sequencing identified a deep intronic *ABCC8* mutation inherited from the unaffected father, which created a cryptic splice site and an out of frame pseudoexon (32). A third possibility is allelic drop-out due to a rare polymorphism within a primer binding site. In this scenario, a maternally inherited coding mutation could escape detection by Sanger sequencing. Besides, some of the missense mutations may be dominantly acting (dominant negative).

As compared with recessive acting mutations where there is defective biogenesis or trafficking of K_{ATP} channel to the β -cell membrane, the dominant acting mutants traffic normally to the plasma membrane but show impaired responsiveness to the channel agonists, MgADP and diazoxide (45). Diazoxide-unresponsive mutations produce a more severe impairment in the expressed channel response to activation by diazoxide and MgADP as compared with diazoxide-responsive mutations (28).

A novel genetic mechanism of diffuse disease due to mosaic paternal uniparental isodisomy in a patient with heterozygous *ABCC8/KCNJ11* mutations was recently described (36). Lastly, it could also very rarely be due to failure of Sanger sequencing to detect the second *ABCC8/KCNJ11* mutation or a missed histological diagnosis of focal CHI in these patients. Although *ABCC8* and *KCNJ11* are not known to be imprinted genes, they are next to the 11p15.5 imprinted region, so another hypothesis could be the involvement of epigenetic mechanisms.

In summary, of the 53 patients associated with paternally inherited *ABCC8/KCNJ11* mutations, 29 (55%) patients had diffuse CHI (18 (34%) patients – diazoxide-responsive; 11 (21%) patients – diazoxide-unresponsive) and 24 (45%) patients had confirmed focal CHI either based on histology or LOH studies. One patient had giant focal lesion based on histology and LOH studies, confusing the interpretation of ^{18}F DOPA–PET CT. From our results, it is clear that paternally inherited heterozygous *ABCC8/KCNJ11* mutations can manifest as a wide spectrum of CHI with focal disease in 45% patients. This heterogeneous clinical picture could be due to modifying genes or other unknown factors, e.g. environmental, that influence the expression of the phenotype in CHI patients.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

References

- 1 Senniappan S, Shanti B, James C & Hussain K. Hyperinsulinaemic hypoglycaemia: genetic mechanisms, diagnosis and management.

- Journal of Inherited Metabolic Disease* 2012 **35** 589–601. (doi:10.1007/s10545-011-9441-2)
- 2 Kapoor RR, Flanagan SE, James C, Shield J, Ellard S & Hussain K. Hyperinsulinaemic hypoglycaemia. *Archives of Disease in Childhood* 2009 **94** 450–457. (doi:10.1136/adc.2008.148171)
- 3 Kapoor RR, Flanagan SE, Arya VB, Shield JP, Ellard S & Hussain K. Clinical and molecular characterisation of 300 patients with congenital hyperinsulinism. *European Journal of Endocrinology* 2013 **168** 557–564. (doi:10.1530/EJE-12-0673)
- 4 Snider KE, Becker S, Boyajian L, Shyng SL, MacMullen C, Hughes N, Ganapathy K, Bhatti T, Stanley CA & Ganguly A. Genotype and phenotype correlations in 417 children with congenital hyperinsulinism. *Journal of Clinical Endocrinology and Metabolism* 2013 **98** E355–E363. (doi:10.1210/jc.2012-2169)
- 5 Nestorowicz A, Wilson BA, Schoor KP, Inoue H, Glaser B, Landau H, Stanley CA, Thornton PS, Clement JP, Bryan J *et al.* Mutations in the sulfonylurea receptor gene are associated with familial hyperinsulinism in Ashkenazi Jews. *Human Molecular Genetics* 1996 **5** 1813–1822. (doi:10.1093/hmg/5.11.1813)
- 6 Greer RM, Shah J, Jeske YW, Brown D, Walker RM, Cowley D, Bowling FG, Liaskou D, Harris M, Thomsett MJ *et al.* Genotype–phenotype associations in patients with severe hyperinsulinism of infancy. *Pediatric and Developmental Pathology* 2007 **10** 25–34. (doi:10.2350/06-04-0083.1)
- 7 Darendeliler F, Fournet JC, Bas F, Junien C, Gross MS, Bundak R, Saka N & Gunoz H. *ABCC8* (SUR1) and *KCNJ11* (KIR6.2) mutations in persistent hyperinsulinemic hypoglycemia of infancy and evaluation of different therapeutic measures. *Journal of Pediatric Endocrinology & Metabolism* 2002 **15** 993–1000.
- 8 Tornovsky S, Crane A, Cosgrove KE, Hussain K, Lavie J, Heyman M, Neshet Y, Kuchinski N, Ben-Shushan E, Shatz O *et al.* Hyperinsulinism of infancy: novel *ABCC8* and *KCNJ11* mutations and evidence for additional locus heterogeneity. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 6224–6234. (doi:10.1210/jc.2004-1233)
- 9 Gloy AL, Siddiqui J & Ellard S. Mutations in the genes encoding the pancreatic β -cell K_{ATP} channel subunits Kir6.2 (*KCNJ11*) and SUR1 (*ABCC8*) in diabetes mellitus and hyperinsulinism. *Human Mutation* 2006 **27** 220–231. (doi:10.1002/humu.20292)
- 10 Fernandez-Marmiesse A, Salas A, Vega A, Fernandez-Lorenzo JR, Barreiro J & Carracedo A. Mutation spectra of *ABCC8* gene in Spanish patients with Hyperinsulinism of Infancy (HI). *Human Mutation* 2006 **27** 214. (doi:10.1002/humu.9401)
- 11 Shimomura K, Flanagan SE, Zadek B, Lethby M, Zubcevic L, Girard CA, Petz O, Mannikko R, Kapoor RR, Hussain K *et al.* Adjacent mutations in the gating loop of Kir6.2 produce neonatal diabetes and hyperinsulinism. *EMBO Molecular Medicine* 2009 **1** 166–177. (doi:10.1002/emmm.200900018)
- 12 Muzyamba M, Farzaneh T, Behe P, Thomas A, Christesen HB, Brusgaard K, Hussain K & Tinker A. Complex *ABCC8* DNA variations in congenital hyperinsulinism: lessons from functional studies. *Clinical Endocrinology* 2007 **67** 115–124. (doi:10.1111/j.1365-2265.2007.02847.x)
- 13 Otonkoski T, Nanto-Salonen K, Seppanen M, Veijola H, Huopio H, Hussain K, Tapanainen P, Eskola O, Parkkola R, Ekstrom K *et al.* Noninvasive diagnosis of focal hyperinsulinism of infancy with [^{18}F]–DOPA positron emission tomography. *Diabetes* 2006 **55** 13–18. (doi:10.2337/diabetes.55.01.06.db05-1128)
- 14 Kassem SA, Ariel I, Thornton PS, Hussain K, Smith V, Lindley KJ, Aynsley-Green A & Glaser B. p57(KIP2) expression in normal islet cells and in hyperinsulinism of infancy. *Diabetes* 2001 **50** 2763–2769. (doi:10.2337/diabetes.50.12.2763)
- 15 Suchi M, MacMullen C, Thornton PS, Ganguly A, Stanley CA & Ruchelli ED. Histopathology of congenital hyperinsulinism: retrospective study with genotype correlations. *Pediatric and*

- Developmental Pathology* 2003 **6** 322–333. (doi:10.1007/s10024-002-0026-9)
- 16 Damaj L, le Lorch M, Verkarre V, Werl C, Hubert L, Nihoul-Fekete C, Aigrain Y, de Keyser Y, Romana SP, Bellanne-Chantelot C *et al.* Chromosome 11p15 paternal isodisomy in focal forms of neonatal hyperinsulinism. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 4941–4947. (doi:10.1210/jc.2008-0673)
 - 17 Verkarre V, Fournet JC, de Lonlay P, Gross-Morand MS, Devillers M, Rahier J, Brunelle F, Robert JJ, Nihoul-Fekete C, Saudubray JM *et al.* Paternal mutation of the sulfonylurea receptor (SUR1) gene and maternal loss of 11p15 imprinted genes lead to persistent hyperinsulinism in focal adenomatous hyperplasia. *Journal of Clinical Investigation* 1998 **102** 1286–1291. (doi:10.1172/JCI4495)
 - 18 Calton EA, Temple IK, Mackay DJ, Lever M, Ellard S, Flanagan SE, Davies JH, Hussain K & Gray JC. Hepatoblastoma in a child with a paternally-inherited *ABCC8* mutation and mosaic paternal uniparental disomy 11p causing focal congenital hyperinsulinism. *European Journal of Medical Genetics* 2013 **56** 114–117. (doi:10.1016/j.ejmg.2012.12.001)
 - 19 Shuman C, Smith AC, Steele L, Ray PN, Clericuzio C, Zackai E, Parisi MA, Meadows AT, Kelly T, Tichauer D *et al.* Constitutional UPD for chromosome 11p15 in individuals with isolated hemihyperplasia is associated with high tumor risk and occurs following assisted reproductive technologies. *American Journal of Medical Genetics. Part A* 2006 **140** 1497–1503. (doi:10.1002/ajmg.a.31323)
 - 20 Giurgea I, Bellanne-Chantelot C, Ribeiro M, Hubert L, Sempoux C, Robert JJ, Blankenstein O, Hussain K, Brunelle F, Nihoul-Fekete C *et al.* Molecular mechanisms of neonatal hyperinsulinism. *Hormone Research* 2006 **66** 289–296. (doi:10.1159/000095938)
 - 21 Fournet JC, Verkarre V, De Lonlay P, Rahier J, Brunelle F, Robert JJ, Nihoul-Fekete C, Saudubray JM & Junien C. Loss of imprinted genes and paternal SUR1 mutations lead to hyperinsulinism in focal adenomatous hyperplasia. *Annales d'Endocrinologie* 1998 **59** 485–491.
 - 22 de Lonlay P, Fournet JC, Rahier J, Gross-Morand MS, Poggi-Travert F, Foussier V, Bonnefont JP, Brusset MC, Brunelle F, Robert JJ *et al.* Somatic deletion of the imprinted 11p15 region in sporadic persistent hyperinsulinemic hypoglycemia of infancy is specific of focal adenomatous hyperplasia and endorses partial pancreatectomy. *Journal of Clinical Investigation* 1997 **100** 802–807. (doi:10.1172/JCI119594)
 - 23 Maiorana A, Barbetti F, Boiani A, Rufini V, Pizzoferrero M, Francalanci P, Faletta F, Nichols CG, Grimaldi C, de Ville de Goyet J *et al.* Focal congenital hyperinsulinism managed by medical treatment: a diagnostic algorithm based on molecular genetic screening. *Clinical Endocrinology* 2014. In press. (doi:10.1111/cen.12400)
 - 24 Hardy OT, Hernandez-Pampaloni M, Saffer JR, Scheuermann JS, Ernst LM, Freifelder R, Zhuang H, MacMullen C, Becker S, Adzick NS *et al.* Accuracy of [¹⁸F]fluorodopa positron emission tomography for diagnosing and localizing focal congenital hyperinsulinism. *Journal of Clinical Endocrinology and Metabolism* 2007 **92** 4706–4711. (doi:10.1210/jc.2007-1637)
 - 25 Stanley CA, Thornton PS, Ganguly A, MacMullen C, Underwood P, Bhatia P, Steinkrauss L, Wanner L, Kaye R, Ruchelli E *et al.* Preoperative evaluation of infants with focal or diffuse congenital hyperinsulinism by intravenous acute insulin response tests and selective pancreatic arterial calcium stimulation. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 288–296. (doi:10.1210/jc.2003-030965)
 - 26 de Lonlay-Debeney P, Poggi-Travert F, Fournet JC, Sempoux C, Vici CD, Brunelle F, Touati G, Rahier J, Junien C, Nihoul-Fekete C *et al.* Clinical features of 52 neonates with hyperinsulinism. *New England Journal of Medicine* 1999 **340** 1169–1175. (doi:10.1056/NEJM199904153401505)
 - 27 Blomberg BA, Moghbel MC, Saboury B, Stanley CA & Alavi A. The value of radiologic interventions and (18)F-DOPA PET in diagnosing and localizing focal congenital hyperinsulinism: systematic review and meta-analysis. *Molecular Imaging and Biology* 2013 **15** 97–105. (doi:10.1007/s11307-012-0572-0)
 - 28 Macmullen CM, Zhou Q, Snider KE, Tewson PH, Becker SA, Aziz AR, Ganguly A, Shyng SL & Stanley CA. Diazoxide-unresponsive congenital hyperinsulinism in children with dominant mutations of the β -cell sulfonylurea receptor SUR1. *Diabetes* 2011 **60** 1797–1804. (doi:10.2337/db10-1631)
 - 29 Bellanne-Chantelot C, Saint-Martin C, Ribeiro MJ, Vauzy C, Verkarre V, Arnoux JB, Valayannopoulos V, Gobrecht S, Sempoux C, Rahier J *et al.* *ABCC8* and *KCNJ11* molecular spectrum of 109 patients with diazoxide-unresponsive congenital hyperinsulinism. *Journal of Medical Genetics* 2010 **47** 752–759. (doi:10.1136/jmg.2009.075416)
 - 30 Mohnike K, Wieland I, Barthlen W, Vogelgesang S, Empting S, Mohnike W, Meissner T & Zenker M. Clinical and genetic evaluation of patients with K channel mutations from the German registry for congenital hyperinsulinism. *Hormone Research in Paediatrics* 2014 **81** 156–168. (doi:10.1159/000356905)
 - 31 Meintjes M, Endozo R, Dickson J, Erlandsson K, Hussain K, Townsend C, Menezes L & Bomanji J. ¹⁸F-DOPA PET enhanced CT imaging for congenital hyperinsulinism: initial UK experience from a technologist's perspective. *Nuclear Medicine Communications* 2013 **34** 601–608. (doi:10.1097/MNM.0b013e32832836069d0)
 - 32 Flanagan SE, Xie W, Caswell R, Damhuis A, Vianey-Saban C, Akcay T, Darendeliler F, Bas F, Guven A, Siklar Z *et al.* Next-generation sequencing reveals deep intronic cryptic *ABCC8* and *HADH* splicing founder mutations causing hyperinsulinism by pseudoexon activation. *American Journal of Human Genetics* 2013 **92** 131–136. (doi:10.1016/j.ajhg.2012.11.017)
 - 33 Huopio H, Reimann F, Ashfield R, Komulainen J, Lenko HL, Rahier J, Vauhkonen I, Kere J, Laakso M, Ashcroft F *et al.* Dominantly inherited hyperinsulinism caused by a mutation in the sulfonylurea receptor type 1. *Journal of Clinical Investigation* 2000 **106** 897–906. (doi:10.1172/JCI9804)
 - 34 Schwarz JM, Rodelsperger C, Schuelke M & Seelow D. MutationTaster evaluates disease-causing potential of sequence alterations. *Nature Methods* 2010 **7** 575–576. (doi:10.1038/nmeth0810-575)
 - 35 Choi Y, Sims GE, Murphy S, Miller JR & Chan AP. Predicting the functional effect of amino acid substitutions and indels. *PLoS ONE* 2012 **7** e46688. (doi:10.1371/journal.pone.0046688)
 - 36 Hussain K, Flanagan SE, Smith VV, Ashworth M, Day M, Pierro A & Ellard S. An *ABCC8* gene mutation and mosaic uniparental isodisomy resulting in atypical diffuse congenital hyperinsulinism. *Diabetes* 2008 **57** 259–263. (doi:10.2337/db07-0998)
 - 37 Flanagan S, Damhuis A, Banerjee I, Rokicki D, Jefferies C, Kapoor R, Hussain K & Ellard S. Partial *ABCC8* gene deletion mutations causing diazoxide-unresponsive hyperinsulinaemic hypoglycaemia. *Pediatric Diabetes* 2012 **13** 285–289. (doi:10.1111/j.1399-5448.2011.00821.x)
 - 38 Banerjee I, Skae M, Flanagan SE, Rigby L, Patel L, Didi M, Blair J, Ehtisham S, Ellard S, Cosgrove KE *et al.* The contribution of rapid K_{ATP} channel gene mutation analysis to the clinical management of children with congenital hyperinsulinism. *European Journal of Endocrinology* 2011 **164** 733–740. (doi:10.1530/EJE-10-1136)
 - 39 Kapoor RR, Flanagan SE, James CT, McKiernan J, Thomas AM, Harmer SC, Shield JP, Tinker A, Ellard S & Hussain K. Hyperinsulinaemic hypoglycaemia and diabetes mellitus due to dominant *ABCC8/KCNJ11* mutations. *Diabetologia* 2011 **54** 2575–2583. (doi:10.1007/s00125-011-2207-4)
 - 40 Thornton PS, MacMullen C, Ganguly A, Ruchelli E, Steinkrauss L, Crane A, Aguilar-Bryan L & Stanley CA. Clinical and molecular characterization of a dominant form of congenital hyperinsulinism caused by a mutation in the high-affinity sulfonylurea receptor. *Diabetes* 2003 **52** 2403–2410. (doi:10.2337/diabetes.52.9.2403)
 - 41 Pinney SE, MacMullen C, Becker S, Lin YW, Hanna C, Thornton P, Ganguly A, Shyng SL & Stanley CA. Clinical characteristics and biochemical mechanisms of congenital hyperinsulinism associated with dominant K_{ATP} channel mutations. *Journal of Clinical Investigation* 2008 **118** 2877–2886. (doi:10.1172/JCI35414)

- 42 Lord K, Dzata E, Snider KE, Gallagher PR & De Leon DD. Clinical presentation and management of children with diffuse and focal hyperinsulinism: a review of 223 cases. *Journal of Clinical Endocrinology and Metabolism* 2013 **98** E1786–E1789. (doi:10.1210/jc.2013-2094)
- 43 Bertrand J, Caquard M, Arnoux JB, Laborde K, Velho G, Verkarre V, Rahier J, Brunelle F, Nihoul-Fekete C, Saudubray JM *et al.* Glucose metabolism in 105 children and adolescents after pancreatectomy for congenital hyperinsulinism. *Diabetes Care* 2012 **35** 198–203. (doi:10.2337/dc11-1296)
- 44 Ismail D, Kapoor RR, Smith VV, Ashworth M, Blankenstein O, Pierro A, Flanagan SE, Ellard S & Hussain K. The heterogeneity of focal forms of congenital hyperinsulinism. *Journal of Clinical Endocrinology and Metabolism* 2012 **97** E94–E99. (doi:10.1210/jc.2011-1628)
- 45 Yan FF, Lin YW, MacMullen C, Ganguly A, Stanley CA & Shyng SL. Congenital hyperinsulinism associated *ABCC8* mutations that cause defective trafficking of ATP-sensitive K⁺ channels: identification and rescue. *Diabetes* 2007 **56** 2339–2348. (doi:10.2337/db07-0150)

Received 1 May 2014

Revised version received 28 July 2014

Accepted 8 September 2014