Clinical and genetic characterization of congenital hyperinsulinism in Spain

R Martínez^{1,*}, C Fernández-Ramos^{2,*}, A Vela³, T Velayos¹, A Aguayo¹, I Urrutia¹, I Rica³ and L Castaño¹ on behalf of the Spanish Congenital Hyperinsulinism Group[†]

¹Endocrinology and Diabetes Research Group, BioCruces Health Research Institute, Cruces University Hospital, CIBERDEM, CIBERER, UPV-EHU, Barakaldo, Spain, ²Pediatric Endocrinology Section, Basurto University Hospital, BioCruces Health Research Institute, UPV/EHU, Bilbao, Spain, ³Pediatric Endocrinology Section, Cruces University Hospital, BioCruces Health Research Institute, CIBERDEM, CIBERER, UPV/EHU, Barakaldo, Spain

* (R Martínez and C Fernández-Ramos contributed equally to this work)

[†] (Details of the Spanish Congenital Hyperinsulinism Group are given in the acknowledgements section)

Correspondence should be addressed to L Castaño

Email
lcastano@osakidetza.net

Abstract

Context: Congenital hyperinsulinism (CHI) is a clinically and genetically heterogeneous disease characterized by severe hypoglycemia caused by inappropriate insulin secretion by pancreatic β -cells.

Objective: To characterize clinically and genetically CHI patients in Spain.

Design and methods: We included 50 patients with CHI from Spain. Clinical information was provided by the referring clinicians. Mutational analysis was carried out for *KCNJ11*, *ABCC8*, and *GCK* genes. The *GLUD1*, *HNFA4*, *HNFA1A*, *UCP2*, and *HADH* genes were sequenced depending on the clinical phenotype.

Results: We identified the genetic etiology in 28 of the 50 CHI patients tested: 21 had a mutation in K_{ATP} channel genes (42%), three in *GLUD1* (6%), and four in *GCK* (8%). Most mutations were found in *ABCC8* (20/50). Half of these patients (10/20) were homozygous or compound heterozygous, with nine being unresponsive to diazoxide treatment. The other half had heterozygous mutations in *ABCC8*, six of them being unresponsive to diazoxide treatment and four being responsive to diazoxide treatment. We identified 22 different mutations in the K_{ATP} channel genes, of which ten were novel. Notably, patients with *ABCC8* mutations were diagnosed earlier, with lower blood glucose levels and required higher doses of diazoxide than those without a genetic diagnosis.

Conclusions: Genetic analysis revealed mutations in 56% of the CHI patients. *ABCC8* mutations are the most frequent cause of CHI in Spain. We found ten novel mutations in the K_{ATP} channel genes. The genetic diagnosis is more likely to be achieved in patients with onset within the first week of life and in those who fail to respond to diazoxide treatment.

European Journal of
Endocrinology
(2016) **174**, 717–726

Introduction

Congenital hyperinsulinism (CHI) is the most frequent cause of persistent hypoglycemia in infancy. It is a rare genetic disorder characterized by severe hypoglycemia caused by inappropriate insulin secretion by pancreatic β -cells (1, 2).

CHI is a heterogeneous disease with differences in clinical presentation, histology, genetics, and response to treatment. To date, it has been associated with defects in nine genes. However, the genetic basis is only known

in approximately 45–55% of patients affected by CHI; hence, other mechanisms of the disease need to be determined (3, 4, 5). The most frequent known causes of CHI are recessive or dominant inactivating mutations in the *ABCC8* and *KCNJ11* genes, coding for SUR1 and Kir6.2 subunits of the ATP-dependent potassium channel (K_{ATP}). Other less frequent causes are mutations in glutamate dehydrogenase (*GLUD1*), glucokinase (*GCK*), and short-chain L-3-hydroxyacyl CoA dehydrogenase

Clinical Study	R Martínez, C Fernández-Ramos and others	Congenital hyperinsulinism in Spain	174:6	718
----------------	--	-------------------------------------	-------	-----

(*HADH*) genes, as well as in the *SLC16A1*, *HNF4A*, *HNF1A*, or *UCP2* genes (2, 6).

The majority of patients with recessive *ABCC8* or *KCNJ11* mutations are medically unresponsive to diazoxide treatment, whereas variable phenotypes and response to treatment have been reported for those with dominant *ABCC8* or *KCNJ11* mutations (7, 8, 9, 10). *GLUD1* mutations are associated with mild-to-moderate hyperammonemia, mild hypoglycemia, and good response to diazoxide treatment (11). Mutations in the *GCK* gene are a rare cause of CHI, and the clinical presentation varies from a mild phenotype that is responsive to drug treatment to a severe disease (12).

There are two main clinically indistinguishable histological subtypes in CHI: diffuse and focal. Diffuse forms affect all the β -cells, are genetically heterogeneous, and may be recessively or dominantly inherited. By contrast, focal CHI is sporadic in inheritance and results from two independent events, a paternally inherited heterozygous mutation in *ABCC8* or *KCNJ11* and the somatic loss of the maternal allele in the 11p15 region (loss of heterozygosity) in which only some β -cells are affected (6, 13).

In this paper, we describe the clinical presentation and the molecular genetic characteristics of a group of patients with CHI in Spain and analyze the data for genotype–phenotype correlations.

Subjects and methods

Patients

In this study, we included 50 patients from 47 families (born between 1972 and 2013) referred between January 2005 and September 2013 to the Molecular Genetics Laboratory at BioCruces Health Research Institute (Bizkaia, Spain) for molecular testing of CHI. Patients with CHI secondary to perinatal stress or associated with syndromic forms were excluded. Biological samples were received from several hospitals across Spain. All families were Spanish Caucasian descent except three families who were of Moroccan, Gambian, and Pakistani descent. Consanguinity was reported in four families. Written informed consent was obtained from all the participants or their parents at each hospital involved in the study. The study was approved by each local ethics committee.

Clinical data

The clinical diagnosis of CHI was based on the presence of non-ketotic hypoglycemia with unsuppressed insulin

secretion, low free fatty acid levels, and a high glucose requirement to correct hypoglycemia. Clinical data were provided by the clinicians responsible for these patients in their hospitals of origin. The following variables were studied: sex, gestational age, type of delivery, birth weight, age at presentation, type of symptoms, blood test results (glycemia and insulin), treatment received (diet, drug treatment, and surgery), drug doses used, duration of medical treatment, occurrence of adverse effects, and responsiveness to diazoxide treatment. Blood insulin and glucose concentrations were measured simultaneously at the time of hypoglycemia. Response to diazoxide treatment was considered satisfactory if glycemic control was achieved with doses of oral diazoxide lower than 20 mg/kg/day.

Patients were classified into three groups as a function of age at presentation of hyperinsulinism, assumed to correspond to the onset of clinical signs and symptoms: group A, in the first month of life; group B, between the first month and 2 years of age; and group C from 2 years of age. We calculated the standard deviation scores (SDS) of birth weights considering anthropometric reference data for the Spanish population (14). We considered infants to be large for gestational age if their birth weight was more than 2 SDS above the mean.

Statistical analysis

IBM SPSS Statistics for Windows (21.0 Version; Armonk, NY, USA) was used for statistical analysis. The independent samples *t*-test was used to compare the means of normally distributed data and the Mann–Whitney U test to compare non-normally distributed data. χ^2 or Fisher's exact tests were performed to compare the frequencies of categorical variables. A *P* value < 0.05 was considered to be statistically significant.

Genetic analysis

Genomic DNA was extracted from peripheral blood leukocytes according to the manufacturer's instructions (QIAamp DNA Blood Mini Kit; Qiagen, Germany). Our current routine for new cases with CHI is to sequence *KCNJ11* and *ABCC8* genes first and, if no mutation is found, *GCK* gene is sequenced next. Furthermore, the *GLUD1* gene is sequenced in patients with hyperammonemia. In patients in whom no mutations were identified, the following genes were also sequenced, depending on the clinical phenotype: *HNF4A* (13 cases), *HNF1A* (eight cases), *UCP2* (nine cases), and *HADH* (six cases).

All promoters, coding exons and intron–exon boundaries of the *ABCC8*, *KCNJ11*, *GCK*, *GLUD1*, *HNF4A*, *HNF1A*, and *HADH* genes were amplified by PCR (primer sequences and conditions are available on request from the authors), purified, bidirectionally sequenced using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), and loaded into an ABI 3130xl Genetic Analyzer (Applied Biosystems). The sequences were analyzed and compared with the wild-type published reference sequences (RefSeq) using SeqScape Software v. 2.5 (Applied Biosystems). If applicable, following identification of a mutation in the proband, parents and affected family members were tested to establish the mode of inheritance. For the *ABCC8* gene, the RefSeq used was NM_001287174.1, corresponding to the isoform (1582 amino acids) that incorporates an additional serine residue in exon 17. For the other genes studied, the RefSeqs used were NM_000525.3 for *KCNJ11*, NM_000162.3 for the pancreatic β -cell isoform of *GCK*, NM_005271.3 for *GLUD1*, NM_175914.4 for *HNF4A*, NM_000545.5 for *HNF1A*, and NM_005327.2 for *HADH*.

Genetic variants were searched against dbSNP, 1000 Genomes, ESP, ExAC, Ensembl, and HGMD databases. The functional consequences of the novel missense variants not listed in these databases were analyzed using *in silico* prediction tools: MutationTaster (www.mutationtaster.org), PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>), and SIFT (<http://sift-dna.org>), whereas for splice site variants we used NNSPLICE (http://www.fruitfly.org/seq_tools/splice.html), NetGene2 (<http://www.cbs.dtu.dk/services/NetGene2/>), and HSF (<http://www.umd.be/HSF/>). DNA mutations were named in accordance with the Human Genomic Variation Society guidelines.

MLPA analysis

When sequencing revealed a single heterozygous *ABCC8* mutation, or no causative mutation was found in *ABCC8* or other genes, the *ABCC8* gene was analyzed by multiplex ligation-dependent probe amplification (MLPA). For this, we used the *ABCC8* SALSA P117-B1 MLPA kit (MRC Holland, Amsterdam, The Netherlands) as recommended by the manufacturer. In two patients, microsatellite markers across the chromosome 11p15.1–11p15.5 region were analyzed to search for somatic events (loss of heterozygosity), using DNA extracted from blood and paraffin-embedded pancreatic tissues. Fragments were then separated by capillary electrophoresis (ABI 3130xl Genetic Analyzer) and analyzed using Gene-Mapper, v.4.0 software (Applied Biosystems).

Results

Clinical characteristics

In this study, the patient cohort was composed of 50 patients with CHI from Spain. Their general clinical and biochemical characteristics are summarized in Table 1. Overall, 64% of patients (30/47) responded to diazoxide treatment. The most common adverse effect was hypertrichosis, observed in 28% of patients. Among the non-responders to diazoxide treatment, 11 patients required pancreatectomy within 1–6 months of age, and these corresponded to eight individuals with *ABCC8* mutation and three with an unknown genetic diagnosis.

Group A patients had lower blood glucose levels (22.2 ± 10 vs 36.3 ± 8.9 mg/dL; $P=0.001$), higher blood insulin levels (37 ± 46 vs 13.5 ± 11 μ U/mL; $P=0.04$), and a lower rate of response to diazoxide treatment (44% (11/25) vs 80% (16/20); $P=0.018$) compared with group B patients. The 27% of group A patients were asymptomatic at diagnosis (27% (7/26) vs 0% (0/21); $P<0.01$), hypoglycemia being detected as part of routine monitoring in neonatal care. Furthermore, in group A, a higher percentage of patients developed hypotonia (46% (12/26) vs 14% (3/21); $P=0.02$) and a lower percentage had convulsions (19% (5/26) vs 57% (12/21); $P=0.014$) than in group B. No other significant differences were found between the groups.

Table 1 Clinical characteristics of patients with CHI at diagnosis.

Clinical characteristics	Results (n = 50)
Females, n (%)	32 (64%)
Mutation positive, n (%)	28 (56%)
Gestational age, weeks (range)	38 $\pm$ 2.5 (31–41)
Birth weight, g	3570 $\pm$ 746
Large for gestational age, n (%)	17 (34%)
Dystocia	28%
Age at presentation, n (%)	
Within 1 first month of life (group A)	26 (52%)
Between 1 month and 2 years (group B)	21 (42%)
From 2 years (group C)	3 (6%)
Blood glucose, mg/dL	29 $\pm$ 12
Serum insulin, μ U/mL	26 $\pm$ 37
Symptomatology	
Seizures	34%
Hypotonia	30%
Loss of consciousness	28%
Patients treated with diazoxide, n (%)	47 (94%)
Diazoxide dose, mg/kg/day (range)	15 $\pm$ 5.2 (7.5–20)
Diazoxide responsive, n (%)	30 (64%)

Data are mean $\pm$ s.d. or percentage.

Table 2 Clinical and genetic characteristics of CHI patients with *ABCC8* and *KCNJ11* mutations. All sequence information is based on the *ABCC8* reference sequence NM_001287174.1, including the alternate exon 17 that contains an additional amino acid, and on the *KCNJ11* reference sequence NM_000525.3.

Patient	Location	Nucleotide change	Protein change	Mutation type	Genetic status	Inheritance	Previously reported	LGA	Age at presentation	Diazoxide responsive	Management
<i>ABCC8 mutations</i>											
1 ^a	Exon 13	c.1829T>G	p.Leu610Arg	Missense	C-htz	Paternal	Novel	Yes	7 days	No	Intensive feeding
	Intron 32	c.3991+2T>C	p.?	Splicing	C-htz	Maternal	Novel	No	2.5 months	No	Intensive feeding
2 ^a	Exon 13	c.1829T>G	p.Leu610Arg	Missense	C-htz	Paternal	Novel	No	2.5 months	No	Intensive feeding
	Intron 32	c.3991+2T>C	p.?	Splicing	C-htz	Maternal	Novel	No	36 h	No	Total pancreatectomy
3	Exon 12	c.1792C>T	p.Arg598*	Nonsense	C-htz ^f	NM	(5, 18, 19, 20, 34)	Yes	20 h	No	Octreotide/nifedipine/partial pancreatectomy. Continuing DZX + Octreotide
4	Exon 36	c.4352T>C	p.Leu1451Pro	Missense	C-htz	Maternal	Novel	Yes	1 h	Yes	Continuing DZX
	Exon 8	c.1317G>A	p.Trp439*	Missense	C-htz	Maternal	Novel	Yes	20 h	No	Octreotide/nifedipine/partial pancreatectomy. Continuing DZX + Octreotide
5	Intron 38	c.4612-2A>T	p.?	Splicing	C-htz	Paternal	(19)	Yes	1 h	Yes	Continuing DZX
	Exon 12	c.1732_1746dup15	p.Ala578_Leu582dup5	In frame duplication	C-htz	Maternal	(5, 19, 20)	Yes	1 h	Yes	Continuing DZX
6	Exon 39	c.4723G>C	p.Ala1575Pro	Missense	Hmz	Paternal	Novel	No	4 h	No	Intensive feeding
	Exon 4	c.453delC	p.Ile152fs	Frameshift	Hmz	Both parents	Novel	No	4 h	No	Intensive feeding
7	Exon 23	c.2800C>T	p.Arg934*	Nonsense	Hmz	Both parents	(4, 5, 19)	Yes	8 h	No	Responsive to octreotide and nifedipine/intensive feeding
8	Exon 23	c.2800C>T	p.Arg934*	Nonsense	Hmz	Both parents	(4, 5, 19)	No	15 h	No	Responsive to octreotide/deceased
9 ^b	Intron 32	c.3991+2T>C	p.?	Splicing	Hmz	Both parents	Novel	Yes	2 months	No	Near total pancreatectomy (90%)
10 ^b	Intron 32	c.3991+2T>C	p.?	Splicing	Hmz	Both parents	Novel	Yes	2 months	No	Near total pancreatectomy (90%)
11	Exon 24	c.2837_2840delAGAG ^s	p.Arg947fs	Frameshift	Htz	Paternal ^c	(5, 35)	Yes	24 h	No	Responsive to octreotide/focal pancreatectomy
12	Exon 29	c.3577delG	p.Asp1193fs	Frameshift	Htz	Paternal ^c	(5, 20, 36)	No	28 h	No	Octreotide/subtotal pancreatectomy
13	Exon 29	c.3626_3628delAAG	p.Glu1209del	In frame deletion	Htz	ND	Novel	No	20 h	No	Subtotal pancreatectomy
14	Exon 5	c.742C>T	p.Arg248*	Nonsense	Htz	Paternal	(4, 5, 19, 20, 37, 38)	No	8 h	No	Responsive to octreotide/deceased
15	Exon 30	c.3751C>T	p.Arg1251*	Nonsense	Htz	Paternal	(4, 5, 18, 19, 20)	No	2 h	No	Intensive feeding
16	Exon 37	c.4519G>T	p.Glu1507*	Nonsense	Htz	Paternal	Novel	Yes	4 h	No	Near total pancreatectomy (95%)

Clinical Study			R Martínez, C Fernández-Ramos and others			Congenital hyperinsulinism in Spain			174:6		721	
17	Exon 6	c.928G>A	p.Asp310Asn	Missense	Htz	Paternal	(5, 8, 19)	No	7 h	Yes	DZX for 9 days/remission	Dextrinomaltese supplements/transient
18	Exon 23	c.2701A>T	p.Ile901Phe	Missense	Htz	Paternal	Novel	No	30 h	Yes	DZX for 7 months/remission	
19	Exon 33	c.4060T>A	p.Tyr1354Asn	Missense	Htz	Maternal	Novel	Yes	22 h	Yes	Continuing DZX low dose	
20	Exon 37	c.4546A>G	p.Thr1516Ala	Missense	Htz	De novo	(39)	Yes	2 h	Yes	DZX for 4 years/remission	
KCNJ11 mutations												
21	Exon 1	c.295G>A	p.Asp99Asn	Missense	C-htz	Maternal	Novel	Yes	4 days	NA		
	Exon 1	c.761C>T	p.Pro254Leu	Missense		Paternal	(40)					
LGA, large for gestational age; Hmz, homozygous; C-htz, compound heterozygous; Htz, heterozygous; DZX, diazoxide. NA, not applicable, as had transient hypoglycemia that did not require treatment with diazoxide; ND, not determined (no sample was available for testing from either parent); NM, no material (no samples were available for testing).												
[†] Presumed compound heterozygote. Paternal sample not available for testing; [‡] Reported as c.2835_2838delAGAG.												
^a Belong to the same family; ^b Belong to the same family; ^c A somatic deletion in <i>ABCC8</i> (loss of heterozygosity) was identified in paraffin-embedded pancreatic tissue.												

Genetic characteristics

The genetic etiology was identified in 56% (28/50) of the patients with CHI: 21 patients having a mutation in the *K_{ATP}* channel genes, three in *GLUD1*, and four in *GCK*.

ABCC8 and *KCNJ11* mutations

Considering the 21 out of 50 patients tested (42%) in whom we identified a *K_{ATP}* channel defect, we found mutations in the *ABCC8* gene in all but one case (20/21) (Table 2). In this exception, a patient presenting with transient CHI, we found a compound heterozygous mutation in the *KCNJ11* gene, which had been inherited from healthy parents. Altogether, 22 different mutations were identified in the *K_{ATP}* channel genes, among which ten were novel. *In silico* analysis of these novel mutations predicts a pathogenic effect, as presented in Table 3. These novel mutations were not present in public databases, including the Exome Aggregation Consortium (ExAC) database that has data from more than 60 000 individuals (<http://exac.broadinstitute.org/>). An exception is the splice site mutation c.3991+2T>C in *ABCC8* gene, which is present in two (0.00165%) of 121 178 alleles in ExAC database (in heterozygosis), indicating that this is a very rare variant. This mutation was present in our patients with CHI in a recessive manner (homozygosis or compound heterozygosis), and the *in silico* software predicts the loss of the splice site, indicating, as a whole, the pathogenic effect of this variant in a recessive manner.

Complete *ABCC8* sequencing revealed biallelic recessive mutations in ten of the patients. Among them, five had a homozygous mutation (two from consanguineous families) and five a compound heterozygous mutation (Table 2). All these patients inherited the mutation from clinically unaffected parents. Two mutations, the novel splice-donor mutation c.3991+2T>C and the nonsense mutation p.Arg934*, were each identified in two unrelated families. Only one patient with a biallelic mutation (patient 5) responded to diazoxide treatment.

Single heterozygous mutations in the *ABCC8* gene were identified in the other ten patients (Table 2). MLPA of the *ABCC8* gene did not disclose any partial or whole gene deletions in these patients. Of these patients, six were unresponsive to diazoxide treatment. Parental samples were available for testing in five cases, all of them showed a paternally inherited mutation from an asymptomatic father. Among them, we only had access to tissue samples in two cases, in which we also

Genetic characteristics

The genetic etiology was identified in 56% (28/50) of the patients with CHI: 21 patients having a mutation in the K_{ATP} channel genes, three in *GLUD1*, and four in *GCK*.

ABCC8 and *KCNJ11* mutations

Considering the 21 out of 50 patients tested (42%) in whom we identified a K_{ATP} channel defect, we found mutations in the *ABCC8* gene in all but one case (20/21) (Table 2). In this exception, a patient presenting with transient CHI, we found a compound heterozygous mutation in the *KCNJ11* gene, which had been inherited from healthy parents. Altogether, 22 different mutations were identified in the K_{ATP} channel genes, among which ten were novel. *In silico* analysis of these novel mutations predicts a pathogenic effect, as presented in Table 3. These novel mutations were not present in public databases, including the Exome Aggregation Consortium (ExAC) database that has data from more than 60000 individuals (<http://exac.broadinstitute.org/>). An exception is the splice site mutation c.3991+2T>C in *ABCC8* gene, which is present in two (0.00165%) of 121178 alleles in ExAC database (in heterozygosity), indicating that this is a very rare variant. This mutation was present in our patients with CHI in a recessive manner (homozygosity or compound heterozygosity), and the *in silico* software predicts the loss of the splice site, indicating, as a whole, the pathogenic effect of the variant in a recessive manner.

Complete *ABCC8* sequencing revealed biallelic recessive mutations in ten of the patients. Among them, five had a homozygous mutation (two from consanguineous families) and five a compound heterozygous mutation (Table 2). All these patients inherited the mutations from clinically unaffected parents. Two mutations, the novel splice-donor mutation c.3991+2T>C and the nonsense mutation p.Arg934*, were each identified in two unrelated families. Only one patient with a biallelic mutation (patient 5) responded to diazoxide treatment.

Single heterozygous mutations in the *ABCC8* gene were identified in the other ten patients (Table 2). MLPA of the *ABCC8* gene did not disclose any partial or whole gene deletions in these patients. Of these patients, six were unresponsive to diazoxide treatment. Parental samples were available for testing in five cases, all of them showed a paternally inherited mutation from an asymptomatic father. Among them, we only had access to tissue samples in two cases, in which we also

Clinical Study	R Martínez, C Fernández-Ramos and others	Congenital hyperinsulinism in Spain	174:6	722
----------------	--	-------------------------------------	-------	-----

Table 3 Characteristics and *in silico* prediction of the deleterious effect of novel mutations detected in patients with CHI. All sequence information is based on the *ABCC8* reference sequence NM_001287174.1, including the alternate exon 17 that contains an additional amino acid, on the *KCNJ11* reference sequence NM_000525.3, and on the *GCK* reference sequence NM_000162.3.

Gene	Mutation			Prediction algorithm		
	Nucleotide change	Protein change	Location	SIFT	PolyPhen-2	MutationTaster
<i>KCNJ11</i>	c.295G>A	p.Asp99Asn	Exon 1	Predict not tolerated	Probably damaging	Disease causing
<i>ABCC8</i>	c.453delC	p.Ile152fs	Exon 4	–	–	–
<i>ABCC8</i>	c.1317G>A	p.Trp439*	Exon 8	–	–	–
<i>ABCC8</i>	c.1829T>G	p.Leu610Arg	Exon 13	Predict not tolerated	Probably damaging	Disease causing
<i>ABCC8</i>	c.2701A>T	p.Ile901Phe	Exon 23	Predict not tolerated	Possibly damaging	Disease causing
<i>ABCC8</i>	c.3626_3628delAAG	p.Glu1209del	Exon 29	–	–	Disease causing
<i>ABCC8</i>	c.4060T>A	p.Tyr1354Asn	Exon 33	Predict not tolerated	Probably damaging	Disease causing
<i>ABCC8</i>	c.4519G>T	p.Glu1507*	Exon 37	–	–	–
<i>ABCC8</i>	c.4723G>C	p.Ala1575Pro	Exon 39	Predict tolerated	Probably damaging	Disease causing
<i>GCK</i>	c.297G>T	p.Trp99Cys	Exon 3	Predict not tolerated	Probably damaging	Disease causing
<i>ABCC8</i>	c.3991+2T>C	p.?	Intron 32	NNSPLICE Donor lost	NetGene2 Donor lost	HSF Site broken

identified a somatic deletion in the *ABCC8* maternal allele in pancreatic tissue, one described as focal (patient 11) and the other, in whom the surgery had been performed in 1997, of unknown form (patient 12). In the four diazoxide-responsive patients, the origins of the mutations were diverse: two of paternal origin (from an asymptomatic father), one of maternal origin, and one *de novo*. Three patients (patients 17, 18, and 20) had non-permanent CHI requiring diazoxide treatment for a variable period of time ranging from days to 4 years. Patient 20 has progressed to diabetes later. This patient needed diazoxide treatment during the fourth year of age due to recurrent hypoglycemia episodes. Hyperglycemia was observed later because of postprandial glucose controls at home. An OGTT was done and diabetes was diagnosed with HbA1C levels between 6 and 7%, negative anti-pancreatic autoantibodies, and normal C-peptide levels. However, patient 19 carrying the unreported p.Tyr1354Asn heterozygous *ABCC8* mutation still required diazoxide administration but at low doses and had inherited the mutation from her mother and grandmother, both were diagnosed with diabetes.

GLUD1 and *GCK* mutations

In the analysis of *GLUD1* gene, dominant missense mutations were identified in three patients who manifested CHI symptoms between 2 and 7 months, had hyperammonemia, and were responsive to diazoxide treatment (Table 4). A *de novo* mutation had arisen in two cases, and in the other, the mutation had been inherited from an affected mother (the phenotypic characteristics of this patient (patient 23) and his mother have been described previously (15)). Only in two sisters

with ammonium levels above 200 $\mu\text{mol/L}$ we have not identified a mutation neither in *GLUD1* nor in *HADH*. We identified four heterozygous missense *GCK* activating mutations (Table 4), one of which has not previously been reported (Table 3). Only one of these mutations was inherited from an affected mother who had required pancreatectomy, while the affected child had a favorable course. This patient was diagnosed before 24 h of age; however, the other three were diagnosed after 3 years of age (Table 4), and all responded to diazoxide treatment.

Genotype–phenotype correlation

In all probands in group A ($n=26$), the onset of hypoglycemia occurred within the first week after birth. Among this group, 73% (19/26) of patients had a mutation, all being mutations in the K_{ATP} channel genes except one case with a mutation in *GCK*. Comparing patients with and without a genetic diagnosis, the only statistically significant difference detected was the percentage of patients who were responsive to diazoxide treatment (33% (6/18) vs 70% (5/7); $P<0.01$).

In group B ($n=21$), 28.6% of patients ($n=6$) had a genetic diagnosis. Three of them were found to have a *GLUD1* mutation and the other three an *ABCC8* mutation, with an age of 4.8 months and 2 months, respectively, at CHI diagnosis. Patients with an unknown genetic diagnosis (71.4%, 15/21) presented with signs and symptoms of CHI at 8 ± 3.8 months of age and the majority (87%) responded to diazoxide treatment.

In group C, all the three cases had a *GCK* mutation. Their clinical characteristics were very different, with mild symptomatology and moderate hypoglycemia, and

Clinical Study	R Martínez, C Fernández-Ramos and others	Congenital hyperinsulinism in Spain	174:6	723
----------------	--	-------------------------------------	-------	-----

Table 4 Clinical and genetic characteristics of CHI patients with dominant *GLUD1* and *GCK* mutations. All sequence information is based on the *GLUD1* reference sequence NM_005271.3, with nucleotide and amino acid numbers starting with the A of the ATG initiation codon. (In parentheses, the mutation is described with amino acid numbers beginning at the start of the mature protein, as is widely used in the literature.) The *GCK* sequence information is based on reference sequence NM_000162.3.

Patient	Gene	Location	Nucleotide change	Protein change	Mutation type	Genetic status	Inheritance	Age at presentation	Management	Described
22	<i>GLUD1</i>	Exon 6	c.820C>T	p.Arg274Cys (p.Arg221Cys)	Missense	Htz	<i>De novo</i>	7 months	DZX/continuing with frequent feeding	(5, 11, 32)
23	<i>GLUD1</i>	Exon 7	c.965G>A	p.Arg322His (p.Arg269His)	Missense	Htz	Maternal	6 months	Continuing DZX	(5, 11, 32)
24	<i>GLUD1</i>	Exon 11	c.1493C>T	p.Ser498Leu (p.Ser445Leu)	Missense	Htz	<i>De novo</i>	2 months	Continuing DZX	(5, 32, 33)
25	<i>GCK</i>	Exon 2	c.194C>T	p.Thr65Ile	Missense	Htz	<i>De novo</i>	3.7 years	Continuing DZX	(30)
26	<i>GCK</i>	Exon 3	c.271G>T	p.Val91Leu	Missense	Htz	Maternal	20 h	Continuing DZX	(41)
27	<i>GCK</i>	Exon 3	c.297G>T	p.Trp99Cys	Missense	Htz	NM	25 years	DZX/continuing with frequent feeding	Novel
28	<i>GCK</i>	Exon 10	c.1324G>A	p.Glu442Lys	Missense	Htz	Paternal	5 years	DZX/lanreotide continuing DZX	(42)

Htz, heterozygous; DZX, diazoxide; NM, no material (no samples were available for testing).

therefore, the diagnosis was made even years after the onset of the signs and symptoms of CHI.

Patients with mutations in the K_{ATP} channel genes were diagnosed earlier (10.7 ± 22.8 vs 164 ± 148 days; $P < 0.01$), had lower blood glucose levels (20.4 ± 8.8 vs 33.7 ± 11 mg/dL; $P < 0.01$), required higher doses of diazoxide (18.5 ± 10.9 vs 12.2 ± 5.6 mg/kg/day; $P < 0.01$), and had lower rates of treatment success (25% (5/20) vs 86% (19/22); $P < 0.01$) than those without a genetic diagnosis.

Discussion

CHI is clinically and genetically a heterogeneous disease with transient and persistent forms. In our study, a genetic diagnosis was obtained in 56% of patients with CHI tested, similar to figures reported in other studies (4, 16, 17, 18), although slightly lower than that found in the only previous study in a Spanish population (19). *ABCC8* inactivating mutations are the leading genetic cause of CHI in our patients. This study has identified ten novel mutations in the K_{ATP} channel genes.

Clinically, we found that patients with CHI secondary to the K_{ATP} channel gene mutations had more severe disease, in line with other data in the literature (6). They had an earlier onset of signs and symptoms, a greater degree of hypoglycemia, and a lower rate of response to treatment.

The likelihood of finding genetic alterations decreases with age at presentation and is lower among individuals

who respond to treatment, as has been reported recently (4, 5, 20). These trends could be explained by the existence of alterations in genes related to CHI that are not known yet and that could cause milder types of hypoglycemia than the currently identified genes. Another possibility is that a burden of hypomorphic mutations or rare variants in several genes could predispose to hyperinsulinism in an individual and may have a role in leading to less severe disease and contribute to the etiology of CHI.

It has been described that hypoglycemia may resolve in as many as a half of newly diagnosed children with CHI (21). A lack of known mutations seems to make remission more likely, ranging from 44% in an Italian cohort (17) to 88% in the study by Banerjee *et al.* (21), and to date 41% have remitted in our series. The variation in the rate may be due to the different follow-up periods across the series. In any case, we should not suspect an incorrect diagnosis, as remission is possible in a variable period of time in individuals with genetic alterations.

In our cohort, half of the patients with *ABCC8* mutations had a biallelic mutation, had a severe phenotype, and did not respond to diazoxide treatment, as has been described in the literature (4, 5, 20). An exception within this group is the patient with compound heterozygous mutation in *ABCC8* (patient 5) who did respond to diazoxide treatment. This patient had a p.Ala578_Leu582dup mutation in the paternal allele, a mutation that has been described to be associated with different situations in two patients with

Clinical Study	R Martínez, C Fernández-Ramos and others	Congenital hyperinsulinism in Spain	174:6	724
----------------	--	-------------------------------------	-------	-----

the mutation in heterozygosis and paternally inherited, who had a severe phenotype and did not respond to diazoxide treatment (5, 20), and in one patient with a compound heterozygous mutation, who was managed with continuous feeding (19). In the maternal allele, our patient has a novel missense mutation p.Ala1575Pro, and *in silico* analysis showed discrepant results (Table 3). Although the majority of biallelic mutations result in diazoxide-unresponsive CHI, some cases have been described with compound heterozygous mutations associated with a diazoxide-responsive CHI (22, 23, 24).

We identified ten heterozygous *ABCC8* mutations. These patients had a mixed phenotype. Arya *et al.* (24) recently evaluated a cohort of CHI patients with paternally inherited heterozygous *ABCC8* mutations, highlighting the clinical and histological heterogeneity of this type of CHI. We showed seven patients to have paternally inherited heterozygous mutations from an asymptomatic father. Of them, four were pancreatectomized but only one was reported to have focal hyperinsulinism. Paternally inherited monoallelic mutations may be recessively associated with somatic loss of the maternal 11p allele linked to a focal CHI, but also with a dominant mutation and diffuse disease.

We found four patients with *ABCC8* heterozygous mutations and diazoxide-responsive CHI, suggestive of a dominant mutation. Two patients inherited the mutation from an unaffected father, one of these patients having the p.Asp310Asn mutation that Pinney *et al.* (7) reported to cause mild, dominantly inherited CHI and having been also inherited from an asymptomatic father. A variable penetrance in dominant mutations has been described previously (7, 9, 10, 25, 26), with adult carriers of a dominant mutation often being asymptomatic. In three patients with *ABCC8* heterozygous mutations who were responsive to diazoxide treatment, the hyperinsulinism resolved after several months. The transient nature of CHI in these patients agrees with previous reports (10, 17, 21). Of these patients, one (patient 20) had transient hypoglycemia that progressed to diabetes later in childhood. Another interesting case is the patient with the novel heterozygous *ABCC8* mutation p.Tyr1354Asn (patient 19), who inherited the mutation from her mother and grandmother, both of whom were diagnosed with diabetes. Progression from hypoglycemia to diabetes has been reported by others authors (8, 10) and has been observed in long-term follow-up of non-pancreatectomized CHI patients with mutations in *ABCC8* (27). Furthermore, various authors have observed diabetes in adult *ABCC8* mutation carriers (8, 10); however, it remains unclear

whether these dominant *ABCC8* gene mutations predispose individuals to diabetes mellitus in adulthood (7). The first dominant heterozygous inactivating *ABCC8* mutation reported, causing a mild form of CHI, was p.Glu1506Lys (p.Glu1507Lys according to reference sequence NM_001287174.1); the authors also reported a loss of insulin secretory capacity in early adulthood and an increased risk of diabetes in middle age in adult carriers (8). This mutation has also been reported in a Brazilian family with CHI in childhood, which developed gestational diabetes, glucose intolerance, and diabetes in adulthood (28). Kapoor *et al.* (10) have shown a progression from hypoglycemia to diabetes later in life in two individuals with *ABCC8* dominant mutations, p.Gly1479Arg and p.Ala1508Pro, but also gestational diabetes or diabetes in *ABCC8* mutation carriers (p.Ala1537Val, p.Gln1459Glu, p.Leu1431Phe, and p.Ala1508Pro). However, it is also clear that not all adult mutation carriers develop diabetes, as was reported in the study of Pinney *et al.* (7), in which from 16 families with dominant *ABCC8* mutations, only 4/29 adults had diabetes. Recently, it has been observed in CHI mouse model that the homeE1506K mutation in *Abcc8* showed an increased insulin secretion in early life, but reduced secretion later, similar to heterozygous patients with this mutation (29).

The finding of mutations in 83% of patients who did not respond to diazoxide treatment in this series is similar to the pattern reported by other authors (4, 5, 17). However, we would like to emphasize that unlike the widely held belief of non-responsiveness, some carriers of the K_{ATP} channel mutations do respond to diazoxide treatment and can even show spontaneous remission, as mentioned earlier.

In our series, activating mutations in the *GCK* gene are the second cause of CHI, and the severity of symptoms and age at presentation vary significantly among the affected individuals, even within the same family. In this sense, our sample generally corroborates what is described in the literature (12, 30, 31). The three patients in our series with dominant mutations in *GLUD1* had moderate hyperinsulinism together with hyperammonemia, normal birth weight, median age of onset of 5 months, and were responsive to diazoxide treatment, similar to other reported cases (11, 32, 33).

A limitation of this study is that patients were diagnosed and treated at different institutions, as this may have affected the uniformity of assessment data and criteria for the treatment of CHI.

In conclusion, genetic analysis revealed a mutation in 56% of patients. Mutations in *ABCC8* are the most

common cause of CHI in Spain. However, the number of cases of CHI with no genetic diagnosis remains high, presenting a challenge for research in molecular genetics and new sequencing technologies. In this study, we present ten patients with mutations not previously described and some cases with a non-classical clinical course, demonstrating the complexity and heterogeneous nature of hyperinsulinism.

Declaration of interest

The authors declare that there is not conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

This work was supported in part by grants from the Department of Health of the Basque Government (GV2010/111185) and the University of the Basque Country UPV/EHU (IT795-13). TV is supported by a personal research fellowship from the researcher training (FPI) program of the University of the Basque Country (IF/UPV12/135).

Acknowledgements

The authors thank all the members of the affected families for their collaborative participation in this study. They also thank A Etxaniz and G Pérez-Nanclares for technical assistance.

The members of the Spanish Congenital Hyperinsulinism Group include the following individuals and institutions: Hospital Universitario (H.U.) Marqués de Valdecilla, Santander (C Luzuriaga); H.U. General de Albacete (R Ruiz); H.U. Miguel Servet, Zaragoza (G M Lou); H.U. Valme, Sevilla (R Espino); H.U. Son Espases, Palma Mallorca (M Caimari); H.U. del Mar, Barcelona (M Bonet); H.U. Virgen Rocío, Sevilla (A L Gómez-Gila); H. de Mérida, Badajoz (M Fernández-Burriel); H.U. Reina Sofía, IMIBIC, Universidad de Córdoba, Córdoba (M Gil-Campos); H.U. Germans Trias i Pujol, Badalona, Barcelona (M Murillo); H.U. Regional Málaga, Málaga (J P López-Sigüero); H.U. Vall d' Hebron, Barcelona (M Clemente); H.U. La Fe, Valencia (M F Moreno); H. General de Granollers, Barcelona (M V Borrás); H.U. Alcalá de Henares Príncipe de Asturias, Madrid (J Ramírez); H.U. Rafael Méndez, Lorca, Murcia (M J Romero); H.U. Príncipe de Asturias, Alcalá de Henares, Madrid (C Blanco); Instituto de Investigación i+12, H.U. 12 de Octubre-CIBERER Grupo U723, Madrid (M T García-Silva); H.U. Cruces, Barakaldo (A Rodríguez); H.U. Getafe, Madrid (B Sagastizabal); H.U. Virgen de la Arrixaca, Murcia (J M Martos); Instituto de Investigación Sanitaria BioCruces-H.U. Cruces, Barakaldo (M Ortiz-Espejo, I Martínez de LaPiscina and G Pérez-Nanclares); and H.U. Ramón y Cajal, Madrid (M Martínez-Pardo).

References

- 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)
- Rahman SA, Nessa A & Hussain K. Molecular mechanisms of congenital hyperinsulinism. *Journal of Molecular Endocrinology* 2015 **54** R119–R129. (doi:10.1530/JME-15-0016)
- Arnoux JB, Verkarre V, Saint-Martin C, Montravers F, Brassier A, Valayannopoulos V, Brunelle F, Fournet JC, Robert JJ, Aigrain Y *et al*. Congenital hyperinsulinism: current trends in diagnosis and therapy. *Orphanet Journal of Rare Diseases* 2011 **6** 63. (doi:10.1186/1750-1172-6-63)
- 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)
- 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 & Metabolism* 2013 **98** E355–E363. (doi:10.1210/jc.2012-2169)
- Lord K & De León DD. Monogenic hyperinsulinemic hypoglycemia: current insights into the pathogenesis and management. *International Journal of Pediatric Endocrinology* 2013 **2013** 3. (doi:10.1186/1687-9856-2013-3)
- 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 KATP channel mutations. *Journal of Clinical Investigation* 2008 **118** 2877–2886. (doi:10.1172/JCI35414)
- Huopio H, Otonkoski T, Vauhkonen I, Reimann F, Ashcroft F & Laakso M. A new subtype of autosomal dominant diabetes attributable to a mutation in the gene for the sulphonylurea receptor 1. *Lancet* 2003 **361** 301–307. (doi:10.1016/S0140-6736(03)12325-2)
- 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 sulphonylurea receptor SUR1. *Diabetes* 2011 **60** 1797–1804. (doi:10.2337/db10-1631)
- 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)
- Kapoor RR, Flanagan SE, Fulton P, Chakrapani A, Chadeaux B, Ben-Omran T, Banerjee I, Shield JP, Ellard S & Hussain K. Hyperinsulinism-hyperammonemia syndrome: novel mutations in the GLUD1 gene and genotype-phenotype correlations. *European Journal of Endocrinology* 2009 **161** 731–735. (doi:10.1530/EJE-09-0615)
- Cuesta-Muñoz AL, Huopio H, Otonkoski T, Gomez-Zumaquero JM, Nääntö-Salonen K, Rahier J, López-Enriquez S, García-Gimeno MA, Sanz P, Soriguer FC *et al*. Severe persistent hyperinsulinemic hypoglycemia due to a de novo glucokinase mutation. *Diabetes* 2004 **53** 2164–2168. (doi:10.2337/diabetes.53.8.2164)
- Arnoux JB, de Lonlay P, Ribeiro MJ, Hussain K, Blankenshtein O, Mohnike K, Valayannopoulos V, Robert JJ, Rahier J, Sempoux C *et al*. Congenital Hyperinsulinism. *Early Human Development* 2010 **86** 287–294. (doi:10.1016/j.earlhumdev.2010.05.003)
- Carrascosa A, Ferrández A, Yeste D, García-Dihinx J, Romo A, Copil A, Almar J, Salcedo S, Gussinyé M & Bague L. Spanish cross-sectional growth study 2008. Part I: weight and height values in newborns of 26–42 weeks of gestational age. *An Pediatr (Barc)* 2008 **68** 544–551. (doi:10.1157/13123286)
- de las Heras J, Garin I, de Nanclares GP, Aguayo A, Rica I, Castaño L & Vela A. Familial hyperinsulinism-hyperammonemia syndrome in a family with seizures: case report. *Journal of Pediatric Endocrinology and Metabolism* 2010 **23** 827–830. (doi:10.1515/jpem.2010.132)
- Faletra F, Athanasakis E, Morgan A, Biarnés X, Fornasier F, Parini R, Furlan F, Boiani A, Maiorana A, Dionisi-Vici C *et al*. Congenital hyperinsulinism: clinical and molecular analysis of a large Italian cohort. *Gen* 2013 **521** 160–165. (doi:10.1016/j.gene.2013.03.021)
- Sogno Valin P, Proverbio MC, Diceglio C, Gessi A, di Candia S, Mariani B, Zamproni I, Mangano E, Asselta R, Battaglia C *et al*. Genetic analysis of Italian patients with congenital hyperinsulinism of infancy. *Hormone Research in Paediatrics* 2013 **79** 236–242. (doi:10.1159/000350827)
- Mohnike K, Wieland I, Barthlen W, Vogelgesang S, Empting S, Mohnike W, Meissner T & Zenker M. Clinical and genetic evaluation of patients with K_{ATP} channel mutations from the German registry for

Clinical Study	R Martínez, C Fernández-Ramos and others	Congenital hyperinsulinism in Spain	174:6	726
----------------	--	-------------------------------------	-------	-----

- congenital hyperinsulinism. *Hormone Research in Paediatrics* 2014 **81** 156–168. (doi:10.1159/000356905)
- 19 Fernández-Marmiesse A, Salas A, Vega A, Fernández-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)
 - 20 Bellanné-Chantelot C, Saint-Martin C, Ribeiro MJ, Vaury 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)
 - 21 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 KATP 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)
 - 22 Dekel B, Lubin D, Modan-Moses D, Quint J, Glaser B & Meyerovitch J. Compound heterozygosity for the common sulfonylurea receptor mutations can cause mild diazoxide-sensitive hyperinsulinism. *Clinical Pediatrics* 2002 **41** 183–186. (doi:10.1177/000992280204100310)
 - 23 Kumaran A, Kapoor RR, Flanagan SE, Ellard S & Hussain K. Congenital hyperinsulinism due to a compound heterozygous ABCC8 mutation with spontaneous resolution at eight weeks. *Hormone Research in Paediatrics* 2010 **73** 287–292. (doi:10.1159/000284394)
 - 24 Arya VB, Aziz Q, Nessa A, Tinker A & Hussain K. Congenital hyperinsulinism: clinical and molecular characterisation of compound heterozygous ABCC8 mutation responsive to Diazoxide therapy. *International Journal of Pediatric Endocrinology* 2014 **2014** 24. (doi:10.1186/1687-9856-2014-24)
 - 25 Shemer R, Avnon Ziv C, Laiba E, Zhou Q, Gay J, Tunovsky-Babaey S, Shyng SL, Glaser B & Zangen DH. Relative expression of a dominant mutated ABCC8 allele determines the clinical manifestation of congenital hyperinsulinism. *Diabetes* 2012 **61** 258–263. (doi:10.2337/db11-0984)
 - 26 Nessa A, Aziz QH, Thomas AM, Harmer SC, Tinker A & Hussain K. Molecular mechanisms of congenital hyperinsulinism due to autosomal dominant mutations in ABCC8. *Human Molecular Genetics* 2015 **24** 5142–5153. (doi:10.1093/hmg/ddv233)
 - 27 Gussinyer M, Clemente M, Cebrián R, Yeste D, Albusu M & Carrascosa A. Glucose intolerance and diabetes are observed in the long-term follow-up of nonpancreatectomized patients with persistent hyperinsulinemic hypoglycemia of infancy due to mutations in the ABCC8 gene. *Diabetes Care* 2008 **31** 1257–1259. (doi:10.2337/dc07-2059)
 - 28 Vieira TC, Bergamin CS, Gurgel LC & Moisés RS. Hyperinsulinemic hypoglycemia evolving to gestational diabetes and diabetes mellitus in a family carrying the inactivating ABCC8 E1506K mutation. *Pediatric Diabetes* 2010 **11** 505–508. (doi:10.1111/pdi.2010.11.issue-7)
 - 29 Shimomura K, Tusa M, Iberl M, Brereton MF, Kaizik S, Proks P, Lahmann C, Yaluri N, Modi S, Huopio H *et al.* A mouse model of human hyperinsulinism produced by the E1506K mutation in the sulphonylurea receptor SUR1. *Diabetes* 2013 **62** 3797–3806. (doi:10.2337/db12-1611)
 - 30 Gloyn AL, Noordam K, Willemsen MA, Ellard S, Lam WW, Campbell IW, Midgley P, Shiota C, Buettger C, Magnuson MA *et al.* Insights into the biochemical and genetic basis of glucokinase activation from naturally occurring hypoglycemia mutations. *Diabetes* 2003 **52** 2433–2440. (doi:10.2337/diabetes.52.9.2433)
 - 31 Hussain K. Mutations in pancreatic β -cell Glucokinase as a cause of hyperinsulinaemic hypoglycaemia and neonatal diabetes mellitus. *Reviews in Endocrine and Metabolic Disorders* 2010 **11** 179–183. (doi:10.1007/s11154-010-9147-z)
 - 32 Santer R, Kinner M, Passarge M, Superti-Furga A, Mayatepek E, Meissner T, Schneppenheim R & Schaub J. Novel missense mutations outside the allosteric domain of glutamate dehydrogenase are prevalent in European patients with the congenital hyperinsulinism-hyperammonemia syndrome. *Human Genetics* 2001 **108** 66–71. (doi:10.1007/s004390000432)
 - 33 Stanley CA, Lieu YK, Hsu BY, Burlina AB, Greenberg CR, Hopwood NJ, Perlman K, Rich BH, Zammarchi E & Poncz M. Hyperinsulinism and hyperammonemia in infants with regulatory mutations of the glutamate dehydrogenase gene. *New England Journal of Medicine* 1998 **338** 1352–1357. (doi:10.1056/NEJM199805073381904)
 - 34 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)
 - 35 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 & Metabolism* 2004 **89** 288–296. (doi:10.1210/jc.2003-030965)
 - 36 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)
 - 37 Aguilar-Bryan L & Bryan J. Molecular biology of adenosine triphosphate-sensitive potassium channels. *Endocrine Reviews* 1999 **20** 101–135. (doi:10.1210/edrv.20.2.0361)
 - 38 Sandal T, Laborie LB, Brusgaard K, Eide SA, Christesen HB, Søvik O, Njølstad PR & Molven A. The spectrum of ABCC8 mutations in Norwegian patients with congenital hyperinsulinism of infancy. *Clinical Genetics* 2009 **75** 440–448. (doi:10.1111/cge.2009.75.issue-5)
 - 39 Bennett JT, Vasta V, Zhang M, Narayanan J, Gerrits P & Hahn SH. Molecular genetic testing of patients with monogenic diabetes and hyperinsulinism. *Molecular Genetics and Metabolism* 2015 **114** 451–458. (doi:10.1016/j.ymgme.2014.12.304)
 - 40 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 & Metabolism* 2004 **89** 6224–6234. (doi:10.1210/jc.2004-1233)
 - 41 Kassem S, Bhandari S, Rodríguez-Bada P, Motaghedi R, Heyman M, García-Gimeno MA, Cobo-Vuilleumier N, Sanz P, Maclaren NK, Rahier J *et al.* Large islets, beta-cell proliferation, and a glucokinase mutation. *New England Journal of Medicine* 2010 **362** 1348–1350. (doi:10.1056/NEJMc0909845)
 - 42 Barbetti F, Cobo-Vuilleumier N, Dionisi-Vici C, Toni S, Ciampalini P, Massa O, Rodríguez-Bada P, Colombo C, Lenzi L, García-Gimeno MA *et al.* Opposite clinical phenotypes of glucokinase disease: description of a novel activating mutation and contiguous inactivating mutations in human glucokinase (GCK) gene. *Molecular Endocrinology* 2009 **23** 1983–1989. (doi:10.1210/me.2009-0094)

Received 11 January 2016

Revised version received 2 March 2016

Accepted 7 March 2016