CLINICAL STUDY

Clinical and molecular characterisation of 300 patients with congenital hyperinsulinism

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Abstract

Background: Congenital hyperinsulinism (CHI) is a clinically heterogeneous condition. Mutations in eight genes (*ABCC8*, *KCNJ11*, *GLUD1*, *GCK*, *HADH*, *SLC16A1*, *HNF4A* and *HNF1A*) are known to cause CHI.
Aim: To characterise the clinical and molecular aspects of a large cohort of patients with CHI.
Methodology: Three hundred patients were recruited and clinical information was collected before genotyping. *ABCC8* and *KCNJ11* genes were analysed in all patients. Mutations in *GLUD1*, *HADH*, *GCK* and *HNF4A* genes were sought in patients with diazoxide-responsive CHI with hyperammonaemia (*GLUD1*), raised 3-hydroxybutyrylcarnitine and/or consanguinity (*HADH*), positive family history (*GCK*) or when CHI was diagnosed within the first week of life (*HNF4A*).
Results: Mutations were identified in 136/300 patients (45.3%). Mutations in *ABCC8/KCNJ11* were the commonest genetic cause identified ($n=109$, 36.3%). Among diazoxide-unresponsive patients ($n=105$), mutations in *ABCC8/KCNJ11* were identified in 92 (87.6%) patients, of whom 63 patients had recessively inherited mutations while four patients had dominantly inherited mutations. A paternal mutation in the *ABCC8/KCNJ11* genes was identified in 23 diazoxide-unresponsive patients, of whom six had diffuse disease. Among the diazoxide-responsive patients ($n=183$), mutations were identified in 41 patients (22.4%). These include mutations in *ABCC8/KCNJ11* ($n=15$), *HNF4A* ($n=7$), *GLUD1* ($n=16$) and *HADH* ($n=3$).
Conclusions: A genetic diagnosis was made for 45.3% of patients in this large series. Mutations in the *ABCC8* gene were the commonest identifiable cause. The vast majority of patients with diazoxide-responsive CHI (77.6%) had no identifiable mutations, suggesting other genetic and/or environmental mechanisms.

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Introduction

Congenital hyperinsulinism (CHI) is a clinically, genetically, morphologically and functionally heterogeneous condition (1, 2, 3). The age of presentation is variable with the severe forms typically presenting in the neonatal period and the milder forms presenting later in infancy or childhood with recurrent symptoms of hypoglycaemia (4). Symptoms generally develop after a period of fasting or when the child is unwell. However, some forms of CHI demonstrate protein and/or leucine sensitivity and symptoms are manifested or aggravated in the postprandial period following a protein-rich meal rather than a fast (5). Macrosomia is a common feature in infants, reflecting fetal hyperinsulinaemia; however, not all babies with CHI are macrosomic (6).

CHI occurs due to defects in key genes involved in regulating insulin secretion from β-cells. So far, mutations in eight different genes (*ABCC8*, *KCNJ11*, *GLUD1*, *GCK*, *HADH*, *SLC16A1*, *HNF4A* and *HNF1A*) that lead to dysregulated secretion of insulin have been described (7, 8). Recessive and dominantly acting mutations in the genes *ABCC8* and *KCNJ11*, encoding the SUR1 and Kir6.2 subunits of the pancreatic β-cell ATP-sensitive K^+ (K_{ATP}) channel respectively, are the most common known cause of CHI (9, 10, 11, 12). While the majority of patients with K_{ATP} channel mutations are medically unresponsive, a number of cases with dominant *ABCC8* or *KCNJ11* mutation(s) who have responded well to therapy have been reported (13). The molecular basis of recessive inactivating *ABCC8* and *KCNJ11* mutations involves multiple defects in channel

biogenesis and turnover, in channel trafficking from the endoplasmic reticulum and Golgi apparatus to the plasma membrane and alterations in the sensitivity of the channel to both magnesium ATP and ADP (14, 15, 16). However, in dominant *ABCC8/KCNJ11* mutations, channel biogenesis and subunit trafficking seem to be normal but with impaired channel activity (13).

Gain-of-function mutations in the *GLUD1* gene, which encodes for the intra-mitochondrial enzyme glutamate dehydrogenase (GDH), form the molecular and biochemical basis of the hyperinsulinism and hyperammonaemia (HI/HA) syndrome and explain the link between leucine hypersensitivity and the HA (17). Heterozygous activating *GCK* mutations cause CHI, which in most cases is medically responsive but in some cases surgery may be required (18, 19, 20). Loss-of-function mutations in the *HADH* gene are associated with CHI and protein sensitivity but with normal serum ammonia levels (21, 22, 23, 24, 25). In some cases, increased levels of plasma 3-hydroxybutyrylcarnitine are observed. Mutations in the promoter region of *SLC16A1* gene have been described in patients with exercise-induced HI (26, 27).

Mutations in the *HNF4A* gene, encoding the hepatocyte nuclear factor 4 alpha (HNF4A), are a novel cause of transient and persistent hyperinsulinae-mic hypoglycaemia (HH), associated with macrosomia (6). Following a remission of the HI patients develop maturity-onset diabetes of the young type 1, charac-terised by autosomal dominant inheritance and impaired glucose-stimulated insulin secretion from pancreatic β-cells (28). While the majority of patients with *HNF4A* mutations have isolated CHI, a recent study reported a case with an *HNF4A* mutation causing CHI and defects in liver glycogen metabolism and renal tubular transport (8). The same study described *HNF1A* as another cause of CHI.

Histologically, CHI is classified into a diffuse form, where the pancreatic β-cells show increased nuclear size throughout the pancreas and a focal form characterised by the presence of a focal adenomatous hyperplasia confined to a single region of the pancreas (29). Focal adenomatous hyperplasia involves the specific loss of the maternal chromosome 11p15 region (loss of hetero-zygosity (LOH)) and a constitutional mutation of a paternally inherited allele of the genes *ABCC8/KCNJ11* encoding the K_{ATP} channel (30). The LOH results from paternal uniparental disomy of chromosome 11p15.5-11p15.1, which unmasks the paternally inherited K_{ATP} channel mutation at 11p15.1 leading to uncontrolled secretion of insulin. Using 18F-ʟ-DOPA-PET/CT, it is now possible to pre-operatively localise the focal lesion (31). The precise preoperative localisation and limited surgical removal of the focal domain 'cures' the patient. By contrast, patients with diffuse disease may require a near total pancreatectomy, which will have lifelong implications (high risk of diabetes mellitus and pancreatic exocrine insufficiency) (32).

The mainstay of medical therapy in CHI is diazoxide – a drug that binds to the intact SUR1 component of the K_{ATP} channels (4). It acts by keeping the K_{ATP} channels open, thereby preventing depolarisation of the β-cell membrane and insulin secretion. Clinically, it is useful to classify children as diazoxide responsive and diazoxide unresponsive. The aims of this study were to investigate genotype/phenotype correlations in relation to birth weight, age at presentation, responsiveness to medical therapy with diazoxide and prediction of focal disease.

Materials and methods
Patients

All patients with CHI recruited into the study were referred to Great Ormond Street Children's Hospital, which is a national and international referral centre for CHI. Three hundred patients with biochemi-cally confirmed CHI (blood glucose concentration < 3.0 mmol/l with detectable concentrations of insulin and/or C-peptide, i.v. glucose infusion rate of > 8 mg/kg per min and inappropriately low concentration non-esterified fatty acids and ketone bodies) were recruited into the study. Clinical and biochemical data were collated via a standard request form (www.diabetes-genes.org), clinical letter of referral or by case note review. Responsiveness to diazoxide was assessed and defined as being able to maintain normoglycaemia (blood glucose > 3.5 mmol/l) following a period of fasting, which is determined by the age of the child (6 h for a neonate, 12 h up to infancy and 18 h in a child). At the end of the fasting period, there should be an appropriate increase in the serum ketone bodies and an appropriate suppression of insulin secretion. Patients with a secondary cause of hyperinsulinaemic hypogly-caemia such as perinatal asphyxia, intra-uterine growth restriction, Rhesus isoimmunisation and syn-dromic forms such as Beckwith–Wiedemann syndrome were excluded from the study (33).

Birth weight SDS were calculated for each patient. The mean birth weight and age of presentation of patients with diazoxide-responsive CHI due to a K_{ATP} channel mutation(s), *GLUD1* mutation, *HADH* mutation or an *HNF4A* mutation were compared using one-way ANOVA followed by the Fisher's least significant difference post-test to test for statistical significance (SPSS, version 20). The study was ethically approved by the Ethics Committee of Great Ormond Street Children's Hospital and the Institute of Child Health.

Sequence analysis strategy of genes known to cause CHI

The DNA samples were analysed for mutations in genes known to cause CHI (*ABCC8*, *KCNJ11*, *HNF4A*, *GCK*, *GLUD1* and *HADH* genes) at the Diagnostic Molecular Genetics Laboratory at Peninsula Medical School

(Exeter, UK), according to the established methods. The pathogenicity of novel variants was considered on an individual basis. Where possible, evidence to support/refute causality was sought by the following: i) co-segregation studies, ii) identification in control chromosomes (dbSNP (November 2010)/1000 genomes databases (May 2011)), iii) SIFT and PolyPhen *in silico* predictions and iv) *in silico* splicing prediction accessed through Alamut Software, version 2.1 (Interactive Biosoftware, Rouen, France).

Diazoxide-unresponsive patients

KCNJ11 and *ABCC8* were sequenced concurrently in all patients. If i) no *ABCC8* or *KCNJ11* mutation was identified, ii) a single mutation inherited from an unaffected mother was found, or iii) a single mutation was identified in a patient with diffuse pancreatic disease, dosage analysis of the *ABCC8* gene by multiplex ligation dependent probe amplification (MLPA) was undertaken. Sequencing analysis of the *GCK* gene was subsequently performed in all patients in whom no mutation in the *ABCC8* and *KCNJ11* genes had been identified.

Diazoxide-responsive patients

In patients with diazoxide-responsive CHI, mutations in *GLUD1* were sought if the patient had HA or leucine sensitivity, and *HADH* was sequenced if there was raised plasma 3-hydroxybutyrylcarnitine or consanguinity was reported. If no mutations were identified and in the absence of the above features, *KCNJ11* and *ABCC8* were sequenced concurrently. MLPA analysis of the *ABCC8* gene was undertaken when a single heterozygous *ABCC8* mutation was identified. If no K_{ATP} channel mutation was identified, *HNF4A* was sequenced. Sequencing of *GCK* was undertaken if there was sufficient DNA and a positive family history of hypoglycaemia or delayed presentation of hypoglycaemia (>6 months). The *SLC16A1* gene was not sequenced as no patients were reported to have exercise-induced HI.

If it was not possible to establish whether a patient with CHI was diazoxide responsive or not ($n=12$), *KCNJ11* and *ABCC8* followed by *HNF4A* sequencing was undertaken.

Results

Three hundred patients with CHI were recruited in this study. The patients were broadly classified into diazoxide-responsive ($n=183$) and diazoxide-unresponsive ($n=105$) groups. Twelve patients with CHI were not able to be grouped within these two broad categories. These included patients with transient CHI that did not require treatment with diazoxide ($n=11$) and one patient who was not treated with diazoxide following an anaphylactic reaction to the drug.

Analysis of *ABCC8* gene mutations

Mutations in the *ABCC8* gene were identified in 98 patients (Fig. 1). Of the 98 patients, 19 were compound heterozygous, 38 were homozygous and 41 were heterozygous. Dominant mutations were confirmed in 17/41 patients with a heterozygous mutation. In the remaining 24 patients, the mutation was presumed to be recessively acting. A variety of missense, frameshift, nonsense, deletion and splice site mutations, located throughout the gene, were identified. Thirty-six novel *ABCC8* mutations were identified in this study. The most common mutations were the splice site mutation c.3992-9G>A and the missense mutation p.G111R, found in six patients each. The *ABCC8* mutations/variants and the associated phenotype are presented in Supplementary Tables 1, 2, 3 and 4, see section on supplementary data given at the end of this article, as i) homozygous or compound heterozygous mutations, ii) heterozygous mutations and iii) variants of uncertain significance.

CHI due to homozygous or compound heterozygous recessively acting* ABCC8 *mutations All patients with two *ABCC8* mutations in *trans* that had been inherited from clinically unaffected parents were unresponsive to diazoxide therapy ($n=57$; Supplementary Tables 1 and 2). Of these, 38 patients underwent a near total pancreatectomy and diffuse disease was confirmed histologically. The remaining 19 patients were treated non-surgically with a combination of glucose polymer in feeds and octreotide therapy. Diazoxide was used as an adjunct in seven patients as there was clinical evidence of partial responsiveness to diazoxide with improvement in blood glucose concentrations. The mean age of presentation of these patients was 20 days (median of 1 day) and the mean birth weight was $+1.67$ SDS.

CHI due to heterozygous* ABCC8 *mutations Forty-one patients were heterozygous for a single *ABCC8* mutation (Supplementary Table 3). In all patients, gene dosage analysis by MLPA had excluded a partial or whole gene deletion on the opposite allele. This heterogeneous group of patients included 25 patients with diazoxide-unresponsive CHI and 14 patients with diazoxide-responsive CHI. A single heterozygous *ABCC8* mutation was also identified in two patients where the diazoxide responsiveness is not known as they had transient CHI that resolved without trial of diazoxide therapy. This included one patient (patient 172) who was heterozygous for a novel p.G1479A mutation and one patient (patient 281) who was heterozygous for the p.R1539Q mutation known to cause mild, dominantly inherited CHI (13). The p.G1479A mutation is likely to be dominantly acting as i) the family member testing demonstrated that the mutation had arisen *de novo*, ii) a second mutation was not detected by sequencing or
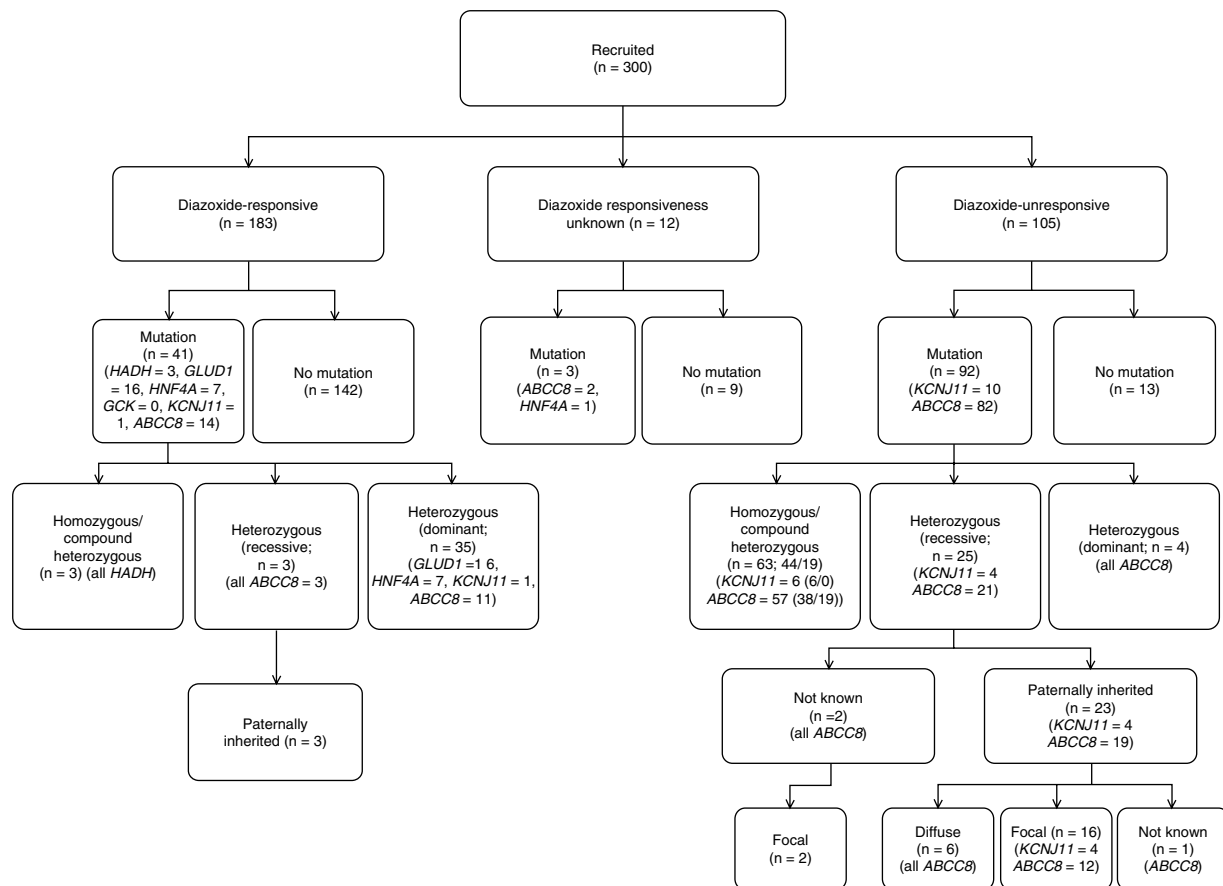
Figure 1 Summary of the genotypes of all patients undergoing sequencing analysis.

MLPA analysis and iii) a different mutation affecting the same residue has been reported previously in patients with dominantly inherited CHI (13, 34). The transient nature of the CHI in these two patients is in keeping with previous reports, which have described a variable penetrance associated with dominantly acting mutations (34).

Diazoxide-unresponsive CHI with a heterozygous ABCC8 mutation Of the 25 with diazoxide-unresponsive CHI and a single *ABCC8* mutation, four had a dominantly acting mutation (patients previously reported in (28)), 19 had a paternal mutation, and in two patients (with histologically confirmed focal disease), the inheritance was not established due to unavailability of the paternal DNA (Supplementary Table 3). All four patients with a dominantly acting mutation had diffuse disease while 12 of the 19 patients with a recessively acting paternal mutation had histologically confirmed focal disease that was managed by resection of the focal lesion/limited pancreatectomy. Six of the remaining patients had diffuse disease with a mutation inherited from an unaffected father. Two of

these (patients 97 and 270) are heterozygous for the same novel mutation (p.A1263T). One other patient with a paternally inherited mutation was treated non-surgically with glucose polymer in feeds and octreotide and it was not determined whether he had a focal lesion or not.

Diazoxide-responsive CHI with a heterozygous ABCC8 mutation Fourteen patients with diazoxide-responsive CHI had a single heterozygous *ABCC8* mutation (Supplementary Table 3). The patients in this category have varying durations of HH, ranging from transient disease of 5-month duration (patient 10) to persistent disease requiring continued diazoxide therapy at 10.7 years of age (patient 194). Similar to the other groups of patients with an *ABCC8* mutation, the mean age of presentation in this group was early at 29.2 days (median 1 day) and the mean birth weight was 1.53 SDS. This category includes 11 patients (four related and seven unrelated) with seven different dominantly inherited mutations. The clinical phenotype of these patients and the functional characterisation of the mutations have been described (34). Apart from the

patients with dominantly inherited mutations, there are three patients where a single mutation was inherited from an asymptomatic parent.

ABCC8 *variants of uncertain clinical significance*

In seven patients, a heterozygous *ABCC8* novel variant of uncertain clinical significance was identified (Supplementary Table 4). All patients were responsive to diazoxide and four of the patients had inherited the variant from an unaffected parent (three maternal and one paternal). In three patients, the parental origin of the variant could not be determined as samples were not available for testing.

Analysis of KCNJ11 *gene mutations*

Eleven different *KCNJ11* mutations were identified, six of which are novel. The genotype and phenotype of these patients are summarised in Supplementary Table 5, see section on supplementary data given at the end of this article. The mean age of presentation of these patients was 5.5 days (median 1 day) and the mean birth weight was +2.02 SDS. Six patients had diazoxide-unresponsive disease associated with a homozygous mutation. Four of these patients underwent a near total pancreatectomy while two were treated non-surgically with a combination of glucose polymer in feeds and octreotide therapy. Diffuse disease was confirmed histologically in the four patients who underwent a near total pancreatectomy and in a further patient via a pancreatic tail biopsy. The histological diagnosis was not established in one patient. Four patients with diazoxide-unresponsive disease and a paternally inherited *KCNJ11* mutation had focal disease, confirmed on histological examination following a limited/sub-total pancreatectomy. In one of these patients (patient 148), who has been reported previously, a giant focal lesion was observed (35).

Diazoxide-responsive disease One patient with diazoxide-responsive disease was heterozygous for a *KCNJ11* mutation (Supplementary Table 5). This patient had inherited the mutation from their affected father. The father had a history of hyperinsulinaemic hypoglycaemia in the neonate, thus confirming dominant inheritance of this mutation.

KCNJ11 *variants of uncertain clinical significance*

In two patients with transient CHI, a novel heterozygous *KCNJ11* variant of uncertain clinical significance was identified (Supplementary Table 6, see section on supplementary data given at the end of this article). One patient had inherited the mutation from an unaffected mother. The inheritance of the mutation in the second patient could not be determined as the mutation was not present in the maternal DNA and a sample from the father was not available for testing.

Diazoxide-responsive CHI without an ABCC8 *or* KCNJ11 *mutation*

The largest category of patients with CHI in this cohort included patients with diazoxide-responsive CHI. Of the 183 patients with diazoxide-responsive disease, a genetic diagnosis was possible in 41 patients (22.4%; Fig. 1).

Twenty patients with diazoxide-responsive CHI (19 patients with HA and one with leucine sensitivity without HA) had sequencing analysis of *GLUD1* gene and mutations were identified in 16 patients. The genotype–phenotype correlations of these patients have been reported (36). Seven of the diazoxide-responsive patients had *HNF4A* mutations. The phenotypic characteristics of these patients have been previously reported (37). No patients with a *GCK* mutation were identified in this cohort.

Clinical characteristics according to genetic aetiology The clinical characteristics of patients with transient and/or diazoxide-responsive CHI were compared according to genetic aetiology. Patients with a *HNF4A* mutation presented earlier and were born heavier than patients with a *GLUD1* mutation (1 day vs 157 days, $P=0.004$, and $+2.36$ birth weight SDS vs -0.10 birth weight SDS, $P=0.001$ respectively). In comparison with patients with a *HADH* mutation, patients with a *HNF4A* mutation were heavier (birth weight SDS $+2.36$ (*HNF4A*) vs -1.08 (*HADH*), $P=0.002$). No difference in the age of presentation was observed (1 day vs 125 days, $P=0.109$). In addition, no differences in the mean age at presentation were observed between patients with a *HNF4A* or K_{ATP} channel mutation(s) with diazoxide-responsive CHI (the range varied from 1 day to 1 year for patients with a K_{ATP} channel mutation). The difference in the birth weights between these two groups were also not significant ($+2.36$ vs $+1.96$ SDS, $P=0.551$).

Within the other groups of patients, the age of presentation and birth weight of patients with a *GLUD1* or *HADH* mutation were similar (157 vs 125 days and birth weight SDS -0.10 vs -1.08, $P=0.63$ and $P=0.32$ respectively). The mean birth weight and age of presentation of the patients with a *GLUD1* mutation was significantly different from patients with a K_{ATP} channel mutation ($P=0.003$ and $P=0.001$ respectively). Patients with a *HADH* mutation were also significantly smaller at birth than patients with a K_{ATP} channel mutation ($P=0.003$). The mean age of presentation of patients with a *HADH* mutation was 125 days in comparison with 27.4 days in patients with K_{ATP} channel mutations; however, the difference was not statistically significant ($P=0.15$).

Discussion

In this study, a genetic diagnosis for CHI was made for 45.3% of patients that were studied. In the category of children who were unresponsive to therapy with diazoxide, mutations in the *ABCC8* gene were the commonest identifiable cause. All patients with two *ABCC8* mutations in *trans* that had been inherited from clinically unaffected parents were unresponsive to diazoxide therapy. Out of the 105 patients who were unresponsive to diazoxide treatment, mutations in *ABCC8/KCNJ11* were found in 92. This suggests that from the clinical point of view, the vast majority of patients who do not respond to therapy with diazoxide are likely to harbour mutations in the *ABCC8/KCNJ11* genes.

By contrast, the phenotype of those patients who inherited a heterozygous *ABCC8* mutation was distinct from those with two *ABCC8* mutations. The heterozygous *ABCC8* mutation carriers were either responsive or unresponsive to therapy with diazoxide. Those that responded to diazoxide mostly had a dominant form of CHI. Thirteen patients (from nine families) were heterozygous for eight different dominantly inherited mutations occurring in the NBD2 area of the SUR1 protein. NBD1 appears to be the principal site for ATP binding, whereas NBD2 binds MgADP (38). These binding domains cooperate with each other in mediating the nucleotide regulation of the pore function (39). MgADP and diazoxide activate K_{ATP} channels in the presence of inhibitory concentrations of ATP, and both processes require Mg^{2+} (40, 41, 42). Mutations in NBD2 can abolish channel activation by diazoxide or MgADP (42). One of these mutations (p.G1479A) is novel. The phenotype associated with these dominantly inherited mutations confirms the reported phenotype associated with dominant mutations in the NBD2 area (13, 43); however, dominant CHI mutations also occur outside the NBD2 (44).

The observations suggest that the clinical presentation of patients with dominant inactivating *ABCC8* missense mutations is variable, ranging from mild medically responsive to severe early-onset CHI requiring a near total pancreatectomy. The clinical presentation of patients at the time of diagnosis cannot distinguish between recessive and dominantly acting *ABCC8* mutations. A genetic diagnosis is important as finding a *de novo* dominant mutation in a patient with diazoxide-unresponsive CHI confers a lower recurrence risk for future siblings (i.e. the risk of germ-line mosaicism) compared with a recessive mutation, but a higher risk of CHI (50%) for the next generation.

This study also demonstrated that a paternal *ABCC8/KCNJ11* mutation in association with diazoxide-unresponsive disease is not synonymous with a focal lesion. Of the 23 patients with diazoxide-unresponsive disease and an identifiable paternal mutation (4, *KCNJ11* and 19, *ABCC8*), 16 had confirmed focal disease, six had diffuse disease and the histological diagnosis was not

confirmed in one. The mechanism of diffuse disease in patients with a heterozygous paternal *ABCC8/KCNJ11* mutation is not understood, but similar observations have been documented in a previous study (45). However, in the previous study, differentiation of diffuse and focal were based on radiological rather than histological grounds, and this might have resulted in the excess of paternal-only cases. It is possible that the sequencing and dosage analysis was unable to detect a second *ABCC8* mutation. It is also possible that there is a second hit within the pancreas, for e.g. loss of a part of the maternal 11p15 affecting the entire pancreas (leading to a large focal lesion) or a second pancreatic specific mutation. Another possibility is that some of these mutations are dominantly acting and the lack of co-segregation with disease in the family reflects incomplete penetrance. Genetic analysis of the pancreatic samples is warranted to test this hypothesis.

Three patients (patients 10, 329 and 129) with diazoxide-responsive CHI were heterozygous for a single *ABCC8* mutation (Supplementary Table 3). These mutations (c.3992-9G>A and p.D1472N) are known to act recessively, and it is possible that a second germ-line, or somatic mutation within the pancreas, has not been identified by Sanger sequencing or dosage analysis. However, the phenotype of these patients does not fit in with recessively inherited CHI that is generally diazoxide unresponsive (as is also seen in this series). This series identified a missense mutation p.G111R in six unrelated patients with severe, diazoxide-unresponsive disease, of Indian background. Two *ABCC8* mutations, c.3992-9G>A and p.F1388del, are associated with CHI in the Ashkenazi Jewish population (11) and the p.V187D mutation has been associated with the Finnish population (46). The recognition of the p.G111R mutation exclusively in patients from an Indian ethnic background suggests a founder effect.

It was striking that those patients with *HNF4A* mutations presented earlier with hypoglycaemia and had a higher birth weight when compared with patients with other genetic aetiologies. However, as an overlap between birth weights and age at presentation was observed between patients with *HNF4A* and K_{ATP} channel mutations, it is not possible to distinguish between the two groups on the basis of these features. Although a family history of diabetes is more likely to suggest a *HNF4A* mutation, it is also not a distinguishing feature. Hence, given the higher prevalence of K_{ATP} channel mutations and the high rate of diabetes phenocopies in the population, we recommend to sequence *KCNJ11* and *ABCC8* initially in all patients with diazoxide-responsive CHI, followed by *HNF4A* if HI is diagnosed within the first week of life.

In contrast to patients with a *HNF4A* or K_{ATP} channel mutation, patients with a *GLUD1/HADH* mutation were diagnosed later and were of normal birth weight. It was not possible to distinguish patients with a *GLUD1* mutation from those with a *HADH* mutation based on

these two features. Patients with *GLUD1* or *HADH* mutation share further similarities in terms of being leucine sensitive. In the vast majority of patients, a serum ammonia concentration would help in distinguishing between the two aetiologies (raised in the majority of patients with a *GLUD1* mutation). The other distinguishing feature would be a history of consanguinity as all *HADH* mutations reported so far have been recessively inherited while *GLUD1* mutations are dominantly inherited. In the category of patients who were responsive to diazoxide, no identifiable mutations in the known genes were found in nearly 80% of patients. This suggests that there may well be other novel genetic/environmental mechanisms involved in regulating insulin secretion. Unravelling the genetic basis of CHI in these patients might provide an opportunity to gain further insights into pancreatic β-cell physiology.

In conclusion, a genetic diagnosis was made for 45.3% of patients in this large series. Mutations in the *ABCC8* gene were the commonest identifiable cause. The vast majority of patients with diazoxide-responsive CHI had no identifiable mutations, suggesting other genetic and/or environmental mechanisms.

Supplementary data

This is linked to the online version of the paper at http://dx.doi.org/10.1530/EJE-12-0673.

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.

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