Clinical and genetic characterization of congenital hyperinsulinism in Spain

R Martínez1,*, C Fernández-Ramos2,*, 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)

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, HNF4A, HNF1A, 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 KATP 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 KATP 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 KATP 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.

Correspondence should be addressed to L Castaño
Email lcastano@osakidetza.net

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 (KATP). Other less frequent causes are mutations in glutamate dehydrogenase (GLUD1), glucokinase (GCK), and short-chain l-3-hydroxyacyl CoA dehydrogenase...
**Clinical Study**

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**Congenital hyperinsulinism in Spain**

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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 nonketotic 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. χ² 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 µU/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>
<thead>
<tr>
<th>Table 1</th>
<th>Clinical characteristics of patients with CHI at diagnosis.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical characteristics</strong></td>
<td><strong>Results (n = 50)</strong></td>
</tr>
<tr>
<td>Females, n (%)</td>
<td>32 (64%)</td>
</tr>
<tr>
<td>Mutation positive, n (%)</td>
<td>28 (56%)</td>
</tr>
<tr>
<td>Gestational age, weeks (range)</td>
<td>38 ± 2.5 (31–41)</td>
</tr>
<tr>
<td>Birth weight, g</td>
<td>3570 ± 746</td>
</tr>
<tr>
<td>Large for gestational age, n (%)</td>
<td>17 (34%)</td>
</tr>
<tr>
<td>Dystocia</td>
<td>28%</td>
</tr>
<tr>
<td>Age at presentation, n (%)</td>
<td></td>
</tr>
<tr>
<td>Within 1st month of life (group A)</td>
<td>26 (52%)</td>
</tr>
<tr>
<td>Between 1 month and 2 years (group B)</td>
<td>21 (42%)</td>
</tr>
<tr>
<td>From 2 years (group C)</td>
<td>3 (6%)</td>
</tr>
<tr>
<td>Blood glucose, mg/dL</td>
<td>29 ± 12</td>
</tr>
<tr>
<td>Serum insulin, µU/mL</td>
<td>26 ± 37</td>
</tr>
<tr>
<td><strong>Symptomatology</strong></td>
<td></td>
</tr>
<tr>
<td>Seizures</td>
<td>34%</td>
</tr>
<tr>
<td>Hypotonia</td>
<td>30%</td>
</tr>
<tr>
<td>Loss of consciousness</td>
<td>28%</td>
</tr>
<tr>
<td>Patients treated with diazoxide, n (%)</td>
<td>47 (94%)</td>
</tr>
<tr>
<td>Diazoxide dose, mg/kg/day (range)</td>
<td>15 ± 5.2 (7.5–20)</td>
</tr>
<tr>
<td>Diazoxide responsive, n (%)</td>
<td>30 (64%)</td>
</tr>
</tbody>
</table>

Data are mean ± 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.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Location</th>
<th>Nucleotide change</th>
<th>Protein change</th>
<th>Mutation type</th>
<th>Genetic status</th>
<th>Inheritance</th>
<th>Previously reported</th>
<th>LGA</th>
<th>Age at presentation</th>
<th>Diazoxide responsive</th>
<th>Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>Exon 13</td>
<td>c.1829T&gt;G</td>
<td>p.Leu610Arg</td>
<td>Missense</td>
<td>Paternal</td>
<td>Maternal</td>
<td>Novel</td>
<td>Yes</td>
<td>7 days</td>
<td>No</td>
<td>Intensive feeding</td>
</tr>
<tr>
<td></td>
<td>Intron 32</td>
<td>c.3991+2T&gt;C</td>
<td>p.?</td>
<td>Splicing</td>
<td>Maternal</td>
<td>Paternal</td>
<td>Novel</td>
<td>No</td>
<td>2.5 months</td>
<td>No</td>
<td>Intensive feeding</td>
</tr>
<tr>
<td>2a</td>
<td>Exon 13</td>
<td>c.1829T&gt;G</td>
<td>p.Leu610Arg</td>
<td>Missense</td>
<td>Paternal</td>
<td>Maternal</td>
<td>Novel</td>
<td>No</td>
<td>6 months</td>
<td>No</td>
<td>Total pancreatectomy</td>
</tr>
<tr>
<td></td>
<td>Intron 32</td>
<td>c.3991+2T&gt;C</td>
<td>p.Arg598*</td>
<td>Nonsense</td>
<td>Paternal</td>
<td>Maternal</td>
<td>Novel</td>
<td>19</td>
<td>36 h</td>
<td>No</td>
<td>Octreotide/nifedipine/ partial pancreatectomy, Continuing DZX+ Octreotide</td>
</tr>
<tr>
<td>3</td>
<td>Exon 36</td>
<td>c.4352T&gt;C</td>
<td>p.Leu1451Pro</td>
<td>Missense</td>
<td>Maternal</td>
<td>Maternal</td>
<td>Yes</td>
<td>20</td>
<td>No</td>
<td>Octreotide/nifedipine/ partial pancreatectomy, Continuing DZX+ Octreotide</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Exon 8</td>
<td>c.1317G&gt;A</td>
<td>p.Trp439*</td>
<td>Missense</td>
<td>Maternal</td>
<td>Maternal</td>
<td>Yes</td>
<td>20</td>
<td>No</td>
<td>Near total pancreatectomy (90%)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Intron 38</td>
<td>c.4612-2A&gt;T</td>
<td>p.?</td>
<td>Splicing</td>
<td>Paternal</td>
<td>Maternal</td>
<td>(19)</td>
<td>Yes</td>
<td>1 h</td>
<td>Yes</td>
<td>Continuing DZX</td>
</tr>
<tr>
<td></td>
<td>Exon 12</td>
<td>c.1732_1746dup15</td>
<td>p.Leu582 duplication</td>
<td>Missense Frameshift</td>
<td>Hmz</td>
<td>Both parents</td>
<td>Novel</td>
<td>No</td>
<td>1 h</td>
<td>No</td>
<td>Intensive feeding</td>
</tr>
<tr>
<td>6</td>
<td>Exon 39</td>
<td>c.4723G&gt;C</td>
<td>p.Ala578_Leu582dup5</td>
<td>Missense Frameshift</td>
<td>Hmz</td>
<td>Both parents</td>
<td>Novel</td>
<td>No</td>
<td>2 months</td>
<td>No</td>
<td>Responsive to octreotide and nifedipine/intensive feeding</td>
</tr>
<tr>
<td></td>
<td>Exon 4</td>
<td>c.453delC</td>
<td>p.Ala1575Pro</td>
<td>Missense</td>
<td>Paternal</td>
<td>Both parents</td>
<td>(4, 5, 19)</td>
<td>Yes</td>
<td>8 h</td>
<td>No</td>
<td>Responsive to octreotide/ deceased</td>
</tr>
<tr>
<td>7</td>
<td>Exon 23</td>
<td>c.2800C&gt;T</td>
<td>p.Arg934*</td>
<td>Nonsense</td>
<td>Hmz</td>
<td>Both parents</td>
<td>Novel</td>
<td>No</td>
<td>15 h</td>
<td>No</td>
<td>Near total pancreatectomy (90%)</td>
</tr>
<tr>
<td>8</td>
<td>Exon 23</td>
<td>c.2800C&gt;T</td>
<td>p.Arg934*</td>
<td>Nonsense</td>
<td>Hmz</td>
<td>Both parents</td>
<td>Novel</td>
<td>No</td>
<td>4 h</td>
<td>No</td>
<td>Near total pancreatectomy (90%)</td>
</tr>
<tr>
<td>9b</td>
<td>Intron 32</td>
<td>c.3991+2T&gt;C</td>
<td>p.?</td>
<td>Splicing</td>
<td>Paternal</td>
<td>Both parents</td>
<td>Novel</td>
<td>No</td>
<td>2 months</td>
<td>No</td>
<td>Responsive to octreotide/ focal pancreatectomy</td>
</tr>
<tr>
<td>10b</td>
<td>Intron 32</td>
<td>c.3991+2T&gt;C</td>
<td>p.?</td>
<td>Splicing</td>
<td>Paternal</td>
<td>Both parents</td>
<td>Novel</td>
<td>No</td>
<td>2 months</td>
<td>No</td>
<td>Responsive to octreotide/ subtotal pancreatectomy</td>
</tr>
<tr>
<td>11</td>
<td>Exon 24</td>
<td>c.2837_2840delAGAG</td>
<td>p.Arg947fs</td>
<td>Frameshift</td>
<td>Htz</td>
<td>Paternal†</td>
<td>(5, 35)</td>
<td>Yes</td>
<td>24 h</td>
<td>No</td>
<td>Octreotide/subtotal pancreatectomy</td>
</tr>
<tr>
<td>12</td>
<td>Exon 29</td>
<td>c.3577delG</td>
<td>p.Asp1193fs</td>
<td>Frameshift</td>
<td>Htz</td>
<td>Paternal†</td>
<td>(5, 20, 36)</td>
<td>No</td>
<td>28 h</td>
<td>No</td>
<td>Octreotide/subtotal pancreatectomy</td>
</tr>
<tr>
<td>13</td>
<td>Exon 29</td>
<td>c.3626_3628delAAG</td>
<td>p.Glu1209del</td>
<td>In frame deletion</td>
<td>Htz</td>
<td>ND</td>
<td>Novel</td>
<td>No</td>
<td>20 h</td>
<td>No</td>
<td>Subtotal pancreatectomy</td>
</tr>
<tr>
<td>14</td>
<td>Exon 5</td>
<td>c.742C&gt;T</td>
<td>p.Arg248*</td>
<td>Nonsense</td>
<td>Htz</td>
<td>Paternal</td>
<td>(4, 5, 19, 20, 37, 38)</td>
<td>No</td>
<td>8 h</td>
<td>No</td>
<td>Responsive to octreotide/ deceased</td>
</tr>
<tr>
<td>15</td>
<td>Exon 30</td>
<td>c.3751C&gt;T</td>
<td>p.Arg1251*</td>
<td>Nonsense</td>
<td>Htz</td>
<td>Paternal</td>
<td>(4, 5, 18, 19, 20)</td>
<td>No</td>
<td>2 h</td>
<td>No</td>
<td>Intensive feeding</td>
</tr>
<tr>
<td>16</td>
<td>Exon 37</td>
<td>c.4519G&gt;T</td>
<td>p.Glu1507*</td>
<td>Nonsense</td>
<td>Htz</td>
<td>Paternal</td>
<td>Yes</td>
<td>4 h</td>
<td>No</td>
<td>Near total pancreatectomy (95%)</td>
<td></td>
</tr>
</tbody>
</table>
Genetic characteristics

The genetic etiology was identified in 56% (28/50) of the patients with CHI: 21 patients having a mutation in the K<sub>ATP</sub> 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<sub>ATP</sub> 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<sub>ATP</sub> 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 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...
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_000525.3, and on the GCK reference sequence NM_000162.3.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Nucleotide change</th>
<th>Protein change</th>
<th>Location</th>
<th>Prediction algorithm</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCNJ11</td>
<td>c.295G&gt;A</td>
<td>p.Asp99Asn</td>
<td>Exon 1</td>
<td>SIFT Predict not tolerated</td>
</tr>
<tr>
<td>ABCC8</td>
<td>c.453delC</td>
<td>p.Ile152fs</td>
<td>Exon 4</td>
<td>PolyPhen-2 Probably damaging</td>
</tr>
<tr>
<td>ABCC8</td>
<td>c.1317G&gt;A</td>
<td>p.Trp439*</td>
<td>Exon 8</td>
<td>MutationTaster Disease causing</td>
</tr>
<tr>
<td>ABCC8</td>
<td>c.1829T&gt;G</td>
<td>p.Leu610Arg</td>
<td>Exon 13</td>
<td></td>
</tr>
<tr>
<td>ABCC8</td>
<td>c.2701A&gt;T</td>
<td>p.Ile901Phe</td>
<td>Exon 23</td>
<td></td>
</tr>
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<td>ABCC8</td>
<td>c.3626_3628delAAG</td>
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<td>Exon 29</td>
<td></td>
</tr>
<tr>
<td>ABCC8</td>
<td>c.4060T&gt;A</td>
<td>p.Tyr1354Asn</td>
<td>Exon 33</td>
<td></td>
</tr>
<tr>
<td>ABCC8</td>
<td>c.4519G&gt;C</td>
<td>p.Leu610Arg</td>
<td>Exon 49</td>
<td></td>
</tr>
<tr>
<td>ABCC8</td>
<td>c.453delC</td>
<td>p.Ile152fs</td>
<td>Exon 4</td>
<td>PolyPhen-2 Possibly damaging</td>
</tr>
<tr>
<td>GCK</td>
<td>c.3991+2T&gt;C</td>
<td>p.?</td>
<td>Intron 32</td>
<td></td>
</tr>
</tbody>
</table>

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 200µmol/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<sub>ATP</sub> 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**

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.

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

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<sub>ATP</sub> 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<sub>ATP</sub> channel genes.

Clinically, we found that patients with CHI secondary to the K<sub>ATP</sub> 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...
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 \textit{ABCC8} mutations. These patients had a mixed phenotype. Arya et al. (24) recently evaluated a cohort of CHI patients with paternally inherited heterozygous \textit{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 \textit{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 \textit{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 \textit{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 \textit{ABCC8} (27). Furthermore, various authors have observed diabetes in adult \textit{ABCC8} mutation carriers (8, 10); however, it remains unclear whether these dominant \textit{ABCC8} gene mutations predispose individuals to diabetes mellitus in adulthood (7). The first dominant heterozygous inactivating \textit{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 \textit{ABCC8} dominant mutations, p.Gly1479Arg and p.Ala1508Pro, but also gestational diabetes or diabetes in \textit{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 \textit{ABCC8} mutations, only 4/29 adults had diabetes. Recently, it has been observed in CHI mouse model that the homE1506K 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 \textit{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 \textit{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 \textit{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 \textit{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.

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References


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