Characterization of ABCC8 and KCNJ11 gene mutations and phenotypes in Korean patients with congenital hyperinsulinism

So Eun Park, Sarah E Flanagan1, Khalid Hussain2, Sian Ellard1, Choong Ho Shin3 and Sei Won Yang3

Department of Pediatrics, College of Medicine, CHA Gangnam Medical Center, CHA University, 650-9 Yeoksam 1-dong, Gangnam-gu, Seoul 135-914, Republic of Korea, 1Peninsula Medical School, Institute of Biomedical and Clinical Science, University of Exeter, Exeter EX2 5DW, UK, 2Developmental Endocrinology Research Group, Molecular Genetics Unit, The Institute of Child Health, London Centre for Paediatric Endocrinology and Metabolism, Great Ormond Street Hospital for Children NHS Trust, University College London, London WC1N 1EH, UK and 3Department of Pediatrics, Seoul National University Children’s Hospital, 28 Yeongeondong, Jongnogu, Seoul 110-744, Republic of Korea

(Correspondence should be addressed to C-H Shin; Email: chshinpd@snu.ac.kr)

Abstract

Objective: Congenital hyperinsulinism (CHI) is characterized by persistent hypoglycemia due to the inappropriate insulin secretion. Inactivating mutations in the ABCC8 and KCNJ11 genes, which encode the sulfonylurea receptor 1 and Kir6.2 subunits of the ATP-sensitive K⁺ (KATP) channel in pancreatic β-cell, are the most common cause of CHI. We studied the genetic etiology and phenotypes of CHI in Korean patients.

Methods: ABCC8 and KCNJ11 mutational analysis was performed in 17 patients with CHI. Medical records were retrospectively reviewed to identify phenotypes.

Results: Mutations (12 ABCC8 and three KCNJ11) were identified in 82% (14/17) of patients. Of these, nine ABCC8 mutations (E100X, W430X, c.1630C>1G, D813N, Q923X, E1087_A1094del salesDKSDT, Q1134H, H1135W, and E1209Rfs) and one KCNJ11 mutation (W91X) were novel. Of the 14 patients, four had confirming recessively inherited CHI. The remaining ten patients had single heterozygous mutations. The majority (12/17) of patients were medically responsive. Of the five diazoxide-responsive patients, four had an ABCC8 mutation. The five patients unresponsive to medical management and one diazoxide-responsive patient underwent pancreatectomy and had diffuse histology. Of the operated six patients, two had recessively inherited mutations; three patients had a single heterozygous mutation (one maternally and two paternally inherited); and one patient had no identifiable KATP channel mutation.

Conclusions: This is the first study to report genotype and phenotype correlations among Korean patients with CHI. Mutations in ABCC8 and KCNJ11 are the most common causes of CHI in Korean patients. Similar to other studies, there is marked genetic heterogeneity and no clear genotype–phenotype correlation.

European Journal of Endocrinology 164 919–926

Introduction

Congenital hyperinsulinism (CHI or persistent hyperinsulinemic hypoglycemia of infancy; OMIM#256450) is characterized by persistent hypoglycemia due to inappropriate insulin secretion and is the most common cause of persistent hypoglycemia in neonates and infants. Early diagnosis and treatment is important to prevent permanent brain damage (1–3). CHI is a rare disorder with an estimated incidence of one in 50 000 live births in outbred communities. CHI is very heterogeneous in its clinical manifestations, genetic etiology, and histological findings (4). There is high variability in disease severity that ranges from drug-responsive mild hypoglycemia to severe CHI requiring pancreatectomy. Infants with CHI are usually large for gestational age and commonly present with seizures.

Mutations in seven genes have been reported in patients with CHI. Recessive inactivating mutations in the ABCC8 (OMIM#600509) and KCNJ11 genes (OMIM#600937) encoding the sulfonylurea receptor 1 (SUR1) and Kir6.2 (potassium inwardly rectifying channel) subunits of the ATP-sensitive K⁺ (KATP) channel, which controls secretion of insulin from pancreatic β-cell, are the most common genetic etiology (1–4). Recently, autosomal dominant mutations in the ABCC8 and KCNJ11 genes have also been reported in patients with CHI (5, 6). To date, more than 175 different ABCC8 and KCNJ11 mutations have been reported (7). Other less frequent genetic etiologies include mutations in the genes encoding glutamate dehydrogenase (GLUD1), glucokinase (GCK), short-chain hydroxyacyl-CoA dehydrogenase (HADH), monocarboxylate transporter-1 (SLC16A1), and hepatocyte...
nuclear factor 4-alpha (HNF4A). However, no genetic etiology has yet been identified in ~50% of patients with CHI (8–10). Founder mutations are reported in certain populations, such as certain ABCC8 gene mutations (c.3992-9G>A and F1389del) in Ashkenazi Jews and V187D and E1507K mutations among the Finnish population. Mutations common to other racial groups have not been reported (5, 8, 11, 12).

CHI can be histologically classified as either diffuse or focal disease. In diffuse CHI, the entire pancreas is affected and patients are usually unresponsive to medical treatment. These patients often require a pancreatectomy and are at high risk of developing diabetes mellitus or pancreatic exocrine insufficiency (13, 14). Focal CHI is characterized by localized adenomatous proliferation in the pancreas. This form of CHI can be cured by excision of the focal lesion (15). Diffuse CHI is inherited in an autosomal recessive or dominant manner, whereas focal lesions result from paternal uniparental disomy (UPD) of chromosome 11p15.5–11p15.1 within a single pancreatic paternal UPD of chromosome 11p15.5–11p15.1 within a single pancreatic β-cell during embryogenesis. UPD un masks the paternal inheritance of ATP channel mutation at 11p15.1 and causes altered expression of imprinted genes that are involved in cell cycle regulation. This altered expression results in clonal expansion of the single cell and dysregulated insulin secretion from the resulting focal lesion (16, 17). Histological diagnosis is very important as it determines whether a near-total pancreatectomy or a lesionectomy is required in patients who show no response to therapy. Although clinical characteristics and mutation spectra of CHI patients have been reported in Western countries and Japan, little is known about CHI in the Korean population.

The objectives of this study were to understand the genetic etiologies of CHI in a population of Korean patients and to explore genotype–phenotype correlations.

Subjects and methods

Subjects

CHI was diagnosed and defined according to previously published criteria (18). Patients with intrauterine growth retardation, asphyxia at birth, or presenting with congenital syndromes were excluded from this study. Of the 33 unrelated patients diagnosed with CHI at the Seoul National University Hospital from January 1989 to July 2009, 17 patients were included in this study. Of the remaining 16 patients, four patients were lost to follow up, one patient was diagnosed with the hyperinsulinism/hyperammonemia syndrome due to a mutation in the GLUD1 gene, and 11 patients did not provide consent for genetic testing.

Mutation testing was undertaken on parental samples when a mutation was identified and DNA was available. This research was carried out in accordance with the research plan approved by the Medical College of Seoul National University Hospital medical research ethics deliberation committee.

Methods

Genomic DNA was extracted from peripheral leukocytes using standard procedures. All exons and intron–exon boundaries of ABCC8, KCNJ11, and GCK genes were amplified by PCR (see Supplementary Tables 1–3, see section on supplementary data given at the end of this article for ABCC8 and KCNJ11 primer details). The products were sequenced using a BigDye Terminator v3.1 Cycle sequencing Kit on an ABI 3730XL Analyzer (Applied Biosystems, Foster City, CA, USA) and sequences were compared with the reference sequence (NM_000525 for KCNJ11, NM_000352.3 for ABCC8, and NM_000162.3 for GCK) using Chromas (V2.01, Technelysium Pty Ltd, Tewantin QLD, Australia) or Mutation Surveyor software V3.24 (Softgenetics, State College, PA, USA). Novel variants were considered pathogenic when they were not identified in a control group composed of 100 healthy Koreans and when they affected an amino acid that was highly conserved across species (human, chimp, mouse, horse, dog, elephant, Xenopus tropicalis, and zebra fish).

ABCC8 gene dosage analysis (multiplex ligation-dependent probe amplification kit P117; MRC Holland, Amsterdam, The Netherlands) was performed on all patients with a heterozygous ABCC8 mutation, with the exception of patients 5 and 10 for whom DNA was limited.

Results

Clinical characteristics

Consanguinity was not reported in any of the families. No patient reported a family history of hypoglycemia. The median follow-up observation period for all patients was 5 years and 4 months (range: 4 months to 20.5 years), while the median age at the time of the last follow-up observation was 6 years (range: 7 months to 20.5 years).

The time of symptom manifestation ranged from immediately following birth to 7 months, with 59% (10/17) of the patients presenting within the first 3 days of life. The median age at diagnosis was 1 month (range: 3 days to 14 months; Table 1). The weight of 13 patients was large for gestational age (average birth weight: 4.080 ± 490 g; range: 3.500–5.000 g), although the average gestational age was 38 weeks and 6 days ± 1 week and 3 days.

One patient underwent surgery due to lack of response to medical treatment after having received only prednisolone (patient 17). Of the remaining 16
<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Sex</th>
<th>GA (weeks) /BWt (g)</th>
<th>Onset age</th>
<th>Diazoxide response</th>
<th>Octreotide response</th>
<th>Age at op/ histology</th>
<th>After op</th>
<th>Follow-up</th>
<th>Age at remission or starting insulin</th>
<th>Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCC8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>M</td>
<td>36 1/3/5000</td>
<td>1 d</td>
<td>ND</td>
<td>ND</td>
<td>1.5 m/diffuse</td>
<td>Octreotide</td>
<td>Remission 44.9 m</td>
<td>Q923X&lt;sup&gt;a&lt;/sup&gt;</td>
<td>R837X</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>40/3500&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6 m</td>
<td>ND</td>
<td>ND</td>
<td>6.7 m/diffuse</td>
<td>Euglycemia</td>
<td>Remission 6.7 m</td>
<td>W430X</td>
<td>H1135W&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>36 1/3/4610</td>
<td>Birth</td>
<td>ND</td>
<td>ND</td>
<td>1.5 m/diffuse</td>
<td>Euglycemia</td>
<td>Diabetes 54.7 m</td>
<td>S1387F</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>38/3650</td>
<td>3 d</td>
<td>ND</td>
<td>ND</td>
<td>13.5 m/diffuse</td>
<td>Diazoxide</td>
<td>Diazoxide</td>
<td>D813N</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>40&lt;sup&gt;1/2&lt;/sup&gt;/3680</td>
<td>3 d</td>
<td>ND</td>
<td>ND</td>
<td>22.0 m/diffuse</td>
<td>Euglycemia</td>
<td>Remission 22.0 m</td>
<td>Q1134H</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>38 1/3/3800</td>
<td>3 d</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Remission 12.2 m</td>
<td>ND</td>
<td>H1135W&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>38 1/3/4320</td>
<td>Birth</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>E1087-A1094 delinsDKSDT&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>36&lt;sup&gt;1/2&lt;/sup&gt;/4500</td>
<td>1 d</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>E100X</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>41&lt;sup&gt;1/2&lt;/sup&gt;/4000</td>
<td>2 d</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>E1209R&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>36/3600</td>
<td>4 m</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>R837X</td>
</tr>
<tr>
<td>11</td>
<td>F</td>
<td>37/3789</td>
<td>1 d</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>R837X</td>
</tr>
<tr>
<td>KCNJ11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>40/4700</td>
<td>6 m</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>W91X</td>
</tr>
<tr>
<td>13</td>
<td>M</td>
<td>40/4000</td>
<td>4 m</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>A187V</td>
</tr>
<tr>
<td>14</td>
<td>M</td>
<td>38&lt;sup&gt;1/2&lt;/sup&gt;/4900</td>
<td>1 d</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>A187V</td>
</tr>
<tr>
<td>No mutation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>M</td>
<td>40/4000</td>
<td>4 m</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>None</td>
</tr>
<tr>
<td>16</td>
<td>M</td>
<td>39/3600</td>
<td>2 m</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>None</td>
</tr>
<tr>
<td>17</td>
<td>M</td>
<td>40/3740&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7 m</td>
<td>ND&lt;sup&gt;e&lt;/sup&gt;</td>
<td>ND&lt;sup&gt;e&lt;/sup&gt;</td>
<td>14.9 m/diffuse</td>
<td>Euglycemia</td>
<td>Diabetes 125.3 m</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

GA, gestational age; BWt, birth weight; ND, not determined; Op, 95% subtotal pancreatectomy.

<sup>a</sup>De novo mutation, probably on the paternal chromosome.

<sup>b</sup>Birth weight is <90th percentile.

<sup>c</sup>De novo heterozygous mutation.

<sup>d</sup>Heterozygous mutation; distribution on parental chromosomes is not known.

<sup>e</sup>Prednisolone was used.
patients, 12 to whom either diazoxide or octreotide was administered responded adequately, which was defined as maintaining normoglycemia and a normal feeding pattern. One of these drug-responsive patients (patient 4) underwent surgery due to the unavailability of diazoxide. The administered dosages of diazoxide and octreotide were 7.5–18 mg/kg per day and 4–27 μg/kg per day respectively. Other than hypertrichosis, no serious drug side effects were reported. Of the patients that presented with CHI within the first 3 days of life, 70% (7/10) exhibited a response to either diazoxide or octreotide (Table 1). In addition, 83% (5/6) of the patients with infantile onset CHI, which was defined as onset after 1 month of age and before 1 year of age, responded to either diazoxide or octreotide. Among the 12 patients who responded to medical treatment, five were treated with diazoxide and seven were treated with octreotide. Administration of the drug could be stopped in the seven patients after a median period of 1 year and 11 months (range: 363 days to 5 years and 10 months) after onset and at a median age of 4 years and 5 months (range: 2 years and 4 months to 6 years and 5 months). Of these seven patients, one was responsive to diazoxide and six were responsive to octreotide. An ABCC8 mutation was identified in seven of the 12 patients who were drug responsive.

A total of six patients underwent subtotal pancreatectomy. The median age at the time of surgery was 304 days (range 46–686 days), and the median time period from diagnosis to surgery was 33 days (range 18–658 days). Histological examination identified diffuse disease in all of these patients. Although systematical neuropsychological development examination was not performed, five of the 17 patients (patients 1, 4–6, and 11) displayed definitive developmental delay.

### Analysis of ABCC8 and KCNJ11 gene mutations

Mutations identified in this research are summarized in Tables 1 and 2. A mutation was identified in 82% of the Korean CHI patients (14/17; ABCC8 (11 patients), KCNJ11 (three patients)). Among the 15 different mutations identified, ten were novel.

### Characterization of ABCC8 and KCNJ11 gene mutations

In 65% (11/17) of the CHI patients, 12 different ABCC8 mutations were discovered.

The mutations were distributed throughout the gene with 17% (2/12) predicted to be within the nucleotide-binding domain 2 (NBD2). We identified five different missense mutations, four nonsense mutations, two frameshift mutations, and one aberrant splicing mutation. Among these, nine mutations (E100X, W430X, c.1630+1G>C, D813N, Q923X, E1087_A1094delinsDKSDT, Q1134H, H1135W, and E1209Rfs) were novel.

The remaining three mutations in ABCC8, namely, S1387F (19), R1539Q (20), and R837X (21–23), have previously been reported. The S1387F mutation is located within NBD2 and has been reported to dissipate the sensitivity of the KATP channel to Mg–ADP (24).

A total of three different KCNJ11 mutations were discovered in 17% (3/17) of the CHI patients (Table 1). These include two different missense mutations and one nonsense mutation. Of the three mutations, one (W91X) was novel, whereas R136C (25) and A187V (26) have previously been reported. Interestingly, different mutations at W91 and R136 have been

### Table 2 ABCC8 and KCNJ11 gene mutations identified in 14 Korean patients with CHI.

<table>
<thead>
<tr>
<th>Location</th>
<th>Nucleotide substitution</th>
<th>Amino acid substitution</th>
<th>Domain</th>
<th>Patient no.</th>
<th>Frequency (%) CHI chromosomes (n=34)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ABCC8 (n=12)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exon 3</td>
<td>c.298G&gt;T</td>
<td>E100X</td>
<td>–</td>
<td>8</td>
<td>3</td>
<td>NR</td>
</tr>
<tr>
<td>Exon 8</td>
<td>c.1289G&gt;A</td>
<td>W430X</td>
<td>–</td>
<td>2</td>
<td>3</td>
<td>NR</td>
</tr>
<tr>
<td>Intron 10</td>
<td>c.1630+1G&gt;C</td>
<td>A</td>
<td>Aberrant splicing</td>
<td>7</td>
<td>3</td>
<td>NR</td>
</tr>
<tr>
<td>Exon 20</td>
<td>c.2437G&gt;A</td>
<td>D813N</td>
<td>NBD1</td>
<td>4</td>
<td>3</td>
<td>NR</td>
</tr>
<tr>
<td>Exon 21</td>
<td>c.2509C&gt;T</td>
<td>R837X</td>
<td>–</td>
<td>1, 11</td>
<td>6</td>
<td>(22–26)</td>
</tr>
<tr>
<td>Exon 23</td>
<td>c.2767C&gt;T</td>
<td>Q923X</td>
<td>–</td>
<td>1</td>
<td>3</td>
<td>NR</td>
</tr>
<tr>
<td>Exon 26</td>
<td>c.3402G&gt;T</td>
<td>Q1134H</td>
<td>CL7</td>
<td>5</td>
<td>3</td>
<td>NR</td>
</tr>
<tr>
<td>Exon 28</td>
<td>c.3403C&gt;T</td>
<td>H1135W</td>
<td>CL7</td>
<td>2, 6</td>
<td>3</td>
<td>NR</td>
</tr>
<tr>
<td>Exon 29</td>
<td>c.3627_3628insCGTA</td>
<td>E1209Rfs</td>
<td>–</td>
<td>9</td>
<td>3</td>
<td>NR</td>
</tr>
<tr>
<td>Exon 34</td>
<td>c.4160C&gt;T</td>
<td>S1387F</td>
<td>NBD2</td>
<td>3</td>
<td>3</td>
<td>(22, 23)</td>
</tr>
<tr>
<td>Exon 39</td>
<td>c.4616G&gt;A</td>
<td>R1539Q</td>
<td>NBD2</td>
<td>10</td>
<td>3</td>
<td>(23)</td>
</tr>
</tbody>
</table>

| **KCNJ11 (n=3)** | | | | | | |
| Exon 1 | c.273 G>A | W91X | – | 12 | 3 | NR |
| Exon 1 | c.406C>T | R136C | – | 14 | 3 | (28) |
| Exon 1 | c.560C>T | A187V | – | 13, 14 | 6 | (29) |

NBD, nucleotide binding domain; CL, cytoplasmic loop; NR, not reported.
reported in patients with hyperinsulinism (W91R (10, 27, 28) and R136L (29)). Each mutation was identified in a single patient, with the exception of the H1135W and R837X mutations in ABCC8 and A187V in KCNJ11, which were each identified in two unrelated probands (Tables 1 and 2).

Inheritance of ABCC8 and KCNJ11 mutations
Mutation testing was performed in parents when DNA was available. In three (patients 2, 7, and 14) of the four patients with two mutations, mutation testing confirmed recessive inheritance. In two of these patients (patients 1 and 2), diffuse pancreatic disease was confirmed following surgery (Table 1). In the remaining ten patients, a single heterozygous mutation was identified. Family member testing demonstrated that the mutation was maternally inherited in three cases (patients 3, 5, and 10) and paternally inherited in five cases (patients 4, 8, 11–13). In one case (patient 9), a de novo mutation was identified. Parental samples were not available for testing in the remaining case (patient 6). Partial or whole ABCC8 gene deletions were not detected in the patients with a single ABCC8 gene mutation with the exception of patients 5 and 10 where there was insufficient DNA.

Familial mutation testing showed that patient 3 inherited the S1387F (19) mutation from their mother who was diagnosed with gestational diabetes at the age of 28 years. A previous study reported the S1387F mutation in a patient with CHI. In this patient, the mutation had arisen de novo and sequencing analysis did not identify a second mutation. Interestingly, a deletion of the S1387 residue has been reported in two families with dominantly inherited hyperinsulinism (20). Because a second mutation of paternal origin was not identified in patient 3 by sequencing or dosage analysis, it seems likely that S1387F is a dominant mutation.

Patient 10 is heterozygous for the R1539Q mutation, a previously reported dominantly acting mutation (20), which causes diazoxide-responsive CHI. This mutation was also present in the patient’s mother who had gestational diabetes at age 31 years and currently has glucose intolerance. A sample from the maternal grandmother, who was diagnosed with diabetes at the age of 30 years, was not available for testing.

We also found that patient 5 is heterozygous for a maternally transmitted mutation, Q1134H, and has diffuse disease. It is possible that this patient has recessively inherited HI with the accompanying mutation being undetected (23, 30).

Patient 4 is heterozygous for a paternally inherited mutation and has diazoxide-responsive diffuse disease. Dominant or recessively inherited HI with the accompanying mutation being undetected is possible.

Patients 8, 11–13 are heterozygous for a paternally inherited mutation and focal CHI remains possible in these patients. Patient 6 (H1135W) is a heterozygote for whom parental hereditary transmission could not be confirmed. Although the parents have no history of hypoglycemia or diabetes, autosomal dominant/recessive hereditary transmission, or if the mutation is paternally inherited, focal CHI is possible.

Genetic analysis of patients without mutations in ABCC8 and KCNJ11
Mutations in ABCC8, KCNJ11, or GCK were not found in patients 15–17. The GLUD1 gene was not sequenced because hyperammonemia had not been reported in any of these patients.

Discussion
In this study, we discovered ABCC8 and KCNJ11 mutations in 82% (14/17) of the CHI patients studied. We found 12 different ABCC8 mutations in 11 patients (65%) and three different KCNJ11 mutations in three patients (17%). This pick-up rate is higher than that in previous reports, which describe ABCC8 or KCNJ11 gene mutations in 30–60% of patients with CHI (31).

Although this study is limited by the relatively small number of subjects, it is possible that the high detection rate of mutations in this study is due to differences in examination method. Mutation-detection rate in a study of Japanese patients with CHI who had mutations in either the ABCC8 or the KCNJ11 genes was 24% (4/17) (32) and 79% (11/14) (33) through single-strand conformation polymorphism analysis and direct sequence method respectively. Accordingly, the high mutation discovery rate in our study may be due to the high sensitivity of direct sequence analysis (21). In addition, the hereditary differences between races and the limited selection of patients cannot be overlooked.

Contrary to previous reports (32, 34, 35) that 60% of ABCC8 mutations are located in NBD2, only 17% (2/12) of the mutations identified in this study were located within this region. The H1135W and R837X mutations of the ABCC8 gene and the A187V mutation of the KCNJ11 gene were each found in two unrelated patients respectively, and the frequencies of the remaining mutations are similar. These results illustrate that the distribution of mutations is highly heterogeneous.

KCNJ11 mutations were discovered in 17% (3/17) of patients with CHI, which account for 21% (3/14) of gene mutations resulting in KATP channel variation. These results are substantially higher than those of 0–8% (30, 32, 36–38) of KCNJ11 mutations resulting in KATP channel variation found in studies that analyzed all mutations of the ABCC8 and KCNJ11 genes.

Of the 14 patients with ABCC8 or KCNJ11 mutations, 28% (4/14) were compound heterozygotes, confirming...
autosomal recessive CHI while a single mutation was identified in 72% of patients (10/14). Of these patients, 50% (5/10) inherited the mutation from their father. Previous studies reported that the single mutation rate was 40–43% in Western countries (37, 39) and 89.5% in Japan (38) and that the paternal inheritance rate was 70 and 84.2% respectively. Our data revealed a high proportion of single mutations with a lower paternal inheritance rate.

A single maternally inherited mutation was identified in three patients. One of these patients had a previously reported dominantly acting mutation and a second patient had the mutation S1387F, for which dominant inheritance is suspected. Results from a recent study reported that 48% of adult carriers with a K\textsubscript{ATP} channel mutation, causing autosomal dominant CHI, demonstrated no hypoglycemia with 86% having no signs of diabetes (20). Patients with autosomal dominant mutations display a diverse range of symptoms ranging from asymptomatic to hyperinsulinism requiring surgery (5, 6, 20, 40). Therefore it is possible that the patients with heterozygous maternally inherited mutations, or a paternally inherited mutation with diffuse CHI, may have a dominant mutation even without a history of hypoglycemia or diabetes. It is also possible that these mutations are recessively acting, but our screening process did not detect the second mutation.

Among four of the five patients with a single paternally inherited K\textsubscript{ATP} channel mutation, who had not undergone surgery, it is possible that a second mutation of the opposite allele remained undetected by sequencing and gene dosage studies or that a focal lesion was present. However, the presence of this lesion could not be confirmed as fluorine-18 dihydroxyphenylalanine-positron emission tomography (PET–CT) facilities were not available.

\textit{ABCC8, KCNJ11}, or \textit{GCK} mutation were not identified in three patients in our cohort. Thus, additional research is necessary to investigate mechanisms of insulin secretion, other gene abnormalities associated with the glucose-sensing pathway, and analysis of \textit{ABCC8} or \textit{KCNJ11} regulatory regions and/or gene expression.

In contrast to a previous study reporting that 16–29% of neonates and 62–69% of infants with CHI responded to medication (15, 30, 41–43), the response rate in our study was 70% (7/10) in neonates and 83% (5/6) in infants. The reasons underlying a higher drug response rate in our study remain unclear and may include ethnic differences in genetic background.

The response of patients with CHI to diazoxide is diverse and has been reported to vary between 15 and 60% (14, 41). In this study, diazoxide responsiveness was observed in 38% (5/13) of patients to whom diazoxide was administered. Of the patients that exhibited a response, 80% (4/5) were positive for an \textit{ABCC8} mutation. K\textsubscript{ATP} channel mutations in patients with diazoxide-responsive HI are associated with dominantly transmitted CHI (5, 6) as well as with some sporadic or autosomal recessively transmitted cases of CHI (31, 32, 44, 45).

All six of the operated patients had the diffuse form of the disease, which is in contrast to previous studies that reported focal disease in 30–65% of surgically treated patients (9, 16). In addition, 80% of patients who underwent surgery in a Japanese study (38) exhibited focal disease and all had a single paternally inherited mutation. Compound heterozygous mutations were found in 33% (2/6) of patients with diffuse CHI, heterozygous mutations were found in 50% (3/6) of these patients, of which the only diazoxide-responsive patient had a paternally inherited mutation, and no mutations were identified in 17% (1/6) of these patients in our study cohort. These data illustrate a higher pick-up rate of heterozygous mutations compared with previous studies in which recessively inherited mutations were found in 36–47% of cases, a single heterozygous mutation in 34–36% of cases, and no mutations in 19–27% (23, 25) of cases.

When integrating the clinical findings and genetic results in this study, it was not possible to predict the response to diazoxide or histological findings through \textit{ABCC8} or \textit{KCNJ11} mutation analysis as reported (8, 18, 44, 46). The data reported in this study represent a single tertiary medical center study and there are some limitations. For example, there was a limited availability of drugs and a PET–CT scan was not available. These factors make it difficult to predict the drug responsiveness and histology through genetic analysis. This study also included a relatively small number of patients. The incidence of CHI in Korean population is not yet known; however, if the incidence is similar to previous estimates in outbred communities (i.e. one in 50,000 live births (4)), we would predict that ~236 pregnancies (based on annual mean live births of 591,508 (range: 435, 031–730, 678) (http://www.kosis.kr)) were affected with CHI between 1989 and 2009. Further work is, therefore, required to identify the remaining patients who may benefit from a genetic diagnosis. Finally, functional studies to verify the biological effects of the variants presumed to be the pathogenic mutations were not performed. Further studies with a larger number of patients should be conducted to reveal greater insight into the CHI in Korean population.

\textbf{Conclusions}

This study reports for the first time that \textit{ABCC8} or \textit{KCNJ11} mutations were present in 82% (14/17) of the Korean CHI patients studied. Furthermore, \textit{ABCC8} or \textit{KCNJ11} mutations were associated with medically responsive CHI in 83% (10/12) of our cohort. A genetic diagnosis is important for patients...
with CHI as it may provide important information regarding the histology of the disease and will provide information regarding the recurrence risk for future generations.

Supplementary data
This is linked to the online version of the paper at http://dx.doi.org/10.1530/EJE-11-0160.

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

Funding
Genetic testing performed in Seoul was supported by Grant no. 04-2007-108 from the SNUH Research Fund. S E Flanagan is the Genetic testing performed in Seoul was supported by Grant no.

Acknowledgments
The authors would like to thank Annet Damhuis for technical assistance.

References

www.eje-online.org

22 Hardy OT, Hernandez-Pampaloni M, Safer JR, Suchi M, Ruchelli E, Zhuang H, Ganguly A, Freifelder R, Adzick NS, Alavi A & Stanley CA. Diagnosis and localization of focal congenital hyperinsulinism by 18F-fluorodeoxy PET scan. *Journal of Pediatrics* 2007 150 140–145. (doi:10.1016/j.jpeds.2006.08.028)


29 Suchi M, MacMullen CM, Thornton PS, Adzick NS, Ganguly A, Ruchelli ED & Stanley CA. Molecular and immunohistochemical analyses of the focal form of congenital hyperinsulinism. *Modern Pathology* 2006 19 122–129. (doi:10.1038/modpathol.3800497)


43 Dekelbab BH & Sperling MA. Recent advances in hyperinsulinemic hypoglycaemia of infancy. *Acta Paediatrica* 2006 95 1157–1164. (doi:10.0380/035250606040414)


46 Durandell F, Fournet JC, Bas F, Junien C, Gross MS, Bundak R, Saka N & Gunoz H. *ABCC8* (SUR1) and *KCNJ11* (KIR6.2) mutations in persistent hyperinsulinemic hypoglycaemia of infancy and evaluation of different therapeutic measures. *Journal of Pediatric Endocrinology & Metabolism* 2002 15 993–1000.