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research article

## Clinical Management and Gene Mutation Analysis of Children with Congenital Hyperinsulinism in South China

**Running Head:** Clinical Management of Congenital Hyperinsulinism

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### What is already known on this topic?

Congenital hyperinsulinism (CHI) is a rare inherited disease characterized by unregulated insulin secretion and profound hypoglycemia. There are few reports pertaining to patients with CHI in south China.

### What this study adds?

This is the first study investigating the clinical features, molecular characteristics, and treatments of CHI in south China and to explore the appropriate therapeutic approaches in these patients.

### Abstract

**Objective:** To explore the clinical presentation and molecular genetics characteristics of a group of CHI patients in southern China and explore the appropriate therapeutic approaches.

**Methods:** We retrospectively reviewed a cohort of 65 children with CHI. Mutational analysis was carried out for *KCNJ11* and *ABCC8* genes. The *GLUD1* gene was sequenced in patients with hyperammonaemia. *GCK* gene sequencing was performed in those patients with no mutation identified in the *ABCC8*, *KCNJ11* or *GLUD1* genes.

**Results:** *ABCC8* mutations were identified in 16 children (25% of the cohort), *GLUD1* mutations were identified in 5 children, and no *KCNJ11* or *GCK* genetic mutations were identified.

Moreover, we found some unique features of *ABCC8* gene mutations in southern Chinese CHI patients with more novel and common mutations. **The deletion/insertion mutation**

**c.3224-3226delACC ins CAGCCAGGAACTG was found in five unrelated patients, which possibly represents a relatively common mutation in southern China. Five compound**

**heterozygous mutations of *ABCC8* gene were identified in this study, and three of them were diazoxide-responsive.** Forty patients were diazoxide-responsive, 13 patients were

diazoxide-unresponsive and 12 patients received dietary treatment only. A pancreatectomy was performed in 10 patients who were unresponsive to medical treatment.

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**Conclusion:** To the best of our knowledge, this is the first study of CHI in south China. Mutations in *ABCC8* are the most common causes of CHI in this cohort. Diazoxide and dietary treatment were effective in most patients. Multicentre studies are necessary to obtain the long-term follow-up characteristics of such patients at the national level.

**Keywords:** Congenital hyperinsulinism, Clinical management, Gene mutation

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**Conflict of interest:** None declared

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## Background

Congenital hyperinsulinism (CHI) is the most frequent cause of persistent hypoglycaemia in neonates and infants. CHI occurs due to the dysregulated and inappropriate secretion of insulin from pancreatic  $\beta$  cells in relation to blood glucose levels (1). The incidence of CHI is estimated to be 1 in 40,000-50,000 live births in northern Europe (2, 3) and 1 in 2,500 births in Saudi Arabia (4). However, there are no nationwide data regarding the incidence of this disorder in China, and the exact percentage of CHI cases is unknown.

**Inappropriate insulin secretion can suppress the production of ketone bodies, which serve as an alternative fuels during hypoglycemia. The lack of glucose and the deprivation alternative fuels for the brain will increase the risk of brain damage in these patients (5). To date, mutations in 14 different genes (*ABCC8*, *KCNJ11*, *GLUD1*, *GCK*, *HADH*, *SLC16A1*, *UCP2*, *HNF1A* (6), *HNF4A* (7), *HK1* (8), *PGM1* (9), *PMM2* (10), *CACNA1D* (11), and *FOXA2* (12)) that lead to dysregulated secretion of insulin had been described.** The most common forms of CHI are due to defects in the genes *ABCC8* and *KCNJ11*, which encode the ATP-sensitive potassium (KATP) channel subunits of the sulfonylurea receptor (SUR1) protein and inwardly rectifying potassium channel (Kir6.2) proteins, respectively (13). Both these genes are localized on chromosome 11p15.1 (14).

The clinical presentation can be varied, ranging from completely asymptomatic to mild or severe disease that is unresponsive to medication and requires surgical intervention (15). Diazoxide is the first-line agent for the treatment of CHI; **it binds to the SUR1 subunits and opens the KATP channel thereby prevents depolarization of** the  $\beta$ -cell membrane and insulin secretion (16).

If necessary, those who are unresponsive to the medical therapy should resort to surgical treatment. Histologically, CHI is divided into **three subgroups: diffuse, focal and atypical forms.** **Children with diffuse CHI may require a near-total pancreatectomy (with the risk of diabetes mellitus and pancreatic exocrine insufficiency) whereas the focal form will only require a limited focal lesionectomy.** Conventional radiological imaging is often used but is unable to distinguish between the two forms (17). <sup>18</sup>F-DOPA PET/CT scanning is an accurate and non-invasive technique to differentiate focal and diffuse types of CHI (18); unfortunately, this method is not available in southern mainland China. **The genetic analysis would be an important tool for CHI study. Children with diffuse form of CHI due to recessive mutations in *ABCC8* and *KCNJ11* usually do not respond to diazoxide. Focal forms are sporadic in inheritance and associated with a paternally inherited mutation in *ABCC8*/*KCNJ11* genes (19).**

Although the clinical characteristics and genetic aetiology of CHI patients have been described in some studies in China (20,21,22), little is known about CHI in southern China. We first reported our experiences with the management of CHI in 12 children in 2009 (23). Furthermore, the objectives of this study were to understand the clinical presentation and molecular genetic characteristics of a group of patients with CHI in southern China and to explore the appropriate therapeutic approaches.

## Subjects and Methods

### Subjects

Enrolled patients included those diagnosed with CHI who were hospitalized in the Guangzhou Women and Children's Medical Center from November 2012 to June 2017. Most of the patients with CHI came from southern China including Guangdong Province, Guangxi Province, Jiangxi Province, Hunan Province, Hubei Province, Yunnan Province and Hainan Province. Serum ammonia levels were checked in all cases. All infants and children were diagnosed with CHI based on clinical and biochemical criteria. The diagnosis of CHI was based on the following criteria, that is, whether serum insulin was simultaneously detectable ( $> 2$  mU/l) with hypoglycaemia (blood glucose  $< 2.6$  mmol/l), along with evidence of elevated glucose requirements (glucose infusion  $> 8$  mg/kg.min), in the absence of ketosis or ketonuria, and an inappropriate glycaemic response to glucagon injection at the time of hypoglycaemia (15). **Patients with a secondary cause of hyperinsulinism hypoglycaemia such as perinatal asphyxia, prematurity, intrauterine growth restriction, maternal diabetes, and syndromic forms were excluded.**

Children were defined as being responsive to diazoxide by maintaining blood glucose above 3.5 mmol/l after a short period of fasting (6) (4 h in neonates, 8 h in infants and 12 h in children). Clinical data details were obtained from medical records. The study was reviewed and approved by the Ethical Committee of Guangzhou Women and Children's Medical Center (2016022210).

### Genetic analysis

Genomic DNA was extracted from peripheral blood leucocytes using a kit according to the manufacturer's instructions (Qiagen, Germany). All exons and intron-exon boundaries of the *ABCC8* and *KCNJ11* genes were PCR amplified, purified and sequenced. The sequences were analysed and compared to the wild-type published reference sequences (NM\_000525 for *KCNJ11* and NM\_000352.3 for *ABCC8*) using Chromas software (Technelysium Pty Ltd, South Brisbane, Australia). The *GLUD1* gene was sequenced in patients with hyperammonaemia, whereas *GCK* gene sequencing was subsequently performed in those patients with no mutation identified in the *ABCC8*, *KCNJ11* or *GLUD1* genes. The new mutation sites were determined by searching the Human Gene Mutation Database (HGMD) and the NCBI database. All variations were identified in this study using the Single Nucleotide Polymorphism database (dbSNP) and the 1000 Genomes Project database. Intronic variants were analysed with GenSCAN. To test the pathogenicity of novel missense mutations, Polymorphism Phenotyping (PolyPhen, <http://genetics.bwh.harvard.edu/pph>) and Sorting Intolerant from Tolerant (SIFT, <http://blocks.fhcrc.org/sift/SIFT.html>) were used.

### Treatment and follow-up

The following aspects of CHI patients were analysed: neonatal history, clinical presentation, treatment and complications. Intravenous glucose infusion to maintain blood glucose levels of > 2.8 mmol/l and nutritional therapy and diazoxide treatment were initiated immediately upon diagnosis. Nutritional therapy included frequent meals enriched with complex carbohydrates and nasogastric feeding at midnight. For those younger than six months of age, a glucose polymer, maltodextrin (Malt Extract, Wakodo, Asahi Group Foods, Ltd., Japan), was utilized, and for those older than six months, supplemental uncooked corn starch was used between meals, before bedtime and for night-time feeding through a nasogastric tube. Five patients with hyperinsulinism/hyperammonaemia (HI/HA) syndrome were **performed a diagnostic tests for protein-sensitive hypoglycemia. The blood glucose concentrations of all 5 patients fell after the protein load and an age-adjusted daily requirement of protein combination with fat and carbohydrate was started.**

The dosage of diazoxide was started at 10 mg/kg.d and did not exceed 15 mg/kg.d in three divided doses. When diazoxide treatment was effective, the dosage was reduced to the effective minimum. Oral hydrochlorothiazide (1-2 mg/kg.d) was used in conjunction with diazoxide to counteract the fluid-retaining properties of diazoxide. All patients treated with diazoxide were carefully monitored for fluid and sodium retention. In three children who were not responsive to diazoxide, octreotide (5-25 µg/kg.d) injections were administered. Pancreatectomy was implemented in patients not responding to medical therapy. Clinical follow-up was initiated one month after hospital discharge and continued at subsequent intervals of 3 months. Self-monitored blood glucose levels were recorded. Brain damage was evaluated at the age of diagnosis and every 3 months of follow-up by a pediatric neurologist.

#### Statistical analysis

The results were analysed using the SPSS 17.0 program (International Business Machines, Armonk, NY, USA) and were expressed as the mean ± standard error of the mean (mean ± SE) and in percentages (%). The Student's t-test and the Wilcoxon test were used for the statistical analysis of the data. All *p* values less than 0.05 were considered significant.

#### Results

In this study, a total of 65 patients (47 males and 18 females) with a diagnosis of CHI ranging from immediately following birth to seven years were included. Twenty-three patients (35.4%) were macrosomic, and their mean birth weight was 3,690 g. Sixty-two patients were born at term. The CHI symptoms occurred during the neonatal period in 29 patients (44.6%), during the infancy period (1–12 months) in 26 patients (40%) and during childhood (> 12 months) in 10 patients (15.4%).

Of the 65 patients, 13 were diazoxide-unresponsive, 40 patients were diazoxide-responsive and 12 received dietary treatment only. The clinical and biochemical characteristics of the patients in the two groups (Group1, diazoxide-unresponsive; Group2, diazoxide-responsive or dietary treatment) are presented in Table 1. The age at onset of CHI was significantly different between these two groups. The neonatal form composed 92.3% of the group 1, but it composed only 32.7% of the group 2. There was a higher incidence of epilepsy in the group 2 than in the group 1 (*p* < 0.05). The time between symptom manifestation and diagnosis ranged from one day to six years, and the duration was longer in the group 2. **A patient (Case 61) in group 2, 6 years old, was initially misdiagnosed as having seizure disorders before the hypoglycemia was detected.**

In our study, *ABCC8* mutations were identified in 16 children (25% of the cohort), and no *KCNJ11* mutations were identified on KATP channel gene mutation analysis. Five patients with persistent hyperammonaemia had mutations in *GLUD1* (Fig.1). No variants were found in the *GCK* gene. Fifteen different *ABCC8* mutations were discovered, five mutations were compound heterozygous, none were homozygous and 11 were heterozygous (Table 2). **Among these children carried compound heterozygous mutations, three children were effective to the treatment of diazoxide, one child was ineffective to the treatment, and one child regulated with diet.** The most common mutation was a deletion/insertion mutation c.3224-3226delACC ins CAGCCAGGAAGT found in five unrelated patients, which possibly represents a relatively common mutation in southern China. Five novel *ABCC8* mutations (p.Phe5SerfsX72,

p.Gln273ArgfsX85, p.Leu724del, p.Asp1447Gly and IVS 25-1G>T) were identified in this study. Of the novel mutations, two were frameshift mutations, one was a deletion mutation, one was a missense mutation and one was a splice site mutation. In accordance with the guidelines from the American College of Medical Genetics and Genomics (ACMG) (24), two variants were perceived as “pathogenic” and three variants were predicted as “likely pathogenic”. **A novel heterozygous variant in *ABCC8* gene was identified in case 22. The patient has now been on therapy with diazoxide for more than 1 year at the dose of 5mg/kg.d and with normal growth and development. In one case (patient 59), two novel mutations were identified. She was macrosomic at birth. She was first found hypoglycemia on day three after birth at a local hospital, and the hypoglycemia was improvement after frequent feeding. But, her parents did not monitor her blood glucose from then on. She admitted to our hospital at 14 months old for the brief generalized shaking several times. Clinical laboratory tests revealed hypoglycemia (blood glucose: 2.5mmol/L) hyperinsulinism (plasma insulin level 5.9μIU/mL) when she had an episode. Four hourly daytime feeds (solids and cow’s milk) and four hourly uncooked cornstarch (1.6g/kg) could maintain the blood glucose above 3.5 mmol/L. During 6 months follow-up, there was no episodes of hypoglycemia. However, she has sustained hypoglycaemic brain injury with global developmental delay.**

In this study, 16 patients underwent genetic test. five patients (patients 1, 5, 10, 14 and 18) had paternally inherited monoallelic mutations. Of the five patients, three were diazoxide-unresponsive, and two were diazoxide-responsive. In the three diazoxide-unresponsive patients (patients 1, 5 and 10), diffuse pancreatic disease was confirmed following surgery. One patient (patient 3) had two heterozygous mutations: one missense mutation c.314A > C (p.His105Pro) in exon 3 inherited from his father and a nonsense mutation c.2800C > T (p.Gln934X) in exon 23 inherited from his mother. He was diazoxide-unresponsive, which we had already reported (25). The 18F-DOPA PET/CT scan indicated a focal lesion in the head of the pancreas whereas the histology of the resected pancreas showed atypical form. The enlargement of pancreatic β-cell nuclei distributed in the head, body, and tail of the pancreas and mainly in the head. The abnormal active endocrine cells were not restricted to a focal lesion nor throughout the entire pancreas.

The *GLUD1* gene was detected in patients with hypoglycaemia, hyperinsulinism and mild hyperammonaemia. Three different heterozygous mutations in the *GLUD1* gene were identified in five patients. The p.Arg322His mutation was found in patients 54, 55 and 56. Patients 55 and 56 were sisters. The mutation was autosomal dominantly inherited from their father, who was an asymptomatic carrier. The p.Ser498Leu mutation was found in patient 52 and the p.Asn463Asp mutation was found in patient 53. All mutations have been previously reported in patients with HI/HA (26,27,28,29). The serum ammonia concentration of this group of patients was 85-184 μmol/l. After a confirmation of the diagnosis of HI/HA syndrome caused by a *GLUD1* genetic defect, the patients were started on a low-protein diet (1.5g/kg.d of natural protein intake). Three patients (patients 54, 55 and 56) were successfully managed through the manipulation of diet alone. They have had no further hypoglycaemia episodes. The other two patients were responsive to diazoxide treatment.

61.5% (40/65) achieved long-term stable glycaemic control by diazoxide alone. Three children who were not responsive to diazoxide, octreotide was administered. Among these three patients, two patients were unresponsive to octreotide, and one patient discontinued this drug due to severe diarrhoea. Side effects of the diazoxide treatment were observed in 32 (80%) patients.

Gastrointestinal disturbances such as nausea, vomiting, severe gastrointestinal upset and poor appetite occurred in 69% of patients. Six patients were fed through a nasogastric tube because of severe gastrointestinal reactions, and their blood glucose levels were kept relatively stable. Different degrees of hypertrichosis occurred in 55% (22/40) of patients during the clinical follow-up.

In one case (patient 43), effective diazoxide therapy had to be stopped because of thrombocytopenic purpura.

Pancreatectomy was performed in 10 patients who were unresponsive to drugs. Nine patients were treated with subtotal pancreatectomy, and one patient received pancreatectomy twice. In patient 3, a second resection of the pancreas was required because of sustained hypoglycaemia (25). Histological examination of the resected pancreatic tissue confirmed diffuse disease in nine

patients and atypical form in one patient. One patient (patient 7) who underwent surgery at two months of age developed diabetes mellitus at 5 years old and was treated with insulin. Case 8 developed diabetes mellitus immediately after surgery and required insulin treatment. Two cases still had mild hypoglycaemia after surgery; one (patient 4) was successfully managed with regular daytime and overnight feedings, and one (patient 9) was treated with diazoxide. **Only one case had malabsorption symptoms.**

**Diazoxide treatment was stopped in 14 of these patients (35%) between six months and four years, and no recurrence of hypoglycaemia was observed.** Eight patients with subtotal resection were able to maintain normal blood glucose and HbA1c levels at the follow-up duration. There were 3 diazoxide-unresponsive patients: one patient died of multiorgan failure and two patients abandoned the treatment and died of severe hypoglycemia after 3 to 7 days at home.

## Discussion

In this study, we reported the clinical characteristics, laboratory data and genetic features of 65 patients with CHI, which was the largest CHI cohort in southern China. Until now, there have been no nationwide data regarding this disorder in China, although several studies have summarized the clinical and genetic characteristics of CHI in northern and eastern China (20,21,22).

Of the patients with CHI from southern China, 32% were noted to have disease-causing mutations in our study: 14 (25%) patients were positive for *ABCC8* mutations, five (7.8%) patients were positive for *GLUD1* mutations and 44 (67%) patients were negative for *GCK*, *GLUD1*, *ABCC8* and *KCNJ11* mutations in the gene analysis. No mutations were found in the *KCNJ11* gene in this study. As described in previous studies, most of the mutations identified have been detected in the KATP channel. The mutation detection rates of *ABCC8* and *KCNJ11* genes reported by Kapoor *et al.* (6) and Snider *et al.* (26) were 36.3% (109/300) and 69% (288/417), respectively. However, **a similar study conducted by a large group of Turkey found that the mutation rate in the *ABCC8/KCNJ11* genes was (48.6%)% (17/35) (30).** This pick-up rate (16/65, 25%) is lower than those in previous reports and differed from those in recent studies in China, which reported mutation rates of approximately 44% (12/27) (31) and 67.6% (25/32) (20) in the *ABCC8* and *KCNJ11* genes. Accordingly, the low mutation discovery rate in our study may be due to the difference in genetic background among most of the cases from southern China. In our study, we found the deletion/insertion mutation c.3224-3226delACC ins CAGCCAGGAACTG in five patients, which may be the most common mutation of CHI in the southern Chinese population. These findings indicate that a geographical distribution difference exists in the mutational spectrum of the *ABCC8* gene in the Chinese population. This mutation causes a frameshift and introduces a premature stop codon 75 codons downstream of the mutation, leading to the loss of the functional domain NBD2 (20). **Five patients carried compound heterozygous mutations in this study. It was previously demonstrated that patients harboring compound heterozygous mutations of *ABCC8* gene were usually associated with medically unresponsive CHI (32). However, in the present study, three patients were effective to the treatment of diazoxide, and one patient regulated with diet. Dekel *et al.* (33) reported that some compound heterozygous mutations may cause milder hyperinsulinism which is responsive to diazoxide. Kumaran *et al.* (34) also reported a case of transient hyperinsulinaemic hypoglycaemia due to a compound heterozygous mutation in *ABCC8*. The mechanisms responsible for this clinical variability may be related to background genetic factors and other unknown factors involved in regulating gene expression (35).**

Five novel mutations were found in the *ABCC8* gene in five patients. One patient (patient 59) was a compound heterozygote with two novel deletion mutations, P.Gln273ArgfsX85 and P.Leu724del, and only dietary treatment could achieve stable glycaemic control.

Current medical management for CHI includes diazoxide combined with chlorothiazide as the first-line therapy (36). Diazoxide binds to the SUR1 subunit of the KATP channel and reduces insulin secretion by hyperpolarisation of the pancreatic  $\beta$ -cell plasma membrane (15). After diagnosis, a therapeutic trial with diazoxide was performed immediately. In our cohort, 40 (61.5%)

CHI patients were diazoxide-responsive. Similar to our results, Kapoor *et al.* (6) recently reported that 64% of their cohort responded to diazoxide treatment. In the cohort reported by Zeynep Şıklar *et al.* (19), 71% (100/141) of Turkish patients with CHI were responsive to diazoxide treatment. The recommended dosage of diazoxide is 5-15 mg/kg.d (37), and the effective dosage of diazoxide is believed to always be lower than 15 mg/kg.d. In our study, the initial dosage of diazoxide was 10 mg/kg.d, and the minimal effective dosage was sought to maintain the stability of blood glucose. If the patient was unresponsive to a dosage of 10 mg/kg.d of diazoxide, further increasing the dosage did not improve the effect but rather increased the risk of serious complications. Gastrointestinal symptoms, such as vomiting, nausea and poor appetite, were common if dosages higher than 10 mg/kg.d were administered. One patient receiving effective diazoxide therapy had to stop treatment due to serious gastrointestinal symptoms, and three cases required nasogastric tube feeding. To improve the effectiveness and reduce side effects, we believe that diazoxide should be used at the minimal effective dosage.

In this study, 35 of 65 patients developed mild mental retardation including 4 patients association with HI/HA syndrome. Both low blood glucose and insufficient treatment increased the risk of neurodevelopmental impairment in CHI (38). HI/HA syndrome is caused by activating mutations in the *GLUD1* gene, which encodes the intramitochondrial enzyme glutamate dehydrogenase (GDH) (39). The epilepsy and developmental problems in HI/HA syndrome are thought to be a result of recurrent hypoglycaemia, chronic hyperammonaemia or decreased brain concentrations of the neurotransmitter GABA due to increased GDH activity (27). The high rate of developmental delay in this study is likely due to the late diagnosis. Given that the clinical symptoms of this disease were not specific, and their hyperinsulinism may be less severe, more infants with CHI were misdiagnosed and not recognized until they presented with hypoglycaemic seizures weeks to months later. The earlier detection of blood glucose and serum insulin levels will be helpful for diagnosis. Therefore, neonates, infants and children should be evaluated for hypoglycaemia (40). Dietary treatment is an important aspect of care for all patients with CHI. In our study, 18% of patients could achieve glycaemic stability with dietary treatment alone. Frequent feedings and specific diets include the provision of adequate carbohydrates to maintain normoglycaemia. Dietary treatment showed a good response in some cases and should be the initial treatment for all CHI patients. Glucose polymer and uncooked corn starch were added based on the patients' age to increase the carbohydrate content. Some infants may require a nasogastric tube for regular and frequent feedings. Patients with HI/HA syndrome require a protein-restricted diet. Feeding problems such as difficulty with sucking, swallowing, vomiting and food refusal occur in a significant proportion of children with CHI, **continuous feeding through nasogastric tube or gastrostomy is required** (41).

A pancreatectomy was implemented in 10 diazoxide-unresponsive CHI patients, accounting for 15.4% of all CHI patients. During surgery, none of our patients were identified to have a focal lesion. A pathological examination of pancreatic tissues revealed the diffuse form of hyperinsulinism in 9 of 10 cases. The diffuse type was the most common type of CHI in our study. The findings were similar to those reported by Bellanne'-Chantelot (58.7% (64/109)) (42) and Li *et al.* (89.5%) (43). In total, 40% (4/10) of patients with CHI unresponsive to diazoxide had *ABCC8* mutations. In these four patients with the diffuse form of CHI proved by histology, three cases carried a single heterozygous *ABCC8* mutation, and one case carried a compound heterozygous mutation; a segregation analysis of both parents showed that the mutation was paternally inherited in three patients and biparentally inherited in one patient. CHI with a single paternally inherited heterozygous mutation in the *ABCC8* gene suggests focal disease (29,37). However, Chandran *et al.* (44) reported that heterozygous paternal mutations cause diffuse CHI. Paternal mutations causing diffuse disease may act via a different mechanism from that of recessive mutations (45).

### Study Limitations

The major limitations of our research is a single-center study other than multiple centers and cross-sectional research. Multicentre studies are necessary to obtain the long-term follow-up characteristics of such patients at the national level in the future.

## Conclusion

In summary, a genetic diagnosis was made for 32% of the patients in this large cohort. Mutations in the *ABCC8* gene were the most common identifiable cause. However, we found some unique features of *ABCC8* gene mutations in southern Chinese CHI patients with more novel and hot-spot mutations. Diazoxide and dietary treatment were effective in most patients. In the remaining 68% of patients, the genetic cause of hypoglycaemia remains unknown, **a targeted gene panel for CHI and even a whole-exome sequencing (WES) analysis** would apply in these patients.

## Ethics

**Ethics Committee Approval:** The study was approved by the ethical committee of Guangzhou Women and Children's Medical Center (approval number: 2016022210).

**Informed Consent:** Consent form was filled out by all parents and participants.

**Peer-review:** Internally peer-reviewed.

## Authorship Contributions

Surgical and Medical Practices: Zhe Wen, Xu A, Zhihong Zhou

Concept: Li Liu, Xiuzhen Li, Xu A,

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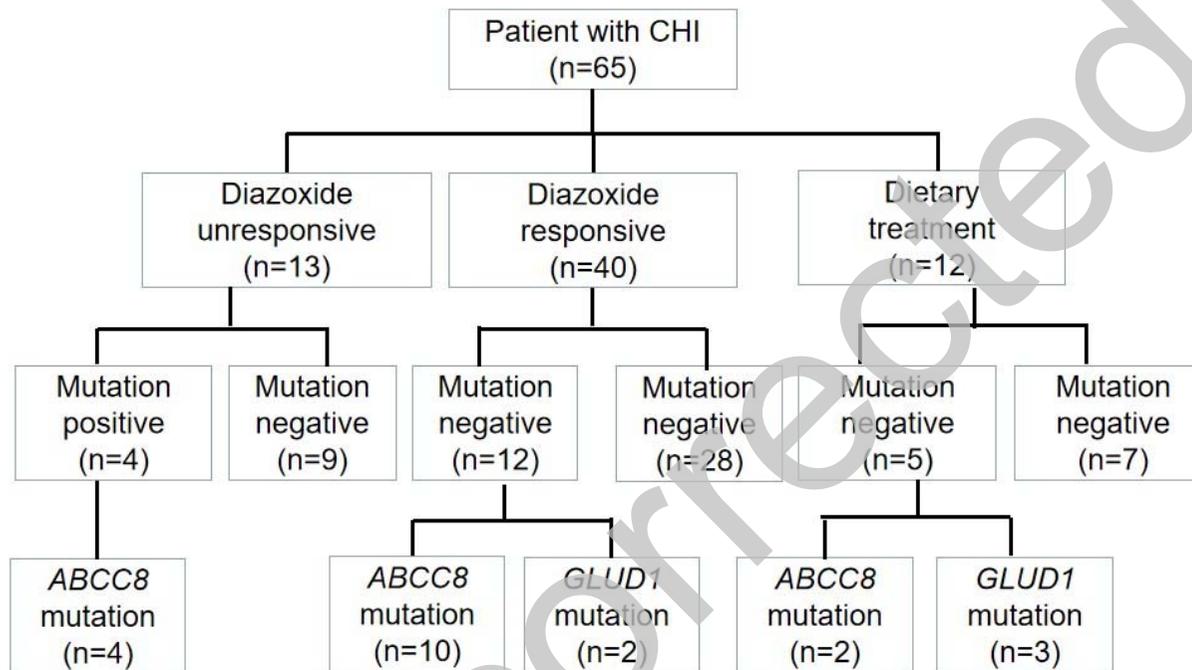
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Figure 1. Distribution of patients according to mutation analysis results and treatment choices for patients with CHI.



	Group1 (n=13)	Group2 (n=52)	<i>p</i>
Sex (Male:Female)	9:4	36:16	
Age of onset			
Neonate (0-4 weeks)	n = 12(92.3%)	n = 17(32.7%)	<0.01
Infancy (1-12 months)	n = 1(7.7%)	n = 25(48.1%)	<0.01
Childhood (>12 months)	0	n = 10(19.2%)	<0.01
Birth weight (kg)	4.0 ± 0.6	3.7± 1.4	
Seizures	n = 4(30.8%)	n = 36(69.2%)	0.02
Other (cyanosis, food refusal, lethargy)	n = 6 (46.2%)	n = 16(30.8%)	
Time to diagnosis (days) <sup>a</sup>	35 (1-120)	163 (1-2610)	0.02
Blood glucose (mmol/l)	1.89 ± 0.71	1.96 ± 0.56	
Plasma insulin (mmol/l) <sup>a</sup>	32.4 (5.7-96.4)	26.6 (4.8-87.1)	
Blood ammonia level (mmol/l)	50.8 ± 12.1	60.1± 28.4	
Neurodevelopmental delay	3(23.1%)	32(61.5%)	0.02
Mutation gene			
<i>ABCC8</i>	5	12	
<i>GLUD1</i>	0	5	

Table 1. Clinical and biochemical characteristics of the CHI patients

Data are the mean ± S.D. or percentage

<sup>a</sup> Median (range)

Group1: diazoxide-unresponsive

Group2: diazoxide-responsive or dietary treatment

Table 2. Genetic and biochemical analysis of 21 patients with mutation-positive CHI

Patient number	Gender	Gestational age( week)	Birth weight (Kg)	Onset age	Glucose levels	Insulin levels	Mutations				Treatment	
					at the diagnosis (mmol/L)	at the diagnosis (μIU/mL)	Gene	Nucleotide	Protein	Inherited from		Previously reported
1	M	38	3.0	1 day	2.4	4.8	<i>ABCC8</i>	c.3224-3226delACC ins CAGCCAGGAACTG	p.Thr1042GlnfsX75	Paternal	Yes	Pancreatectomy
3	M	35	4.1	1 day	0.6	38.3	<i>ABCC8</i>	c.314A > G c.2800C > T	p.His105 Pro p.Gln934X	Bilateral	Yes	Pancreatectomy
5	M	39	3.5	3 months	2.1	21.6	<i>ABCC8</i>	c.3224-3226delACCinsC AGCCAGGAACTG	p.Thr1042GlnfsX75	Paternal	Yes	Pancreatectomy
10	M	38	4.0	1 day	1.3	25.9	<i>ABCC8</i>	c.2113C > T	p.Arg705X	Paternal	Yes	Pancreatectomy
14	F	39	2.8	3 days	2.4	30.5	<i>ABCC8</i>	c.1879delC	p.His627MetfsX20	Paternal	Yes	Diazoxide
16	M	38	4.1	1 day	2.6	26.7	<i>ABCC8</i>	c.1990C > T	p.Gln664X	No sample	Yes	Diazoxide

18	M	37	4.8	3 days	1.6	16.4	<i>ABCC8</i>	c.3224-3226delACC insCAGCCAGGAACTG	p.Thr1042GlnfsX75	Paternal	Yes	Diazoxide
21	M	38	4.8	3 days	0.6	42.9	<i>ABCC8</i>	c.12delc	p.Phe5SerfsX72	No sample	No	Diazoxide
								c.3224-3226delACC insCAGCCAGGAACTG	p.Thr1042GlnfsX75		Yes	
22	M	39	3.5	4 months	1.8	8.7	<i>ABCC8</i>	c.4340A > G	p.Asp1447Gly	–	No	Diazoxide
23	M	40	4.35	3 days	1.7	5.8	<i>ABCC8</i>	c.3224-3226delACC insCAGCCAGGAACTG	p.Thr1042GlnfsX75	–	Yes	Diazoxide
31	M	39	3.55	1 month	2.6	7.3	<i>ABCC8</i>	c.4213G > A	p.Asp1405Asn	–	Yes	Diazoxide
47	M	34	3.4	1 day	2.2	8.7	<i>ABCC8</i>	c.4211T > C	p.Leu1404Thr	Maternal	Yes	Diazoxide
48	F	39	4.95	1 day	1.7	13.1	<i>ABCC8</i>	c.1792C > T	p.Arg598X	No sample	Yes	Diazoxide
								c.3641G > A	p.Arg1214Gln		Yes	
49	M	38	4.1	1 day	2.8	9.9	<i>ABCC8</i>	c.1792C > T	p.Arg598X	No sample	Yes	Diazoxide
								IVS 25-1G > T			No	
57	F	41	3.5	4 days	2.3	7.1	<i>ABCC8</i>	c.12delc	p.Phe5serfsX72	Maternal	No	Dietary
59	F	40	4.2	3 days	2.5	5.9	<i>ABCC8</i>	c.817delC	p.Gln273Argfsx85	No sample	No	Dietary
								c.2169-2171delTCT	p.Leu724del		No	
52	M	38	3.6	7 months	2.6	8.3	<i>GLUD1</i>	c.1493C > T	p.Ser498Leu	–	Yes	Diazoxide
53	F	Yes	3.8	13 months	2.4	10.7	<i>GLUD1</i>	c.1387A > G	p.Asn463Asp	–	Yes	Diazoxide

54	F	Yes	2.5	11 months	2.5	4.8	<i>GLUD1</i>	c.965G > A	p.Arg322His	Maternal	Yes	Dietary
55	F	Yes	3.2	10 months	2.4	5.7	<i>GLUD1</i>	c.965G > A	p.Arg322His	Paternal	Yes	Dietary
56	F	Yes	3.3	2 months	2.7	6.9	<i>GLUD1</i>	c.965G > A	p.Arg322His	Paternal	Yes	Dietary

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Uncorrected proof