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Investigating Genetic Mutations in a Large Cohort of Iranian Patients with Congenital Hyperinsulinism

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What is already known on this topic?

It is well known that congenital hyperinsulinism (CHI) is the most frequent cause of severe and persistent hypoglycaemia in the neonatal period, infancy, and childhood. To date, mutations in at least nine different genes have been reported to cause CHI*: ABCC8, KCNJ11, GLUD1, GCK, HADH, HNF4A, SLC16A1, HNF1A* and *UCP2*. Data are mainly limited to European populations while the occurrence of the pathogenic mutations underlying CHI are higher in consanguineous families which are more prevalent in Asian societies.

What this study adds?

We report the frequency of causal gene mutations in a cohort of Iranian children with a diagnosis of CHI and add five novel mutations. Based on our findings we recommend screening of *HADH* gene variants in all patients with diazoxide-responsive CHI if there is no access to targeted next generation sequencing.

Abstract

Objective: Congenital hyperinsulinism (CHI) is the most frequent cause of severe and persistent hypoglycaemia from birth. Understanding the pathophysiology and genetic defects behind hyperinsulinism and its complications provides clues to timely diagnosis and management. The aim of this study was to evaluate the underlying genetic aetiology of a specific Iranian pediatric cohort with CHI.

Methods: A total of 44 unrelated children, 20 girls and 24 boys, with an initial diagnosis or history of CHI from all regions of Iran were recruited between 2016 and 2019. Targeted next generation sequencing (tNGS) was performed for the genes found in about half of CHI patients.

Results: Mutations were identified in 24 cases (55%). Patients with a confirmed genetic cause were mainly diagnosed below age of one year old ($p = 0.01$), had fewer other syndromic features, excluding seizure, ($p = 0.03$), were less diazoxide responsive ($p = 0.04$) and were more diazoxide unresponsive leading to pancreatectomy ($p = 0.007$) compared to those with no identified mutations. Among 24 patients with identified genetic mutations, 17 (71%) had a mutation in *ABCC8*, 3 (12%) in *KCNJ11*, 3 (12%) in *HADH*, and 1 patient had a mutation in *KMT2D*. These included five novel mutations in *ABCC8*, *KCNJ11*, and *KMT2D*.

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Conclusion: This is the biggest genetic study of CHI in Iran. A high frequency of recessive forms of CHI, especially HADH mutations, in our study could be due to a high rate of consanguineous marriage. We recommend tNGS to screen for all the CHI genes.
Keywords: Congenital hyperinsulinism, genetic mutations, diazoxide, targeted next generation sequencing

Introduction

Congenital hyperinsulinism (CHI) is the most frequent cause of severe and persistent hypoglycaemia and the most common metabolic abnormality in the neonatal period, infancy, and childhood (1,2). There is an unregulated secretion of insulin from pancreatic β-cells in the course of low blood glucose in CHI patients, leading to severe and persistent hypoglycaemia due to genetic defects (3,4). CHI is rare but a very high incidence has been reported in isolated European populations and also communities with a high rate of consanguinity (5,6,7). About 60 % of infants with CHI develop hypoglycaemia during the first months of life. This condition typically goes through remission and flare-up cycles due to hypoglycaemia. Age of onset is variable and symptoms can range from asymptomatic and mild to severe symptoms, including medically unresponsive hypoglycaemia (5,6).

The clinical manifestations, histological subtypes and underlying molecular mechanisms of CHI are heterogeneous (4). Based on histological assessments, there are two major subtypes of CHI including diffuse and focal forms (3,8). The differentiation between these two subtypes is important for clinical management. The diffuse form, where abnormality is in all pancreatic β-cells, is inherited in an autosomal recessive or dominant mode, most commonly due to mutations in *ABCC8* and *KCNJ11*. Recessive mutations in *ABCC8/KCNJ11* are usually severe and require high concentrations of intravenous glucose to maintain normoglycemia while dominant mutations usually cause milder disease. The focal form is confined to a small region of the pancreas with sporadic inheritance. This subtype results from a heterozygous paternal mutation in *ABCC8/KCNJ11* and somatic loss of maternal chromosome 11p15 in the focal pancreatic lesion (9).

Understanding the pathophysiology and genetic defects behind hyperinsulinism and its complications has provided clues to diagnosis and management of the disease. Since CHI causes a set of complex and heterogenous metabolic complications, cases will have different clinical presentation with different age of onset and prognosis. The conservative treatment includes diazoxide as first choice. However, some children, mainly those with the recessive mutations in *ABCC8/KCNJ11*, develop a form of disease unresponsive to medical therapy and so pancreatectomy is the only option (3,10).

Since severe hypoglycaemia has a negative effect on neural system function, especially during the early years of life, early diagnosis and treatment are important. In recent decades there has been a substantial expansion in information of genetic defects leading to CHI. To date, mutations in at least nine different genes have been reported to cause CHI: *ABCC8, KCNJ11, GLUD1, GCK, HADH, HNF4A, SLC16A1, HNF1A* and *UCP2* (4,9). These genes are involved in regulating insulin secretion from β-cells (4). Data about CHI are mainly limited to European populations. However, the occurrence of the pathogenic mutations underlying CHI is increased in consanguineous families compared with non-consanguineous families (4). Therefore, investigating the aetiologies of this disorder is even more momentous in Asian countries with highly consanguineous populations, including Iran.

The aim of this study was to assess genetic mutations underlying CHI by recruiting individuals diagnosed with CHI. All the genetic and clinical data was combined to provide an Iranian CHI database for patients and specialists. We also report the frequency of causal gene mutations and describe a number of novel mutations.

Methods

Study Participants

Unrelated participants diagnosed with CHI were recruited to the study. Patients were from all regions of Iran and who had been referred to two centres in Iran, Imam Reza Hospital, Mashhad, Iran and the Division of Endocrinology and Metabolism in the Department of Paediatrics at the Children's Medical Centre in Tehran, Iran (Table 1). Clinical information was supplied by the referring clinicians. Informed consent was obtained from parents on behalf of their children. Peripheral blood samples were collected from affected participants and their parents at the time of referral and used to perform genetic testing.

Clinical Data

CHI was defined as fasting hypoglycaemia [glucose < 50 mg/dL (2.8 mmol/l)] occurring simultaneously with an inappropriately detectable plasma insulin (> 2.0 µU/mL) (11). Paediatric subjects whose hyperinsulinemic hypoglycemia (HH) did not remit after at least three months follow-up were eligible to be enrolled in this study. A detailed clinical

and demographical history, along with EDTA blood sample from the affected individual, both parents and affected siblings, were obtained. In the clinical history, diazoxide-responsiveness was defined as the ability to achieve elevated intravenous glucose and maintain normoglycemia (12). Low birth weight was determined based on birth weight adjusted by the gestational age (13).

Genetic Analysis

The genetic testing and variant interpretation were performed by the Exeter Molecular Genetics Laboratory (Exeter, UK). Briefly, DNA was extracted using standard methods and the samples were analysed for coding and flanking intronic regions of the *KCNJ11* (NM_000525.3) and *ABCC8* (NM_001287174.1) genes by Sanger sequencing. If no mutation was found, targeted next generation sequencing (tNGS) (Agilent custom capture v5.3/Illumina NextSeq500) for the coding regions and exon/intron boundaries of the genes found in about half of CHI patients (*ABCC8, KCNJ11, AKT2, GLUD1, GCK, GPC3, HADH, HNF4A, KDM6A, KMT2D, SLC16A1, CACNA1D, PMM2, TRMT10A and HNF1A*) (14) was performed. This assay can also detect partial/whole gene deletions and duplications (15). For a patient with mosaicism in *KMT2D*, a confirmatory dosage analysis of exons 51-54 of the *KMT2D* genes (NM_003482.3) by Droplet Digital PCR using EvaGreen was performed.

Variants were classified according to the American College of Medical Genetics and Genomics and the Association for Molecular Pathology standards and guidelines for the interpretation of sequence variants (16). The frequencies of the identified variants were checked in GnomAD [>120 000 individuals (http://gnomad.broadinstitute.org)] and in human variant and mutation databases, such as ClinVar and Human Gene Mutation Database, as well as in the literature via PubMed and Google searches. The *in silico* tools SIFT, PolyPhen-2 and Align-GVGD were used to assess the pathogenicity of missense variant effects, and the prediction of variant effect on mRNA splicing was made using SpliceSiteFinder-like, MaxEntScan, GeneSplice, NNSPLICE and Human Splicing Finder. All *in silico* programs were accessed through the ALAMUT Visual software version 2.7.1 (Interactive Biosoftware, Rouen, France). Conservation of amino acids and nucleotides across multiple species was performed using the University of California Santa Cruz genome browser (http://genome.ucsc.edu).

Ethical Considerations

The study was approved by the Ethical Committee of the Endocrinology and Metabolism Research Institute (ethical code: IR.TUMS.EMRI.REC.1397.009, date: 18.07.2018). The consent form was signed by all participants, or in the case of minors, the consent form was signed by their parents or legal guardian. A signed, written consent form was separately obtained for genetic testing. All procedures performed in this study were in accordance with the ethical standards of the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Statistical Analysis

We used the chi-square test to assess the differences between patients with confirmed genetic cause and those with no identified mutations groups.

Table 1. The clinical features of congenital hyperinsulinism patients in our study

	n (%)	Gender n		Clinical features n							Novel mutation n
		Girls	Boys	Low birth weight	Hypoglycaemia	Diagnosed < 1 year	Positive consanguinity	Other clinical features	Diazoxide responsive	Pancreatectomy	
Total	44 (100%)	20	24	8	30	34	21	7	35	8	-
No mutation	20 (45%)	8	12	7	14	12	7	7	20	0	-
With mutation	24 (55%)	12	12	1	16	22	14	0	15	8	5
ABCC8	17 (71%)	9	8	1	11	16	10	0	9	7	2
KCNJ11	3 (12%)	3	0	0	3	3	2	0	2	1	2
HADH	3 (12%)	0	3	0	1	3	2	0	3	0	0
KMT2D	1 (4%)	0	1	0	1	0	0	0	1	0	1

Result

Patient Characteristics

Forty-four unrelated children, 20 (45.45%) girls, with an initial diagnosis or history of CHI from all regions of Iran, diagnosed between 2016 and 2019 were recruited. Of the 44, 21 children (48%) came from consanguineous families (Table 1). Age of diagnosis varied but 36 (81.8%) were diagnosed before their first birthday. Only two (4.5%) patients had a positive family history of hypoglycaemia and 13 (29.5%) had a family history of diabetes. Low birth weight was reported for 10 (22.7%) patients. Insulin level ranged from 7.2 to 147 mU/L within our population and pancreatectomy had already been performed in eight (18.2%). Other symptoms, including hypokalaemia, autism, renal failure, seizure, oesophageal atresia, and failure to thrive were observed in 14 (31.8%). Nine out of 44 patients (20.45%) were Diazoxide-unresponsive including eight patients who had a history of pancreatectomy.

Disease causing mutations were identified in 24 cases (55%). Patients with a confirmed genetic cause in any of the known genes, were mainly diagnosed below the age of one year old ($p = 0.01$), had fewer other syndromic features, excluding seizure ($p = 0.03$), were less diazoxide responsive ($p = 0.04$) and were more diazoxide unresponsive leading to pancreatectomy ($p = 0.007$) compared to those with no identified mutations. Birth weight, hypoglycaemia and consanguinity rate was similar between the two groups (Tables 1, 2, 3).

Genetic Findings

Among 24 patients with identified genetic mutations, 17 (71%) had a mutation in *ABCC8*, 3 (12%) in *KCNJ11*, 3 (12%) in *HADH*, and one patient had a mutation in *KMT2D* (Table 1). These included five novel mutations in *ABCC8, KCNJ11,* and *KMT2D*. Details of the mutations and the clinical features of the patients are described below and in Table 2.

Patients with *ABCC8* mutations. A total of 17 probands, including eight males and nine females were found with mutations in *ABCC8* gene, of whom two patients were carrying novel mutations; a homozygous in-frame deletion (c4724-4732del) and a heterozygous missense *de novo* mutation (c.1109G > C; p.Arg370Thr). Both of these patients were diazoxide responsive. In total, five patients had heterozygous mutations, one compound heterozygous and 11 patients had homozygous mutations. Ten patients (59%) were from consanguineous families. The median (interquartile range) birth weight was 3550 (3200-4200) g. Eight patients (47%) were diazoxide unresponsive and had undergone pancreatectomy. Other clinical features included seizure in two (11.8%) patients.

Patients with *KCNJ11* mutations. All three patients were homozygous, including two with novel missense mutations (c.362T > G; p.Phe121Cys and c.370T > A; p.Ser124Thr) who were both diazoxide responsive. Birth weight was between 3800 g and 4880 g. The patient with c.287_288delinsTG mutation (p.Ala96Val) had a history of seizure, was diazoxide unresponsive and underwent pancreatectomy.

Patients with *HADH* mutations. Three cases were detected with *HADH* gene mutations, of whom one case was homozygous for a frameshift variant (c.617del; p.Lys206fs) and the other two were homozygous for a nonsense variant (c.706C > T; p.Arg236Ter). Two patients were from consanguineous families and had seizures. Birth weight ranged from 3250g to 4800g. The response to diazoxide was good in all the patients.

Patient with *KMT2D* mutation. One patient was mosaic for a *KMT2D* partial gene deletion of exons 51-54. The level of mosaicism within his leukocyte DNA was estimated to be at least 20%, consistent with a post-zygotic origin. Genetic testing in the parents indicated a *de novo* change. The patient was from a non-consanguineous family and was diagnosed with hyperinsulinism at the age of two years. Birth weight was 4500g and the response to diazoxide was good. The patient had a history of seizure and did not have any facial dysmorphism.

Diazoxide Responsiveness and Pancreatectomy

Diazoxide unresponsiveness was seen in nine (38%) of the patients with genetic mutations. These included eight patients (89%) with mutations in *ABCC8* and one with *KCNJ11* mutation. This figure means 45% of those with K_{ATP}-channel mutations were diazoxide-unresponsive. Seven out of eight (88%) patients who had had pancreatectomy had *ABCC8* gene mutations and one had *KCNJ11* mutation. Pathogenic mutation was invariably found in diazoxide-unresponsive patients (9/9; 100%), although more than half of cases in the diazoxide-responsive group (20/35; 57%) had no genetic variant identified in the genes investigated (Figure 1).

Discussion

We described the spectrum of genetic mutations in CHI in an Iranian population, as well as the frequency of each mutation and their related clinical features. Genetic mutations were found in 55% of our patients, consistent with previous studies which identified mutations in 12 out of 19 (63%) patients in Turkey (10) or in only 47% (56 of 118)

Table 2. Mutation description and clinical features of patients with identified genetic cause

ID	Gene variant	Location	Effect	Inheritance	DNA description	Protein description	Novel	Consanguineous	Clinical features	Pancreatectomy	Age of diagnosis	BW	Response to diazoxide
HI 1	*ABCC8*	Exon 21	Nonsense	Homozygous	c.2524C > T	p.Arg842Ter	No	Yes	Seizure	No	< 1 year	4500	Good
HI 12	*ABCC8*	Exon 1	Missense	Heterozygous	c.96C > G	p.Asn32Lys	No	No	Seizure	No	< 1 year	3590	Good
HI 2	*ABCC8*	Exon 1	Missense	Homozygous	c.96C > G	p.Asn32Lys	No	Yes	No	Yes	< 1 year	3000	Poor
HI 4	*ABCC8*	Exon 16	Missense	Heterozygous	c.2159C > T	p.Ser720Phe	No	No	No	Yes	< 1 year	4100	Poor
M-10	*ABCC8*	Exon 3	Missense	Homozygous	c.331G > A	p.Gly111Arg	No	Yes	No	No	< 1 year	4200	Poor
M-12	*ABCC8*	Intron 14	Aberrant splicing	Homozygous	c.2041-21G > A	p.?	No	Yes	No	No	< 1 year	3400	Good
M-15	*ABCC8*	Exon 28	Frameshift	Heterozygous	c.3438dup	p.Thr1147fs	No	No	No	Yes	< 1 year	3300	Poor
M-17	*ABCC8*	Intron 11	Aberrant Splicing	Homozygous	c.1672-5C > G	p.?	No	Yes	No	Yes	< 1 year	5500	Poor
M-23	*ABCC8*	Exon 25	Frameshift	Homozygous	c.3151dup	p.Cys1051fs	No	Yes	No	No	< 1 year	5530	Good
M-24	*ABCC8*	Exon 28	Frameshift	Heterozygous	c.3438dup	p.Thr1147fs	No	No	No	Yes	< 1 year	3550	Poor
M-28	*ABCC8*	Intron 14	Aberrant splicing	Homozygous	c.2041-21G > A	p.?	No	No	No	No	< 1 year	2000	Good
M-3	*ABCC8*	Intron 11	Aberrant splicing	Homozygous	c.1671 + 1G > A	p.?	No	Yes	No	Yes	< 1 year	4350	Poor
M-30	*ABCC8*	Intron 14	Aberrant splicing	Homozygous	c.2041-21G > A	p.?	No	Yes	No	No	< 1 year	3600	Good
M-5	*ABCC8*	Exon 23	Nonsense	Homozygous	c.2809C > T	p.Cln937Ter	No	No	No	Yes	< 1 year	2600	Poor
M-6	*ABCC8*	Intron 14	Aberrant splicing and missense	Compound heterozygous	c.2041-21G > A / c.96C > G	p.? / p.Asn32Lys, c.96C > G	No	Yes	No	No	< 1 year	3150	Good
HI 3	*ABCC8*	Exon39	In-Frame Deletion	Homozygous	c4724-4732del	p.A1575-F1577	Yes	Yes	No	No	< 1 year	3500	Good
M-21	*ABCC8*	Exon 7	Missense	Heterozygous	c.1109G > C	p.Arg370Thr	Yes	No	No	No	< 1 year	3200	Good
HI 17	*HADH*	Exon 6	Nonsense	Homozygous	c.706C > T	p.Arg236Ter	No	Yes	Seizure	No	< 1 year	4800	Good
HI 5	*HADH*	Exon 5	Frameshift	Homozygous	c.617del	p.Lys206fs	No	Yes	Seizure	No	< 1 year	3600	Good
M-19	*HADH*	Exon 6	Nonsense	Homozygous	c.706C > T	p.Arg236Ter	No	No	No	No	< 1 year	3250	Good
M-14	*KCNJ11*	Exon 1	Missense	Homozygous	c.287_288delinsTG	p.Ala96Val	No	Yes	Seizure	Yes	< 1 year	3800	Poor
M-20	*KCNJ11*	Exon 1	Missense	Homozygous	c.362T > G	p.Phe121Cys	Yes	No	No	No	< 1 year	3950	Good
M-32	*KCNJ11*	Exon 1	Missense	Homozygous	c.370T > A	p.Ser124Thr	Yes	Yes	No	No	< 1 year	4880	Good
HI 13	*KMT2D*	Exons 51-54	Partial Deletion	Post-zygotic	Chr12 (GRCh37): g.(49415449) (49416715)del		Yes	No	Seizure	No	2 years	4500	Good

of the diazoxide-responsive cases in the US (17). This figure is lower than some studies, which could reflect differences in referral patterns and inclusion or exclusion criteria (18).

Consistent with previous studies (17,19,20), mutations in K_{ATP}-channel including SUR1 (*ABCC8*) and Kir6.2 (*KCNJ11*), accounted for 83 % of all CHI-causing mutations in our study. Mutations in the *ABCC8* gene with 71 % occurrence was the most frequent cause of CHI in our population. In addition, eleven participants were homozygous for a mutation in *ABCC8* which confirmed a diagnosis of autosomal recessive CHI. One patient was homozygous for a novel, in-frame deletion in *ABCC8*, which requires further investigation to determine its clinical significance.

The majority of recessive mutations in the K_{ATP}-channel has been shown to lead to medically unresponsive CHI (21,22), but in our study, six out of 11 patients with homozygous *ABCC8* mutations and two out of three patients with homozygous *KCNJ11* mutations were diazoxide-responsive. Although Salomon-Estebanez et al (23) had suggested that this may be due to the reduction of severity of disease over time, it is not reasonably likely and may be due to variable criteria used to determine diazoxide responsiveness. In contrast, *ABCC8* and *KCNJ11* heterozygous mutations were characterized by various presentations and treatment responses; three out of six (50 %) patients with heterozygous mutations were diazoxide-unresponsive which is in agreement with other studies (21). In our study, all heterozygous mutations were in the *ABCC8* gene, which may indicate a dominant pattern of inheritance for certain mutations in the *ABCC8* gene.

Table 3. Clinical features of patients with no identified genetic cause

ID	Genetic test	Consanguineous	Clinical features	Pancreatectomy	Age of diagnosis	BW	Response to diazoxide
HI 11	tNGS	No	No	No	4 years	3800	Good
HI 14	tNGS	Yes	Renal cyst, abnormal internal genitalia	No	13 months	NA	Good
HI 6	tNGS	Yes	Hypocalcemia, autism, renal failure, nephrectomy	No	10 years	3700	Good
M-1	tNGS	No	No	No	2 years	1930	NA
M-11	tNGS	Yes	No	No	Diagnosed < 1 year	3200	Good
M-18	tNGS	Yes	No	No	After birth	3250	Good
M-2	tNGS	No	No	No	4.5 months	3300	Good
M-25	tNGS	No	No	No	2 years	3500	Good
M-27	tNGS	Yes	Precocious puberty, hypothyroidism	No	4 years	3100	Good
M-9	tNGS	No	GI obstruction	No	7.5 years	1890	Good
HI 10	*ABCC8* & *KCNJ11*	Yes	No	No	Diagnosed < 1 year	3500	Good
HI 9	*ABCC8* & *KCNJ11*	No	Esophageal atresia	No	Diagnosed < 1 year	1069	Good
M-13	*ABCC8* & *KCNJ11*	No	No	No	Diagnosed < 1 year	3050	Good
M-16	*ABCC8* & *KCNJ11*	Yes	Macroglossia		Diagnosed < 1 year	4200	Good
M-22	*ABCC8* & *KCNJ11*	No	No	No	2 years	1700	Good
M-26	*ABCC8* & *KCNJ11*	No	No	No	Diagnosed < 1 year	3970	NA
M-29	*ABCC8* & *KCNJ11*	No	No	No	Diagnosed < 1 year	1600	Good
M-31	*ABCC8* & *KCNJ11*	No	No	No	Diagnosed < 1 year	2200	Good
M-4	*ABCC8* & *KCNJ11*	No	No	No	14 month	4070	Good
M-7	*ABCC8* & *KCNJ11*	No	IUGR	No	Diagnosed < 1 year	1900	Good

tNGS: targeted next generation sequencing

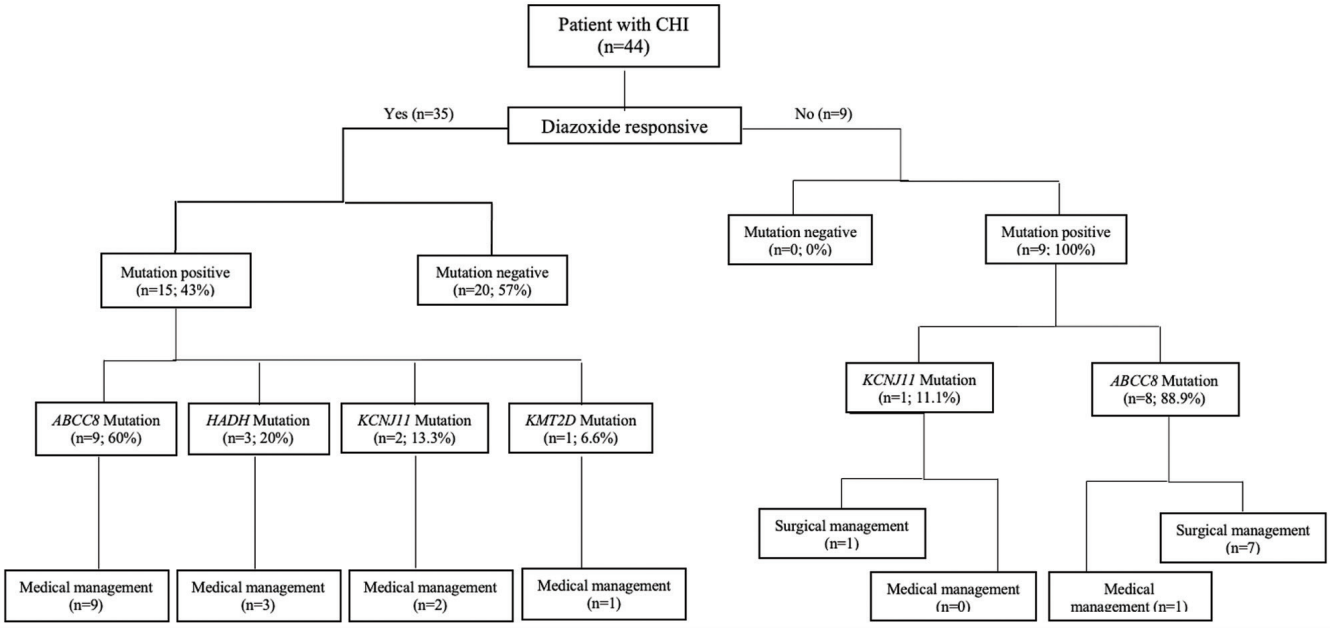

Figure 1. Mutation analysis results and treatment choices for patients with diazoxide-responsive congenital hyperinsulinism (CHI) vs. diazoxide-unresponsive CHI

Although, a subtotal pancreatectomy is necessary in most cases of CHI (24), the vast majority of our participants were diazoxide-responsive (79%). Previous studies have reported different rates of diazoxide responsiveness in their cohorts. In studies of de Lonlay et al (8) and Snider et al (17) the ratio of diazoxide-responsive participants were 28% and 34%, respectively. In a study from Turkey 59% (13/22) of patients were diazoxide-responsive (10). In 27 infants who were born small-for-gestational age in the UK and developed HH, diazoxide-response was 100% (25). Diazoxide-responsiveness in two studies of Iranian patients has been reported to be 18/23 and 3/6 (18,26). This discrepancy is probably due to the number of cases in some studies, variable criteria used to define diazoxide responsiveness, differences in sensitivity of the methods used for detecting the mutations and/or occurrence of mutations in combination with a second mutant allele that complicated the prediction of consequences. Our results, however, are consistent with previous studies (8,27), and indicate that *ABCC8* gene defects are the most important cause of diazoxide-unresponsive CHI (8/9 of diazoxide-unresponsive patients) in Iranian children.

The three patients with *HADH* homozygous mutations were all diazoxide-responsive. This is consistent with other studies showing hyperinsulinism due to *HADH* gene mutations responds relatively well to diazoxide (12,18,24). Mutations in the *HADH* gene result in a diffuse form of CHI (28). Interestingly, the rate of observed *HADH* mutations in our study was the same as for *KCNJ11* mutations. This result is in concordance with some other studies (18,26). A high frequency of *HADH* mutations in our study could be due to a high rate of consanguineous marriage. Based on our results, screening for *HADH* gene variants is recommended in all patients with diazoxide-responsive CHI.

One patient in our cohort was mosaic for a *KMT2D* partial gene deletion. Pathogenic variants in the *KMT2D* gene cause Kabuki syndrome (29). People with Kabuki syndrome also have facial and some other specific congenital anomalies, including growth delays, mental retardation and skeletal abnormalities, which were not observed in our participant.

In nearly 20% of patients, no mutation was detected using tNGS, which may miss large deletions and chromosomal rearrangements. Furthermore, negative results do not exclude a monogenic aetiology and further unidentified mutations in other unidentified genes should be considered as new aetiologies.

Study Limitations

The strength of our study is that we have collected genetic data on the largest cohort of CHI in an Iranian population to date and identified novel variants causing the disease. Our samples were referred from different parts of Iran. Study limitations include the retrospective collection of clinical data and the lack of follow-up data to assess detailed clinical significance of novel findings.

Conclusion

Most novel mutations identified in this study were inherited in a homozygous fashion. Variation in reported rates of diazoxide responsiveness suggest a need for revision of the criteria used to define diazoxide responsiveness. All novel mutations that we report for the first time were medically responsive, and yet should now be considered in CHI analysis. More studies on the molecular basis of CHI are necessary for societies with highly consanguineous families. We recommend tNGS for all CHI patients but screening of *HADH* gene variants in patients with diazoxide-responsive CHI seems to be required if there is no access to tNGS.

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Ethics

Ethics Committee Approval: The study was approved by the Ethical Committee of the Endocrinology and Metabolism Research Institute (ethical code: IR.TUMS. EMRI.REC.1397.009, date: 18.07.2018).

Informed Consent: Informed consent was obtained from parents on behalf of their children.

Peer-review: Externally peer-reviewed.

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