Clinical characteristics and phenotype–genotype analysis in Turkish patients with congenital hyperinsulinism; predominance of recessive K_{ATP} channel mutations

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Abstract

Objective: Congenital hyperinsulinism (CHI) is the commonest cause of hyperinsulinaemic hypoglycaemia in the neonatal, infancy and childhood periods. Its clinical presentation, histology and underlying molecular biology are extremely heterogeneous. The aim of this study was to describe the clinical characteristics, analyse the genotype–phenotype correlations and describe the treatment outcome of Turkish CHI patients.

Design and methods: A total of 35 patients with CHI were retrospectively recruited from four large paediatric endocrine centres in Turkey. Detailed clinical, biochemical and genotype information was collected.

Results: Diazoxide unresponsiveness was observed in nearly half of the patients ($n=17$; 48.5%). Among diazoxide-unresponsive patients, mutations in *ABCC8*/*KCNJ11* were identified in 16 (94%) patients. Among diazoxide-responsive patients ($n=18$), mutations were identified in two patients (11%). Genotype–phenotype correlation revealed that mutations in *ABCC8*/*KCNJ11* were associated with an increased birth weight and early age of presentation. Five patients had p.L1171fs (c.3512del) *ABCC8* mutations, suggestive of a founder effect. The rate of detection of a pathogenic mutation was higher in consanguineous families compared with non-consanguineous families (87.5 vs 21%; $P<0.0001$).

Among the diazoxide-unresponsive group, ten patients were medically managed with octreotide therapy and carbohydrate-rich feeds and six patients underwent subtotal pancreatectomy. There was a high incidence of developmental delay and cerebral palsy among diazoxide-unresponsive patients.

Conclusions: This is the largest study to report genotype–phenotype correlations among Turkish patients with CHI. Mutations in *ABCC8* and *KCNJ11* are the commonest causes of CHI in Turkish patients (48.6%). There is a higher likelihood of genetic diagnosis in patients with early age of presentation, higher birth weight and from consanguineous pedigrees.

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Introduction

Hyperinsulinaemic hypoglycaemia (HH) is the commonest cause of hypoglycaemia in the neonatal, infancy and childhood periods (1, 2, 3). It occurs due to the unregulated secretion of insulin from pancreatic β-cells leading to severe and persistent hypoglycaemia. HH can be congenital (congenital hyperinsulinism, CHI) or transient due to risk factors such as perinatal asphyxia, intrauterine growth restriction (IUGR) and maternal diabetes mellitus (gestational or non-gestational) (1, 2, 3).

CHI refers to a group of conditions that are extremely heterogeneous in terms of clinical presentation, histological subgroups and underlying molecular biology. The molecular basis of CHI involves genetic defects in nine different genes (*ABCC8*, *KCNJ11*, *GLUD1*, *GCK*, *HADH*, *SLC16A1*, *HNF4A*, *HNF1A* and *UCP2*) that are involved in regulating insulin secretion from β-cells (1, 3). Mutations in the *ABCC8* and *KCNJ11* genes that encode the two subunits (SUR1 and Kir6.2) of ATP-sensitive potassium (K_{ATP}) channel are the commonest cause of CHI (4, 5).

At a histological level, two major subtypes of CHI (diffuse and focal) have been described. The differentiation of these two histological subtypes is important from the management point of view (6). The diffuse form 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. The frequency of focal disease has been reported to be 30–40% of all CHI patients in different series (7, 8). Genetic analysis and specialised positron emission tomography scan using the isotope Fluorine 18 L-3,4-dihydroxyphenyalanine (^{18}F-DOPA-PET) can be helpful in differentiating focal and diffuse diseases (9, 10). PET scan can also localise the focal lesions within the pancreas. Regarding their underlying genetic basis, the diffuse form is inherited in an autosomal recessive (or dominant) manner, whereas the focal form is sporadic in inheritance.

Besides differentiation of focal and diffuse diseases, documentation of protein sensitivity constitutes another important issue in the management of CHI. Mutations in *GLUD1* and *HADH* are associated with protein-sensitive CHI (11, 12). Protein-restricted diet may increase the success of medical therapy (13).

Although the incidence of CHI in the general population is one in 35 000–40 000, it rises to one in 2500 in the communities with the high rates of consanguinity (14). Turkey also has a high rate of consanguineous marriage (15). Thus, interpretation of clinical characteristics, genetic basis and phenotype–genotype relation of Turkish patients with CHI could bring a new perspective in understanding genetic basis, genotype–phenotype correlation and management of CHI. However, to our knowledge, to date, except for two small studies reporting the clinical and genetic characteristics of five and 13 Turkish patients with CHI, there is no large-scale study reporting clinical and genetic characteristics of Turkish children with CHI (16, 17). In this study, we describe clinical characteristics, genetic results and treatment outcome of a large cohort of CHI patients from Turkey.

Subjects and methods

Patients

Patients presenting with CHI to four large paediatric endocrine centres in Turkey were included in this study. CHI was defined as a detectable insulin level ($\geq$2 mU/l) during an episode of spontaneous or provoked hypoglycaemia (blood glucose $<$3.0 mmol/l). Patients with secondary HH due to IUGR, evidence of perinatal asphyxia and maternal diabetes mellitus were excluded. Detailed clinical and biochemical information was collected from the responsible clinician of these patients.

Written informed consent was obtained from parents of all participants for genetic mutation analysis. Diazoxide (5–15 mg/kg per day) was commenced as first line for the management of CHI. In diazoxide-unresponsive patients, octreotide (5–40 μg/kg per day) was tried. Patients unresponsive to medical therapy were managed with open near-total pancreatectomy. As ^{18}F-DOPA-PET–CT is not currently available in Turkey, a near-total pancreatectomy was performed for all patients who required surgical therapy irrespective of the results of molecular genetic analysis.

Mutation analysis

Genomic DNA was extracted from peripheral leukocytes using standard procedures and the coding regions and intron/exon boundaries of the *ABCC8* and *KCNJ11* genes were amplified by PCR (primers available on request) in all patients. Amplicons were subsequently sequenced using the Big Dye Terminator Cycler Sequencing Kit v3.1 (Applied Biosystems) according to the manufacturer's instruction and reactions were analysed on an ABI 3730 Capillary sequencer (Applied Biosystems). Sequences were compared with the reference sequences (NM_000525.3

and NM_000352.3) using the Mutation Surveyor v3.24 software (SoftGenetics, State College, PA, USA). If no mutations in *ABCC8* and *KCNJ11* were identified, the coding regions of *HADH* (only in diazoxide-responsive patients and those with abnormal acylcarnitine and/or urine organic acid profile) and *HNF4A* were amplified and sequenced as described previously (18, 19).

Statistical analyses

IBM SPSS statistics 21.0 for Windows software package program was used for statistical analyses. The Kolmogorov–Smirnov test was used to test normality for distribution of data. The independent samples *t*-test was used to compare mean of normally distributed data and the Mann–Whitney *U* test for non-normally distributed data. The χ^2-test was used to compare the ratios. A *P* value of <0.05 was considered statistically significant.

Results

A total of 35 patients presented with CHI between April 2002 and October 2013. The clinical and biochemical characteristics at presentation are summarised in Table 1. The median follow-up period for this cohort of patients was 2 years and 3 months (range: 1 month–10.5 years).

Mutation analysis, genotype–phenotype relation and treatment outcome

Mutation analysis ▸ Molecular genetic analysis identified pathogenic mutations in 51.4% of Turkish CHI patients (18/35; *ABCC8* (14 patients), *KCNJ11* (three patients) and *HADH* (one patient)) (Table 2). The K_{ATP} channel mutations included homozygous (13), compound heterozygous (2) and paternally inherited heterozygous (1) mutations. Maternal DNA was unavailable for testing to confirm the inheritance pattern in one patient with heterozygous *ABCC8* mutation.

Table 1 Clinical characteristics of CHI patients.

Clinical characteristics	Results
Number of patients, *n*	35
Males, *n* (%)	20 (57.1)
Gestational age (weeks)[a]	38 (29–40)
Birth weight (g)[b]	3407 ± 789
Large for gestational age (>90th percentile), *n* (%)	14 (40)
Age at presentation (weeks)[a]	1 (1–48)
Presentation within first week of life, *n* (%)	19 (58)
Consanguinity, *n* (%)	16 (45.7)
Family history of CHI, *n* (%)	7 (20)
Hypoglycaemia screen[b]	
Blood glucose (mmol/l)	1.7 ± 0.5
Serum insulin (mU/l)	32.7 ± 35.9
Hyperammonaemia[c]	0

[a]Median (range).
[b]Mean ± s.d.
[c]Serum ammonia more than twice the normal upper limit.

While a mutation was identified in 14 out of 16 patients (87.5%) from consanguineous families, it was identified only in four out of 19 patients (21%) from non-consanguineous families ($P<0.0001$).

In 40% (14/35) of the CHI patients, eight different *ABCC8* mutations were identified. One of the commonest mutations in our cohort was p.L1171fs (c.3512del), a frameshift mutation on exon 28 of *ABCC8* gene (five patients). Four unrelated patients from consanguineous families were homozygous for this mutation and one was heterozygous (inherited from unaffected father). Another common mutation identified was c.3554C>A (p.Ala1185Glu). This was a novel mutation identified in the homozygous state in four first cousins and a second unrelated proband.

The remaining six *ABCC8* mutations, p.R168C, p.N188S, p.L533P, p.W232G, p.R842Q and p.F591L were each identified in a single patient. Among these, p.L533P and p.W232G were novel mutations. These novel variants are not listed in various sequence variant databases (dbSNP, Exome Variant Server and 1000 Genomes) and the nucleotide at this position is conserved across all species. Additionally, *in silico* analysis by SIFT, PolyPhen2, AlignGVGD and Grantham distance predicts these novel variants to be likely pathogenic.

In 8.6% (3/35) of the CHI patients, three different *KCNJ11* mutations were identified. These included two missense (p.E126K, and p.R34H) and one nonsense (p.W91X) mutation. Among these, p.E126K was a novel mutation. The p.E126K mutation was identified in two probands in our cohort. Conservation across species, *in silico* analysis and comparison with various sequence databases predict this variant to be likely pathogenic. The remaining two mutations in *KCNJ11*, p.W91X and p.R34H, were identified *in trans* in a single patient and have been reported previously (20).

A previously described homozygous nonsense mutation in exon 6 of *HADH* (p.R236X) was identified in one patient. A protein-loading test showed protein-sensitive HH in this patient.

Sequencing of *ABCC8*, *KCNJ11*, *HNF4A* and *HADH* did not identify a causative mutation in the remaining 15 patients.

Table 2 Genetic analysis and treatment outcome of 18 patients with mutation-positive CHI.

Gene	Current age (year)	Exon/intron	DNA description	Protein description	Consequence	Transmission	Treatment			Follow-up	Developmental delay	Comments
							Diazoxide responsive	Octreotide responsive	Pancreatectomy (histology)			
ABCC8												
1	3.9	Exon 28	c.3554C>A	p.Ala1185Glu	Missense	Homozygous	−	+	−	Octreotide	+++	Novel mutation
2	0.7	Exon 28	c.3554C>A	p.Ala1185Glu	Missense	Homozygous	−	+	−	Octreotide		Novel mutation
3	9.1	Exon 28	c.3554C>A	p.Ala1185Glu	Missense	Homozygous	−	−	−	Irregular	++++	Novel mutation
4	0.7	Exon 28	c.3554C>A	p.Ala1185Glu	Missense	Homozygous	−	+	−	Octreotide	+	Novel mutation
5	0.2	Exon 28	c.3554C>A	p.Ala1185Glu	Missense	Homozygous	−	+	−	Octreotide		Novel mutation
6	0.7	Exon 28	c.3512delT	p.Leu1171fs	Frameshift	Homozygous	−	−	+ (diffuse)	Remission		
7	0.7	Exon 28	c.3512delT	p.Leu1171fs	Frameshift	Homozygous	−	+	+ (diffuse)	Octreotide		
8	Died	Exon 28	c.3512del	p.Leu1171fs	Frameshift	Heterozygous paternal	−	−	+ (diffuse)	Died		
9	5.8	Exon 28	c.3512delT	p.Leu1171fs	Frameshift	Homozygous	−	+	−	Octreotide	+++	Ectodermal dysplasia
10	9.6	Exon 28	c.3512delT	p.Leu1171fs	Frameshift	Homozygous	−	+	−	Octreotide	+++	
11	0.7	Exon 4	c.502C>T c.563A>G	p.Arg168Cys/ p.Asn188Ser	Missense	Compound heterozygous	−	+	+ (diffuse)	Octreotide	−	
12	Died	Exon 10	c.1598T>C	p.Leu533Pro	Missense	Homozygous	−	+	−	Died		Novel mutation
13	10.6	Exon 5/ exon 21	c.694T>G/ c.2525G>A	p.Trp232Gly/ p.Arg842Gln	Missense/ Missense	Compound heterozygous	−	+	−	Octreotide	++	
14	5.5	Exon 12	c.1771T>C	p.Phe591Leu	Missense	Heterozygous	+		−	Diazoxide	−	
KCNJ11												
15	2.4	Exon 1	c.101G>A/ c.376G>A	p.Arg34His/ p.Glu126Lys	Missense/ Missense	Compound heterozygous	−	+	−	Octreotide	−	
16	3.3	Exon 1	c.272G>A	p.Trp91X	Nonsense	Homozygous	−	+	+ (diffuse)	Octreotide	++	
17	3.2	Exon 1	c.376G>A	p.Glu126Lys	Missense	Homozygous	−	+	+ (diffuse)	Octreotide	++	
HADH												
18	4.4	Exon 6	c.706C>T	p.Arg236X	Nonsense	Homozygous	+			Diazoxide	+	

Table 3 Clinical characteristics of CHI patients with and without mutation at presentation. Data are presented as mean ± S.D.

Characteristics	Mutation positive	Mutation negative	*P* value
Birth weight (g)	3725 ± 664	3070 ± 788	0.012
Gestational age (weeks)	38.6 ± 1.6	37.6 ± 3.1	0.532
Age of presentation (weeks)	3.1 ± 6.8	10.3 ± 13.8	0.032
Serum insulin (mU/l)	36.1 ± 34.4	29.2 ± 38.1	0.355
Blood glucose level (mmol/l)	1.7 ± 0.5	1.8 ± 0.6	0.456

$P<0.05$ was considered statistically significant.

Genotype–phenotype correlation ▸ Comparison between K_{ATP} mutation-positive and K_{ATP} mutation-negative groups highlighted a statistically significant increased birth weight and younger age of presentation in K_{ATP} mutation-positive group as compared with K_{ATP} mutation-negative patients (Table 3).

Rate of detection of a pathogenic mutation in diazoxide-unresponsive patients (16/17; 94.1%) was higher than that of diazoxide-responsive group (2/18, 11.1% ($P<0.0001$) (Fig. 1).

Treatment outcome ▸ Of the total number of patients, 18 (51.4%; median age 22 months (range 3–128 months)) were responsive to diazoxide treatment (Fig. 1). Children were defined as being diazoxide responsive if they demonstrated age-appropriate fasting tolerance or evidence of appropriate hyperketonaemia before developing hypoglycaemia on diazoxide at doses <15 mg/kg per day. Administration of diazoxide could be successfully stopped in four of the diazoxide-responsive CHI patients at a median age of 3.5 months (range, 3–15 months). Of these, a pathogenic mutation was identified in only two patients (monoallelic *ABCC8* – 1 and biallelic *HADH* – 1).

Of the diazoxide-unresponsive group (17), six patients underwent pancreatectomy (five subtotal and one near-total) and ten patients were managed with octreotide treatment. One patient was lost to follow-up and represented at a later age with severe learning disability due to uncontrolled severe hypoglycaemia. A pathogenic mutation was identified in 16 out of 17 patients (94.1%; biallelic K_{ATP} – 15 and paternally inherited K_{ATP} – 1).

The median age at pancreatectomy was 1.5 months (range 1–2 months). Histological examination identified typical diffuse disease (abnormal large β-cell nuclei in pancreatic islets and low nuclear crowding in the whole pancreas) in all of these patients. After pancreatectomy, one patient unfortunately died because of sepsis and four patients required octreotide treatment to maintain euglycaemia. In only one patient who underwent near-total pancreatectomy, normoglycaemia was achieved without the need for additional medical therapy. There was no correlation between the type of mutation and the severity of CHI.

Long-term neurological sequelae such as developmental delay, cerebral palsy and epilepsy were higher in diazoxide-unresponsive patients (9/17) as compared with diazoxide-responsive (3/18) patients (52.9 vs 16.6%; $P=0.035$). This is likely to be due to difficulties in controlling hypoglycaemia in the diazoxide-unresponsive group.

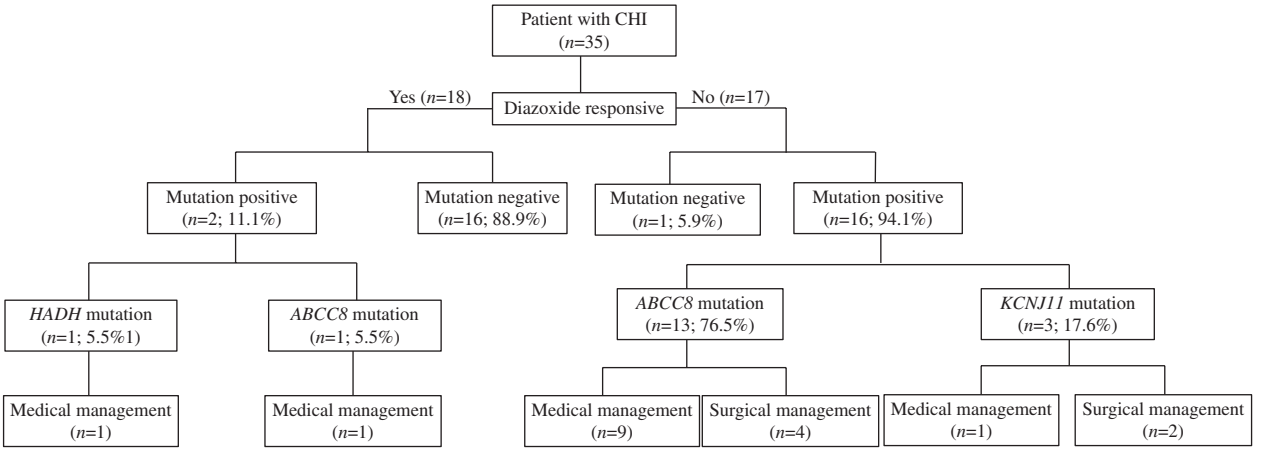

Figure 1
Mutation analysis results and treatment choices for patients with diazoxide-responsive CHI vs diazoxide-unresponsive CHI.

Discussion

In this study, 18 out of 35 patients (51.4%) had a genetically confirmed diagnosis of CHI (biallelic – 15 and monoallelic – 3). In two recent large studies of patients with CHI, genetic mutations were identified in 45.3 and 78.8% (21, 22). Mutations in the K_{ATP} channel genes were the commonest identifiable cause in our cohort (biallelic *ABCC8* – 12, monoallelic *ABCC8* – 2 and biallelic *KCNJ11* – 3).

All patients with biallelic K_{ATP} channel mutations in our cohort ($n=15$; 43%) were unresponsive to diazoxide treatment. Despite high doses of diazoxide for an adequate period of time, these patients could not be weaned off high-concentration dextrose fluids and experienced random episodes of hypoglycaemia. Similar findings have been reported by other investigators (Table 4). In the studies by Kapoor *et al.* (21) and Snider *et al.* (22), all patients with biallelic K_{ATP} mutations (63/300 (21%) and 84/417 (20%)) were unresponsive to diazoxide. The prevalence of homozygous K_{ATP} mutations in these studies (15 and 3.6%) was less when compared with our study (42.8%), possibly due to high consanguinity in our cohort. The studies by Kapoor *et al.* (21) and Snider *et al.* (22) have not mentioned the percentage of patients born of consanguineous marriage in their cohorts.

Five patients with biallelic K_{ATP} mutations were treated with near-total pancreatectomy and the remaining patients were treated with s.c. octreotide injections and carbohydrate-rich feeds. All the patients with biallelic K_{ATP} channel mutations who were pancreatectomised had diffuse disease on histological examination of the pancreatic tissue.

Of the two patients with monoallelic *ABCC8* mutations, the mutation was paternally inherited in one patient (p.Leu1171fs). This patient was medically unresponsive and was treated with near-total pancreatectomy. The histology of the resected pancreatic tissue showed diffuse disease, although it is likely that the focal lesion could have been missed. As this particular *ABCC8* mutation was present in monoallelic state in clinically unaffected parents (although not biochemically evaluated) of four different patients in our cohort and it is a null mutation, it seems likely that this was a recessive *ABCC8* mutation which has been unmasked by paternal uniparental disomy within the pancreas. Although there is no family history of hyperinsulinism or early-onset diabetes mellitus on the maternal side, the milder clinical phenotype (diazoxide responsiveness) of the second patient with monoallelic non-paternal *ABCC8* mutation (maternal DNA unavailable for testing) was suggestive of dominant *ABCC8* mutation.

Table 4 Summary of studies showing mutation detection rate, diazoxide responsiveness and histological subtype of CHI patients.

Reference	Year	*n*	Mutation detection rate *n* (%)			K_{ATP} channel mutations *n* (%)		Other mutations[b]	Histology[a]		Country
			DZ responsive	DZ unresponsive	Overall	Monoallelic	Biallelic		Focal	Diffuse	
This study	2014	35	2/18 (11)	16/17 (94)	18/35 (51)	1 (6)	15 (94)	1	0 (0)	6 (100)	Turkey
(17)	2002	13	0/10 (0)	3/3 (100)	3/13 (23)	0 (0)	3 (100)	–	0 (0)	3 (100)	Turkey
(20)	2011	17	4/5 (80)	7/8 (87)	14/17[c] (82)	10/14 (71)	4/14 (29)	–	0 (0)	6 (100)	Korea
(23)	2011	36	NS	NS	24/36 (67)	17/19 (84)	2/19 (15)	5	1 (10)	9 (90%)	Japan
(25)	2009	26	NS	NS	16/26 (58)	6 (40)	9 (60)	1	3 (33)	6 (67)	Norway
(26)	2013	36	12/25 (48)	9/11 (82)	20/36 (55)	12[d] (92)	1 (8)	8	3 (100)	0 (0)	Italy
(21)	2013	300	41/183 (22)	92/105 (87.6)	136/300[e] (45)	46 (42)	63 (58)	27	NS	NS	UK
(22)	2013	417	56/118 (47)	272/292 (91)	328/417 (79)	200 (69)	88 (31)	40	149 (53)	122[f] (43)	USA

n, number; DZ, diazoxide; NS, not specified.
[a]Mutations detected in other genes (*HADH*, *HNF4A*, *HNF1A*, *GCK*, *UCP2* and *GLUD1*).
[b]Histology proven after surgery.
[c]Diazoxide not tried in three patients.
[d]One patient had monoallelic K_{ATP} as well as *GCK* mutation.
[e]Diazoxide responsiveness not determined in 12 patients.
[f]Histology normal in four patients and atypical diffuse disease in 11 patients.

The other interesting observation in our study was the low prevalence of monoallelic K_{ATP} mutation (2/35; 5.7%). This is in sharp contrast to observations from genetic analysis of patients from Korea and Japan, where the single mutation rate was between 50 and 60% (Table 4) (20, 23). In the study by Kapoor *et al.*, monoallelic K_{ATP} mutation was present in 14.6% of patients, whereas 48% of patients had monoallelic K_{ATP} mutation in the study by Snider *et al.* (21, 22).

In our cohort, about half of the patients were diazoxide responsive ($n=18$). Only two patients from this group had a pathogenic mutation (monoallelic *ABCC8* – 1 and biallelic *HADH* – 1). No mutation could be identified in the remaining 16 out of 18 (88.9%) diazoxide-responsive patients. The mutation detection rate in diazoxide-responsive category in our cohort is less when compared with other studies (21, 22). This may be due to the fact that sequencing of *GLUD1*, *GCK* and *HADH* genes was not performed in these patients unless indicated by the clinical phenotype. Novel genetic mechanisms might be responsible for CHI in these patients.

In our study, the majority of diazoxide-unresponsive patients (16/17; 94.1%) had a K_{ATP} channel mutation. This has been established in large cohort studies by Kapoor *et al.* (21) and Snider *et al.* (22) (87.6 and 91% respectively). Our results are in line with these larger studies.

This study identified a number of novel *ABCC8* and *KCNJ11* mutations (Table 2). One particular novel *ABCC8* missense mutation (p.A1185E, c.3554C>A), co-segregating with disease in the family, was found in four affected cousins from one family and another unrelated patient. In addition to this mutation, another *ABCC8* mutation (p.L1171fs, c.3512delT) was also detected in five patients from four different families, possibly suggesting a founder effect.

In this study, integration of the clinical findings and genetic results suggests the likelihood of K_{ATP} mutations in patients with CHI in the presence of increased birth weight and earlier age of presentation. It was striking that those patients with K_{ATP} channel mutations presented earlier and had a higher birth weight when compared with patients without a K_{ATP} channel mutation. Previous studies have shown that there may be an overlap between birth weight and age of presentation between patients with *HNF4A* and K_{ATP} channel mutations (21). However, our study clearly showed the difference in age of presentation and birth weight between K_{ATP} channel-positive and -negative groups. These observations can be very helpful in the clinical management of patients with CHI, especially if the clinicians do not have access to urgent molecular genetic analysis.

Lastly, in our cohort, 12 out of 35 (34%) patients had long-term neurological sequelae such as developmental delay, cerebral palsy and epilepsy. This is similar to what has been reported in the recent literature from Turkey (24). The likelihood of adverse neurological sequelae was significantly higher in the diazoxide-unresponsive group.

Conclusions

In conclusion, in this largest Turkish cohort with CHI, K_{ATP} channel mutations were detected in 48.6% (17/35) of the patients studied. The likelihood of long-term neurological sequelae was higher in the diazoxide-unresponsive group, highlighting the need for management of these complex patients in highly specialised centres. Additional research to identify novel genetic mechanisms for patients with diazoxide-responsive CHI is required.

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

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Author contribution statement
H Demirbilek contributed to the conceptualisation of the manuscript, collection of data, data analysis and writing of the manuscript; V B Arya, collection of data, data analysis and writing of the manuscript; M N Ozbek, A Akinci, M Dogan, F Demirel, S Kaba, F Guzel, R T Baran, S Unal and S Tekkes, collection of data and review of the manuscript; J Houghton, S E Flanagan and S Ellard, genetic analysis and review of the manuscript and K Hussain, conceptualisation of the manuscript, review of the manuscript and guarantor of the manuscript.

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