TITLE

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

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OBJECTIVES

Congenital hyperinsulinism (CHI) is the most common cause of hyperinsulinaemic hypoglycaemia (HH) in the neonatal, infancy and childhood periods. Its clinical presentation, histology and underlying molecular biology are extremely heterogeneous. The aim of present study was to describe the clinical characteristics, analyse the genotype-phenotype correlations and describe the treatment outcome of Turkish patients with the diagnosis of CHI.

METHODS

HH was defined and diagnosed according to previously published standards. Patients with intrauterine growth restriction (IUGR), evidence of perinatal asphyxia, and maternal diabetes were excluded. Thirty-five patients with CHI were retrospectively recruited from four large paediatric endocrine centres in Turkey. Detailed clinical, biochemical and genotype information was collected. Diagnosis of HH was considered as a detectable insulin level (>2 IU/L) in course of clinical and/or biochemical hypoglycemia (plasma glucose<2.8 mmol/L). A written informed consent was obtained from parents of all participants for mutation analysis. Diazoxide (5-15 mg/kg/day) was commenced as first line for the management of HHI. In diazoxide unresponsive patients, octreotide and nifedipine were tried. Patients unresponsive to medical therapy were managed with near-total laparatomic pancreatectomy. Since 18F-DOPA-PET analysis is not available in Turkey yet, a near-total pancreatectomy was performed for all patients who required surgical therapy irrespective of results of molecular genetic analysis.

RESULTS

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

Table 1: Clinical Characteristics of CHI patients

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

Molecular genetic analysis identified pathogenic mutations in 51.4% of Turkish CHI patients (18/35; *ABCC8* [14 patients], *KCNJ11* [3 patients], HADH [1 patient]) (Table 3). The K_{ATP} channel mutations included homozygous (13), compound heterozygous (2), and paternally inherited heterozygous (1) mutations. While a mutation was identified in 14/16 patients (87.5%) from consanguineous families, it was identified only in 4/19 patients (21%) from non-consanguineous families (p<0.0001).

In 40% (14/35) of the CHI patients, 8 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 (5 patients). Four unrelated patients from consanguineous families were homozygous for this mutation and one was heterozygous (inherited from unaffected father). The other commonest 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.

In 8.6% (3/35) of the CHI patients, 3 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 predicts 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 hyperinsulinaemic hypoglycaemia in this patient.

Genotype-Phenotype Correlation

Comparison between K_{ATP} mutation-positive and K_{ATP} mutation-negative group highlighted statistically significant increased birth weight and younger age of presentation in K_{ATP} mutation-positive group as compared to K_{ATP} mutation-negative patients (Table 3).

Table 3: Clinical characteristics of CHI patients with and without mutation at presentation

Characteristics	Mutation (+)	Mutation (-)	P
Birth weight (grams)	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

Data are presented as Mean ± SD, p<0.05 was considered as statistically significant.

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) (Figure 1).

Treatment Outcome (Figure 1)

Eighteen patients (51.4%; median age 22 months [range 3 – 128 months]) were responsive to diazoxide treatment. 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 <15mg/kg/d. Administration of diazoxide could be successfully stopped in 4 of diazoxide responsive CHI patients at a median age of 3.5 months (range 3 months to 15 months). Of these, a pathogenic mutation was identified in only two patients (monoallelic *ABCC8* – 1, biallelic *HADH* – 1).

Of the diazoxide-unresponsive group (17), six patients underwent pancreatectomy (5 sub-total and 1 near-total) and 10 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 16/17 patients (94.1%; biallelic K_{ATP} – 15, paternally inherited K_{ATP} – 1).

The median age at pancreatectomy was 1.5 months (range 1 to 2 months). Histological examination identified typical diffuse disease (abnormal large β-cell nuclei in a pancreatic islets and low nuclear crowding in the whole pancreas) in all of these patients. Post-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 of 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, was higher in diazoxide-unresponsive patients (9/17) as compared to diazoxide- responsive (3/18) patients (52.9% vs. 16.6%; p=0.035). This is likely to be due to difficulties in controlling the hypoglycaemia in the diazoxide-unresponsive group.

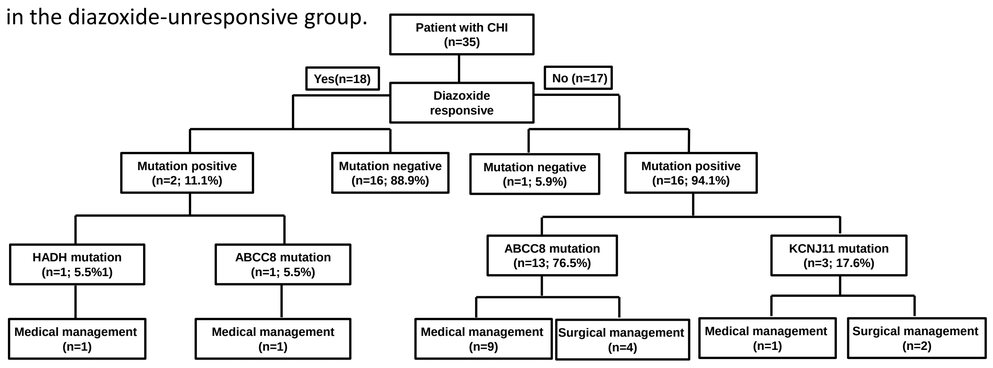

Figure 1. Mutation analysis results and treatment choices for diazoxide responsive vs unresponsive CHI patients

Table 3. Genetic analysis and treatment outcome of 18 patients with mutation positive CHI

	Current age (year)	Exon/ Intron	NA 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	+	

CONCLUSIONS

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 specialized centres. Additional research to identify novel genetic mechanisms for patients with diazoxide responsive CHI is required