

Seizures and diagnostic difficulties in hyperinsulinism-hyperammonemia syndrome

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Hyperinsulinism/hyperammonemia (HI/HA) syndrome is a rare disorder presented with recurrent hypoglycemia and elevated serum ammonia, which may lead to development delays, permanent neurologic damages, if it remains underdiagnosed. It is caused by activating mutations in the *GLUD1* gene which encodes the intra-mitochondrial enzyme glutamate dehydrogenase (GDH).

HI/HA syndrome is considered the second most common form of hyperinsulinism (HI), and usually associated with epileptic seizures, mental retardation and generalized dystonia.

We reported a patient who was diagnosed as HI/HA with multiple episodes of seizures; and previously had been diagnosed and treated for epilepsy. She has heterozygous mutation in *GLUD1* gene. Treatment with diazoxide enabled complete resolution of the seizures.

One year later, when her brother was six months old, he was also diagnosed with HI/HA. Later, the same mutation of *GLUD1* was detected in both her father and brother too.

Key words: hyperinsulinism, hyperammonemia.

Congenital hyperinsulinism is one of the most complicated and challenging disorders faced by pediatric endocrinologists. The potential for preventing permanent brain damage caused by persistent hypoglycemia, makes it extremely important to identify and treat these children early. The hyperinsulinism/hyperammonemia syndrome (HI/HA) is the second most common cause of hyperinsulinemic hypoglycemia in children¹. It is a rare genetic disease caused by activating mutations in *GLUD1*, a gene located on chromosome 10q23.3, composed of 13 exons that encode the mitochondrial enzyme glutamate dehydrogenase (GDH). Although the majority of patients are carriers of a *de novo* mutation, some familial cases have demonstrated autosomal dominant inheritance. From a clinical perspective, most children manifest hypoglycemic symptoms after 4-6 months of age, triggered by fasting or high-

protein meals, together with elevated serum ammonia. The severity of hypoglycemia is variable and it can be controlled by treatment with diazoxide². In general, the high risk of brain damage appears to be due to delays in diagnosis and treatment rather than a consequence of the genetic defects and thus, is potentially preventable. The neurological abnormalities (absence epilepsy, ADHD-like behavioral problems, and developmental delay in many cases) appear to be unrelated to hypoglycemia or elevated ammonia levels, probably related to GDH-activation in the brain. In our case, a two-year-old girl was diagnosed and treated for epilepsy. Her seizures occurred during her treatment period and finally was diagnosed with HI/HA syndrome. This case illustrates the relationship between seizures in HI/HA syndrome that can create diagnostic difficulties and delay the diagnosis.

Case Report

A 2-year-old female infant was referred with intractable seizure since the age of 9 months. She had been diagnosed with epilepsy and was treated with antiepileptic medications phenobarbital and valproic acid. She was born at term with normal length and normal weight for gestational age. Developmental milestones were normal in early infancy. She sat with support at 6 months and walked at 13 months.

At age 2, she was hospitalized during a seizure episode and biochemical work-up revealed hypoglycemia and hyperammonemia (glucose 45 mg/dl, ammonia 111 μ g/dl). Hyperammonemia had been previously recognized but interpreted as a side effect of valproic acid. At admission, physical examination was normal. She was overweight (height, 91 cm [50-75 p]; weight, 15.6 kg [97 p]). Following hospitalization, she showed hypoglycemic episodes both after overnight fasting and during postprandial period. Blood collected in one of the episodes has been shown in Table I.

Insulin/glucose ratio was greater than 0.3. Administration of glucagon elevated plasma glucose from 40 mg/dl (i.e. delta 70 mg/dl) to 110 mg/dl (6.1 mM). Abdominal CT scan excluded pancreatic insulinoma. A diagnosis of hyperinsulinemic hypoglycemia was established. Other laboratory test results, including liver and renal function tests, tandem mass spectrometer, EEG and cranial MRI were normal.

Repeated serum ammonia concentrations in two occasions were 174.8 and 155.5 μ g/dl (reference range, 0-86 μ g/dl). The serum ammonia concentrations of the parents were normal. Diazoxide (10 mg/kg) was started for

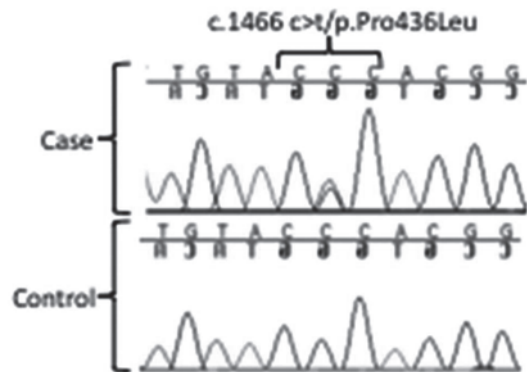


Fig. 1. A heterozygous change, c.1466 c>t in exon 11 was found in the *GLUD1* gene

HI/HA syndrome. The patient's mother was instructed to avoid high protein meals and leucine. The seizure disappeared in three days following treatment.

The patient's genomic DNA was analyzed at The Children's Hospital of Philadelphia for mutations in *GLUD1* that cause HI/HA syndrome. Coding sequences and intron/exon splice junctions were PCR amplified and directly sequenced on an ABI 3730 capillary DNA analyzer (Applied Biosystems, Carlsbad, California). Resulting sequences were analyzed (Gene Codes Corp., Ann Arbor, MI) and compared with the published *GLUD1* reference sequence NM_005271.3 (<http://www.ncbi.nlm.nih.gov>). Nucleotides were numbered beginning with the ATG transcription start site in exon 1 and amino acids were numbered beginning at the start of the mature protein, omitting the 53-amino acid leader sequence.

A heterozygous change, c.1466 c>t in exon 11 was identified (Fig. 1). This mutation is predicted to cause a proline to leucine amino acid substitution at codon 436 of the GDH protein.

A few months after she was diagnosed with HI/HA syndrome and had started treatment for it, her brother also had seizures along with hypoglycemia. Therefore, her family member's genomic DNA was analyzed for mutations *GLUD1*. He was also found to be heterozygous for his sister's reported *GLUD1* missense mutation p.P436L. Her father also had *GLUD1* missense mutation p.P436L while her mother did not.

After her father's family history was searched

Table I. Results of Laboratory Investigations at the Time of Hypoglycemia

Plasma glucose	40 mg/dl
Serum insulin	21.7 μ U/ml
C-peptide	3.9 ng/dl
Cortisol	19 μ g/dl
Growth hormone	15 ng/ml
Lactic dehydrogenase	293 U/L
Ammonia	212 μ g/dl
Blood ketone bodies (strip test)	negative

thoroughly, it was learned that her father sometimes had hypoglycemic symptoms which continued until he was 3 years old.

Discussion

Activating mutations of GDH lead to HI/HA syndrome, while activating mutations of glucokinase (GK), the 'glucose sensor' of the beta cell, cause hyperinsulinism with variable clinical phenotypes. HI/HA syndrome was first described in 1977 by Weinzimmer et al³. In 1998, Stanley et al.¹ studied eight children with the syndrome, and identified the causative gene as *GLUD1*. In 2002, in a multicenter series of 175 patients, hyperammonemia was found in 12 out of 69 patients tested with hyperinsulinemic hypoglycemia. In the presence of activating mutations in the gene encoding GDH, the sensitivity of the enzyme to allosteric inhibition by GTP and ATP is reduced. Increase in GDH activity, alone or as a response to allosteric activation by leucine, increases deamination of glutamate and consequent rise in ATP production, which is a cause for excessive insulin secretion from beta cells in presence of glutamate and leucine.

These events explain hyperinsulinemic hypoglycemia that occurs during fasting, and particularly in the postprandial period after protein ingestion^{4,5,6}. The chronic mild hyperammonemia in HI/HA syndrome is most likely due to increased renal ammoniogenesis caused by the hyperactivity of GDH in the kidney⁷. This finding enables diagnosis of the disease, although ammonia concentrations may vary in the same patient and normal values may occasionally occur.

HI/HA is a disease rarely considered in the differential diagnosis of hypoglycemic seizures. Accurate and timely diagnosis depends on a critical sample collection during a hypoglycemic episode and a glucagon stimulation test performed when plasma glucose is <50 mg/dl. An interesting clinical aspect of HI/HA syndrome is that epilepsy is an unusually frequent finding; in a cohort of 16 patients, 15 presented with seizures and 43% later developed epilepsy (8). Potential explanations of this include recurrent hypoglycemia or chronic hyperammonemia, but especially decreased brain concentrations of the neurotransmitter GABA due to increased GDH activity in the

brain (8). To date, *GLUD1* mutations have been described in the exons 6, 7, 10, 11 and 12 which all encode amino acids in the allosteric regions of GDH (9). It was reported that epilepsy is associated more frequently with mutations in exons 6 and 7 (10); but in our case, the c.1466 c>t/p. Pro436Leu mutation was observed in exon 11 of the *GLUD1* gene. This mutation has been previously reported in a girl presenting at age 1 with a tonic-clonic seizure associated with HI. She had a normal serum ammonia concentration on presentation and in repeated measurements during childhood. She responded to diazoxide, however, she continued to experience intermittent hypoglycaemic episodes in the postprandial period even on a high dose of diazoxide. She was hence evaluated for leucine sensitivity at the age of 13, by an oral leucine tolerance test. In response to an oral leucine load, the patient developed symptomatic hypoglycemia (8). Our patient, in contrast, had a high serum ammonia concentration on presentation which persisted in the following measurements. Moreover, her seizures disappeared completely after diazoxide treatment and avoiding leucine.

The girl in our case and her brother who has the same mutation, had similarities on the appearance time and form of the symptoms. However, the father hadn't any symptoms excluding the history of his intermittent hypoglycemic episodes until he was 3 years old. Despite having the same genetic mutation, the clinical differences between father and his children makes us think that HI/HA syndrome doesn't have any genotype - phenotype correlation.

In conclusion, the findings in this patient contradict the previous claims of a genotype-phenotype correlation in HI/HA syndrome. This case emphasizes the importance of HI/HA syndrome in the differential diagnosis of unexplained seizures with hypoglycemia.

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