Hyperinsulinism/hyperammonemia (HI/HA) syndrome due to a mutation in the glutamate dehydrogenase gene

Síndrome de hiperinsulinemia/hiperamonemia devido a uma mutação no gene da glutamato desidrogenase

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SUMMARY

The hyperinsulinism/hyperammonemia (HI/HA) syndrome is a rare autosomal dominant disease manifested by hypoglycemic symptoms triggered by fasting or high-protein meals, and by elevated serum ammonia. HI/HA is the second most common cause of hyperinsulinemic hypoglycemia of infancy, and it is caused by activating mutations in *GLUD1*, the gene that encodes mitochondrial enzyme glutamate dehydrogenase (GDH). Biochemical evaluation, as well as direct sequencing of exons and exon-intron boundary regions of the *GLUD1* gene, were performed in a 6-year old female patient presenting fasting hypoglycemia and hyperammonemia. The patient was found to be heterozygous for one *de novo* missense mutation (c.1491A>G; p.Il497Met) previously reported in a Japanese patient. Treatment with diazoxide 100 mg/day promoted complete resolution of the hypoglycemic episodes. Arq Bras Endocrinol Metab. 2012;56(8):485-9

SUMÁRIO

A síndrome de hiperinsulinemia/hiperamonemia (HI/HA) é uma condição rara, de herança autossômica dominante, que se manifesta por sintomas de hipoglicemia desencadeada por jejum ou refeições de alto conteúdo proteico, juntamente com elevação da concentração de amônia sérica. HI/HA é a segunda causa de hipoglicemia hiperinsulinêmica da infância e é causada por mutações ativadoras no *GLUD1*, o gene que codifica a enzima mitocondrial glutamato desidrogenase (GDH). A avaliação bioquímica, bem como o sequenciamento direto dos éxons e junções éxon-íntron do gene *GLUD1*, foi realizada em uma paciente de 6 anos de idade com hipoglicemia de jejum e hiperamonemia. A paciente apresentava uma mutação *de novo* missense (c.1491A>G; p.Il497Met) em heterozigose, que havia sido previamente relatada em um paciente japonês. O tratamento com diazóxido 100 mg/dia promoveu resolução completa dos episódios hipoglicêmicos. Arq Bras Endocrinol Metab. 2012;56(8):485-9

INTRODUCTION

The hyperinsulinism/hyperammonemia syndrome (HI/HA) is the second most common cause of hyperinsulinemic hypoglycemia of infancy. 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). Most patients are carriers of a *de novo* mutation, but some familial cases show 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 (1,2). The severity of hypoglycemia is variable, and it is generally corrected by the administration of diazoxide (2).

In the present report, we describe the case of a Brazilian patient with HI/HA syndrome carrying an activating mutation in the *GLUD1* gene.

CASE REPORT

A 6-year-old Caucasoid girl presented generalized tonic-clonic seizures since the age of 7 months. Initially, she was diagnosed with epilepsy, and treatment with anticonvulsants was instituted, although without improvement in clinical status. At the age of 3 years, during a convulsive episode, she was hospitalized and biochemical hypoglycemia was documented on that occasion. Glucose administration prevented seizures, which relapsed when the child received her usual daily feeding routine. At the age of 6 years, she was referred to Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HC-FMUSP). The child was delivered at term, with normal length and adequate weight for gestational age according to the parents (exact data not available), and without perinatal complications. Initially, developmental milestones appeared to be normal (the patient sat with support at the age of 5 months and walked at 14 months), but they were later compromised. She started babbling at the age of three years, and at admission she presented severe cognitive impairment. She was unable to speak complete sentences. There was no family history of hypoglycemia or epilepsy. At admission, physical examination was normal, except for overweight (height, 123 cm [Z-score, +1.89]; weight, 32 kg [Z-score, +4.1]). Laboratory test results, including liver and renal function tests and concentrations of GH, IGF-I, cortisol and ACTH were normal (data not shown). The child presented hypoglycemic episodes after overnight fasting, as well as in the postprandial period. During an episode suggestive of fasting hypoglycemia, the following results were observed: plasma glucose, 42 mg/dL (2.3 mM); serum insulin, 7.7 µU/mL; C-peptide, 3.4 ng/dL; blood ketone bodies (strip test), negative. Administration of glucagon elevated plasma glucose to 113 mg/dL (6.3 mM).

Following demonstration of hyperinsulinemic hypoglycemia, an abdominal CT scan was carried out to exclude pancreatic neuroendocrine tumor (NET), and blood was collected to determine ammonia concentrations. CT scan was normal and ammonia concentrations in two occasions were 97 and 166 µmol/L (reference range, 11-32 µmol/L). Both parents presented normal serum ammonia concentrations. With the diagnosis of HI/HA syndrome, the patient's mother was instructed to avoid offering high protein meals and the patient was started on diazoxide 100 mg/day, with complete resolution of the hypoglycemic episodes.

Direct sequencing of the coding region of the *GLUD1* gene revealed that the affected child was heterozygous for a missense mutation in exon 11 (c.1491A>G; p.Ile497Met; based on NCBI Accession Number NM_005271.3). Neither parents carried this variant (Figure 1), suggesting a "de novo" mutation, which could not be definitively confirmed because a paternity test was not performed.

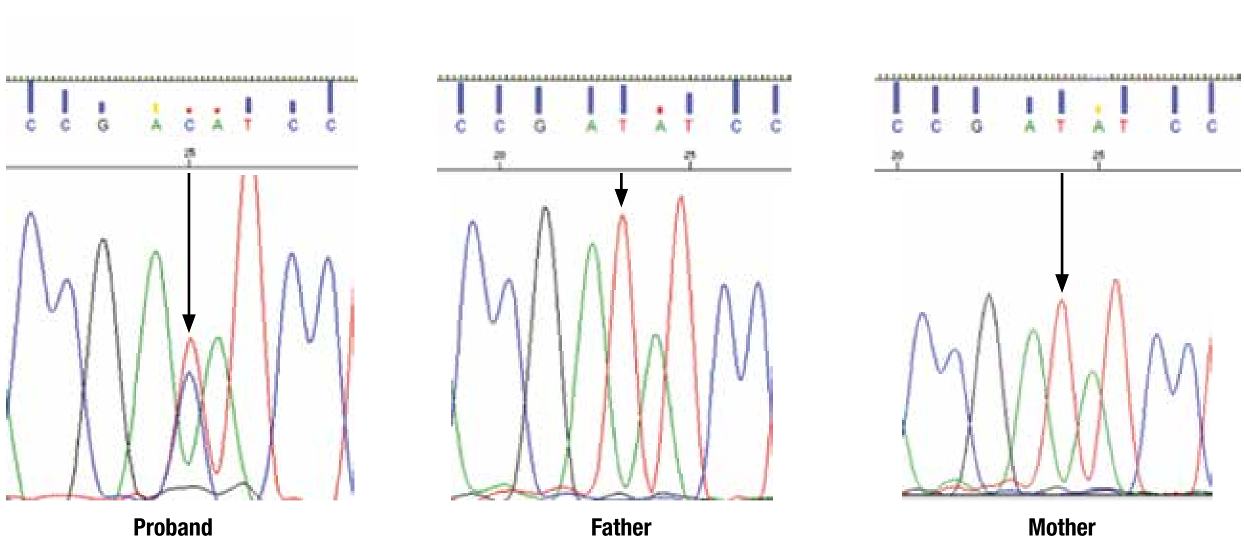

Figure 1. Reverse strand sequencing electropherogram showing the substitution of T by C at nucleotide 1491 (c.1491>G), resulting in the replacement of isoleucine by methionine at codon 497 (p.I497M) of the *GLUD1* gene in the proband. The mutation was absent in the parents.

DISCUSSION

Hyperinsulinism is one of the most common causes of neonatal and childhood hypoglycemia (3). Insulin secretion by pancreatic beta cells is triggered by increased intracellular calcium concentrations. On the surface of these cells, potassium channels composed of Kir6.2 and SUR1 proteins control the polarization of the cell membrane by opening and closing its channels in response to increased or decreased concentration of intracellular adenosine triphosphate (ATP), respectively. When these channels are closed, the cell depolarizes, enabling the opening of calcium channels, increased intracellular concentrations of this ion, and consequent release of insulin (4) (Figure 2).

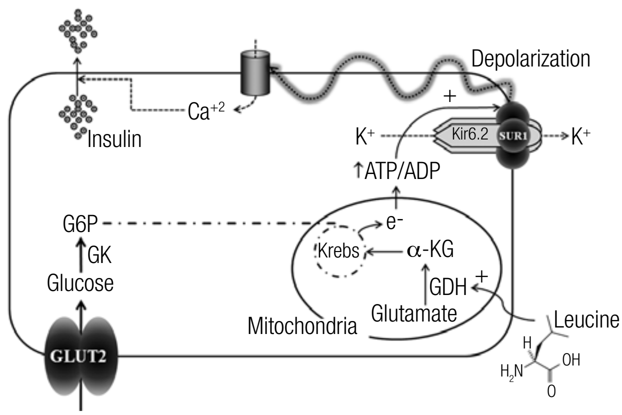

Figure 2. Mechanisms of insulin secretion in pancreatic β-cells. α-KG, α-ketoglutarate; ADP, adenosine diphosphate; ATP, adenosine triphosphate; Ca^{+2}, calcium ion; e-, electron; G6P, glucose 6-phosphate; GDH, glutamate dehydrogenase; GLUT2, glucose transporter type 2; GK, glucokinase; K^+, potassium ion; Kir6.2, inward-rectifier potassium ion channel subunit 6.2; SUR1, sulfonylurea receptor 1. Adapted from Giurgea and cols. (5).

There are two basic mechanisms associated with abnormal increase of insulin secretion by the beta cells: (1) defects in potassium channels (channelopathies) due to inactivating mutations in *ABCC8* and *KCNJ11* genes, which encode, respectively, the proteins SUR1 and Kir6.2. These mutations lead to constitutive closure of potassium channels, so that beta cell membranes remain continuously depolarized, allowing constant insulin secretion irrespective of intracellular concentrations of ATP. These mutations are usually inherited in an autosomal recessive manner, and result in severe hypoglycemia during the neonatal period. In such situations, diazoxide, a drug that acts on potassium channels, is ineffective; and (2) increased generation of mitochondrial ATP (metabolopathies), with consequent closure of potassium channels and increased insulin secretion. In this case, the administration of diazoxide causes opening of potassium channels (which is normal), and corrects hypoglycemia (Figure 2) (5).

The major regulator of insulin secretion is glucose which, in its metabolism, generates ATP and guanosine triphosphate (GTP). In addition to glucose, other substrates may also generate ATP and stimulate insulin secretion, such as fatty acids and the amino acids glutamate and leucine. Glutamate is the substrate of GDH, an enzyme that catalyzes its oxidative deamination to alpha-ketoglutarate (α-KG) and ammonia. Within pancreatic beta cells, α-KG enters the Krebs' cycle, leading to an increased generation of ATP. Leucine, which is present in almost all proteins ingested, is a direct stimulator of GDH (Figure 3) (2,4). Under hyperglycemic conditions, however, the amino acids do not stimulate insulin release, as ATP, and mostly GTP, both generated during glucose metabolization, inhibit intracellular GDH (6). Thus, GDH is allosterically activated by leucine and inhibited by GTP (7).

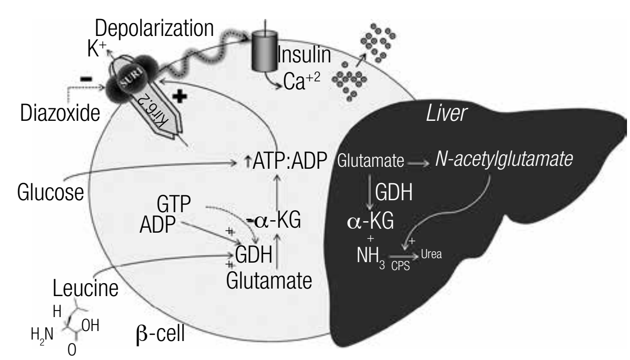

Figure 3. Mechanisms of hyperinsulinism and hyperammonemia in HI/HA syndrome. α-KG, α-ketoglutarate; ADP, adenosine diphosphate; ATP, adenosine triphosphate; Ca^{+2}, calcium ion; CPS, carbamoyl phosphate synthetase; GDH, glutamate dehydrogenase; GTP, guanosine triphosphate; K^+, potassium ion; Kir6.2, inward-rectifier potassium ion channel subunit 6.2; SUR1, sulfonylurea receptor 1. Adapted from Stanley and cols. (11).

In the presence of activating mutations in the gene encoding GDH, there is a reduction in the sensitivity of the enzyme to allosteric inhibition by GTP and ATP, followed by increased response of GDH to leucine, increased deamination of glutamate, and consequent rise in ATP production, which causes excessive insulin secretion from beta cells in presence of glutamate and leucine. This sequence of events explains hyperinsulinemic hypoglycemia that occurs during fasting, and particularly in the postprandial period after protein ingestion (5,6,8).

The hyperactivity of GDH also results in increased serum concentrations of ammonia – which enable the diagnosis of the disease – and in impaired urea production. In the liver, increased GDH activity converts all glutamate into ammonia and α-KG. Urea synthesis from ammonia is carried out by the action of carbamoyl phosphate synthetase (CPS), an enzyme activated by N-acetylglutamate (NAG), which is decreased as a result of GDH overactivity (2,9).

HI-HA syndrome was first described in 1977 by Weinzimer and cols. (10) in two boys with severe hyperinsulinemic hypoglycemia together with hyperammonemia. In 1998, Stanley and cols. studied eight children with the syndrome, identified the gene *GLUD1* and unraveled the pathophysiological mechanisms involved in hyperinsulinemia and hyperammonemia (11). In 2002, in a multicenter series of 175 patients, hyperammonaemia was found in 12 out of 69 tested patients with hyperinsulinemic hypoglycemia (12). Although the disease is rare, several case reports and some reviews have been published (2,9,13-18). An interesting clinical aspect of HI-HA syndrome is that epilepsy is a frequent finding; in a cohort of 16 patients, 15 presented seizures and 43% of them developed epilepsy. This is probably explained not only by recurrent hypoglycemia, but also by chronic hyperammonaemia and by decreased brain concentrations of the neurotransmitter GABA due to increased GDH activity (15).

To date, *GLUD1* mutations described were located in exons 6, 7, 10, 11 and 12, encoding the catalytic and allosteric domains of GDH (15). The mutation c.1491A>G; p.Ile497Met found at exon 11 of the Brazilian patient was also described in a Japanese patient (the mutation was previously named c.1504A>G; p.Ile444Met), and the functional study revealed that the inhibitory effects of GTP on GDH activity were decreased in presence of this mutation (18).

In conclusion, HI/HA syndrome is a serious condition with harmful consequences related to permanent brain damage that occurs when diagnosis is delayed. Diagnosis should be considered in all infants with hyperinsulinemic hypoglycemia, and should motivate the determination of ammonia concentrations. It is worthy commenting that the accuracy of ammonia measurement is extremely dependent on sample collection. Blood samples should be collected from a stasis-free vein into an EDTA evacuated tube, which must be immediately placed on ice and delivered to the lab as quickly as possible; plasma should be separated from the sample without delay, and ammonia analysis performed within 30 minutes. Some rare patients may exhibit serum ammonia within the normal range. If HI/HA is highly suspected in presence of normal serum ammonia, the sensitivity to leucine can be assessed by an oral leucine tolerance test, with administration of 0.15 g/kg of leucine after a 4-hour fasting and determination of plasma glucose and serum insulin at times -30, 0, 30, 60, 90 and 120 minutes. Patients with HI/HA syndrome develop hypoglycemia induced by leucine (15). For definitive diagnosis, direct sequencing of the *GLUD1* gene should be performed.

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