Identification of a diffuse form of hyperinsulinemic hypoglycemia by 18-fluoro-L-3,4 dihydroxyphenylalanine positron emission tomography/CT in a patient carrying a novel mutation of the HADH gene

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Abstract

Objective: Congenital hyperinsulinism is the most common cause of persistent hypoglycemia in infancy (HI), leading to severe neurologic disabilities if not promptly treated. The recent application of positron emission tomography (PET)/computed tomography (CT) scanning with 18-fluoro-L-3,4 dihydroxyphenylalanine improved the ability to distinguish the two histopathologic forms of HI (focal and diffuse), whose differentiation heavily influences the therapeutic management of the patient.

Case report: We describe the case of a patient presenting with severe hypoglycemia from infancy. High concentration of insulin suggested the diagnosis of congenital hyperinsulinism. No metabolic disorders related to amino acid, organic acids or fatty acid oxidation were detected. Medical treatment was able to obtain a satisfactory metabolic response.

Results: The patient underwent PET/CT scanning, revealing a diffuse form of the disease. The absence of mutations in KCNJ11 and ABCC8 genes (responsible for 50% of HI cases), and whole genome single nucleotide polymorphisms analysis by microarray suggested the HADH gene as a likely candidate. Sequence analysis revealed a novel homozygous nonsense mutation (R236X) in HADH gene.

Conclusions: This case indicates that mutations of the HADH gene should be sought in hyperinsulinemic patients in whom diffuse form of HI and autosomal recessive inheritance can be presumed when KCNJ11 and ABCC8 genes mutational screening is negative, even in the absence of altered organic acids and acylcarnitines concentration.

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of hepatic dysfunction, cardiomyopathy or effects on skeletal muscle (3) that are typically present in patients with all other defects in fatty acid oxidation. The clinical presentation is heterogeneous, with either mild late onset hypoglycemia or severe neonatal hypoglycemia that is responsive to medical therapy with diazoxide. The mechanism leading to altered insulin secretion due to HADH gene mutations is still unclear.

We describe a patient carrying a novel mutation of the HADH gene, who underwent positron emission tomography (PET)/CT scanning and genome-wide single nucleotide polymorphism (SNP) analysis by microarray.

Case report

The propositus is an Italian boy born from consanguineous parents (Fig. 1). He was born at 40 weeks of gestation, and his birth weight was 3900 g. He fed poorly from birth and he was described as lethargic. At the age of 2 months, he showed a fixed stare for a few minutes, right rotation of the head, and clonic movements of upper limbs. A neurologic disease was excluded. A blood glucose concentration of 0.94 mmol/l was measured. During a subsequent palpebral clonic episode that lasted about 30 s, the patient’s blood glucose concentration was 1.22 mmol/l. He received a bolus of 20% glucose (2 ml/kg) and his glycemias rose to 7.16 mmol/l. Further investigations performed during an episode of hypoketotic hypoglycemia (glycemia 2.38 mmol/l) showed inappropriately elevated serum insulin concentration (8.43 mU/l/ml). A glucose infusion rate of 8 mg/kg per min (0.43 mmol/kg per min) was initially necessary, and after 24 h it was decreased due to the good response. A 5 mg/kg per min (0.27 mmol/kg per min) glucose infusion rate was sufficient to maintain blood glucose between 3.33 and 5.3 mmol/l. He was evaluated for inborn errors of metabolism, and no metabolic disorders related to amino acid, organic acids or fatty acid oxidation was detected.

The clinical characteristics suggested the diagnosis of hyperinsulinemic hypoglycemia, and therefore the proband was referred to our centre for appropriate treatment. The patient was initially treated with

![Figure 1](http://dx.doi.org/10.1530/EJE-08-0945)
diazoxide (17 mg/kg per day) leading to a satisfying clinical response. Diazoxide requirement to maintain normal blood glucose and insulin concentrations was progressively reduced during follow-up, and it stabilized to a daily dose of 5.6 mg/kg. Two attempts to interrupt treatment were made, but asymptomatic hypoglycemia (glucose concentration: 1.16 mmol/l; insulin concentration: 10 mU/ml) occurred 3–4 days after suspension.

Mutation scanning of KCNJ11 and ABCC8 genes was performed, as previously described (5), but no mutations were found in the coding region of the two genes.

The absence of mutations in KCNJ11 and ABCC8 genes prompted the search for new susceptibility loci using genome-wide 250K SNP mapping array technology (Affymetrix, Santa Clara, CA, USA). The family profiles did not show any chromosomal aberrations, but we found a long contiguous trait of homozygosity of 79 Mb on the patient’s chromosome 4 (4q13.2–4q31.22, Fig. 1). Proband’s parents were heterozygous in the same chromosomal segment indicating homozygous by descent trait nature. Additionally, transmission analysis of parents’ haplotypes in the homozygous stretch excluded the presence of uniparental disomy (autozygosity). The autozygous segment contains 312 known genes including the HADH gene located at 4q25. Consequently, we sequenced the complete coding region of the HADH gene, including intron–exon boundaries. A novel homozygous mutation was identified at codon 236 (706 C>T) in exon 6 of the patient’s HADH gene, resulting in the novel nonsense mutation R236X, while the parents were found heterozygous for this mutation (Fig. 1). To verify the effect of the nonsense mutation on HADH transcription, a PCR product covering exons 3–8 was amplified from reverse-transcribed peripheral lymphocytes mRNA using appropriate primer pairs. Agarose gel electrophoresis analysis of amplified product yielded an amplicon of 622 bp which was directly sequenced, showing the skipping of the mutated exon 6 (data not shown).

At the age of 7 years, the patient was readmitted to our center for a diagnostic re-evaluation and he underwent PET/CT scan analysis with 18-fluoro-L-3,4 dihydroxyphenylalanine ([18F]-DOPA) PET/CT to differentiate the HI form. Therapy with diazoxide was suspended 3 days before PET/CT scan. The imaging investigation included an abdominal ultrasound, abdominal CT, and whole-body [18F]-DOPA PET/CT. There were no intrapancreatic lesions identified in the ultrasound or CT examinations. PET/CT demonstrated intense and homogenous uptake of [18F]-DOPA (Fig. 2), thus suggesting a diffuse form of congenital hyperinsulinemia. This finding indicates increased metabolic activity in the islets, similar to that observed in patients with HI due to mutations in the...
ABCC8 and KCNJ11 genes (6). After the examination, the patient restarted the therapy with diazoxide because of asymptomatic hypoglycemia.

Urinary metabolites were re-evaluated showing an elevated excretion of 3-hydroxybutyric acid, a slight rise of dicarboxylic aciduria, while the level of acylcarnitine in blood was normal.

Discussion

We report the first case of a hyperinsulinemic patient bearing a nonsense mutation (R236X) of HADH gene. Mutations of the HADH gene causing HI have been described in three other families of different ethnic backgrounds (2–4): a missense mutation P258L, a six base pair deletion at the start of HADH exon 5 and a splice site mutation IVS6-2.

Age at onset of the reported cases varied from a few days to 4 months (2–4). Hypoglycemia led to seizures in two cases (3, 4), and to hypotonia and cyanosis in one (2). Blood glucose concentration at diagnosis ranged from 0.8 to 1.4 mmol/l (2–4). Our patient presented with a fixed stare and clonic movements at the age of 2 months, with a blood glucose concentration of 0.94 mmol/l, indicating a highly variable clinical presentation of the disease. A common finding in all cases, including the present one, was the good response to medical treatment.

Unlike all other reported cases (2–4), neither specific increases of hydroxybutyryl-carnitine in blood nor of urinary 3-hydroxyglutarate were observed in our patient. It is difficult to interpret these findings. However, in vitro studies that silenced HADH gene expression in β-cells showed increased insulin release, which was not caused by increased rate of glucose metabolism or inhibition of fatty acid oxidation (7). Moreover, a similar case has been recently communicated (8). Elevated levels of urinary 3-hydroxybutyric acid, and slightly increased dicarboxylic aciduria were evident at his diagnostic re-evaluation. The HADH protein is known to act in a common branch of the cyclically organized β-oxidation pathway, converting l-3-hydroxyacyl-CoA of variable chain length to their corresponding 3-ketoacyl-CoA: the mitochondrial β-oxidation role in the regulation of glucose-induced insulin release has been examined in a recent work (7), suggesting that this enzyme could play a regulatory role between glucose metabolism and acyl-CoA-derived signals. This view is supported by a moderate increase in glucose-induced NADH in severely HADH suppressed cells, a glucose-induced accumulation of l-3-hydroxybutyryl-CoA, the unbalance between HADH expression and l-3-hydroxyacyl profiles, and finally the complex influence of HADH expression on β-oxidation (7). The urinary metabolic profile of our patient is consistent with a slow down of β-oxidation at level of HADH, leading to increased accumulation of medium and long l-3-hydroxyacyl-CoA species, and upstream long-chain acyl-CoAs, which stimulate insulin release (9). Long chain-acyl-CoAs or other yet unidentified (l-3-hydroxy-¸) acyl-CoAs derived molecules could represent metabolic signals that stimulate insulin secretion in a K+ ATP independent way.

Considering that the nonsense mutation R236X leads to a protein lacking an important portion of the C-terminal domain, involved in subunit dimerization (10), it is reasonable to assume that a non-functional protein is generated. An easy tool to verify this hypothesis would have been to measure HADH enzyme activity in skin fibroblasts. However, the proband’s parents refused to give their consent for skin biopsy.

In summary, we report the case of a new HADH gene R236X mutation in a hyperinsulinemic Italian patient with a diffuse form of HI. High-density microarray analysis revealed HADH gene to be a likely candidate in this patient and direct sequencing confirmed the presence of causative mutation.

This case indicates that mutations of HADH gene should be sought in hyperinsulinemic patients in whom a diffuse form of HI and autosomal recessive inheritance can be presumed when KCNJ11 and ABCC8 genes mutational screening is negative, independently from the organic acids and acylcarnitine metabolism.

Declaration of interest

The authors do not have any conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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