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Original article

Two compound frame-shift mutations in succinate dehydrogenase gene of a Chinese boy with encephalopathy

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Abstract

Objective: To investigate respiratory chain complex II deficiency resulted from mutation in succinate dehydrogenase gene (SDH) encoding complex II subunits in China. *Methods:* An 11-year-old boy was admitted to our hospital. He had a history of progressive psychomotor regression and weakness since the age of 4 years. His cranial magnetic resonance imaging revealed focal, bilaterally symmetrical lesions in the basal ganglia and thalamus, indicating mitochondrial encephalopathy. The activities of mitochondrial respiratory chain enzymes I–V in peripheral leukocytes were determined via spectrophotometry. Mitochondrial DNA and the succinate dehydrogenase A (*SDHA*) gene were analyzed by direct sequencing. *Results:* Complex II activity in the leukocytes had decreased to 33.07 nmol/min/mg mitochondrial protein (normal control 71.8 ± 12.9); the activities of complexes I, III, IV and V were normal. The entire sequence of the mitochondrial DNA was normal. The *SDHA* gene showed two heterozygous frame-shift mutations: c.G117G/del in exon 2 and c.T220T/insT in exon 3, which resulted in stop codons at residues 56 and 81, respectively. *Conclusions:* We have described the first Chinese case of mitochondrial respiratory chain complex II deficiency, which was diagnosed using enzyme assays and gene analysis. Two novel, compound, frame-shift mutations, c.G117G/del in exon 2 and c.T220T/insT in exon 3 of the *SDHA* gene, were found in our patient.

Keywords: Respiratory chain complex II deficiency; *SDHA* gene; Frame-shift mutation

1. Introduction

Mitochondrial diseases are a group of inherited diseases that present with clinically heterogeneous manifestations and usually emerge in infancy and childhood [1]. Mitochondrial respiratory chain complex deficiencies are a main cause of mitochondrial diseases. Deficiencies can occur in isolated or multiple complexes, and a wide spectrum of biochemical and genetic data related to these deficiencies has been reported [1,2]. Respiratory chain complex I and complex IV deficiencies, resulting from mutations of the mitochondrial and nuclear genes, account for most cases of mitochondrial diseases. In contrast, isolated complex II deficiency is rare.

Respiratory chain complex II, also known as succinate dehydrogenase (SDH; EC 1.3.5.1), is composed of

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four subunits, including two membrane-bound proteins (SDHC and SDHD), a flavoprotein (SDHA) and an iron-sulfur subunit (SDHB). All four subunits are encoded by nuclear DNA. Subunits SDHA and SDHB catalyze the oxidation of succinate to fumarate and transfer electrons to ubiquinone in the respiratory chain [3]. Complex II deficiency can result from mutations in the *SDHA* gene on chromosome 5p [4] or in *SDHAF1* gene on chromosome 19q [5]. Patients with mutations in mitochondrial or nuclear genes encoding complexes I, III, IV or V have been observed consecutively in China. However, pathogenic mutations in the *SDHA* gene have not yet been reported from the Chinese population. In this study, two novel frame-shift mutations in the *SDHA* gene were detected in a Chinese boy with encephalopathy.

2. Methods

2.1. Patient

An 11-year-old boy was admitted to our hospital. He had a history of progressive psychomotor regression and weakness since the age of 4 years. He was the first child of non-consanguineous and healthy parents. His family history was negative. He developed normally until the age of 4 years. At age 4, however, a gradual loss of major motor skills occurred, and this worsened after infections. These symptoms could be relieved temporarily. At the age of 8 years, he presented with instability of gait, weakness and psychomotor regression.

His head circumference was within the normal range, and he had no obvious dysmorphic features. His external genitalia, limbs and skin appeared normal, and his deep tendon reflexes were brisk.

Routine laboratory examinations yielded normal results. However, the serum lactate level was increased (2.9–4.5 mmol/l vs. 0.5–2.0 mmol/l in a normal control).

Magnetic resonance imaging (MRI) of the brain revealed focal, bilaterally symmetrical, hypointense lesions on T1-weighted images and hyperintense lesions on T2-weighted images in globus pallidus (Fig. 1), which indicated Leigh-like syndrome due to metabolic disorder.

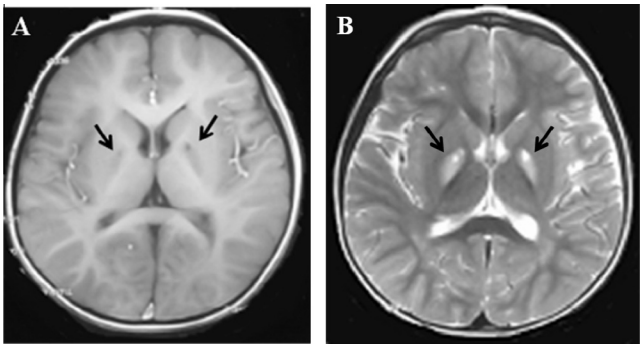

Fig. 1. Brain magnetic resonance image (MRI) of the patient. Hypointense lesions on T1-weighted images (A) and hyperintense lesions on T2-weighted images (B) can be seen in the basal ganglia and thalamus.

Typical aminoacidopathies, organic acidurias and mitochondrial fatty acid beta-oxidation defects were excluded via the analysis of urinary organic acids, and blood amino acids and acylcarnitines [6,7].

The patient was administered carnitine, coenzyme, thiamine and vitamins C and E for empirical treatment of mitochondrial disease. He is currently 11 years of age, and his psychomotor development has shown intermittent improvement. His serum lactate level has decreased to the normal level. Although only a slight improvement was observed on his brain MRI, he now has an almost normal school life.

The patient's parents provided informed consent, and this study was approved by the hospital ethics committee.

2.2. Assays for mitochondrial respiratory chain complexes I–IV and ATP synthase (complex V)

The activities of mitochondrial respiratory chain enzymes I–IV and ATP synthase in peripheral leukocytes were determined via spectrophotometry. The activity of each complex was expressed as the rate (nmol/min/mg mitochondrial protein) and as the ratio to the rate of citrate synthase, as previously reported [2].

2.3. Analysis of nuclear genes encoding respiratory chain complex II

Sequence analysis of four genes encoding the complex II subunits SDHA, SDHB, SDHC and SDHD was performed. Total DNA was extracted from the blood by the proteinase-K-chloroform method. Primers for coding region amplification were designed on the basis of genomic sequences (GenBank accession numbers: SDHA, NG_012339.1; SDHB, NG_012340.1; SDHC, NG_012767.1; SDHD, NG_012337.2). Polymerase chain reaction (PCR) was performed in a 50-μl system containing 50 ng DNA template, 2.5 U Taq DNA polymerase, 10 × Taq buffer, dNTP and primers. The reaction was carried out with an initial denaturation step at 95 °C for 5 min, followed by 35 cycles at 95 °C for 40 s, 60 °C for 40 s and 72 °C for 1 min, and a final elongation step at 72 °C for 5 min. Sequence analysis was performed using ABI-3730XL (Applied Biosystems, California, USA). Two copies of SDHA were found. The SDHA copy on Chr 3q probably represents a pseudogene. A mutation on Chr 5p was detected using restriction fragment length polymorphism.

3. Results

In the peripheral leukocytes of the patient, complex II activity was reduced to 33.07 nmol/min/mg mitochon-