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Case Report

A fetus with mitochondrial trifunctional protein deficiency: Elevation of 3-OH-acylcarnitines in amniotic fluid functionally assured the genetic diagnosis

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ABSTRACT

Mitochondrial trifunctional protein (TFP) is a multienzyme complex that catalyzes the last three steps of the β-oxidation cycle of long-chain fatty acids. In the prenatal diagnosis of TFP deficiency, acylcarnitine (AC) analysis has been considered difficult because of limited excretion of long-chain ACs into the fetal urine and hence into the amniotic fluid. Here, we report our experience with prenatally diagnosing TFP deficiency using AC analysis of amniotic fluid. The index case was a boy born at 38 weeks gestation and weighing 2588 g. He suddenly became unconscious and hypoglycemic and died on day 6 of life. Postmortem blood AC analysis and gene sequencing revealed TFP deficiency. Therefore, the parents underwent prenatal diagnoses for their subsequent 2 pregnancies. Mutation analysis suggested that one (Case 1) was affected and the other (Case 2) was not. AC analysis also demonstrated identical results, with significantly elevated 3-hydroxy-AC levels in the amniotic fluid of the affected pregnancy compared with those of heterozygotes and normal controls ($n = 2$ for heterozygotes and $n = 8$ for normal controls). Our findings suggest that AC analysis can functionally confirm results even in families with unidentified mutations, without raising issues related to maternal cell contamination. During prenatal diagnosis, misdiagnosis has to be avoided, and combining AC analysis with gene sequencing may result in more accurate prenatal diagnosis of TFP deficiency.

1. Introduction

Mitochondrial trifunctional protein (TFP) is a multienzyme complex consisting of trans-2,3-long-chain enoyl-CoA hydratase (LCEH, EC 4.2.1.74), long-chain 3-OH-acyl-CoA dehydrogenase (LCHAD, EC 1.1.1.211) located in the TFP α-subunit (HADHA, OMIM: 600890), and long-chain 3-ketoacyl-CoA thiolase (LCKT, EC 2.3.1.16) located in the TFP β-subunit (HADHB, OMIM: 143450). These enzymes catalyze the last three steps of the β-oxidation cycle of long-chain fatty acids [1,2]. TFP deficiency is clinically classified into three types: 1) lethal type (neonatal-onset form), which includes the development of profound hypoglycemia, lactic acidosis and cardiomyopathy during the neonatal period; 2) intermediate type (infant-onset form), which is accompanied by hypoketotic hypoglycemia that is generally observed following infection or long periods of fasting during the infantile period; and 3) myopathic type (adult-onset form), which includes muscular symptoms, such as intermittent myalgia or rhabdomyolysis, that are associated with prolonged exercise after adolescence. The neonatal form is normally lethal during the neonatal period, irrespective of any intensive treatments [3]. Therefore, families who have had such an affected child often undergo genetic counseling for prenatal diagnosis during subsequent pregnancies.

TFP deficiency is usually diagnosed based on increased levels of long-chain 3-OH-acylcarnitines (3-OH-ACs), such as C16-OH or C18:1-OH, which can be measured by blood acylcarnitine (AC) analysis using tandem mass spectrometry (MS/MS). However, instead of AC analysis, gene analysis is usually performed for the prenatal diagnosis of TFP deficiency [4]. Herein, we report our experience with prenatally diagnosing TFP deficiency using AC analysis and gene analysis. Our data indicate that AC analysis of amniotic fluid is useful for the prenatal diagnosis of TFP deficiency.

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2. Materials and methods

The protocol for this study was approved by the Ethical Committee of Shimane University Faculty of Medicine.

2.1. Case

The index case was a boy born at 38 weeks gestation via vaginal delivery and weighing 2588 g. He was the second child of non-consanguineous parents, and his elder brother was healthy (Fig. 1).

His mother had no abnormalities during the pregnancy, including no HELLP (hemolysis, elevated liver enzymes, and low platelet counts) syndrome or AFLP (acute fatty liver of pregnancy). On the 2nd day after birth, the boy suddenly became unconscious and hypotonic, accompanied by severe hypoglycemia and lactic acidosis. Despite various treatments, including continuous hemodiafiltration that was performed to address the potential of septic shock, his clinical condition deteriorated, and he died of heart failure on the 6th day. Postmortem blood AC analysis revealed the accumulation of 3-OH-ACs, suggesting that the boy had the lethal type of TFP deficiency. Gene analysis and Western blotting confirmed the diagnosis. Therefore, the parents underwent prenatal diagnoses for their subsequent 2 pregnancies (Cases 1 and 2).

After obtaining informed consent from the parents, AC analysis of the amniotic fluid was performed as well as gene analysis and Western blotting.

2.2. Amniotic fluid

Amniotic fluid was collected at 16 and 18 weeks of gestation for Cases 1 and 2, respectively. For comparison purposes, amniotic fluid samples were obtained after 15 weeks from 8 normal controls and from 2 heterozygotes for TFP deficiency who had both undergone prenatal genetic testing (heterozygote-A: c.442+614 A>G and heterozygote-B: c.1364T>G in the *HADHB* gene).

2.3. Gene analysis

Genomic DNA was extracted from the pellets of centrifuged amniotic fluid using the QIAamp DNA Micro Kit (Qiagen GmbH, Hilden, Germany). Both the *HADHA* and the *HADHB* genes, which encode TFP, were sequenced as previously reported [5].

2.4. Western blot analysis

Western blot analysis of cultured fibroblasts or amniocytes was performed using a rabbit polyclonal antibody raised against both the α- and β-subunits of TFP; this antibody was kindly provided by Dr. T. Hashimoto, Professor Emeritus, Shinshu University, Matsumoto, Japan. The signals were visualized using the ImmunoPure NBT/BCIP Substrate Kit™ (Promega, Madison, WI, USA), as previously described [6].

2.5. Acylcarnitine analysis

AC analysis was performed according to the modification of the method as reported [7]. Briefly, 10 μL of amniotic fluid supernatant was obtained after centrifugation at 3000 rpm for 5 min. The sample was then subjected to butyl derivatization using API 3000 triple–quadrupole tandem mass spectrometer (MS/MS) in combination with an SIL-HTc autosampler (Shimadzu, Kyoto, Japan).

3. Results

3.1. Gene analysis

Direct DNA sequencing of the index case revealed the compound heterozygote mutations c.1392+1G>A and c.1689+2T>G in the *HADHA* gene, both of which induce mRNA splicing errors. The same mutations were identified in Case 1, whereas no mutations were identified in Case 2.

3.2. Western blot analysis

Western blot analysis of TFP in the cultured amniotic cells showed that both the α- and β-subunits were detected in Case 2, but not in Case 1, whereas the very-long-chain acyl-CoA dehydrogenase (VLCAD, EC 1.3.8.9) protein was expressed normally in both cases (Fig. 2).

3.3. Acylcarnitine analysis

AC analysis of the amniotic fluid of Case 1 demonstrated significant elevations of 3-hydroxy-ACs: C14-OH measured at 55 nmol/L (control, 3.9 ± 5.0; +10.3 SD), C16-OH measured at 120 nmol/L (control, 0.8 ± 1.5; +77.6 SD), C18-OH measured at 31 nmol/L (control, 3.2 ±

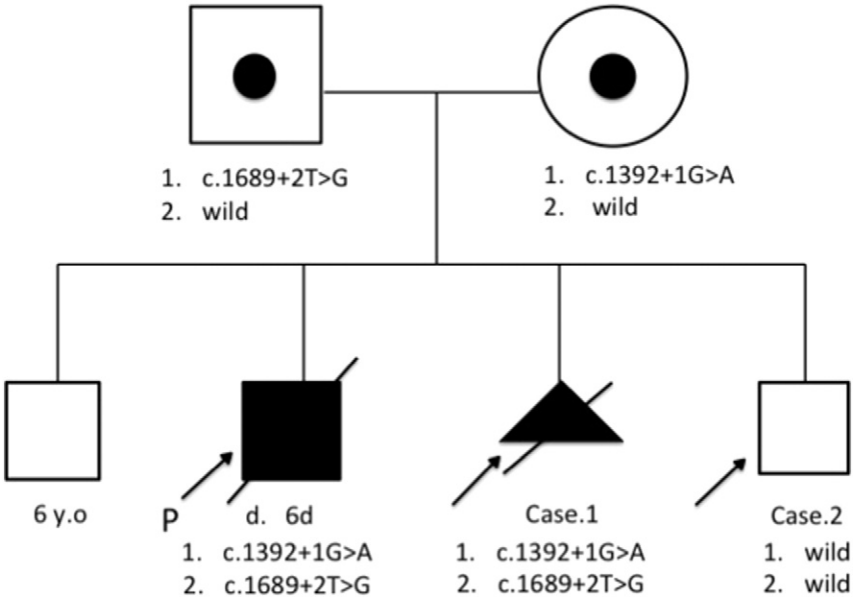

Fig. 1. Family tree The age of each patient and sibling is described for the time at which the amniotic fluid of Case 2 was obtained.