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The First Case of Korean Boy with Mitochondrial Trifunctional Protein Deficiency Diagnosed by Acylcarnitine Profiles and DNA analysis : A Novel Mutation in the α-subunit of the Mitochondrial Trifunctional Protein and a Unusual Intergenic Sequence with Two Polymorphisms

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Summary

A premature baby boy with mitochondrial trifunctional protein(TFP) deficiency, the first Korean case, presented neonatally with refractory respiratory distress syndrome, severe metabolic acidosis with hypotension, oliguric renal failure, cardiomyopathy. On the 8th day of life, he died despite intensive treatment. Blood acylcarnitine analysis by tandem mass spectrometry showed the diagnosis of the long chain 3-hydroxyacyl-CoA dehydrogenase(LCHAD) deficiency or TFP deficiency. DNA analysis revealed a novel mutation in the α-subunit of the TFP and a unusual intergenic sequence(C/C and G/G) with two polymorphisms.

Human mitochondrial trifunctional protein (TFP) is a hetero-octamer of four α- and four β-subunits that catalyzes three steps in the β-oxidation spiral of long-chain fatty acids(Kamijo et al, 1993) and includes the long chain 3-hydroxyacyl-CoA dehydrogenase (LCHAD). TFP deficiency causes a Reye-like syndrome, cardiomyopathy, or sudden infant death. As the importance of acylcarnitine analysis by tandem mass spectrometry (MS/MS) for diagnosis has been recognized recently, a number of fatty acid β-oxidation disorders have been disclosed(Matern et al, 1999). We report the first Korean patient with TFP deficiency diagnosed by acylcarnitine analysis in whom molecular diagnosis revealed a novel mutation.

A preterm baby boy was born with body weight of 1,600 g at 32 weeks gestational age to non-consanguineous parents. The first baby in this family was alive and well, but the second and the third siblings succumbed to suspected sepsis at days 7 and 15 after birth, respectively.

After birth, the propositus received ventilatory support and surfactant therapy because of respiratory distress syndrome(RDS) without any symptomatic improvement. On the second day of life,

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severe metabolic acidosis(pH 7.0, bicarbonate 4 mmol/L, base exess -19.8 mmol/L), hypotension (BP 25/10 mmHg), and oliguria developed. A patent ductus arteriosus with left to right shunting was demonstrated on echocardiography. Intravenous administration of dopamine, dobutamine, bicarbonate and glucose induced no clinical improvement. The next day, hypotension and metabolic acidosis persisted and anuria with generalized edema occurred. Acute peritoneal dialysis was initiated and continuous administration of epinephrine was added. The baby became increasingly ill and was transferred to our hospital.

Initial laboratory findings were serum ammonia of 207 μg/dl (normal<150), BUN 46.9 mg/dl, creatinine 2.9 mg/dl, AST 170 IU/L, and ALT 35 IU/L. There was metabolic acidosis (pH 7.1, bicarbonate 9.0 mmol/L, base excess -16.8 mmol/L) with lactic acidosis (lactic acid 28.1 mmol/L, normal <2.2), but no hypoketotic hypoglycemia. Then, the patient developed frequent seizures. Brain ultrasonogram showed grade I subependymal hemorrhage with diffuse brain edema. Echocardiography revealed poor contractility of both ventricles and persistent fetal circulation. A chest radiograph showed cardiomegaly (CT ratio 0.72) and air bronchograms with granular density in both lungs. Repeated exogenous surfactant therapy and high-frequency ventilation with nitric oxide inhalation were added. However, the clinical course got rapidly worsened, and the infant died on the 8 th day of life. Unfortunately, the parents denied permission about autopsy and skin biopsy for fibroblast culture.

Blood acylcarnitine analysis by MS/MS showed elevated excretion of 3-OH-tetradecanoylcarnitine, 3-OH-hexadecenoylcarnitine, 3-OH-hexadecanoylcarnitine, and 3-OH-oleylcarnitine, consistent with the diagnosis of LCHAD or TFP deficiency. In addition, we also detected elevation of hexanoylcarnitine and 3-OH-stearoylcarnitine.

TFP deficiency can be diagnosed by direct assay of the three enzyme activities in cultured fibroblast extracts. Unfortunately, lack of parental permission precluded performance of these enzyme assays.

The only source of DNA for molecular analysis was the limited amount of the newborn blood spot obtained from the patient. Single-stranded conformation variance analysis of amplified DNA of all 20 exons of α-subunit of TFP revealed a single, heterozygous 2-bp-deletion(bp 1793-4) in exon 17 (frame shift mutation), creating an internal termination codon. Exon 15, the site of the common mutation of LCHAD deficiency, G1528C, was normal and Sequences of all β-subunit exons were normal. This 2 bp deletion has been previously found in two American patients, both of whom are of Hispanic ancestry(A. Strauss, unpublished). We did find that this patient has a quite unusual (about 3% of the normal population in a sample of 100 American individuals) intergenic sequence in that this individual is C/C and G/G. The intergenic region is a 300 bp sequence that lies between the two subunit genes. There are two polymorphisms among these. We do not yet know if these polymorphisms affect transcription of the genes. We know that this region is essential for regulation in vivo and in vitro of expression of these two subunits. Also we have found this unusual haplotype in several patients with TFP deficiency in whom we can only find one mutation. So, we postulate that this haplotype reduce transcription.

Parental mutational analysis could not be performed due to their refusal. We believe that this unfortunate male infant is the first Korean case with TFP deficiency and the first report with this novel mutation in the α-subunit of the TFP with an Asian ethnic background.

Review of the clinical course of our patient revealed that cardiomyopathy related to TFP defici-

ency might have induced persistent hypotension and oliguric renal failure. Refractory RDS, probably combined pulmonary edema, probably caused his demise.

In conclusion, we report the first Korean case of TFP deficiency with a novel mutation in the α-subunit of the TFP and a unusual intergenic sequence between the two subunit genes with two polymorphisms, diagnosed through newborn screening by MS/MS and DNA analysis.

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