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CASE REPORT

Glutaric acidemia type Ⅱ patient with thalassemia minor and novel electron transfer flavoprotein-A gene mutations: A case report and review of literature

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Abstract

Glutaric acidemia type Ⅱ (GAⅡ), also known as multiple acyl-CoA dehydrogenase deficiency, is an autosomal recessive inborn error of amino acid and fatty acid metabolism. We report a case of GAⅡ with novel electron transfer flavoprotein (ETF)-A mutations in a 2-year-old female with thalassemia minor. The patient developed an episode of hypoglycemia and hypotonicity

on the postnatal first day. Laboratory investigations revealed elevations of multiple acyl carnitines indicating glutaric acidemia type Ⅱ in newborn screening analysis. Urinary organic acids were evaluated for the confirmation and revealed a high glutaric acid excretion. Genetic analysis revealed two novel mutations in the ETF-A gene, which are considered to be compound heterozygote. At the 8 mo of life ketone therapy was added, which significantly increased the neuromotor development. The patient had been closely followed for two years with carnitine, riboflavin, coenzyme Q10, and ketone supplementation in addition to a high carbohydrate diet. Although the patient had comorbidity like thalassemia minor, her neuromotor development was normal for her age and had no major health problems. This specific case expands the previously reported spectrum of this disease.

Key words: Electron transfer flavoprotein-A mutation; Newborn screening; Glutaric acidemia type Ⅱ; Inborn error of metabolism; Ketone bodies; Case report

Core tip: Multiple acyl-CoA dehydrogenase deficiency (GAⅡ) is an autosomal recessive inborn error of amino acid and fatty acid metabolism. We report a case of GAⅡ with novel electron transfer flavoprotein (ETF)-A mutations in a 2-year-old female with thalassemia minor. Genetic analysis revealed two novel mutations in the ETF-A gene, which are considered to be the etiology for the disease. Most neonatal-onset patients of GAⅡ may not survive due to progressive deterioration despite aggressive treatment. However, at 8 mo of age our patient, with the experimentally added ketone therapy, had no major health problems, and neuromotor development was normal for her age. The present case report is the only one reporting a patient with both GAⅡ and thalassemia minor.

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INTRODUCTION

Glutaric acidemia type Ⅱ (GAⅡ), also known as multiple acyl-CoA dehydrogenase deficiency, is an autosomal recessive inborn error of amino acid and fatty acid metabolism[1]. GAⅡ is caused by a defect in the electron transfer flavoprotein (ETF) or ETF dehydrogenase (ETFDH) resulting in deficiencies in multiple acyl-CoA dehydrogenases[2]. The ETF/ETFDH deficiencies are responsible for multiple defects of the dehydrogenation system because they block not only fatty acid oxidation, but also the oxidation of branched-chain amino acids and of glutaryl-CoA on the catabolic pathway of lysine, hydroxylysine, and tryptophan[3].

There are three clinical phenotypes in GAⅡ: neonatal form with congenital anomalies (most commonly cystic or dysplastic kidneys)[1]; neonatal form without congenital anomalies; and late onset form with myopathic phenotype and rarely metabolic acidosis[4]. All forms of GAⅡ can be caused by defects in the genes encoding the ETF α and β subunits (ETF-A and ETF-B, respectively) or in the gene encoding ETF-QO (ETFDH). The majority of patients with ETF deficiency are found to have mutations in the ETF-A gene[1].

Neonatal onset form is usually fatal and characterized by severe non-ketotic hypoglycemia, metabolic acidosis, excretion of large amounts of fatty acid, and amino acid-derived metabolites with congenital anomalies[5]. In addition to hypoglycemia and metabolic acidosis, routine laboratory findings may include hyperammonemia and elevated liver transaminases. Cardiomyopathy may be present in some cases. Pathologic abnormalities include fatty infiltration of the liver, heart, and kidneys[1].

Treatment consists of a diet low in protein and fat together with carnitine, ubiquinone, and riboflavin supplementation. However, most neonatal-onset patients may not survive due to progressive deterioration despite aggressive treatment[1]. We hereby present a case of GAⅡ, a neonatal form without congenital anomalies with a novel ETF-A mutation in a 2-year-old female with thalassemia minor.

CASE REPORT

The patient was born at full-term to nonconsanguineous parents after an uneventful pregnancy as the first child of the family with no history of metabolic diseases. The birth weight was 3192 g and height was 50 cm. Initial physical examination revealed a healthy infant with no dysmorphic features. An episode of hypoglycemia and hypotonicity occurred on the postnatal first day, and the infant was admitted to the neonatal intensive care unit. Initial laboratory results revealed significant hypoglycemia with elevated transaminase levels.

An expanded newborn screening (NBS) sample obtained in the first week was reported to be positive for glutaric acidemia type Ⅱ with elevations of multiple acylcarnitines. In particular, C4 butyrylcarnitine levels were extremely high, accompanied by elevated C5, C6, C8, C10, C14, and C5-DC glutaryl carnitine levels. Second-tier testing was carried out for NBS and urinary organic acids were evaluated for confirmation. Glutaric acid excretion was extremely high with increased levels of lactic acid, adipic acid, 5-hydroxyhexanoic acid, 2-hydroxy glutaric acid, and suberic acid. Radiological examinations revealed normal cranial Doppler

Table 1 Laboratory tests on the 22nd day after birth

Biochemical data	Patient results	Reference range
ALT (IU/L)	55 ↑	13-45
AST (IU/L)	63	18-69
ALP (IU/L)	252	107-474
CK (IU/L)	139	43-474
Urea (mg/dL)	14	2.1-34
CRE (mg/dL)	0.3	0.3-0.8
Lactate (mmol/L)	23.4	17-24
Ammonia (μmol/L)	103 ↑	16-68
HCO_3 (mmol/L)	18.3	17-24
Ca (mg/dL)	10.7	8.4-11.9
P (mg/dL)	5.9	3.1-7.7

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; CK: Creatine kinase; CRE: Creatinine.

Table 2 Dried blood spot acylcarnitine results

Blood acylcarnitine concentrations (μmol/L)	Patient	Reference range
Free carnitine (C0)	5.46 ↓	8.60-90.0
Acetylcarnitine (C2)	3.86 ↓	8.00-73.4
Propionylcarnitine (C3)	0.20 N	< 6.800
Butyrlycarnitine (C4)	2.25 ↑	< 1.200
Isovalerylcarnitine (C5)	0.18 N	< 0.600
Glutarylcarnitine (C5DC)	0.27 ↑	< 0.210
Hexanoylcarnitine (C6)	0.52 ↑	< 0.210
Octanoylcarnitine (C8)	2.77 ↑	< 0.320
Decanoylcarnitine (C10)	2.18 ↑	< 0.480
Dodecanoylcarnitine (C12)	1.37 ↑	< 0.690
Tetradecanoylcarnitine (C14)	0.57 N	< 0.800
Hexadecanoylcarnitine (C16)	0.69 N	< 8.700
Octadecenoylcarnitine (C18)	0.76 N	< 2.240

ultrasonography (USG) and normal cranial magnetic resonance findings. There were no pathologic findings aside from minimal hepatomegaly in the liver in USG (craniocaudal length 79.6 mm) and peripheral pulmonary stenosis was detected in echocardiographic evaluation.

The patient was transferred to the department of nutrition and metabolism of the university hospital with GA Ⅱ pre-diagnosis on the 22nd day after birth and was followed up for further examination and treatment regimen. Her general condition was good, and no abnormality was identified during the physical examination upon arrival at the university hospital. The laboratory findings and acylcarnitine profile are shown in Tables 1 and 2, respectively.

At the university hospital, a nasogastric tube was inserted and the patient was started on a diet of 6 mg/kg/min glucose with total parenteral nutrition containing 13% protein, 63% carbohydrate, 23% lipid, with totally 116 kcal/kg per day energy balance. After a week nutritional education was provided to the caregivers, the patient was discharged on carnitine (50 mg/kg per day), coenzyme Q10 (100 mg/d) and riboflavin (100 mg/d) supplementation with a high carbohydrate oral diet. At a follow-up after six months, ammonia levels were found to be high and sodium benzoate therapy was initiated. Meanwhile, a genetic analysis revealed, two different novel mutations in the ETF-A gene [NM_000126.3 p.L67P (c.200T>C) and Q285L (c.854A>T), compound heterozygote] and these mutations were considered to be the cause of the disease according to SIFT, PolyPhen-2, and PROVEAN databases[6-8].

At the sixth month follow-up, although the Denver test had revealed normal findings in terms of neuromotor development before, a slight delay in neuromotor development was observed. At the age of eight months, sodium 3-hydroxybutyrate (NaHB) administration was started and gradually increased from 360 mg/kg to 1400 mg/kg by the end of the 12th month, together with the high carbohydrate diet. Diet challenge was undertaken according to the plasma acylcarnitine and ketone body levels. After the diet alignments, the patient's neuromotor development was noted to increase significantly.

In the second year, follow-up laboratory tests identified low mean corpuscular volume (< 65 fl) and high red blood cell (5.5 × 10^6/μL). A hemoglobin electrophoresis was performed and results confirmed the diagnosis of thalassemia minor. Following these diagnostic tests, a percutaneous endoscopic gastrostomy (PEG) tube was inserted. The patient has been closely followed with carnitine, riboflavin, coenzyme Q10, and 1600 mg/kg per day ketone treatment in addition to a high carbohydrate diet. Patient is followed with routine controls and infections are monitored and treated immediately. Although she is slightly overweight, her neuromotor development is normal for her age and she has no major health problems.

DISCUSSION

Glutaric acidemia type Ⅱ is among the severe inborn errors of metabolism and it may be associated with significant morbidity and mortality, particularly in the neonatal-onset patients[1]. Neonatal-onset form patients may develop severe respiratory failure, cardiomyopathy, hypotonia, metabolic acidosis, and profound hypoglycemia soon after birth, which corresponds with short life expectancy[2].

The patient in the present case report had severe hypoglycemia on the postnatal first day, and diagnosis was achieved by the expanded newborn screening analysis. Acylcarnitines in dried blood spots were analyzed using a tandem mass spectrometry after butyl-derivatization. Urine organic acids were analyzed by gas chromatograph mass spectrometer and showed increased excretion of GA Ⅱ characteristic compounds, such as adipate, suberate, glutarate, 2-hydroxyglutarate, ethylmalonate, or isovalerylglycine, which are the corresponding metabolites derived from defective acyl-CoA dehydrogenase reaction steps[9]. As a result of these findings, together with those of genetic analysis, our patient was diagnosed with GA Ⅱ, neonatal-onset form without congenital anomalies.

The most important goal of newborn screening for inborn errors of metabolism is to reduce morbidity and mortality by early interventions such as dietary and pharmacologic treatments[1]. In recent years, expanded NBS using tandem mass spectrometry eased the detection of many inborn errors of metabolism in asymptomatic newborns. Screening for GAⅡ, which is estimated to have a prevalence of 1/200000, is also very important given the potential for early detection and intervention such as our case. The patient in the present study was diagnosed through the use of advanced technologies and the timely application of therapies enabled the patient to reach the second year of life without major health problems.

Our patient was diagnosed with thalassemia minor in the second year of life. In thalassemia minor (Tm), hemoglobin synthesis is decreased, but individuals are generally considered healthy. However, Tm is thought to be a potential risk for cardiovascular, neurological, metabolic, and vascular complications[10], and as a result of increased iron absorption, ineffective erythropoiesis, erythroid hyperplasia, and decreased antioxidant capacity, Tm affects health expectations[10]. Coexistence of GAⅡ and thalassemia minor in our patient may make her prone to infections and a decreased tolerance to complications may be expected.

Pharmacological therapy for GAⅡ is based on the administration of L-carnitine, Coenzyme Q10, and riboflavin (vitamin B2)[11]. Unfortunately, response is often poor to these treatments. Although ketone therapy is still at the experimental stage, sodium 3-hydroxybutyrate (NaHB) administration has emerged as a promising form of treatment, enabling the replacement of the deficient endogenous ketone body production needed not only for energy supply, but also for the synthesis of complex cell and tissue components such as myelin in the central nervous system[12]. Studies have shown that NaHB treatment results in the improvement of cardiomyopathy, hypotonia, and feeding tolerance in GAⅡ patients[13].

In our patient, in addition to routine therapy, ketone supplementation was started at eight months and the dose was increased gradually from 360 mg/kg/d to 1600 mg/kg/d, which significantly increased neuromotor development in a short time. Ketone body treatment is also thought to increase resistance to infection in GAⅡ patients[12-14]. Moreover a PEG tube insertion was another advantage for the patient because it allowed the family and caregivers to easily apply the planned diet and medications and prevent hypoglycemia attacks.

In conclusion, expanded NBS by tandem MS has made it possible to diagnose GAⅡ in this particular patient and enabled us to take measures and make early interventions for the patient who additionally had thalassemia minor. To our knowledge the present case report is the only one reporting a patient with both GAⅡ and thalassemia minor. Ketone body treatment should be considered to be part of routine therapy in GAⅡ patients with existing comorbidities, such as in our case. Finally, this patient's genetic analysis added two novel mutations of the ETF-A gene to the literature.

ARTICLE HIGHLIGHTS

Case characteristics

An episode of hypoglycemia and hypotonicity occurred on the postnatal first day, and the patient was admitted to neonatal intensive care unit.

Clinical diagnosis

Patient's general condition was good. No abnormality was identified during the physical examination.

Differential diagnosis

Metabolic diseases were investigated with tandem mass spectrometry.

Laboratory diagnosis

Tandem mass spectrometry, high pressure liquid chromatography, and mass spectrometry measurements in newborn screening enabled us to diagnose the patient very early.

Imaging diagnosis

Cranial Doppler ultrasonography (USG) and cranial magnetic resonance findings were normal, and there were no pathologic findings aside from minimal hepatomegaly in the liver.

Treatment

Patient was treated with carnitine, riboflavin, Coenzyme Q10, and ketone treatment in addition to a high carbohydrate diet.

Related reports

Glutaric academia type Ⅱ (GAⅡ) was first described in 1976 and is estimated to have a prevalence of 1/200000 live births. There are a few case reports describing the neonatal-onset form of the disease in the literature since the life-expectancy is short. There are also some cases reports describing adult-onset forms, which are mild with the patients living until adulthood.

Term explanation

Tandem mass spectrometer: High pressure liquid chromatography and mass spectrometry.

Experiences and lessons

Expanded new born screening by tandem mass spectrometry has made it possible to detect GAⅡ in this particular patient and enabled us to make early interventions for the patient. Moreover ketone treatment seems to be advantageous in GAⅡ patients, particularly in cases with comorbidities.

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