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증 례

아이소발레린산혈증 신생아에서 발견된 새로운 돌연변이

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Neonatal Onset Isovaleric Acidemia with Novel Mutation

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Isovaleric acidemia is autosomal-recessively inherited and an inborn error of metabolism caused by abnormal leucine metabolism due to the genetic defect of IVD (Isovaleryl-CoA dehydrogenase). IVD corresponds to mitochondrial matrix enzyme that acts on converting isovaleryl-CoA into 3-methylcrotonyl-CoA in the leucine catabolism. The IVD gene is located at Chromosome 15q14-q15, particularly between base pair 40,405,485 and base pair 40,435,948. It consists of 12 exons and has been reported to cause over 50 diseases so far. We conducted IVD gene test on the patient with acute isovaleric acidemia and confirmed a new type of mutation for the first time. As a result of analyzing the IVD gene sequence, we found out that c.129T>G(p.Asn43Lys) and c.1033A>G(p.Asn345Asp) mutations exist as heterozygosity at Exon 1 and Exon 10 respectively, novel mutation.

Key words: Isovaleric acidemia, Isovaleryl-CoA dehydrogenase, Organic acid analysis

Introduction

Isovaleric acidemia is a disease that isovaleric acid remarkably increases in the blood due to the abnormality of isovaleryl-CoA dehydrogenase in the process of leucine metabolism[1)]. It causes several different clinical symptoms, such as vomiting, anorexia, seizure, lethargy, deteriorated intelligence and hypothermia. The clinical aspects were divided into two types, acute type showing serious symptoms in neonatal period and chronic type showing intermittent symptoms. The IVD gene is located at Chromosome 15q14-q15, and over 50 mutations related to the IVD gene have been reported so far[2-4)]. Such IVD gene mutations cause to increase the concentration of isovaleric acid in the blood by causing abnormality in the leucine metabolism. We confirmed an patient who had isovaleric acidemia through tandem mass spectrometry and urine organic acid analysis and molecular analysis with novel mutation.

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Case Report

The patient showed an intermittent symptom of vomiting after birth. The patient was born in vaginal delivery at the 37th week, weighing 3.1 kg. The infant did not have any familial medical

history of inherited metabolic disorders, while not showing other specific symptom than intermittent vomiting and irritability. When the neonate was 48 hours old, we conducted tandem mass spectrometry and found out there was an increase in C5-acylcarnitine. In the test of complete blood count, the white blood cell count was 12,360/mm^3 (neutrophils: 58% and lymphocyte: 29.5%), the hemoglobin 14.5 g/dL, the platelet count 394,000/mm^3 all of which were normal. The chemistry test demonstrate AST 34 IU/L, ALT 5 IU/L, blood urea nitrogen 18.7 mg/dL, creatinine 0.9 mg/dL, total protein 5.4 g/dL, albumin 3.6 g/dL, uric acid 7.4 mg/dL, calcium 8.5 mg/dL, phosphorus 8.3 mg/dL and total bilirubin 7.0 mg/dL. It was found that the blood ammonia increased to 181 μmol/L (reference value: 21–90 μmol/L). In the analysis of blood gases, it was found that pH was 7.39, and pCO_2 26.1 mmHg, pO_2 83.0 mmHg, HCO_3 16.1 mmol/L and TCO_2 16.3 mmol/L. Besides, in the test of urine organic acid, it was found that isovalerylglycine greatly increased to 4,591 mmol/mol creatinine (reference value: <5 mmol/mol creatinine) as well as lactic acid to 280 mmol/mol creatinine (reference value: <150 mmol/mol creatinine). Based on the results above, we diagnose this case as isovaleric acidemia and conducted an IVD gene test. To find out a mutation from the infant's IVD gene, we extracted genomic DNA through peripheral blood leukocytes and analyzed the gene sequence of Exon 1 to 12 through direct sequencing technique. As a result of this analysis, we found out that c.129T>G(p.Asn43Lys) and c.1033A>G(p.Asn345Asp) mutations existed as heterozygosity at Exon 1 and Exon 10 respectively (Fig. 1), and both the mutations were new ones that had not been reported before[2-4].

After being diagnosed as isovaleric acidemia, the patient's intake of leucine was limited to 80 mg/kg/d, and we also administered IV 10% dextrose to provide calorie of 100 kca/kg. At the same time, we had the patient constantly take 100 mg/kg/d of carnitine and glycine each. 4 weeks after birth, we conducted blood test for ammonia and blood gas analysis again, and the blood ammonia was 45 μmol/L (reference value: 21–90 μmol/L), and pH was 7.35 mg/dL, and pCO_2 40.5 mmHg, pO_2 27.6 mmHg, HCO_3 22.2 mmol/L and TCO_2 22.8 mmol/L. In the test of urine organic acid, isovalerylglycine greatly decreased to 56 mmol/mol creatinine (reference value: <5 mmol/mol creatinine), and the lactic acid also decreased to

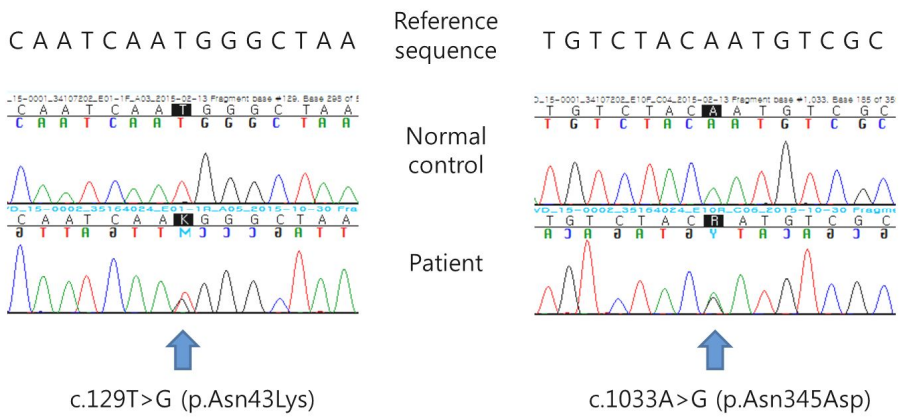

Fig. 1. Sequencing result of IVD gene show heterozygote mutations including c.129T>G (p.Asn43Lys) and c.1033A>G (p.Asn345Asp).

Table 1. The Sequential Result of Urine Organic Acid Analysis

Organic acids (mmol/mol creatinine)	At diagnosis	3 days after diagnosis	1 weeks after diagnosis	2 weeks after diagnosis	3 weeks after diagnosis	4 weeks after diagnosis
Isovalerylglycine	4,591	2,691	1,091	238	92	56
Lactic acid	280	258	56	85	68	40

40 mmol/mol creatinine (reference value: <150 mmol/mol creatinine) (Table 1). In addition, the patient' weight increasing speed changed for the better, and the amount of breast-feeding increased. Since then, we have observed the patient' health progress without any specific symptom.

Discussion

Isovaleric acidemia is a disease occurring when genetic defect of IVD take place, further failing to convert Isovaleryl-CoA into 3-methylcrotonyl-CoA in mitochondria and leading to abnormal leucine metabolism[1]. As a result, several kinds of metabolic materials including isovaleric acid remarkably increase, causing such clinical symptoms as vomiting, anorexia, seizure, deteriorated intelligence and hypothermia. It can be classified into two types, acute neonatal type and chronic intermittent type, depending on the clinical aspect. In the acute type, newborn don't show specific symptoms at birth, but within several days (3 to 6 days on average and 15 days to the maximum), they show serious acidosis and hypothermia with constant vomiting, then leading to anorexia, lethargy, seizure and even death. In the chronic intermittent type, infants show such characters as intermittent vomiting, anorexia, acidosis and lethargy after several months to years after birth. And these symptoms may become severe with catabolic state like fever caused by upper respiratory infection or dehydration caused by serious enteritis, and occur when patient take leucine protein excessively[7].

It may appear severe metabolic acidosis, lactic acidemia, hyperammonemia and ketonuria in the laboratory findings, some of which may also appear in hypocalcemia and hyperglycemia due to hormonal action. Besides, when either neutropenia or thrombocytopenia becomes serious, it may lead to pancytopenia, such a hamatological disorder occurs when the maturity of progenitors in the bone marrow is suppressed[5]. The diagnosis is made by confirming isovaleric acid and other metabolites, such as isovalerylglycine, 3-hydroxyisovaleric acid, 4-hydroxyisovaleric acid, methylsuccinic acid, methylfumaric acid, isovalerylglucuronide, isovalerylglutamic acid, isovalerylalanine and isovalerylsarcosine through blood and urinary laboratory finding. This disorder also can be diagnosed by measuring enzymes in the culture of fibroblasts, and its perinatal diagnosis can be possibly made by measuring isovalerylglycine from the amniotic fluid[6].

In the acute type, it is important to reduce the catabolic effect of protein by providing calories through glucose for treatment. At the same time, it is needed to restrict patients' intake of leucine-included protein, and correct dehydration and metabolic acidosis. As medication to administrate, there are two kinds, glycine (250 mg/kg/d) and carnitine (100 mg/kg/d). Glycine converts isovaleric acid into isovalerylglycine through glycine N-acylase, and carnitine converts isovaleric acid into isovalerylcarnitine through carnitine acetyltransferase. Two converted substances are easily

discharged in the urine[7].

Out of all the isovaleryl-CoA dehydrogenase genes, over 50 mutations likely to cause diseases have been reported so far. The IVD gene is located at Chromosome 15q14-q15, particularly between base pair 40,405,485 and base pair 40,435,948[3]. Thus, the IVD gene mutation acts as a cause for isovaleric acidemia by causing abnormality in the leucine metabolism. In this case, we analyzed the patients' IVD gene sequence and found out that c.129T>G(p.Asn43Lys) and c.1033 A>G(p.Asn345Asp) mutations exist as heterozygosity at Exon 1 and Exon 10 respectively, and these two kinds of mutations are new ones that have never been reported before[2-4].

In this case, the patient constantly showed vomiting after birth, and during the process of evaluation, we found an increase in C5-acylcarnitine through tandem mass spectrometry[8]. Through the test of urine organic acid, we analyzed the gene sequence, which made it possible to make a genetic diagnosis. As a result, the patient was diagnosed as acute-type isovaleric acidemia, accompanied with symptoms even from the neonatal period. In the long term, it is important that training to take protein dietary restrictions and the right amount of leucine together according to age and weight. When an acute illness accompanied, careful follow-up is required that patient have a metabolic disorder occur, such as metabolic acidosis or hyperammonemia. If patient use an overdose of glycine and carnitine for a long term, it may reduce the excretion of metabolic product of leucine[9]. So it is important to maintain appropriate capacity to periodic inspection by serum and urine organic acid test. If the patient is taking the carnitine continuously, it may cause a side effect of increasing the red blood cell destruction by activating a calpain (intracellular nonlysosomal calcium activiated cysteine proteinase). In this regard, long-term studies are needed to establish guidelines of isovaleric acidemia for chronic treatment[10].

Summary

Isovaleric acidemia is inherited metabolic disease caused when a certain problem takes place in the leucine metabolism due to the genetic defect of IVD. We found an patient who showed a constant symptom of vomiting after birth. And during the process of evaluation, we confirmed an increase in C5-acylcarnitine through tandem mass spectrometry[8]. Additionally through the test of urine organic acid, we found an increase in both isovalerylglycine and lactic acid, so we confirmed isovaleric acidemia. Afterwards, in the IVD gene test, we found out that c.129T>G(p.Asn43Lys) and c.1033A>G(p.Asn345Asp) mutations exist as heterozygosity at Exon 1 and Exon 10 respectively, novel mutation[2-4].

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