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증 례

유전자패널 시퀀싱으로 진단된 성인형 very-long-chain acyl-coenzyme A dehydrogenase (VLCAD) 결핍증 증례

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A Case of Late-onset Episodic Myopathic Form with Intermittent Rhabdomyolysis of Very-long-chain acyl-coenzyme A Dehydrogenase (VLCAD) Deficiency Diagnosed by Multigene Panel Sequencing

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Very-long-chain acyl-CoA dehydrogenase (VLCAD) deficiency (OMIM#201475) is an autosomal recessively inherited metabolic disorder of mitochondrial long-chain fatty acid oxidation. The clinical features of VLCAD deficiency is classified by three clinical forms according to the severity. Here, we report a case of later-onset episodic myopathic form of VLCAD deficiency whose diagnosis was confirmed by plasma acylcarnitine analysis and" multigene panel multigene panel sequencing. A 34-year old female patient visited genetics clinic for genetic evaluation for history of recurrent myopathy with intermittent rhabdomyolysis. She suffered first episode of rhabdomyolysis with acute renal failure requiring hemodialysis at twelve years old. After then, she suffered several times of recurrent rhabdomyolysis provoked by prolonged exercise or fasting. Physical and neurologic exam was normal. Serum AST/ALT and creatinine kinase (CK) levels were mildly elevated. However, according to her previous medical records, her AST/ALT, CK were highly elevated when she had rhabdomyolysis. In suspicion of fatty acid oxidation disorder, multigene panel sequencing and plasma acylcarnitine analysis were performed in non-fasting, asymptomatic condition for the differential diagnosis. Plasma acylcarnitine analysis revealed elevated levels of C14:1 (1.453 μmol/L; reference, 0.044-0.285), and C14:2 (0.323 μmol/L; 0.032-0.301) and upper normal level of C14 (0.841 μmol/L; 0.065 -0.920). Two heterozygous mutation in *ACADVL* were detected by multigene panel sequencing and confirmed by Sanger sequencing: c.[1202G>A(;) 1349G>A] (p.[(Ser 401Asn)(;)(Arg450His)]). Diagnosis of VLCAD deficiency was confirmed and frequent meal with low-fat diet was educated for preventing acute metabolic derangement. Fatty acid oxidation disorders have diagnostic challenges due to their intermittent clinical and laboratorial presentations, especially in milder late-onset forms. We suggest that multigene panel sequencing could be a useful diagnostic tool for the genetically and clinically heterogeneous fatty acid oxidation disorders.

Key words: Very-long-chain acyl-CoA dehydrogenase deficiency, Multigene panel sequencing, *ACADVL*

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Introduction

Very-long-chain acyl-CoA dehydrogenase (VLCAD) deficiency (OMIM#201475) is an autosomal recessively inherited metabolic disorder of mitochondrial long-chain fatty acid oxidation. VLCAD is an enzyme that catalyzes the dehydrogenation of long-chain fatty acids in the first step of mitochondrial fatty acid oxidation encoded by *ACADVL* located on chromosome 17p13.1[1]. The clinical features of VLCAD deficiency is classified by three clinical forms: the severe early-onset cardiac and multiorgan failure form presented in infant with cardiomyopathy, pericardial effusion, hypotonia, hepatomegaly, and intermittent hypoglycemia. The hepatic or hypoketotic hypoglycemic form typically presents during early childhood with hypoketotic hypoglycemia and hepatomegaly, but without cardiomyopathy. The late-onset episodic myopathic form presents with exercise intolerance, muscle cramps, intermittent rhabdomyolysis provoked by exercise. Hypoglycemia is not typically present in the hepatic or hypoketotic hypoglycemic form and the later-onset episodic myopathic form[2,3].

In Korea, since the introduction of expanded newborn screening test using tandem mass spectrometry, the majority of patients with VLCAD deficiency can be diagnosed and treated early. There are several reports demonstrating Korean patients with early-onset or asymptomatic VLCAD deficiency found in newborn screening[4-6]. However, as far as we were able to determine, the Korean patient with late-onset VLCAD deficiency has not been reported. Here, we report the late-onset VLCAD deficiency patient diagnosed by plasma acylcarnitine analysis and multigene panel sequencing for fatty acid oxidation disorders.

Case Report

A 34-year-old female patient was referred for evaluation of recurrent myopathy accompanied by intermittent episode of rhabdomyolysis since childhood. She was 3rd child of healthy, nonconsanguineous Korean parents. She had two older sister and one younger brother. One of her older sister had similar symptom. She suffered first episode of rhabdomyolysis after vigorous exercise at twelve years old. Rhabdomyolysis complicated with acute renal failure requiring dialysis and admitted intensive care unit. After then, she suffered several times of recurrent rhabdomyolysis provoked by prolonged exercise or fasting. Although she was asymptomatic between acute episodes, she had exercise intolerance, muscle cramps and intermittent myoglobinuria triggered by exercise or fasting. She also complained the weight control because of her difficulties of fasting and exercise intolerance.

Her height was 175 cm, weight was 80 kg and body mass index (BMI) was 26.1 kg/m^2. Hepatosplenomegaly was not noted. Neurologic exam was normal. Her hemoglobin, white blood cell counts, and platelet counts were 11.5 g/dL (normal range, 11.0-14.0 g/dL), 5.1×10^3/μL (4-13×10^3/μL), and 259×10^3/μL (134-387×10^3/μL). Serum glucose was 98 mg/dL. Serum aspartate aminotransferase (AST) was 53 IU/L (5-40 IU/L) and alanine aminotransferase (ALT) was 51 IU/L (8-41 IU/L). Serum BUN was 10.6 mg/dL (6-20 mg/dL) and creatinine was 0.62 mg/dL (0.5-0.9 mg/dL). Serum creatine kinase (CK) was 263 IU/L (26-192 IU/L). No hematuria or proteinuria was found in random urine analysis. However, according to her previous medical records, her AST/ALT were elevated to 465/406 IU/L and CK was elevated up to 21254 IU/L when she had rhabdomyolysis.

In suspicion of fatty acid oxidation disorder, multigene panel sequencing and plasma acylcarnitine analysis were performed simultaneously in non-fasting asymptomatic condition for the differential diagnosis. The plasma acylcarnitine profile was analyzed by tandem mass spectrometry. Multigene panel for fatty acid oxidation disorder included *ACADVL, ACADM, ACADS, HADHA, HADH, HADHB, ACAD9, SLC22A5, CPT1A, SLC25A20*, and *CPT2*. Briefly, genomic DNA was extracted from the peripheral blood. Library was prepared using the xGen Inherited Disease Panel (IIntegrated DNA Technologies, Inc., Coralville, Iowa). Massively parallel sequencing was performed on the Illumina NextSeq platform. Average coverage of depth of the entire panel was 168x, and 99% of targeted bases were covered by 10x sequence reads. Sequence reads were aligned to hg19 with Burrow-Wheeler Aligner (version 0.7.12, MEM algorithm). Duplicate reads were removed by using Picard-tools1.96. Local realignment and base quality recalibration was done by The Genome Analysis Toolkit (GATK version 3.5-46). Variant calling was performed by GATK Haplotype Caller. Variants were annotated by Variant Effect Predictor (88) and dbNSFP (3.3).

Plasma acylcarnitine analysis revealed elevated levels of C14:1 (1.453 μmol/L; reference, 0.044-0.285), and C14:2 (0.323 μmol/L; reference, 0.032-0.301) and upper normal level of C14 (0.841 μmol/L; reference, 0.065-0.920) (Table 1). Two heterozygous variants in *ACADVL* were detected by multigene panel sequencing and confirmed by Sanger sequencing: c.[1202G>A(;)1349G>A] (p.[(Ser401Asn)(;)(Arg450His)]) (Fig. 1).

Table 1. Results of Plasam Acylcarnitine Analysis

Plasma acylcarnitine	Result (μmol/L)	Reference (μmol/L)
Tetradecadienoylcarnitine (C14:2)	0.323	0.032-0.301
Tetradecenoylcarnitine (C14:1)	1.453	0.044-0.285
Myristoylcarnitine (C14)	0.841	0.065-0.920
Dodecenoylcarnitine (C12:1)	0.109	0.063-0.420

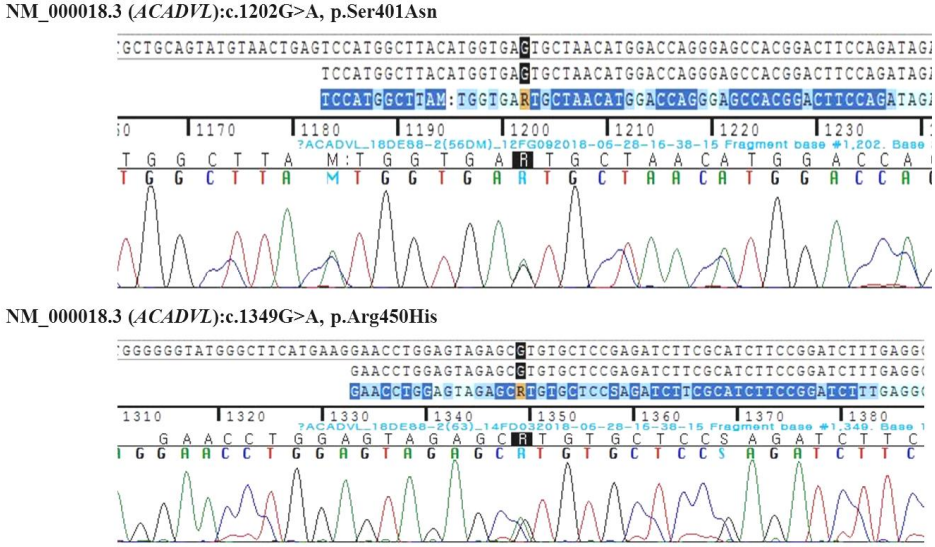

Fig. 1. *ACADVL* variants detected by multigene panel and Sanger sequencing. The multigene panel sequencing and confirmatory Sanger sequencing identified two heterozygous mutation in *ACADVL*: c.[1202G>A(;)1349G>A] (p.[(Ser401 Asn)(;)(Arg450His)]).

c.1349G>A was a pathogenic variant recurrently reported in patients with late-onset VLCAD deficiency[2,7,8]. c.1202G>A was a novel variant which was not identified in general population and Korean Reference Genome Database (KRGDB). The variant was consistently predicted as damaging protein structure in various *in silico* analysis (SIFT, PolyPhen-2, and MutationTaster). Based on the results of plasma acylcarnitine analysis and genetic analysis, she was diagnosed VLCAD deficiency. Echocardiogram and EKG for surveillance showed normal cardiac structure and function. Frequent meal with low-fat diet was educated for preventing acute metabolic derangement. The genetic counselling for the family was provided, especially for her older sister who has the similar clinical symptoms.

Discussion

Newborn screening using tandem mass spectrometry enables early diagnosis of asymptomatic patients. However, late-onset adult forms of VLCAD deficiency remain rare and challenging for diagnosis. Here, we reported a Korean adult case with VLCAD deficiency of intermittent rhabdomyolysis whose molecular diagnosis were confirmed by multigene panel sequencing.

The late-onset VLCAD deficiency remains diagnostic challenge resulting from the non-specific and episodic symptoms of muscle pain, myoglobinuria, and intermittent rhabdomyoloysis. The patients are usually asymptomatic in between the episodes. Furthermore, the clinicians are hard to suspect the inborn errors of metabolism for the adult patients. The clinical differential diagnosis of various types of fatty acid oxidation disorders could be difficult especially in the cases with late-onset mild presentation. Multigene panel sequencing of the candidate genes is a useful diagnostic tool and enables to shorten the diagnostic odyssey of the diseases in rare inherited metabolic diseases. In this report, although fatty acid oxidation disorder was clinically suspected, the clinical presentation of the patient was uncertain to differentiate the types. Therefore, multigene panel sequencing provided a useful diagnostic option in this situation.

Plasma acylcarnitine analysis is the simple and useful diagnostic test for VLCAD deficiency[9]. It should be noted that the urinary organic acid profile could not diagnostic for the mild form of VLCAD deficiency, even when the patients have acute metabolic derangement with rhabdomyolysis [7]. There are many reports demonstrate that hypoketotic dicarboxylic aciduria was not found even in situation of highly elevated serum CK[7,8,10]. The dicarboxylic aciduria was only detected after more than 36-hour fast[7]. On the other hand, C14:1 carnitine was abnormal under even asymptomatic conditions in patients with adult form, and consistent in our patient, too. Therefore, acylcarnitine analysis should be considered for patients with mild clinical presentation[7,11]. Because the genetic analysis is not influenced by the patients' condition, it can evidently support the confirmatory diagnosis. Therefore, we performed the plasma acylcarnitine analysis and multigene panel test simultaneously.

Andresen et al.[2] suggested a clear correlation of genotype and phenotype of VLCAD deficiency. The authors reported that most patients with the null mutations presented as severe infantile or childhood form whereas the patients with missense mutations had retained residual enzyme activity and resulted mild late-onset clinical presentation. Our patient also showed the genotype-phenotype correlation, she had two missense mu-

tations (p.[(Ser401Asn)(;)(Arg450His)]) and presented with late-onset episodic myopathic form.

In terms of management, emergent high intravenous glucose as an energy source should be provided in acute episodes. During rhabdomyolysis, alkalization and hydration should be done or dialysis can be performed in case of acute renal insufficiency. Current long-term managements for VLCAD deficiency are based on low long-chain fatty acid diet and supplementation with medium-chain triglycerides and carnitine. Education for the avoidance of precipitating factors including fasting, dehydration, and prolonged exercise are advisable[11-13]. The use of carnitine supplementation is controversial[13]. The clinical trials using triheptanoin, the triglyceride composed of three seven-carbon fatty acid (C7:0), are in progress[3]. A phase II open-label trail of the effect of triheptanoin on exercise tolerance showed some potential benefits[14]. The major adverse event was diarrhea[15].

In conclusion, late-onset myopathic form VLCAD deficiency is a rare condition. The detailed observation of the clinical features, the awareness of a phenotype is necessary for the prompt diagnosis. The plasma acylcarnitine analysis and multigene panel sequencing could be a useful diagnostic tool. A precise molecular diagnosis can provide appropriate therapy and genetic counseling for the family.

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요 약

Very-long-chain acyl-CoA dehydrogenase (VLCAD) 결핍증은 상염색체 열성으로 유전되는 유전성대사질환으로 미토콘드리아에서 장쇄지방산의 산화장애이다. VLCAD 결핍증의 임상증상은 중증도 및 발현 시기에 따라 심각한 심장 이상을 동반하는 신생아기 발현형, 소아기 발현형, 지발형의 세 가지로 분류할 수 있다. 저자들은 혈장 아실카르니틴 분석과 유전자패널 염기서열분석 방법으로 확진된 성인기 발현형 VLCAD 결핍증 1례를 경험하였기에 보고하고자 한다. 34세 여자가 반복되는 근육통증과 간헐적 횡문근융해증의 병력을 주소로 내원하였다. 환자는 12세에 처음으로 운동 후 횡문근융해증으로 급성 신부전이 발생하여 혈액투석을 받고 회복하였다. 이후 환자는 장시간의 운동이나 금식 후에 반복적으로 근육통증과 횡문근융해증이 발생하였다. 내원 시 신체 검진과 신경학적 검진은 정상이었다. 내원시 혈장 AST/ALT, Creatinine kinase (CK)는 약간 상승해있었으나, 이전 의무기록에 의하면 횡문근융해증이 있을 당시 AST/ALT, CK는 매우 상승하였다. 환자의 병력을 토대로 지방산대사장애 의심하에 감별진단을 위하여, 유전자 패널 염기서열 분석과 혈장 아실카르니틴 분석을 시행하였다. 혈장 아실카르니틴 분석결과 C14:1 (1.453 μmol/L; 참고치, 0.044-0.285)와 C14:2 (0.323 μmol/L; 0.032-0.301)가 증가였고, C14 (0.841 μmol/L; 0.065-0.920)는 높은 정상이었다. 유전자패널 염기서열분석에서는 ACADVL 유전자에서 두 개의 병원성변이가 이형접합으로 발견되었으며, 이는 Sanger 염기서열 분석으로 확진되었다: c.[1202G>A(;)1349G>A] (p.[(Ser401Asn)(;)(Arg450His)]). 환자는 생화학적, 유전학적 검사결과를 바탕으로 지발형 VLCAD 결핍증으로 확진되어 저지방식이와 급성 대사장애를 예방하기 위한 영양 교육을 받았다. 경증의 지발형 지방산 대사장애는 임상증상과 생화학적 검사 이상이 간헐적으로 발생하기 때문에 진단이 어렵다. 유전자패널 염기서열 분석은 지방산대사 장애와 같이 임상증상과 원인 유전자 이상이 다양한 대사이상질환에서 유용한 진단법이 될 수 있다.

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