学 位 論 文 の 要 旨

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学 位 論 文 名	Clinical, Biochemical and Molecular Investigation of Adult-onset Glutaric Acidemia Type II: Characteristics in Comparison With Pediatric Cases
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論 文 内 容 の 要 旨

INTRODUCTION

Glutaric acidemia type II (GA2) is one of fatty acid oxidation disorders (FAODs), and an autosomal recessive disease caused by a defect in electron transfer flavoprotein (ETF) or ETF dehydrogenase (ETFDH), resulting in deficiencies in multiple acyl-CoA dehydrogenases, such as short-, medium-, and long-chain acyl CoA dehydrogenases, and so on. GA2 has been clinically classified into 2 types of the neonatal-onset and late-onset. Patients with the neonatal-onset type of GA2 develop severe respiratory failure, cardiomyopathy, and hypoglycemia soon after birth, and they often are fatal in early infancy. In the late-onset type, intermittent attacks of lethargy and hypoglycemia, or, occasionally, acute encephalopathy or sudden death triggered by infection or long fasting are seen starting in early childhood.

Recently, several adult-onset GA2 cases have been also reported. In our study, the clinical, biochemical, and pathological characteristics of 2 cases of adult-onset GA2 were investigated and compared with those of pediatric cases.

MATERIALS AND METHODS

Case 1 was a 58-year-old male with episodic myalgia and muscle weakness. His younger brother died unexpectedly from an unknown cause in his 30s. The patient became symptomatic in his 40s. He began to use a wheelchair because of persistent muscular symptoms, and had 3 episodes of unconsciousness in his 50s. Then, as he repeatedly developed liver dysfunction and rhabdomyolysis, he was hospitalized at age 58 for detailed examination. On admission, no abnormality was found, except for liver dysfunction and elevation of creatine kinase (CK).

Case 2 was a 31-year-old male with episodic muscle weakness and myalgia similar to case 1. No abnormalities in his past and family history were noted. He was formerly a baseball player on a non-professional team, but he developed muscle weakness at 29 years of age. Then, his muscular symptoms worsened gradually. He was hospitalized to undergo further examination at 31 years of age. Blood examination just indicated a slight elevation of liver enzymes and CK.

Both cases received several close examinations, such as urinary organic acid analysis, blood acylcarnitine (AC) analysis, muscle biopsies, immunoblotting of ETFA, ETFB, and ETFDH, gene analysis of *ETFDH*, and in vitro probe acylcarnitine (IVP) assay which can evaluate the β-oxidation capacity. Briefly, confluent fibroblasts derived from patients were cultured onto 6-well microplates with fresh medium until confluence. Thereafter, cells were washed twice with D-PBS and cultured at 37°C in 1 mL of experimental MEM containing 0.4% essential fatty acid-free BSA, 0.4 mmol/L L-carnitine, and 1% penicillin/streptomycin with 0.2 mmol/L unlabeled palmitic acid. The concentration of ACs in 10 μL of the culture medium after incubation for 96 hours was determined by MS/MS.

The study protocol was approved by the Ethics Committee of Shimane University and written informed consent was obtained from all subjects.

RESULTS AND DISCUSSION

In both cases, ETFDH protein was not detected in immunoblotting. Mutation analysis of *ETFDH* revealed that case 1 was a homozygote of c.1367C>T (p.P456L), and case 2 was a compound heterozygote of c.890G>T (p.W297L) and c.950C>G (p.P317R). However, it is not easy to establish the correct diagnosis.

Urinary organic acid analysis showed no obvious abnormalities in both cases. Moreover, in AC analysis of dried blood spots (DBS), there were no abnormalities and slight elevation from C4 to C18 in cases1 and 2, respectively. These results suggested that a biochemical diagnosis of adult-onset GA2 is challenging compared

with those of pediatric cases.

IVP assay also revealed no obvious abnormalities in both cases, although the elevation of short- to long-chain ACs is mostly observed in pediatric cases of GA2. Biochemical abnormality of case 1 was milder than those of case 2, while clinical features of Case 1 were not milder. Poor correlation between the clinical severity and biochemical abnormality was suggested.

By contrast, in the serum AC analysis, obvious elevation of medium- to long-chain AC was observed in both cases. Serum AC analysis appeared to be more informative than DBS for diagnosing adult-onset GA2.

Muscle tissues stained with Oil-Red O revealed fat deposition, which provided an initial clue for the diagnosis of GA2 in both cases. If fatty degeneration is revealed by muscle biopsy in patients with myopathy of unknown cause, the possibility of FAODs should be considered, even in the absence of biochemical abnormalities.

The clinical course of the "late-onset type" differs substantially among individuals; some cases have encephalopathy or sudden death during the childhood, while others may only have muscular symptoms in adulthood. Therefore, we propose to distinguish the late-onset type of GA2 between the intermediate and myopathic forms according to the results of the IVP assay as well as age at onset, fatality, and clinical characteristics. The intermediate (juvenile-onset) form, with elevation of short- to long-chain ACs in the IVP assay, exhibits intermittent attacks, including hypotonia, hypoglycemia, hyperammonemia, and acute encephalopathy-like attack, with typical biochemical abnormalities and relatively high mortality following metabolic stress in infancy or young childhood. The myopathic (adult-onset) form, in which typical findings in the IVP assay are not detected, primarily presents intermittent muscular symptoms after adolescence or adulthood with normal intelligence, and offers a favorable life prognosis in many cases.

CONCLUSION

We reported several characteristics in clinical and biochemical finding in adult-onset GA2, comparing with those in pediatrics cases. Muscle biopsy and serum AC analysis can be clues to make a diagnosis adult-onset GA2. We propose the more detailed classification of GA2, such as neonatal-onset, juvenile-onset, and adult-onset forms, based on clinical and biochemical features, and the profile of IVP assay. This classification can also be used for preclinical risk control of GA2 detected in neonatal mass screening. Moreover, it is considered that making diagnosis using IVP assay is useful because clinical form cannot be predicted only by the genotype.

論文審査及び最終試験又は学力の確認の結果の要旨

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論文審査の結果の要旨

脂肪酸代謝異常症の一つであるグルタル酸血症Ⅱ型（GA2）は、電子伝達フラビンタンパク質（ETF）または ETF 脱水素酵素（ETFDH）の欠損により起こる。従来新生児期に発症し致死的な経過をとる新生児型と、低血糖や肝機能障害などの間欠発作あるいは急性脳症様の症状で発症する遅発型に大別されているが、成人発症の報告例は少なく不明な点が多い。申請者が検討した二例の成人 GA2 において、小児 GA2 の有用な診断法である尿中有機酸分析、ろ紙血中アシルカルニチン分析、*in vitro* aclycarnitine （IVP）assay では異常の検出が困難であったが、筋への脂肪沈着に加え、血清アシルカルニチンの増加と繊維芽細胞における ETFDH タンパク質の消失が認められ、同遺伝子にアミノ酸置換をもたらす点突然変異が検出された。本論文はこれらの知見にもとづき GA2 の新たな分類を提唱するとともに、成人発症のミオパチーであっても同疾患を始めとする脂肪酸代謝における先天異常をも考慮する必要があることを指摘した意義のある論文である。

最終試験又は学力の確認の結果の要旨

申請者は、成人発症型 GA2 をその若年発症型と比較して異同を示し、より詳細な疾患分類を提案するとともに原因不明の成人発症筋疾患への関与についての注意喚起を行った。関連分野の知識も十分であり学位授与に値すると認める。（主査：土屋美加子）

申請者は筋症状を初発とする成人発症型 GA2 の臨床症候と生化学的所見の特性を明らかにするとともに乳児期～小児期発症型と比較検討することにより、より細分化した疾患分類を提起した。関連領域の知識も豊富であり、学位授与に値すると判断した。（副査：杉本利嗣）

申請者は、成人発症型 GA2 の臨床的・生化学的特徴を質量分析、筋生検、イムノブロッティング、遺伝子解析、IVP assay を用いて明らかにした。本疾患の分類に新たな示唆を与え、学位授与に値するものと考える。（副査：内尾祐司）

（備考）要旨は、それぞれ４００字程度とする。