学 位 論 文 の 要 旨

氏名　四 本 由 郁

学 位 論 文 名	**Clinical and Molecular Investigations of Japanese Cases of Glutaric Acidemia Type 2**
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著 者 名	**Yuka Yotsumoto, Yuki Hasegawa, Seiji Fukuda, Hironori Kobayashi, Mitsuru Endo, Toshiyuki Fukao, Seiji Yamaguchi**

INTRODUCTION

Glutaric acidemia type 2 (GA2) is an inherited autosomal recessive disorder of fatty and organic acid metabolism caused by a defect of electron transfer flavoprotein (ETF), consisting of alpha and beta subunits (ETFα and ETFβ, respectively) or ETF dehydrogenase (ETFDH). GA2 is roughly divided into 2 clinical forms: a neonatal onset form (severe form) and a late onset form (milder form). In Japanese cases of fatty acid disorders, GA2 is relatively common, following VLCAD deficiency and CPT2 deficiency in terms of frequency. In the acute phase of the disease, hypoketotic dicarboxylic aciduria are noted on urine organic acid analysis using GC/MS. However, they may not be detected in the stable phase of many cases. In this study, we investigated the relationship between clinical and molecular aspects of Japanese patients with GA2, in which typical profile of urinary organic acids were observed at least in the acute stage, and found 15 mutations in 11 Japanese patients, referring 4 previously reported cases.

MATERIALS AND METHODS

GA2 was newly diagnosed in 11 Japanese children, based on the characteristic symptoms and metabolic profiles of urinary organic acids analyzed using gas chromatography/mass spectrometry (GC/MS). Additionally, 4 Japanese patients previously reported were compared. Skin fibroblasts obtained from the patients, Immunoblot analysis was carried out according to

the Bio-Rad protein assay protocol (Bio-Rad Laboratories, Hercules, CA, USA).

The genomic DNAs were isolated from fibroblasts using a Qiamp DNA Microkit (QIAGEN GmbH, Hilden, Germany). Control genomic DNA from 50 unaffected Japanese individuals was obtained from peripheral blood lymphocytes using Blood and Cell Culture DNA Midi Kits (QIAGEN GmbH, Hilden, Germany). Each exon of genes for ETFα (*ETFA*), ETFβ (*ETFB*), and ETFDH (*ETFDH*) including intron/exon boundaries was PCR-amplified for 30 cycles. The PCR products were purified by a QIAquick PCR Purification Kit (QIAGEN GmbH, Hilden, Germany) and sequenced using ABI PRISM 310 Genetic Analyzer (PE Applied Biosystems, Foster City, CA, USA) or CEQ 8000 Genetic Analysis System (Beckman Coulter Inc., Fullerton, CA, USA).

RESULTS AND DISCUSSION

In this study, 3 patients with the neonatal onset form and 8 cases with the late onset form were identified in Japanese. Biochemical and genetic analyses, including 4 previously reported cases, revealed that there were 4 cases each with ETFα deficiency and ETFβ deficiency, and 7 with ETFDH deficiency. A total 15 genetic alterations; 14 novel and 1 reported mutations were identified in *ETFA*, *ETFB* and *ETFDH* gene. The number of subjects limited, the frequency of ETFα, ETFβ, and ETFDH deficiency in Japanese patients is 27%, 27%, and 47%, respectively, in our studies. The previous study have shown similar results, although patients from various ethnic groups were analyzed (11%, 33%, and 56%, respectively). All neonatal forms were either ETFα or ETFβ deficiency, but cases 6 and 15 with ETFα deficiency showed the late onset form. No cases of ETFDH deficiency showed the neonatal onset form in our study. Previous reports, however, identified patients with the neonatal onset form with ETFDH deficiency.

In addition to the current study, our previous studies revealed 4 novel mutations in the *ETFA* gene and 2 mutations in the *ETFB* gene in 4 Japanese patients. In the 15 cases studied, 2 cases each with ETFα deficiency and ETFDH deficiency had homozygous mutations, while the rest of them were compound heterozygotes. There were no common mutations except for L366F in the ETFDH gene that was shared by 2 unrelated patients. Other mutations were heterogenous in each case, except for the sibling cases. In addition, mutations identified in our study were different from those reported in other countries except for P456L. These findings suggest that Japanese patients with GA2 show allelic heterogeneity without frequent mutations. Similarly, multiple mutations were identified in *ETFA*, *ETFB*, and *ETFDH* genes in non-Japanese populations (19, 7, and 29 mutations, respectively), though T266M in the *ETFA* gene was

reported as the most frequent mutation associated with a severe phenotype. All mutations described in this report are novel, except for P456L. Furthermore, none of the mutations were found in 100 alleles from normal Japanese controls. These findings strongly suggest that they are specifically associated with the disease and are the most likely disease-producing mutations. These results suggest that some mutations may be associated with a specific phenotype, although it is necessary to accumulate additional cases with the same genotype to identify a definitive relationship.

These findings suggest that the severity of the disease is unlikely associated with particular enzyme deficiency. Even if the late onset form appears to be predominant in GA2, the neonatal form may be underestimated, since children with the neonatal onset form die early in the neonatal period before the diagnosis of GA2 is made. It is not feasible to make a definitive diagnosis for GA2 by GC/MS or ESI-MS/MS, although the technique is useful to detect the disease. While immunoblot analysis is useful to identify defective proteins, it does not rule out the functional deficiency of enzymes. Since separate measurement of the activity of ETF and ETFDH may be impossible on a routine basis due to technical difficulties, the easiest and most reliable means to determine specific enzyme deficiency is a genetic diagnosis. Furthermore, it may be of use for a prenatal diagnosis.

CONCLUSION

Our results demonstrate that the mutational spectrum is heterogeneous in Japanese patients with GA2, and their phenotypes were not associated with a specific enzyme deficiency. Although no common mutation was found, some mutations appeared to be associated with a specific phenotype. Immunoblot analysis can clearly separate which the GA2 patients has a defect in ETF (ETFα or ETFβ) or ETFDH. A genetic diagnosis may help to predict the potential outcome of patients and provide more accurate diagnostic information for patients and families with GA2. Since one of the three genes is affected in GA2, it is essential to accumulate information on genetic mutations to determine any genotype/phenotype correlation and to identify defective enzymes for an accurate diagnosis/prenatal diagnosis of GA2.