学 位 論 文 の 要 旨

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学 位 論 文 名　**Clinical and Molecular Investigation of 19 Japanese Cases of Glutaric Acidemia Type 1**

発 表 雑 誌 名　**Molecular Genetics and Metabolism (in press)**
（巻，初頁～終頁，年）

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論 文 内 容 の 要 旨

INTRODUCTION

Glutaric aciduria type 1 (GA1) is an autosomal recessive disorder of organic acid metabolism caused by deficiency of glutaryl-CoA dehydrogenase (GCDH), which is involved in the catabolic pathway of lysine, hydroxylysine, and tryptophan. Untreated patients mostly develop severe striatal degeneration. More than 200 mutations have been reported in the *GCDH* gene, and common R402W and IVS10-2A>C were found in Caucasian and Chinese/Taiwanese, respectively. In Japan, however, it is not known whether there are common mutations and a phenotype/genotype correlation in Japanese GA1 cases. We investigated the clinical and molecular aspects of 19 Japanese patients with GA1.

MATERIALS AND METHODS

We studied 19 Japanese patients who were diagnosed with GA1 based on the urinary organic acid profiles and/or blood acylcarnitine analysis. The diagnoses were confirmed by analysis of *GCDH* gene and/or GCDH activity. The mutations of 6 cases were reported previously. In this study, we analyzed the mutations on GCDH gene in 13 cases. No family demonstrated consanguineous marriage.

Genomic DNA was isolated from skin fibroblasts using a Qiamp DNA Microkit (QIAGEN GmbH, Hilden, Germany) and from peripheral blood lymphocytes using the DNA Quick Ⅱ kit (Dainippon Pharmaceuticals, Osaka, Japan). Each exon of *GCDH* including the intron/exon boundaries was PCR-amplified for 30 cycles. The PCR products were purified using a QIAquick PCR Purification Kit (QIAGEN GmbH, Hilden, Germany) and sequenced using the ABI PRISM 310 Genetic Analyzer (PE Applied Biosystems, Foster City, CA, USA) or the CEQ 8000 Genetic Analysis System (Beckman Coulter Inc., Fullerton, CA, USA).

RESULTS AND DISCUSSION

Clinical characteristics

Fifteen of the 19 cases were detected after symptomatic onset. Eleven patients were severely impaired (three patients died), three had mild impairment, and five showed normal development. Four of 5 patients that developed normally were detected in the presymptomatic stage by neonatal or sibling screening. All symptomatic cases except for one case had mild impairment or severe handicap, indicating that the neurological prognosis of symptomatic cases is poor in Japanese GA1 patients, as reported in previous cases. All cases showed high urinary glutaric acid excretion. Frequency (31.6 %: 6/19 cases) of macrocephaly of this study is lower than that of other reports (65-75 %). This may represent unique phenotype in Japanese patients with GA1, which have genetic backgrounds distinct from the other countries. One of a pair of sisters with the same mutations (M339V/S305L) lacking residual activity was severely retarded, whereas the older girl remains asymptomatic at 22 years of age, indicating that genotype does not necessarily predict GA1 phenotype. Importantly, children with mild impairment were diagnosed and treated earlier than severely impaired cases {4.7 ± 2.5 months (range: 2 to 8 months) vs 11.6± 12.7 months (range: 4 to 51 months)}, suggesting that a better outcome was induced by early diagnosis. The reason for the better outcome seen in the patients who were diagnosed younger age was considered that early diagnosis led to an earlier initiation of

treatment and/or intervention in a timely manner for any medical conditions, which in turn prevented patients from neurological impairment.

Gene mutations in *GCDH*

Nineteen mutations in 26 alleles examined in this study were identified, and eight of them (89 or 90delC, Y155C, IVS4+2T>C, G244S, Q352X, G354A, K361E, and 1144-1145delGC) were novel. Only two unrelated patients out of 19 cases had homozygous mutations (Q352X, R355H). S305L mutation was found in several cases (12.1%, 4/34 alleles), suggesting that this mutation is a common mutation. In contrast, R402W was not identified and IVS10-2A>C was found in only one allele, suggesting that Japanese patients with GA1 show allelic heterogeneity and have different genetic background compared with patients of other countries. Although all 19 cases were assumed to have barely detectable enzyme activity, their clinical outcomes were diverse, ranging from normal development, through mild impairment, to severe handicap. We consistently found that there was no association between genotype and phenotype.

Taken together, these findings strongly suggest that early diagnosis and treatment but not genotype are associated with a favorable patient outcome, reinforcing the importance of neonatal screening for early detection.

CONCLUSIONS

We investigated the relationship between clinical and mutational spectrums of 19 Japanese patients with GA1. We found a few common mutations distinct from other countries. We also found that mutations in Japanese cases are different from what have been reported in the Caucasian cases, indicating specific genetic information unique for Japanese cases are crucial for their diagnosis in the future. The current study also indicates that earlier detection of the disease followed by appropriate intervention is crucial for the better outcome than the genotype, reinforcing the importance of neonatal screening for GA1. This is a first report that studied the largest cohort of Japanese patients with GA1.