学 位 論 文 の 要 旨

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学 位 論 文 名 Clinical and Molecular Aspects of Japanese Patients With Mitochondrial Trifunctional Protein Deficiency
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論 文 内 容 の 要 旨

INTRODUCTION

Mitochondrial trifunctional protein (MTP), binding to the mitochondrial inner membrane, plays a significant role in the β-oxidation cycle of long-chain acyl-CoAs, and it is an $\alpha_4\beta_4$ hetero-octamer encoded by 2 different genes of HADHA and HADHB. The α-subunit (HADHA) encodes trans-2,3-long-chain enoyl-CoA hydratase (LCEH) and long-chain 3-OH-acyl-CoA dehydrogenase (LCHAD), while β-subunit (HADHB) encodes long-chain 3-ketoacyl-CoA thiolase (LCKT).

MTP deficiency is clinically classified into the three forms: 1) lethal phenotype with neonatal onset (severe form), 2) hepatic phenotype with infant onset (intermediate form), and 3) myopathic phenotype with late-adolescent onset (mild form).

Since the first case of MTP deficiency with the decreased activity of all 3 enzymes was reported, the molecular basis of MTP deficiency has been identified in more than 50 cases so far. Twenty-six of them showed mutations of the HADHB gene, suggesting that the frequency of mutations of the HADHA and HADHB genes in MTP deficiency is likely similar. However, isolated LCHAD deficiency is common in Caucasian, and usually caused by a common mutation, 1528G>C, in the HADHA.

In this study, we newly characterized 5 novel mutations in 3 Japanese patients with MTP deficiency, and investigated the clinical and molecular aspects of 5 Japanese patients including 2 previously reported cases. Further, we employed a sensitive transient expression analysis to determine the residual activities of 4 missense mutations in the HADHB gene identified in Japanese patients with MTP deficiency.

MATERIALS AND METHODS

Subjects: Five Japanese children (3 boys and 2 girls) were studied. The age at onset of the patients ranged from 0 days to 15 years. Of the 5 patients, 2 each showed lethal (severe form) and hepatic (intermediate form) phenotypes, respectively, and one had a myopathic phenotype (mild form).

Enzyme assay: Mitochondrial LCKT activity was determined in fibroblasts from the patients and SV40-transformed fibroblasts after expression analysis. LCKT activity was measured with 10 μM 3-ketopalmitoyl-CoA at 303 nm.

Western blot analysis: Western blotting was performed using rabbit polyclonal antibody against α- and ß- subunits of MTP as the primary antibody, and visualized using the ImmunoPure NBT/BCIP Substrate Kit™.

Mutation analysis: Mutations were checked using primers of HADHA and HADHB genes to amplify each exon including 5' and 3' splice sites, respectively. In the replicate experiments, four overlapping fragments for the entire coding region of HADHB cDNA were amplified.

Construction of eukaryote transient expression vectors: cDNA synthesis was performed using total RNA from control fibroblasts with a mixture of the HADHA-specific and HADHB-specific antisense primers. Wild-type (WT) of HADHA and HADHB cDNAs including the full coding sequence were amplified using the specific sense and antisense primers. Amplified HADHA and HADHB cDNAs were ligated into the pUC118 *Hinc* II/BAP vector, and designated as pUC118-WTα and pUC118-WTß, respectively. Four mutant HADHB cDNAs, H346R, V422G, R214C, and R411K, were introduced into the pUC118-WTß by mutagenesis using a QuikChange Site-Directed Mutagenesis kit. After the mutations were confirmed by sequencing, pUC118-WTα, pUC118-WTß cDNA, and pUC118-mutant HADHB cDNA fragments were subcloned into the pCAGGS eukaryote expression vector, and designated as pCAGGS-WTα, pCAGGS-WTß, and pCAGGS-mutant ß, respectively.

Transient expression analysis of mutant cDNAs: Recipient cells, SV40-transformed fibroblasts, from patient 1 with the severe form were prepared. Wild-type (pCAGGS-WTß) or pCAGGS-mutant ß expression vectors (4 μg) were co-transfected with 4 μg of pCAGGS-WTα and 1 μg of cytosolic acetoacetyl-CoA thiolase (CT) cDNA-expressing vector (pCAGGSct) into ~ 5 x 10^5 recipient cells employing Lipofectamine 2000. Cells were incubated at 37 °C for 24 h, followed by additional 48-h incubation at 30 °C or 37 °C.

RESULTS AND DISCISSIONS

Clinical course, results of tandem MS analysis, enzyme assay, and Western blot analysis confirmed MTP deficiency in the 3 Japanese patients, who were analyzed in our laboratory. We identified 5 novel mutations, R214C, R346H, V422G, an exonic c.817delG and deep intronic mutation, g.33627A to G (IVS7+ 614A to G) in the HADHB gene from the 3 Japanese patients with MTP deficiency. All the 5 patients showed mutations in HADHB but not HADHA, suggesting that ß-subunit mutation may be more common in Japanese patients with MTP deficiency. However, there were not any common mutations.

Transient expression analysis of mutant cDNAs: To investigate the biological significance of the missense mutations, R214C, H346R, R411K, and V422G, we performed transient expression analysis of the mutant cDNAs. Hence transient expression of pCAGGS-WTß alone was insufficient to achieve significant LCKT activity, we performed transient co-expression of pCAGGS-WTα and pCAGGS-WTß or pCAGGS-mutantß expression vectors in the same cell line. Co-transfection with pCAGGS-WTα and pCAGGS-WTß cDNAs increased the enzyme activity 4-fold compared to control fibroblasts

in this system. Since we used an SV40-transformed MTP-deficient cell line as recipient cells, the background activity was nearly nil. Moreover, we used two different incubation temperatures of 30°C and 37°C to determine the temperature sensitivity.

In the 5 Japanese patients, one had mild and two each had intermediate and severe forms, respectively. The patient with the mild form (patient 5) was homozygous for the "mildest" mutation, R411K. Patients with the intermediate form (patients 3 and 4) had R411K or the "mild" mutation R214C, which retained residual activity at both 30°C and 37°C, at least in one allele. Patients with the severe form (patients 1 and 2) showed missense mutations, H346R and V422G, which did not retain any enzyme activity at 37°C. However, patient 2 showed a V422G mutation that retained some residual enzyme activity at 30°C, and survived until 3 months of age, whereas patient 1 died on the 8th day after birth. These findings may explain the longer survival of patient 2 compared to patient 1. Although the number of patients was still limited, our analysis suggests that there is a phenotype-genotype correlation in MTP deficiency.

The molecular basis of more than 50 patients with MTP deficiency has been characterized worldwide to date. Of them, 26 patients showed mutations of the HADHB gene, and clinical presentation of the 25 patients with HADHB mutations were described. Those previous reports emphasized that the myopathic phenotype is relatively common in MTP deficiency. In Japan, only one patient with myopathic form was found. This might be due to an ethnic difference. Alternatively, the frequency of the mild phenotype of MTP deficiency may be underestimated in Japan, since patients with the myopathic form may remain asymptomatic for a long period without any diagnosis being made.

There have been only a few reports of the molecular basis of LCHAD or MTP deficiency from Asian countries, although LCHAD deficiency, which is caused by a 1528G>C mutation, is more common in Caucasian. The common 1528G>C mutation has not been found in the Japanese patients so far. These results suggest that ethnic differences may exist in the genes affected in MTP deficiency.

CONCLUSION

In summary, our results found no HADHA mutations but variety of HADHB mutations among Japanese patients with MTP deficiency, and the mutational spectrum is likely heterogeneous. The phenotypes were likely correlated with the genotypes, and a combination of two mutant alleles. The present findings show that all missense mutations described in this study are disease-causing, and add three missense mutations (R214C, H346R and V422G), an exonic c.817G single deletion (240 frameshift) and deep intronic mutation, g.33627A to G (IVS7+ 614A to G) to the catalogue of HADHB gene mutations. A genetic diagnosis may help to predict the clinical severity and provide more accurate diagnostic information for patients and families with MTP deficiency.