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論文審査の結果の要旨及び担当者

(申請者氏名) Islam Mohammad Saiful

論文審査担当者	(職)	氏名
主査	大阪大学教授	大薗恵一
副査	大阪大学教授	下村伊一郎
副査	大阪大学教授	金井好克

論文審査の結果の要旨

Monocarboxylate transporter 8 (MCT8) facilitates T3 uptake into cells. Mutations in MCT8 lead to Allan-Herndon-Dudley syndrome (AHDS), which is characterized by severe psychomotor retardation and abnormal thyroid hormone profile. Nine uncharacterized MCT8 mutations in Japanese patients with severe neurocognitive impairment and elevated serum T3 levels were studied regarding the transport of T3 in wild-type (WT) or mutant hMCT8-transfected human placental choriocarcinoma cells (JEG3) by visualizing the locations of the proteins in the cells, detecting specific proteins, and measuring T3 uptake. We identified 6 missense (p.Arg445Ser, p.Asp498Asn, p.Gly276Arg, p.Gly196Glu, p.Gly401Arg, and p.Gly312Arg), 2 frameshift (p.Arg355Profs*64 and p.Tyr550Serfs*17), and 1 deletion (p.Pro561del) mutation(s) in the hMCT8 gene. All patients exhibited clinical characteristics of AHDS with high free T3, low-normal free T4, and normal-elevated TSH levels. All tested mutants were expressed at the protein level, except p.Arg355Profs*64 and p.Tyr550Serfs*17, which were truncated, and were inactive in T3 uptake, excluding p.Arg445Ser and p.Pro561del mutants, compared with WT-hMCT8. Immunocytochemistry revealed plasma membrane localization of p.Arg445Ser and p.Pro561del mutants similar with WT-hMCT8. The other mutants failed to localize in significant amount(s) in the plasma membrane and instead localized in the cytoplasm. These data indicate that p.Arg445Ser and p.Pro561del mutants preserve residual function, whereas p.Asp498Asn, p.Gly276Arg, p.Gly196Glu, p.Gly401Arg, p.Gly312Arg, p.Arg355Profs*64, and p.Tyr550Serfs*17 mutants lack function. These findings suggest that the mutations in MCT8 cause loss of function by reducing protein expression, impairing trafficking of protein to plasma membrane, and disrupting substrate channel.

It is evaluated that deserving of a doctoral degree (medicine) is worth.

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論　文　内　容　の　要　旨
Synopsis of Thesis

氏　名 Name	Islam Mohammad Saiful
論文題名 Title	Functional analysis of monocarboxylate transporter 8 mutations in Japanese Allan-Herndon-Dudley syndrome patients（日本人Allan-Herndon-Dudley症候群患者におけるモノカルボキシレートトランスポーター8変異の機能解析）

論文内容の要旨

〔目　的(Purpose)〕

Monocarboxylate transporter 8 (MCT8) facilitates triiodothyronine (T3) uptake into cells. Mutations in MCT8 lead to Allan-Herndon-Dudley syndrome (AHDS), which is characterized by severe psychomotor retardation and abnormal thyroid hormone profile. Nine uncharacterized MCT8 mutations [6 missense (p.Arg445Ser, p.Asp498Asn, p.Gly276Arg, p.Gly196Glu, p.Gly401Arg, and p.Gly312Arg), 2 frameshift (p.Arg355Profs*64 and p.Tyr550Serfs*17), and 1 deletion (p.Pro561del) mutation(s)] in Japanese patients with severe neurocognitive impairment and elevated serum T3 levels were studied regarding the transport of T3.

〔方法ならびに成績(Methods/Results)〕

(Methods)

Genetic Analysis Genomic DNA was extracted from peripheral white blood cells of the patients. The entire coding region and exon-intron boundaries of the MCT8 gene were amplified from the genomic DNA by PCR (polymerase chain reactions) with the specific primers. PCR products were subsequently sequenced and the forward and reverse primers from the PCR amplification. Direct sequencing in both directions was performed. **Functional analysis** Human MCT8 (hMCT8) function was studied in wild-type (WT) or mutant hMCT8-transfected human placental choriocarcinoma cells (JEG3, derived from human placental choriocarcinoma), which do not express endogenous MCT8 by visualizing the locations of the proteins in the cells, detecting specific proteins, and measuring T3 uptake.

(Results)

Nine uncharacterized MCT8 gene mutations in 13 boys of differing ages showed severe mental retardation. Although most patients had hypotonia and dyskinetic cerebral palsy, seizures were less common. The TSH level was normal to elevated, whereas serum fT3 was markedly elevated and fT4 was below the normal range. All tested mutants were expressed at the protein level, except p.Arg355Profs*64 and p.Tyr550Serfs*17, which were truncated, and were inactive in T3 uptake, excluding p.Arg445Ser and p.Pro561del mutants, compared with WT-hMCT8. Immunocytochemistry revealed plasma membrane localization of p.Arg445Ser and p.Pro561del mutants similar with WT-hMCT8. The other mutants failed to localize in significant amount(s) in the plasma membrane and instead localized in the cytoplasm. These data indicate that p.Arg445Ser and p.Pro561del mutants preserve residual function, whereas p.Asp498Asn, p.Gly276Arg, p.Gly196Glu, p.Gly401Arg, p.Gly312Arg, p.Arg355Profs*64, and p.Tyr550Serfs*17 mutants lack function. These findings suggest that the mutations in MCT8 cause loss of function by reducing protein expression, impairing trafficking of protein to plasma membrane, and disrupting substrate channel.

〔総　括(Conclusion)〕

Our study underscores that the functional defects of mutant-hMCT8 proteins are the result of protein expression reduction, impairment in trafficking to the plasma membrane, substrate channel disruption, and modification of helices conformation. Mutant-hMCT8s that localize to the plasma membrane will more likely have residual activity and result in a less severe phenotype.