論文の内容の要旨

論文題目　**Investigation of genetic background in the Japanese children with Acute encephalopathy with biphasic seizures and late reduced diffusion(AESD)**

（日本人小児けいれん重積型急性脳症（ＡＥＳＤ）患者の遺伝的背景）

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INTRODUCTION

Acute encephalopathy is the most serious complication of pediatric viral infections. Acute encephalopathy with biphasic seizures and late reduced diffusion (AESD) is the most frequent type of acute encephalopathy in Japan. However its etiology and pathogenesis remain unclear. Its incidence is highest in infancy and early childhood. The antecedent infections are caused by various viruses and bacteria. However, there is no specific difference between the antecedent infections and the type of acute encephalopathy. In addition, the frequency of acute encephalopathy in East Asians is higher than that in Caucasians. This suggests that genetic factors play an important role in the etiology of acute encephalopathy.

OBJECTS

Acute encephalopathy has been considered multifactorial syndrome, caused by multiple genetic and environmental factors. However, most of these risk factors, especially for genetic factors, have been unknown. In order to develop the effective treatments and the prevention methods for AESD, it is necessary to investigate genetic background in the patients with AESD. It has recently been reported that thermolabile haplotype 352C-368I-647M of the *CPT2* (carnitine palmitoyl transferase 2 gene) is associated with influenza encephalopathy with poor prognosis. There is no report conducting *CPT2* variations analysis in a large number of AESD patients with various antecedent infections. In addition, I hypothesized that the adenosine-mediated signal pathway may be altered in AESD. Adenosine is an

endogenous anticonvulsant. And theophylline, which is non-selective A_1R and $A_{2A}R$ antagonist, is considered one environmental risk factor of AESD. Dysfunction of adenosine pathway might be caused excitotoxicity and predispose AESD. Therefore, this study focused on three genes, carnitine palmitoyltransferase 2 gene (*CPT2*), Adenosine A_1R and $A_{2a}R$ gene (*ADORA1* and *ADORA2A*) in AESD patients.

MATERIALS

85 patients with AESD from hospitals in Japan and 100 normal controls from Human science research resources bank participated in genetic analysis. And in order to investigate *ADORA2A* mRNA expression level, 100 human brain cDNA and RNA samples were obtained from Stanley Medical Research Institute (SMRI) (Bethesda, MD, USA). In order to investigate mRNA and protein expression level and estimate cAMP accumulation, 15 lymphoblast cell lines from control Japanese adults, obtained from control subjects at the University of Tokyo Hospital for functional analysis.

METHODS

After informed consent, genomic DNA of patients was extracted from whole blood using standard protocols. First, three *CPT2* SNPs (rs2229291 c.1055T>G p.F352C and rs1799821 c.1102A>G p.I368V in exon4, and rs1799822 c.1939A>G p.M647V in exon5) were analyzed in 85 patients with AESD and 100 controls. TA cloning was performed, when patients and controls showed heterozygosity in two SNPs, rs2229291 and rs1799821, of exon 4. Second, *ADORA1* and *ADORA2A* in 85 patients with AESD and 100 normal controls were analyzed. For statistical analysis, goodness-of-fit to the Hardy-Weinberg equilibrium in patients and control subjects were examined by χ^2 test using Microsoft Office Excel 2010. Differences in the demographic characteristics of the genotypes were assessed by Pearson's χ^2 test and Fisher's exact test for categorical data. Odds ratio (OR) together with the 95% confidence interval (CI) for each allele and haplotype frequency with AESD were calculated by Microsoft Office Excel 2010. Bonferroni correction was used to adjust for multiple testing. Significant differences were defined as a corrected p value in each conditional analysis. Finally, the mRNA expression in brain samples, mRNA and protein expression in lymphoblasts, as well as the production of cyclic adenosine monophosphate (cAMP) by lymphoblasts in response to adenosine was compared among *ADORA2A* diplotypes. The differences in mRNA and protein expression levels and cellular cAMP accumulation, expressed as the mean ± SEM, were calculated using analysis of variance (ANOVA) followed by the Tukey-Kramer test in the case of multiple comparisons.

RESULTS

By *CPT2* analysis, the frequency of rs2229291 G (p.352 C) allele in *CPT2* exon 4 was significantly higher in patients than that in controls (p=0.013, OR=2.09, 95%CI=1.22-3.57). 352 C-CPT2 decreases CPT2 activity to 50% of 352 F-CPT2 and is related to the severity of acute encephalopathy. However, the frequency of cases positive rs2229291 G (p.352 C) allele did not show difference between good and poor prognosis (p=0.177).

By A_1R and $A_{2A}R$ analysis, I found *ADORA2A* AA diplotypes, containing four variations in *ADORA2A,* was associated with AESD. The frequency of haplotype A (rs2298383 C, rs5751876 T, rs35320474 deletion and rs4822492 C) in patients was significantly higher than in controls (p=0.005, OR=1.70, 95%CI=1.17-2.45). $A_{2A}R$ mRNA expression was significantly higher in AA than AB and BB diplotypes, both in the brain (p=0.003 and 0.002, respectively). In lymphoblasts, $A_{2A}R$ protein expression (p=0.028), as well as cellular cAMP production (p=0.0006), was significantly higher in AA than BB diplotype.

DISCUSSION

This study found two genetic risk factors. One is thermolabile 352C-CPT2 variation, and another is *ADORA2A* AA diplotype, consisting of four *ADORA2A* genetic variants. When cases with thermolabile 352C-CPT2 are provoked by infection and high fever, they may suffer from energy failure and AESD. High A2AR expression level and high cAMP accumulation, caused by *ADORA2A* AA diplotypes, may predispose children to AESD by altering the intracellular adenosine/cAMP signal cascade. Based on these findings on molecular pathomechanism, further clinical and laboratory studies are needed to find an appropriate biomarker for early diagnosis, and to develop effective treatments and preventive measures for AESD.