学 位 論 文 の 要 旨

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学 位 論 文 名	Association Between Cystatin C Gene Polymorphism and the Prevalence of White Matter Lesion in Elderly Healthy Subjects
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論 文 内 容 の 要 旨

INTRODUCTION

Cystatin C (CST3) is a 13-kDa protein consisting of 120 amino acids, encoded by a 7.3-kb gene located in chromosome 20. It is expressed in all types of cells, where it is located in the lysosome, Golgi apparatus and endoplasmic reticulum. It is also a secreted protein and found in all types of body fluid. Particularly, CST3 concentration is high in cerebrospinal fluid (CSF), signifying its high expression and important function in central nervous system. It can inhibit the activity of cysteine proteases including cathepsin B, L and H, and is considered to be the main endogenous inhibitor of those enzymes. Since these proteases have important roles in various pathophysiological processes including inflammation and immune regulation, vascular remodeling and cell migration, CST3 could affect those processes by modulating their activities. Indeed, it is demonstrated that by modulating the activities of cysteine proteases, CST3 is shown to play an important role in the pathology of several disease processes including atherosclerosis, tumor metastasis, chronic kidney diseases and inflammatory conditions.

Several nucleotide changes in the promoter and coding sequence of *CST3* gene have been reported. Among single nucleotide polymorphisms (SNP) in *CST3* gene, -82G/C (rs5030707) located in the 5'-promoter region has been reported to affect promoter activity. Another SNP +148G/A (rs1064039) located in the coding region causes the changes in CST3 secretion. Furthermore, a haplotype of 3 SNPs containing -82G/C, +4A/C (rs4994881) and +148G/A has been reported to be associated with CST3 levels in serum and CSF. Therefore, the aim of this study is to examine the relation of white matter diseases with *CST3* SNPs in Japanese healthy

population and confirm whether plasma CST3 levels are affected by the SNPs.

MATERIALS AND METHODS

A total of 1,795 subjects (1,002 males, 793 females) with a mean age of 60.1 ± 8.9 years (range 29 to 95) were included in this study. All subjects were voluntarily participated in a brain health screening program at Shimane Health Science Center between October 2001 and July 2013. The screening program included general medical and neurological examinations, MRI scans of head and blood tests. Plasma CST3 was measured using immunoassay kit and an automatic biochemical analyzer at Central Clinical Laboratory division of Shimane University Hospital. Genotyping analyzed 7 SNPs of *CST3*, including -82G/C, -78T/G (rs71334202), -5G/A (rs113065546), +4A/C, +87C/T (rs1055084), +148G/A and +213G/A (rs201089355). The genotyping was done with a TaqMan genotyping assay kit, using PCR system. The study protocol was approved by the Research Ethics Committee of Shimane University.

RESULTS AND DISCUSSION

To determine the effect of the genetic variations in *CST3* gene on cerebral white matter changes, 7 polymorphisms (-82G/C, -78T/G, -5G/A, +4A/C, +87C/T, +148G/A and +213G/A) in *CST3* gene have been analyzed, and checked their relation with laboratory data, cognitive impairment and MRI findings in healthy Japanese subjects. The analysis revealed that in the study population, there was no polymorphism at -5, +87 and +213 positions in the gene. Since -78T/G and -82G/C was haplotype, 3 polymorphisms at -82G/C, +4A/C and +148G/A were chosen for further analysis. Haplotype analysis at position -82, +4 and +148 revealed a major allele haplotype of G/A/G (allele A), and a minor allele haplotype of C/C/A (allele B). Our analysis demonstrated that the polymorphism at these three positions was the haplotype of *CST3* gene that affected the plasma concentration and brain white matter lesions.

In our study, the population with carriers of the B allele had lower plasma CST3 level. However, the subject group with white matter disturbance demonstrated higher plasma CST3 levels. This subject group is aged, and eGFR is lower than that of the subject group with no white matter disturbance. Since plasma CST3 is exclusively cleared by the kidney, higher CST3 level in the subject group with cerebral white matter disturbance could be explained by decreased renal function. Interestingly, after adjustment, logistic regression revealed an increased risk of periventricular hyperintensity (PVH) and deep and subcortical white matter hyperintensity in B allele. B allele can affect the production and secretion of CST3. Then its level in cerebrovascular tissue could decrease despite high plasma concentration. Taken together, these findings suggest a relation of cerebral small vessel disease and the alteration of CST3 expression due to polymorphism that is independent of kidney-mediated clearance.

Progression of white matter lesion such as PVH is a predictor of cognitive impairment risk in healthy elderly individuals. Although initiation of white matter damage is more likely to be induced in carriers of the B allele, any of the cognitive test performances were not different between the groups. The reason might be the tests parameters were not affected at the early stage of white matter damage. It would be interesting to know whether the relation of white matter damage and decline of cognitive performance is evident in carriers of the B allele in next follow-up study. There are also other limitations in this study. Our study recognized that although carriers of the B allele have lower level of plasma CST3, it might not be the same in its CSF level. In future study, we need to confirm the effect of polymorphism on CSF CST3 levels as well. Another important aspect can be explored is *CST3* gene relationship with Alzheimer's disease (AD) and *APOE* genotype. Moreover, other class II cystatins could be important for cerebral small vessel disease pathology. Particularly cystatin F is increased in the CSF of AD patients, indicating its relation with neurodegenerative conditions. It will be interesting to explore its relation with cerebral small vessel diseases.

CONCLUSION

The carriers of the B allele (-82C/+4C/+148A) have lower levels of serum CST3. The population did present MRI evidence of cerebral small vessel disease without the decline of cognitive performance, indicating the involvement of CST3 from the early stage of the disease. Such findings provide a new insight into the pathological mechanisms of the white matter disease.