42nd Mihara Award Lecture

Title: Identification of new genetic factors for moyamoya disease using long-read sequencing

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Background and Objectives

Moyamoya disease can cause stroke, including cerebral infarction and cerebral hemorrhage, and commonly occurs in individuals from East Asian countries such as Japan. *RNF213* has been identified as a susceptibility gene for moyamoya disease, and the missense variant c.14429G>A (p.Arg4810Lys) in *RNF213* has been shown to be associated with moyamoya disease. However, the p.Arg4810Lys variant is present in 1.5–2% of the population, whereas the prevalence of moyamoya disease is 3–10 per 100,000 people. Therefore, this variant alone cannot explain the development of this disease, and a definitive pathogenic mechanism remains to be elucidated.

We have conducted and published genetic analysis research on moyamoya disease and intracranial artery stenosis (*Stroke* 2012, 2013; *J Stroke Cerebrovasc Dis* 2015, 2017, 2018; *Sci rep* 2020*; Transl Stroke Res* 2022; *Lancet Neurol* 2022). We performed a phenome-wide association study of *RNF213* p.Arg4810Lys and found that it is associated with coronary artery stenosis and hypertension (*Transl Stroke Res* 2022). Thus, *RNF213* p.Arg4810Lys is associated with various phenotypes of systemic vascular diseases, but the determinants of disease onset and phenotype are unknown and need to be identified.

In this study, we aim to use long-read sequencing to identify repetitive sequences and genomic structural variations that are difficult to analyze through short-read sequencing using conventional next-generation sequencers. This study was designed to identify modifiers that coexist with the p.Arg4810Lys mutation and new genetic structural abnormalities such as repeat sequences and to elucidate the pathogenic mechanisms of moyamoya disease and cerebrovascular stenosis.

Research Plan

At present, we have already collected DNA samples from over 300 patients with moyamoya disease. A research team comprising sample collecting facility staff, experts in genetic analysis, experts in bioinformatics, and genetic statisticians has already been established.

First, we will perform long-read sequencing of a region containing *RNF213* with a linkage disequilibrium block of approximately 1 MB. Target DNA enrichment will be performed using the hybridization capture method. Next, we will analyze the long-read sequencing results of the whole-genome region. Japanese research groups have performed linkage analysis of moyamoya disease and identified the 3p24.2 - p26, 6q25, 8q22, and 12p13 regions as candidate regions in addition to *RNF213.* A Chinese research group conducted a genome-wide association study of moyamoya disease and identified 15 new disease-related gene regions in addition to *RNF213.* However, no specific causative genes were identified in these regions. Through whole-genome analysis, we aim to identify phenotypically relevant structural abnormalities such as repetitive sequences in these regions. Furthermore, we will analyze the relationship between the identified novel genetic factors and clinical features to clarify the clinical significance of the disease.