RNF213-Related Vasculopathy: Various Systemic Vascular Diseases Involving RNF213 Gene Mutations: Review

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Moyamoya disease (MMD) is a cerebrovascular disorder that is predominantly observed in women of East Asian descent, and is characterized by progressive stenosis of the internal carotid artery, beginning in early childhood, and a distinctive network of collateral vessels known as “moyamoya vessels” in the basal ganglia. Additionally, a prevalent genetic variant found in most MMD cases is the p.R4810K polymorphism of RNF213 on chromosome 17q25.3. Recent studies have revealed that RNF213 mutations are associated not only with MMD, but also with other systemic vascular disorders, including intracranial atherosclerosis and systemic vascular abnormalities such as pulmonary artery stenosis and coronary artery diseases. Therefore, the concept of “RNF213-related vasculopathy” has been proposed. This review focuses on polymorphisms in the RNF213 gene and describes a wide range of clinical and genetic phenotypes associated with RNF213-related vasculopathy. The RNF213 gene has been suggested to play an important role in the pathogenesis of vascular diseases and developing new therapies. Therefore, further research and knowledge sharing through collaboration between clinicians and researchers are required. (J Nippon Med Sch 2024; 91: 140-145)

Key words: internal carotid artery, RNF213, moyamoya disease, vasculopathy

Introduction
Moyamoya disease (MMD) is characterized by cerebral ischemia resulting from the stenosis or occlusion of the terminal portion of the bilateral internal carotid artery and the formation of a network of small abnormal vessels in the basal ganglia that serve as collateral blood vessels. MMD is typically diagnosed based on distinctive MRI, MRA, and angiographic findings. The annual incidence of MMD is low in Europe and the United States but high in East Asia, with recent increases in Japan, Korea, and China. Since MMD often occurs within families, genetic mutations are considered to play a role. Herein, we discuss the detection of RNF213 and its association with systemic vascular diseases. We also provide an overview of current research and summarize the functions of the RNF213 protein and its role in “RNF213-related vasculopathy,” particularly clinical phenotypes, with the goal of contributing to research in multiple fields.

Creation of the “RNF213-Related Vasculopathy” Concept
After the discovery that chromosome 17q25.3 is involved in moyamoya disease, the R4810K polymorphism (p.Arg4810Lys) in the ring finger protein 213 (RNF213, myostatin) gene on chromosome 17q25.3 was identified as a
susceptibility gene for MMD in East Asian populations using genome-wide linkage and exome analysis\textsuperscript{25}. The p.
R4810K polymorphism (\textit{RNF213} c.14576G>A, rs\n112735431), the founder of moyamoya disease in Asia, including
Japan, is a missense mutation that changes the
4,810th amino acid residue of \textit{RNF213} (arginine) to lys-
ine\textsuperscript{2,9}. \textit{RNF213} or its polymorphism is found in ap-
proximately 80\% of Japanese\textsuperscript{11} patients with moyamoya
disease, 80-90\% of Korean patients, and 20-30\% of Chi-
nese patients, but it is not prevalent in Western coun-
tries\textsuperscript{12,14}. R4810K is more prevalent among Japanese indi-
viduals, while A4399T is more frequent in individuals of
Chinese descent\textsuperscript{15}. Thus, \textit{RNF213}-related vasculopathy includes a range of genetic
polymorphisms. However, the precise details of these
variants remain unclear. The R4810K polymorphism is
absent in Caucasians in the general population but is
present in 1.5\% of carriers in Japan and Korea and 0.5\%
in China, suggesting that it is a variant specific to East
Asia\textsuperscript{11}. In addition to the p.R4810K polymorphism, sev-
eral missense mutations, including p.P4007R and p.T4589
P, have been identified in Chinese families with
moyamoya disease\textsuperscript{2,11,15-17,18}. In contrast, a rare variant of
\textit{RNF213}, which accumulates in the C-terminal region of
the RING finger domain (amino acid numbers: 3997-
4093), has been reported to occur in Caucasian individuals
with MMD\textsuperscript{11,21}. Therefore, \textit{RNF213} is a disease susceptibili-
ty polymorphism shared across racial groups, although
exhibiting variations in its mutations\textsuperscript{12,16,20}.

This polymorphism has been associated with intracra-
nial arterial stenosis, occlusion, and atherothrombotic
stroke in East Asia, even in cases that did not meet the
diagnostic criteria for MMD\textsuperscript{11,21,22}. Recently, the \textit{RNF213}
polymorphism has been shown to be associated with
both intracranial arteries and arteries throughout the
body, including the cervical\textsuperscript{2,14}, coronary\textsuperscript{25}, pulmonary\textsuperscript{26-28},
abdominal visceral\textsuperscript{29}, and peripheral arteries, and has
been reported to be associated with vascular diseases
throughout the body, resulting in \textit{RNF213}-related vascu-
lopathy, which may be the most significant risk factor for
vascular diseases\textsuperscript{5,12,30,31} (Table 1). Most lesions are stenotic
and have vascular origins. However, they are also associ-
ated with aneurysms\textsuperscript{2,22}. Based on this, Okazaki et al.\textsuperscript{21}
proposed \textquotedblright \textit{RNF213}-related vasculopathy\textquotedblright as a new dis-
ease spectrum in 2019. More recently, moyamoya disease
and intracranial carotid stenosis, as well as lesions of the
coronary arteries, pulmonary arteries, and abdominal
aorta have been identified as \textquotedblright \textit{RNF213}-related vasculopa-
thy\textquotedblright \textsuperscript{21,33,34}. Analysis of these systemic cardiovascular dis-
eases based on the prevalence of \textit{RNF213} variants may
lead to a more detailed classification of cardiovascular
diseases and the elucidation of their underlying patho-
physiologies. In this article, we review the functions of
\textit{RNF213} encoded by the \textit{RNF213} gene and discuss the re-
lationship between \textit{RNF213} polymorphisms and diseases
in various vascular regions. It has been proposed that in-
tegrating \textit{RNF213}-related vasculopathy into fields such as
clinical medicine and genetic diagnostics will help ad-
advance research.

### \textit{RNF213} Gene (Mysterin) Function

Moyamoya disease leads to progressive arterial stenosis
and occlusion of limited intracranial vessels bilaterally,
resulting in cerebral ischemia and infarction in pediatric
patients, and hemorrhage due to the disruption of collat-
eral channels in adult patients\textsuperscript{13,35}. The lesions exhibit an
abnormal proliferation of vascular smooth muscle cells
and infiltration of the vascular intima, resulting in inti-
mal thickening and lumen narrowing\textsuperscript{6,27}. The \textit{RNF213}
gene comprises 5,256 amino acids and is located on the
long arm of chromosome 17\textsuperscript{7}. Molecular cloning led to
the discovery, naming, and functional analysis of mys-
terin (MMD-associated AAA+ and RNF protein), also

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<td>Brain</td>
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<td>Moyamoya disease</td>
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<td>Heart</td>
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Table 1 Systemic vascular lesions reported to be associated with \textit{RNF213}

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known as RNF213 and ALO17\textsuperscript{28}. Encoding a giant protein with a molecular mass of 591 kDa, RNF213 contains a core sequence with a RING finger domain, characteristic of E3 ubiquitin ligase, and AAA ATPase domain\textsuperscript{29,30}. E3 ubiquitin ligase ubiquititates substrate proteins to facilitate their degradation by the proteasome, and AAA ATPase is involved in this process\textsuperscript{31,32}. Zebrafish phenotypes expressing mysterin suppressors were analyzed, and intracellular binding proteins were sought to identify mysterin substrates and functions. Many early developmental abnormalities have been detected, and mysterin-stabilizing factors were successfully identified\textsuperscript{33}. Mysterin is also found in intracellular lipid droplets and is believed to stabilize them by excluding adipose triglyceride lipase (ATGL), inhibiting lipolysis\textsuperscript{34}. No functional difference was detected between wild-type mysterin and the East Asian p.R4810K mutant\textsuperscript{35}.

RNF213 is thought to help regulate vascular endothelial function and angiogenesis, as RNF213 knockout zebrafish show defects in eye and brain vessel formation\textsuperscript{36,37}. In contrast, the RNF213 gene is believed to induce vascular abnormalities in collaboration with environmental factors, such as infection and autoimmunity, or with other genes, as RNF213 knockout mouse models develop normally without exhibiting the intracranial vascular phenotype characteristic of moyamoya disease\textsuperscript{38,39}.

The clinical effects of this rare RNF213 variant have also been reported\textsuperscript{40}. Patients without p.R4810K had an earlier age of onset (7.1±3.7 vs. 4.4±0.9 years), beginning in infancy, and a higher percentage of infarcts (24.0 vs. 7.6%) and lower percentage of transient ischemic attacks (36.0 vs. 71.7%) than those with the heterozygous variant. Eight rare mutations other than p.R4810K were isolated from 25 patients without p.R4810K mutations. Three of the four pediatric-onset patients had variants other than p.R4810K, with more severe functional effects\textsuperscript{41}. Similarly, Ishigami et al.\textsuperscript{42} reported more bilateral lesions (p = 0.008) and progressive condition (Suzuki grade ≥ 4; p = 0.017) in the heterozygous (GA) group. Multivariate logistic regression analysis showed that younger age at diagnosis (p < 0.001; odds ratio [OR], 0.936; 95% CI, 0.914-0.959) and GA (p = 0.017; OR, 3.326; 95% CI, 1.237-8.941) were significantly associated with bilateral lesions. Patients with MMD and RNF213 heterozygous mutations are at risk of contralateral cerebral infarction if one side of the brain is symptomatic. Torazawa et al.\textsuperscript{43} reported that 72% of patients were GA for p.Arg4810Lys and 28% were wild-type (GG). The GG and p.Ala4399Thr hemispheres had more bleeding events (p = 0.028), and GG was more likely to cause de novo bleeding in the asymptomatic hemisphere than GA (adjusted hazard ratio [aHR], 5.36). This indicates that GG with p.Arg4810Lys is a risk factor for de novo bleeding in asymptomatic hemispheres with MMD.

**RNF213 Variants and Head and Neck Vascular Disease**

RNF213 p.Arg4810Lys variant is associated with ischemic stroke, particularly large artery stroke. In moyamoya disease, p.Arg4810Lys is a risk factor for ischemic stroke in general\textsuperscript{44}. Intracranial atherosclerosis and moyamoya disease are prevalent in Asian populations. Limited data are available regarding the role of RNF213 in atherosclerotic diseases, and no genetic factors specific to intracranial atherosclerosis have been reported. In East Asia, including Japan, cerebral infarction attributed to atherothrombotic intracranial artery stenosis is more frequent than in Western populations. While this regional difference was initially thought to be influenced by environmental factors including diet, the higher incidence of cerebral infarction due to intracranial atherosclerosis among East Asians in the U.S. suggests a possible genetic contribution. Similar findings have been reported in Korean and Chinese populations, suggesting a role for RNF213 p.R4810K polymorphism in the increased prevalence of intracranial arterial stenosis across East Asia\textsuperscript{45}.

Miyawaki et al.\textsuperscript{46}, Uemura et al.\textsuperscript{47}, and Murai et al.\textsuperscript{48} examined the presence of the R4810K mutation in patients with intracranial aortic stenosis without signs of MMD. This variant was detected in 21.9-24.3% of patients with non-MMD intracranial stenosis\textsuperscript{49,50}. Among the patients with intracranial atherosclerosis, R4810K carriers tended to be younger, more frequently female, and had a higher likelihood of a family history of proximal anterior circulation stenosis than noncarriers\textsuperscript{51}. Ischemic stroke patients with the p.R4810K were recently found to have smaller vasculature in the middle cerebral artery and reduced diameters in the common carotid, cervical internal carotid, and cervical vertebral arteries\textsuperscript{52}.

The influence of RNF213 polymorphisms extends beyond stenotic lesions in head and neck vessels. In a study involving a small number of Korean patients, p.R4810K was associated with intracranial artery dissection\textsuperscript{53}. Additionally, in a study of French Canadian participants, the RNF213 genetic end polymorphism (rs6565666) was linked to intracranial aneurysms\textsuperscript{54}. While some reports from Japan have refuted the connection between cerebral aneurysms and RNF213\textsuperscript{55}, others have identified an association between RNF213 and saccular aneurysms of the
internal carotid artery. Given that moyamoya disease primarily affects the terminal portion of the internal carotid artery, this suggests a potential vascular specificity for the action of this mutation in summary, RNF213 polymorphisms have been implicated in various vascular diseases of the head and neck region. This should be considered when evaluating and managing cerebrovascular disorders.

**RNF213 Gene and Coronary Artery Disease**

In a recent case-control study involving Japanese participants, patients with coronary artery disease had significantly more minor variant alleles than controls (2.04 vs. 0.98%; OR, 2.11; p = 0.017). After adjustment for risk factors, the association remained significant (OR, 2.90; 95% CI: 1.37-6.61; p = 0.005). In a replication study, the association remained significant after adjusting for age and sex (OR, 4.99; 95% CI: 1.16-21.53; p = 0.031), but not after further adjustments for risk factors (OR, 3.82; 95% CI: 0.87-16.77; p = 0.076). However, a subsequent genome-wide association analysis of 168,000 individuals demonstrated an association between p.R4810K and coronary disease. This association was identified alongside PCSK9, APOB, HHIPL1, and LDLR (OR, 1.61; 95% CI: 1.46-1.78; P = 2.3 x 10-21). Subsequent reports have been sporadic.

**RNF213 and Pulmonary Artery Disease**

Peripheral pulmonary artery stenosis and pulmonary arterial hypertension (PAH) are associated with RNF213-related lung vasculopathy. In a Japanese case of pulmonary arterial hypertension (PAH) are associated with peripheral pulmonary artery stenosis and pulmonary arterial disorders.

**Conclusion and Future Perspective**

In this report, we outline the relationship between RNF213, a disease susceptibility gene for moyamoya disease, and systemic diseases. The emerging concept of RNF213-related vasculopathy is gaining acceptance due to increased disease recognition and advances in genetic analysis. The p.R4810K polymorphism can lead to various disease phenotypes depending on other genetic and environmental factors. Therefore, elucidating the function of mysterin and developing disease prevention and treatment methods based on this understanding is highly desirable. Moyamoya disease presents as cerebral infarction in children, and the incidence of intracranial hemorrhage increases with progression into adulthood. Recently, surgical revascularization has been effective in preventing recurrent intracranial hemorrhage; however, its relationship with RNF213 remains unclear. This requires further research.

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**References**


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