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Filamin A Variant as a Possible Second-Hit Gene Promoting Moyamoya Disease–like Vascular Formation Associated With *RNF213* p.R4810K Variant

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Abstract

Background and Objective

The objective of this case report was to identify a second-hit gene that may promote Moyamoya disease (MMD)–like vascular formation in an individual having the *RNF213* p.R4810K variant.

Methods

We performed magnetic resonance imaging and genetic analyses of *RNF213* and *FLNA* in a 21-year-old woman, who showed Ehlers-Danlos–like symptoms and developed a first-ever unprovoked seizure, and of her healthy parents.

Results

We identified bilateral periventricular nodular heterotopia (PNH) as the cause of seizures and MMD-like vascular formation in the patient. The patient had the *RNF213* p.R4810K variant. Exome analysis identified c.4868delG in the X-linked *FLNA* gene encoding filamin A p.G1623V fs*41, which could explain PNH and Ehlers-Danlos–like symptoms. Her mother had the same *FLNA* variant and had asymptomatic bilateral PNH, whereas her father had the *RNF213* variant and had normal cerebrovascular structure.

Discussion

The family study suggested that the *FLNA* variant promoted MMD-like vascular formation in a patient having the *RNF213* variant, while the *RNF213* variant amplified the phenotypic changes elicited by the *FLNA* abnormality. Collectively, we identified a gene abnormality in filamin A, a target of RNF213-mediated proteasomal degradation, that may promote MMD-like vascular formation as a possible second-hit gene in individuals having the *RNF213* p.R4810K variant.

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Moyamoya disease (MMD) is characterized by chronic stenosis or occlusion of the terminal portion of the internal carotid artery accompanied by the formation of abnormal vascular networks. Its familial and regional prevalence in East Asia suggests genetic involvement: A genome-wide association study identified the *RNF213* (NM_001256071.3) c.14429G>A (p.R4810K) variant as a susceptible gene for MMD.[1] This variant is found in 80% of patients with MMD in Japan, whereas 1.8% of healthy controls have this variant.[2] Because MMD develops in approximately 1 per 10,000 Japanese individuals, it can be estimated that 1%–2% of patients having the variant would develop MMD. However, recent reports have demonstrated that the *RNF213* variant is frequently identified in stroke and nonstroke patients with large-artery atherosclerosis, thereby expanding its impact beyond MMD.[2] Experiments in mice with genetic deletion of *Rnf213* or carrying human *RNF213* c.14429G>A knock-in did not show any abnormal cerebrovascular structures. Therefore, it is believed that additional genetic or environmental factors are required to develop MMD in individuals having *RNF213* p.R4810K variant, that is, the two-hit theory in MMD.[3]

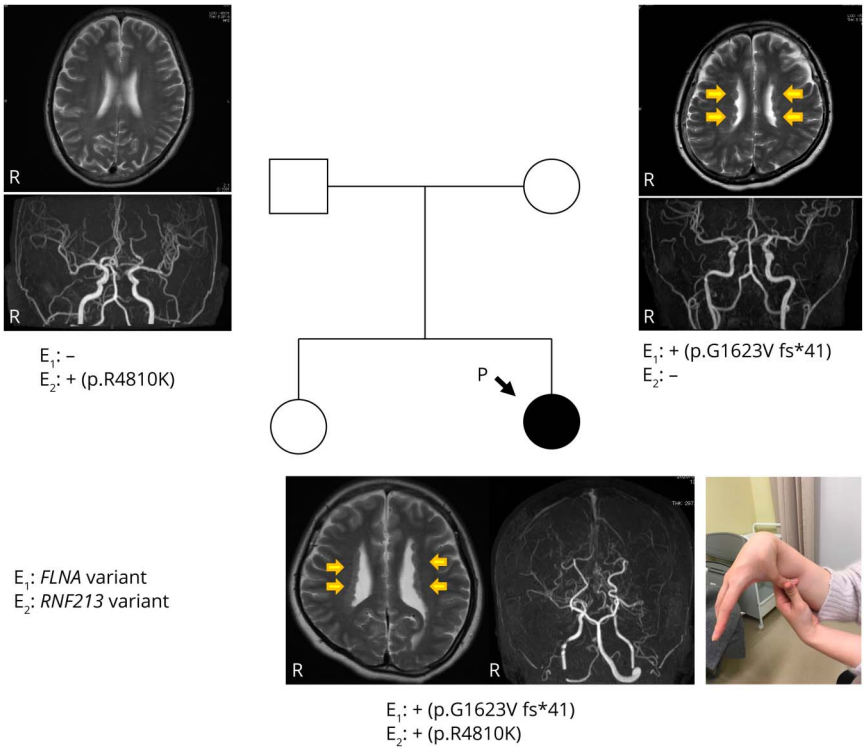
Case Description

A 21-year-old woman developed generalized tonic-clonic seizures when she visited our hospital due to headaches and transient loss of consciousness. IV administration of diazepam easily controlled seizures. She had no mental retardation or known CNS disorders except depression and migraine. We observed joint hypermobility (Figure 1) and ecchymoma in her lower limbs, with a medical history of patellar luxation and spontaneous pneumothorax. MRI revealed bilateral periventricular nodular heterotopia (PNH) (Figure 1). Magnetic resonance angiography (MRA) revealed stenosis of the terminal portion of the bilateral internal carotid artery and MMD-like abnormal vascular networks (Figure 1). Transthoracic echocardiography revealed mild aortic regurgitation. Bilateral PNH accompanied by Ehlers-Danlos–like symptoms resembled the variant phenotype of the X-linked gene *FLNA* (NM_001456.4), encoding filamin A.[4] An exome analysis of the patient identified a previously unknown heterozygous variant in *FLNA* (c.4868delG), creating a protein with Gly1623Val substitution with early termination, omitting the C-terminal 983 amino acids (p.G1623V fs*41) (Figure 2). We also sequenced *RNF213* and found the heterozygous c.14429G>A variant (Figure 2). We further performed brain MRI/MRA and genetic analyses in the patient's parents. Her mother had the same *FLNA* variant, but not the *RNF213* variant, with asymptomatic bilateral PNH and normal cerebrovascular structure (Figures 1 and 2). By contrast, her father did not show any abnormalities on MRI and MRA while having the heterozygous *RNF213* variant (Figures 1 and 2).

Discussion

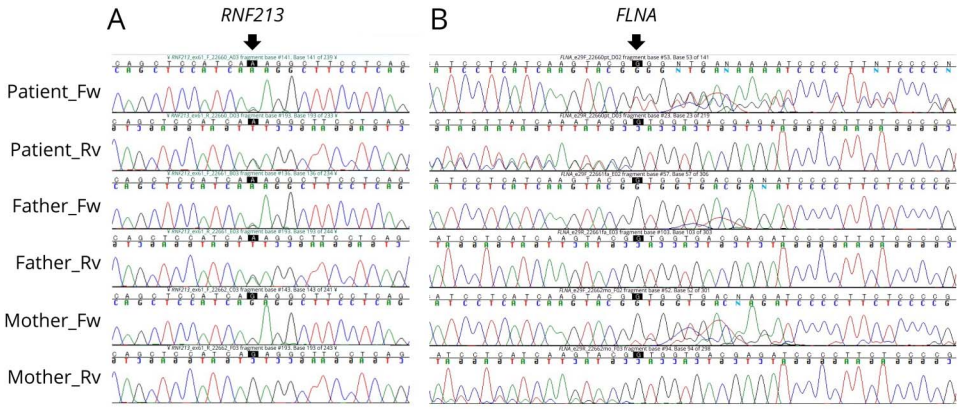
In this study, we clearly demonstrated that the patient inherited the *RNF213* variant from her father and the *FLNA* variant from her mother (Figures 1 and 2). Because only the

Figure 1 Pedigree and Neuroimaging



(Patient) Bilateral PNH (yellow arrows) on T2-weighted images and narrowing of the bilateral internal carotid artery terminal portion accompanied by MMD-like vascular formation on MRA. Hypermobility of the thumb is shown. (Mother) Asymptomatic bilateral PNH (yellow arrows) without MMD-like vascular formation and Ehlers-Danlos–like symptoms. (Father) No abnormalities on MRI/MRA. MMD = Moyamoya disease; MRA = magnetic resonance angiography; PNH = periventricular nodular heterotopia.

Figure 2 Sequencing of *RNF213* and *FLNA*

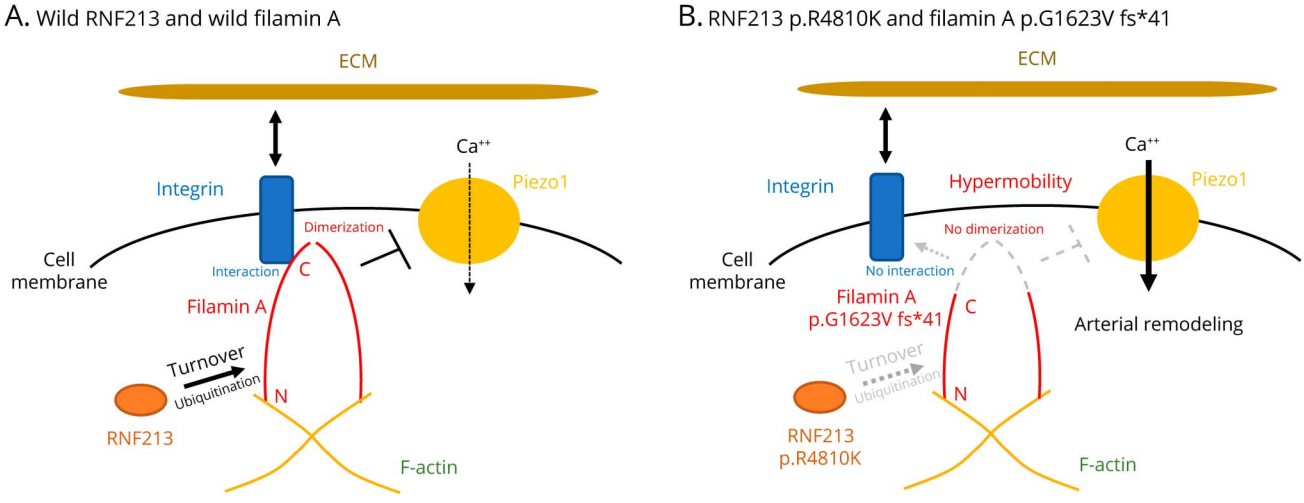


(A) A single nucleotide substitution of G to A at 14429 in the *RNF213* gene (c.14429G>A, p.Arg4810Lys) is identified in the patient and her father. (B) A single nucleotide deletion at 4868 in the *FLNA* gene (c.4868delG), leading to p.Gly1623Val fs*41 is identified in the patient and her mother. Fw = forward; Rv = reverse.

patient having the 2 variants manifested symptomatic PNH accompanied by Ehlers-Danlos–like symptoms and MMD-like vascular formation, each gene abnormality alone may cause only subtle phenotypic changes while their coexistence may mutually amplify them. It is important that the direct interaction between the 2 molecules is known: Filamin A undergoes RNF213-mediated ubiquitination and proteasomal degradation for its turnover.[2] Because ligase activity of *RNF213* p.R4810K is decreased, the abnormal filamin A may accumulate in cells expressing RNF213 and filamin A.[2] Filamin A regulates cellular mobility through its dimerization and interaction with various molecules, including integrin, via its C-terminal portion, which further interacts with extracellular matrix proteins, while the N-terminal portion participates in actin-binding and ubiquitination (Figure 3).[4] The C-terminally truncated filamin A may impair the migration of newborn neurons from the periventricular zone to the cortex during development, resulting in PNH. In adults, the filamin A variant may cause flow-mediated or pressure-mediated hypermobility of vascular smooth muscle cells, resulting in increased calcium influx through the mechanosensor channel, thereby inducing arterial remodeling and stenosis of cerebral arteries (Figure 3).[5]

We cannot completely exclude that the *FLNA* abnormality coexists independently of MMD in the patient. Even if it is related to MMD, it may be uncommon in MMD. There has

Figure 3 Putative Mechanism Underlying Hypermobility of Vascular Smooth Muscle Cells Leading to Arterial Remodeling in the Patient Having RNF213 p.R4810K and FLNA p.G1623V fs*41



(A) Filamin A forms a homodimer and interacts directly with integrin via its C-terminal portion, while its N-terminal portion interacts with filamentous actin (F-actin) and undergoes RNF213-mediated ubiquitination for its turnover. Integrin interacts with ECM, thereby preventing hypermobilization of vascular smooth muscle cells. (B) The filamin A variant with p.G1623V fs*41, which lacks its C-terminal portion, can neither form a homodimer nor interact with integrin, thereby causing hypermobilization of vascular smooth muscle cells. The hypermobilization of the cells increases the calcium influx through the mechanosensor channel Piezo1 that leads to arterial remodeling. Because the RNF213 p.R4810K variant has a decreased ubiquitin ligase activity that is required for the degradation and turnover of filamin A, the filamin A variant would accumulate and amplify its phenotypic changes as a dominant-negative form in the cells expressing the RNF213 p.R4810K variant. ECM = extracellular matrix proteins.

been 1 case series that briefly described a 3-year-old male patient having an *FLNA* abnormality among 54 patients with MMD.[6] Although *FLNA* variants presenting with PNH often show X-linked dominant inheritance, we should note that some *FLNA* variants are transmitted in X-linked recessive mode and their phenotypic changes may be affected by X-inactivation.[7]

Collectively, we identify *FLNA* as a possible second-hit gene that may affect MMD-like vascular formation in an individual having the *RNF213* p.R4810K variant. RNF213 also functions as an E3-ubiquitin ligase for itself and other molecules, including NFAT.[2] Moreover, RNF213 contributes to lipid metabolism.[2] Thus, besides *FLNA*, there may be other *RNF213*-related genes whose abnormality can elicit MMD-like vascular formation associated with the *RNF213* p.R4810K variant.

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