# RNF213 in Moyamoya Disease Pathogenesis

The aim of a retrospective study was to investigate the RNF213 genotype in patients with MMD and to determine their genotype-phenotype associations.

The study involved 165 Korean MMD patients from 155 unrelated families who were diagnosed with MMD at a single center from 1995 to 2013. Their demographic, radiological, and clinical findings were evaluated. Direct sequencing of the major RNF213 single nucleotide polymorphisms was performed. The association of the common RNF213 variant with MMD risk was evaluated using historical controls for comparison. Correlations between RNF213 genotype and phenotype were statistically analyzed.

The c.14429G>A (p.R4810K) variant was identified in 125 (75.8%) of 165 MMD patients. Most patients (112) were heterozygous, and 13 patients had 2 copies of the c.14429G>A variant. A novel heterozygous variant, c.12086A>G (p.Q4029R), was found in 1 additional patient. The minor allele frequency of the c.14429G>A variant was significantly higher in the MMD group (138 [41.8%] of 330 patients) than in the control group (8 [1.36%] of 588 subjects; $p < 0.001$). The c.14429G>A (p.R4810K) variant significantly increased the risk of MMD in Korean patients, with an OR of 52.11 ($p < 0.001$) compared with controls. Moreover, c.14429G>A (p.R4810K) genotypes occurred more frequently in patients with a family history of MMD. The homozygous variant was highly associated with early-onset MMD (age at onset < 5 years), cerebral infarction at diagnosis, and cognitive impairment in long-term outcome.

The findings indicate that the c.14429G>A (p.R4810K) allele of RNF213 is strongly associated with Korean patients with MMD. The homozygous c.14429G>A (p.R4810K) variant is particularly related to early-onset MMD, severe symptomatic manifestations at diagnosis, and poor prognosis. This genotypic variant may be a useful biomarker for early-onset MMD or unstable MMD with cerebral infarction, which requires early diagnosis and revascularization treatment [1].

---

Ito et al. aimed to investigate the association between RNF213 founder polymorphism (p.R4810K) and each direct and indirect collateral development. By qualitative and quantitative evaluations of direct and indirect surgical collaterals using time-of-flight MR angiography, the postoperative development of each type of bypass was evaluated independently into two categories. Multivariate logistic regression analysis was performed to study the contributing factors for the development of each surgical collateral. Excellent development of postoperative direct and indirect bypass was observed in 65 hemispheres (70%) by qualitative evaluation, which was confirmed by quantitative evaluation. Multivariate logistic regression analysis of excellent indirect bypass development revealed a significant positive correlation with the p.R4810K (odds ratio, OR4.0; 95%-confidence interval, CI 1.2-16), advanced MR angiographic stage (OR9.5; 95%CI 1.7-73), and preoperative middle meningeal artery caliber (OR6.8; 95%CI 1.8-35), but a significant negative correlation was found with the excellent direct bypass development (OR0.17; 95%CI 0.03-0.75). No significant correlation was observed between excellent direct bypass development and the p.R4810K (OR0.95; 95%CI 0.37-2.4).In conclusion, excellent development of indirect collaterals after STA-MCA anastomosis combined with indirect pial synangiosis occurs more frequently in adult Moyamoya disease (MMD) with the RNF213 founder polymorphism, suggesting a role of the p.R4810K variant for marked in-growth of indirect collaterals and the utility of preoperative genetic analysis [2].

A variant in the Ring Finger 213 gene (RNF213), altering arginine at position 4810 (p.R4810K), is associated with Moyamoya disease MMD in Asian populations.

However, there are a lack of data on the role of RNF213 in patients with MMD of additional ethnicities and diasporic Asian populations.

Cecchi et al., investigate the contribution of RNF213 alterations to MMD in an ethnically diverse population based in the United States.

Results confirm that alterations in RNF213 predispose patients of diverse ethnicities to MMD, and that the p.R4810K variant predisposes individuals of Asian descent in the United States to MMD [3].

The exact mechanism by which the RNF213 abnormality leads to MMD remains unknown. Ito et al. sought to clarify the role of RNF213 in angiogenesis under ischemic conditions using conventional RNF213 knockout mice and assessed the infarction volume, cerebral edema, and vascular density in the ischemic brain after transient middle cerebral artery occlusion (tMCAO). To further evaluate systemic angiogenesis following chronic ischemia, they investigated blood flow recovery using laser speckle flowmetry, the severity of ambulatory impairments, and vascular density in the hind-limb after permanent femoral artery ligation. Results were compared between homozygous RNF213 knockout mice (RNF213 -/-) and wild-type littermates (Wt). No significant differences were observed in infarction volume or the formation of edema following tMCAO, or in vascular density 28 days after tMCAO between RNF213 -/- and Wt. Blood flow recovery was significantly improved in RNF213 -/- from 3 to 28 days after femoral artery ligation, and angiogenesis as shown by vascular density in the hind-limb was significantly enhanced in RNF213 -/- at 28 days. The amelioration of ambulatory impairments was also evident in RNF213 -/-. Angiogenesis was enhanced in mice lacking RNF213 after chronic hind-limb ischemia, which suggested the potential role of the RNF213 abnormality in the development of pathological vascular networks in chronic ischemia [4].

---

The p.R4810K (rs112735431) variant is a founder polymorphism that is strongly associated with moyamoya disease in East Asia. Many non-p.R4810K rare variants of RNF213 have been identified in white moyamoya disease patients, although the ethnic mutations have not been investigated in this population. In the present study, we screened for RNF213 variants in 19 Slovakian and Czech moyamoya disease patients. A total of 69 RNF213 coding exons were directly sequenced in 18 probands and one relative who suffered from moyamoya disease in Slovakia and the Czech Republic. We previously reported one proband harboring RNF213 p.D4013N. Results from the present study identified four rare variants other than p.D4013N (p.R4019C, p.E4042K, p.V4146A, and p.W4677L) in four of the patients. P.V4146A was determined to be a novel de novo mutation, and p.R4019C and p.E4042K were identified as double mutations inherited on the same allele. P.W4677L, found in two moyamoya disease patients and an unaffected subject in the same pedigree, was a rare single nucleotide polymorphism. Functional analysis showed that RNF213 p.D4013N, p.R4019C and p.V4146A-transfected human umbilical vein endothelial cells displayed significant lowered migration, and RNF213 p.V4146A significantly reduced tube formation, indicating that these are disease-causing mutations. Results from the present study identified RNF213 rare variants in 22.2% (4/18 probands) of Slovakian and Czech moyamoya disease patients, confirming that RNF213 may also be a major causative gene in a relative large population of white patients [5].

Although RNF213 is a risk factor for moyamoya disease in East Asians, Zhou et al., demonstrated that it might also be a risk factor for IA in the FC population. It, therefore, appears that the function of RNF213 can be differently altered to predispose distinct populations to dissimilar neurovascular conditions, highlighting the importance of a population's background in genetic studies of heterogeneous disease [6].

1)
Kim EH, Yum MS, Ra YS, Park JB, Ahn JS, Kim GH, Goo HW, Ko TS, Yoo HW. Importance of RNF213 polymorphism on clinical features and long-term outcome in moyamoya disease. J Neurosurg. 2015 Oct 2:1-7. [Epub ahead of print] PubMed PMID: 26430847.
2)
Ito M, Kawabori M, Sugiyama T, Tokairin K, Tatezawa R, Uchino H, Kazumata K, Houkin K, Fujimura M. Impact of RNF213 founder polymorphism (p.R4810K) on the postoperative development of indirect pial synangiosis after direct/indirect combined revascularization surgery for adult Moyamoya disease. Neurosurg Rev. 2022 Feb 7. doi: 10.1007/s10143-022-01749-9. Epub ahead of print. PMID: 35128622.
3)
Cecchi AC, Guo D, Ren Z, Flynn K, Santos-Cortez RL, Leal SM, Wang GT, Regalado ES, Steinberg GK, Shendure J, Bamshad MJ; University of Washington Center for Mendelian Genomics, Grotta JC, Nickerson DA, Pannu H, Milewicz DM. RNF213 rare variants in an ethnically diverse population with Moyamoya disease. Stroke. 2014 Nov;45(11):3200-7. doi: 10.1161/STROKEAHA.114.006244. Epub 2014 Oct 2. PubMed PMID: 25278557.
4)
Ito A, Fujimura M, Niizuma K, Kanoke A, Sakata H, Morita-Fujimura Y, Kikuchi A, Kure S, Tominaga T. Enhanced post-ischemic angiogenesis in mice lacking RNF213; a susceptibility gene for moyamoya disease. Brain Res. 2015 Jan 12;1594:310-20. doi: 10.1016/j.brainres.2014.11.014. Epub 2014 Nov 13. PubMed PMID: 25446450.
5)
Kobayashi H, Brozman M, Kyselová K, Viszlayová D, Morimoto T, Roubec M, Školoudík D, Petrovičová A, Juskanič D, Strauss J, Halaj M, Kurray P, Hranai M, Harada KH, Inoue S, Yoshida Y, Habu T, Herzig R, Youssefian S, Koizumi A. RNF213 Rare Variants in Slovakian and Czech Moyamoya Disease Patients. PLoS One. 2016 Oct 13;11(10):e0164759. doi: 10.1371/journal.pone.0164759. PubMed PMID: 27736983.
6)
Zhou S, Ambalavanan A, Rochefort D, Xie P, Bourassa CV, Hince P, Dionne-Laporte A, Spiegelman D, Gan-Or Z, Mirarchi C, Zaharieva V, Dupré N, Kobayashi H, Hitomi T, Harada K, Koizumi A, Xiong L, Dion PA, Rouleau GA. RNF213 Is Associated with Intracranial Aneurysms in the French-Canadian Population. Am J Hum Genet. 2016 Sep 30. pii: S0002-9297(16)30379-2. doi: 10.1016/j.ajhg.2016.09.001. PubMed PMID: 27745834.