



Pathophysiology of Moyamoya Disease

6

Seung-Ki Kim, Ji Yeoun Lee, and Kyu-Chang Wang

6.1 Introduction

Moyamoya disease (MMD) is an occlusive cerebrovascular disease that is characterized by idiopathic and progressive stenosis of the distal portion of major intracranial arteries with abnormal basal collaterals (“moyamoya” vessels) [1]. The internal carotid artery (ICA) and its major branches (anterior carotid artery [ACA] and middle cerebral artery [MCA]) are mainly involved, but basilar artery (BA) and posterior cerebral artery (PCA) stenosis is also detected in some patients, especially during follow-up [2]. When the vascular changes are associated with well-recognized conditions, it is called moyamoya syndrome (MMS). As the disease is defined by the radiological morphology of vessels, MMD may not be a homogeneous entity but may instead comprise a heterogeneous group of conditions.

A diagnosis is made based on symptoms and radiologic evaluation. The vessel morphology and presence of infarction are evaluated using brain magnetic resonance imaging (MRI) and magnetic resonance angiography (MRA) [3, 4]. Transfemoral cerebral angiography is the gold standard modality to confirm the diagnosis. The regional blood flow status of the brain is usually checked with perfusion MRI or single photon emission computed tomography (SPECT).

The prevalence of MMD shows a bimodal age pattern: once in mid-childhood and again during the late 40s [3]. The presentation and treatment policy of pediatric and adult MMD are different. Hemorrhagic presentation is very rare in children, but it is not uncommon in adult patients [5].

The common sites of intracranial hemorrhage are the basal ganglia, thalamus, and near the lateral ventricle wall, so possible role of moyamoya vessels has been suspected. Some reports have shown that in cases with no moyamoya vessel dilatation, other arteries such as anterior choroidal artery or posterior communicating artery supplying the hemorrhage area showed dilatation [6]. According to morphometric evaluation using autopsy specimens, the dilated arteries in MMD seem to have fibrosis and attenuation of the media, with irregular segmentation of the elastic lamina. Under the condition of hemodynamic

S.-K. Kim (✉) · K.-C. Wang
Department of Neurosurgery, Seoul National University Hospital, Seoul National University College of Medicine, Seoul, South Korea
e-mail: nstomas@snu.ac.kr

J. Y. Lee
Department of Anatomy, Seoul National University College of Medicine, Seoul, South Korea

Department of Neurosurgery, Seoul National University Hospital, Seoul, South Korea

stress or aging, the attenuated walls in these dilated arteries may be predisposing factor for microaneurysm formation. The rupture of the microaneurysm is hypothesized to cause bleeding in MMD patients [7].

For pediatric patients, indirect revascularization is the primary treatment of choice, but in adults, it is controversial whether indirect revascularization is equivalent to direct bypass procedures [5, 8–14].

Much work has been done to elucidate the pathophysiology of MMD over the years. Due to the inaccessibility of the involved vessel during surgery, blood, cerebrospinal fluid (CSF), and urine have been used for molecular and genomic analyses. The attempts to establish an animal model of the disease have yet to be successful, but recent technical advances in genomic analysis have shed light on the field. This chapter covers the current knowledge of the pathophysiology of MMD in various aspects: proteins (growth factors and cytokines), cells, autoimmunity, biomechanics, and genes.

6.2 Pathology

Autopsy specimens of the stenotic arteries revealed that the fibrocellular thickening of the intima, an irregular undulation (“waving”) of the internal elastic lamina, and attenuation of media are the main histopathological findings of MMD. Smooth muscle cell hyperplasia is typically found in the thickened intima. These smooth muscle cells are considered to be the synthetic type and to migrate from the media [15–17]. In contrast to atherosclerosis, inflammatory cell infiltration and the presence of lipid-laden foamy macrophages are not found in the stenotic vessels of MMD.

The moyamoya vessels, the basal collaterals that are dilated perforating arteries, show fibrin deposits in the wall, fragmented elastic lamina, attenuated media, and the formation of microaneurysm [16, 18].

6.3 Cytokines

As the hallmark of the vessel pathology is the proliferation of smooth muscle cells in the intima of the arteries, the involvement of growth factors has been suspected. In addition, both the formation of basal collaterals during disease progression and the extensive neovascularization that is seen after indirect bypass surgery, which involves the mere relocation of a vascularized tissue on the surface of the brain cortex, imply the potential role of an aberrant “abundance” of angiogenic factors in MMD patients [19]. Hence, one of the earliest studies to investigate the pathophysiology of MMD examined the expression levels of various cytokines that were quantified in the CSF, serum, or tissue of MMD patients.

Increased levels of growth factors such as basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF), hypoxia-inducing factor-1 α (HIF-1 α), granulocyte colony-stimulating factor (GCSF), transforming growth factor- β (TGF- β), and vascular endothelial growth factor (VEGF) have been observed in the CSF and serum of MMD patients [20–24].

The cytokines associated with angiogenesis and vascular repair have also been evaluated. Matrix metalloproteinases-9 (MMP-9) was increased, and the tissue inhibitor of metalloproteinase (TIMP1) was decreased in the serum of MMD patients, suggesting a disruption in the balance between the two. The imbalance may cause aberrant vascular smooth muscle cell dynamics, which can lead to MMD [20, 21].

Increased levels of the cellular retinoic acid-binding protein-1 (CRABP-1) were detected in the CSF of MMD patients [23]. CRABP-1 is one of the proteins that mediates the biological activity of retinoic acid (RA) and may provide a link between RA signaling and MMD pathogenesis. As retinoids are known to negatively regulate growth factor-stimulated vascular smooth muscle cell proliferation and differentiation, it can be hypothesized that increases in CRABP-1 may inhibit the RA signaling action, resulting in an

increase in vascular smooth muscle cell proliferation [23].

It should be noted that although an abundant amount of data implies an important role for the various cytokines in MMD, there is possibility that the differences in the expression levels of cytokines may actually be a compensatory response to the pathology rather than the cause.

6.4 Circulating Progenitor Cells

Investigating the vessel wall cells (endothelial-lineage or smooth muscle type) of MMD patients may be a direct approach to evaluate the pathogenesis of the disease. However, it is nearly impossible to obtain the cells directly from patients. Fortunately, vascular progenitor cells can be derived from the peripheral blood of patients, thus providing an experimental cell model (Fig. 6.1) [25].

Endothelial progenitor cells (EPCs) have been evaluated extensively in many vascular diseases before MMD because these cells are known to originate from the bone marrow and help maintain the vasculature and blood flow in infarcted areas [26–30]. EPCs are commonly characterized by the expression of the surface proteins CD34, CD133, and vascular endothelial growth factor receptor-2 (VEGFR-2). Some studies also con-

sider CD45 and CD114 to be additional markers for defining EPC [31, 32]. Two studies have reported on the EPC count in the peripheral blood of MMD patients. One study, which included mainly adult patients, showed that MMD patients had more EPCs than the normal controls did [29], whereas another study with pediatric patients demonstrated a decreased number of EPCs in patients [33]. The contradictory results may be due to differences in the patients' age groups or in the markers used to isolate EPCs [34]. Additionally, the number of colony-forming units (CFU) of EPCs was shown to be decreased and that of outgrowth cells was increased in MMD patients; these changes were more profound in those patients with advanced diseases. Further, the tube formation ability was significantly decreased in the late EPCs from MMD patients, suggesting the dysfunction of the EPCs of MMD patients [29, 33]. More direct evidence on the contribution of EPCs in MMD was provided when cells expressing CD34 and VEGFR2 were found in the thickened intima of distal ICA samples collected from MMD patients [35].

The role of EPCs in MMD was further analyzed in a recent study that compared the gene expression profiles of EPCs from MMD patients and normal controls. A downregulation of retinaldehyde dehydrogenase 2 (Raldh2), which is one of the enzymes in the physiological process

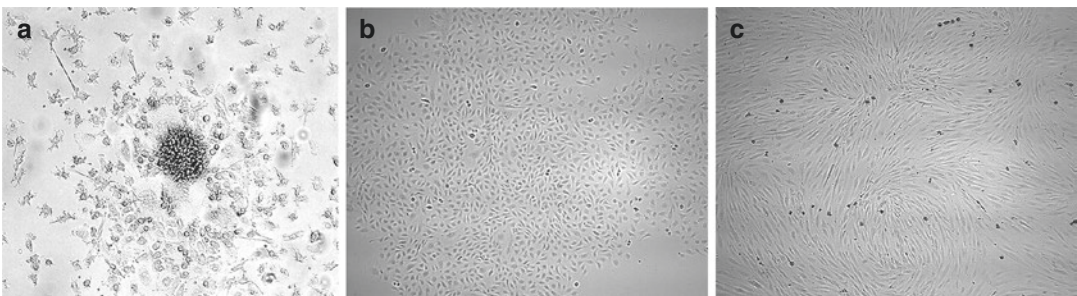


Fig. 6.1 Vascular progenitor cells. (a) Early endothelial progenitor cells (EPCs), also called colony-forming units or cell clusters ($\times 200$). This cell cluster is composed of a central core of round cells that are surrounded by spindle-shaped cells. (b) Late EPCs, also known as endothelial

outgrowth cells ($\times 40$). The cells are arranged in cobblestone-like formations. (c) Smooth muscle progenitor cells (SPCs) ($\times 40$). These cells appear elongated and have a typical hill-and-valley appearance. Adapted from Journal of Korean Neurosurgical Society [25]

to convert RA from retinol, was noted. Based on the previous link between RA signaling and MMD, the effect of Raldh2 on the function of EPCs was analyzed. It was shown that decreased levels of Raldh2 in normal EPCs resulted in poor tube formation, similar to EPCs from MMD. Furthermore, the disrupted tube formation capacity was restored by supplementation with exogenous RA in both MMD EPCs and the normal EPCs with downregulated Raldh2 [36].

The possible role of smooth muscle progenitor cells (SPCs) in MMD has recently been evaluated to determine the origin of smooth muscle cell hyperplasia in the thickened intima. SPCs differ from EPCs in the appearance of the outgrowth cells, namely, “hill-and-valley” for the former and “cobblestone” for the latter (Fig. 6.1) [37]. Additionally, SPCs stain positive for smooth muscle-specific markers, such as smooth muscle actin- α , smooth muscle myosin heavy chain (MHC), and calponin [38]. The SPCs from MMD expressed lower levels of platelet-derived growth factor receptor (PDGF)- α , MHC, and calponin than did normal controls. Furthermore, in the tube formation assay, MMD SPCs made more irregular and thick tubes, reminiscent of the pathologic arteries in MMD patients (Fig. 6.2) [39]. These findings suggest that SPCs of MMD patients may

have a defect in the cell maturation process that results in dysfunctional vasculogenesis.

A recent study investigated the interaction between the precursors of the two major cellular components’ vessel walls. The results showed that EPCs are mostly responsible for the dysfunction of both EPCs and SPCs in MMD and that the enhanced migration of the SPCs to the EPCs is mediated by the release of chemokine (C-C motif) ligand 5 (CCL5) by the EPCs [40].

6.5 Immunologic Factors

The role of immune function in the pathogenesis in MMD has been hypothesized based on its association with immune diseases [41]. Many studies have reported on cases of MMS associated with Graves’ disease, an autoimmune disease that causes hyperthyroidism [42]. A recent meta-analysis on the literature has clearly demonstrated a close relationship between thyroid function and MMS [43]. Most of the cases were females, and most of the patients were suffering from active hyperthyroidism when they were diagnosed with MMS. Moreover, recurrence or aggravation of the MMS symptoms was more common in patients with poorly controlled thy-

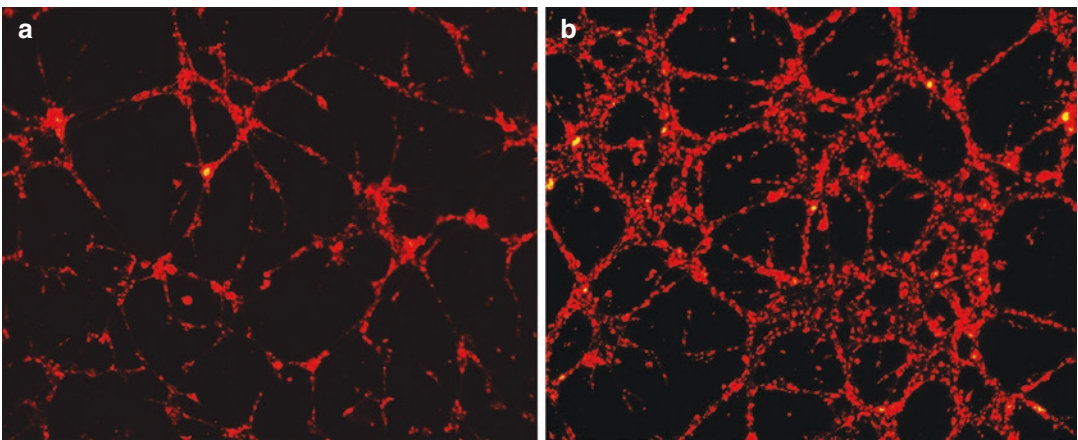


Fig. 6.2 The networks formed by smooth muscle progenitor cells (SPCs). Photomicrographs showing immunofluorescence staining and tubule formation of SPCs on Matrigel. The cells are labeled with red fluorescent dye (PKH26). Compared with the SPCs obtained from a

healthy volunteer (a), the SPCs obtained from a moyamoya disease patient (b) have rather irregularly arranged tubules of varying sizes. In some areas, thickened tubules are noted. Adapted from Journal of Korean Neurosurgical Society [25]

roid function [43]. The fact that abnormal cerebrovascular autoregulation associated with sympathetic nerve excitation has been known to occur in patients with hyperthyroidism suggests a potential mechanism underlying the role of autoimmunity in MMD pathogenesis [44]. However, the possibility that hemodynamic stress caused by the thyrotoxicosis instead of autoimmune dysfunction still stands as the cause of vascular changes in these patients.

Antiphospholipid syndrome (APS) consists of various conditions that are caused by the presence of antiphospholipid (APL) autoantibodies, and systemic lupus erythematosus (SLE) is a representative disease in APS. MMS patients with SLE or increased APL autoantibodies have been reported [45]. Also some SLE patients with “lupus headaches” have been investigated for the possibility of underlying cerebral ischemia and MMS-like changes [46].

Studies to search for more direct evidence of the involvement of immunity in MMD have been performed over the last several years. An increase in the number of thyroid-related autoantibodies (but with normal thyroid function) in MMD patients has been demonstrated in both Asians and Caucasians. The recent reports are different from previous ones, in which the patients showed both hyperthyroidism and increased autoantibodies, thus providing supportive evidence that autoimmunity, instead of thyroid dysfunction, may be the main contributor in MMS [42].

6.6 Biomechanical Factors

The regional predilection of MMD to only involve the distal portion of major intracranial arteries (ICA and BA) is a distinguishing characteristic of the disease [44]. However, most studies on the pathophysiology of MMD have only been able to investigate systemic factors, which does not address the question of why certain arteries are involved. Biomechanical factors, including hemodynamic changes and the properties of the intracranial vasculature, have been hypothesized as one of the etiologies [4]. Many techniques, such as perfusion MRI, SPECT, and transcranial

Doppler, have been used to predict the regional blood perfusion and hemodynamic changes in MMD, but the data were only useful for detecting the “resulting” changes in regional cerebral perfusion [47–49].

Computerized mathematical models have been used to analyze the hemodynamics of cerebral arteries [50]. Only one study is available in the literature, and it utilized computational modeling to elucidate the underlying mechanism of the regional specificity of MMD [51]. Based on data obtained for cardiovascular diseases, the authors hypothesized that a chronic, constant state of relatively low shear stress causing turbulent flow to the vessel wall may result in the stimulation and proliferation of smooth muscle cells of the intima of the vessel. After establishing models of ICA and BA with the respective distal branches of ACA/MCA and PCA, the shear stress at various points, including the predisposing areas of stenosis at the bifurcation of distal branches, was determined. The stimulation results revealed that the predisposed areas showed lower values of shear stress than do regions of ICA or BA that are more distant from the bifurcation. Although the computational models have a critical limitation in recapitulating the real *in vivo* phenomenon, this study is noteworthy because it shows the potential biomechanics to explain the regional predilection of MMD. A retrospective clinical investigation on the delayed involvement of PCA suggested that a smaller angle between PCA and BA is significantly associated with the progressive stenosis of PCA [52]. This is in line with the computational modeling, as a smaller angle between the vessels may result in less shear stress.

6.7 Genetics

A strong predisposition for ethnicity and the existence of familial cases (10–15%) suggest a genetic susceptibility of the disease [34, 44]. Different modes of inheritance have been proposed, including an autosomal dominant pattern with incomplete penetrance in several parent-child cases and an autosomal recessive pattern in

sibling pedigrees [53]. Various technical methods have been applied and have provided clues (candidate loci or gene) for the genetic etiology since the late 1990s. The interpretation of the genetic findings was difficult because the results were infrequently replicated in following studies. Additionally, genetic analysis on Caucasians has yet to be successful in identifying associated genes. Recent high-throughput genomic analysis has yielded a gene (*Ring finger 213* [RNF213]) with a strong robust association, but the mechanism of the mutation in the pathogenesis of MMD remains unknown.

Linkage studies using genome-wide markers or previously reported loci have revealed several candidate loci: 3p24-26, 6q25, 8q21-22, 12p12-13, and 17q25 [54–57]. Only the 17q25 locus was replicated, and further genome-wide linkage analysis narrowed the candidate locus to 17q25.3. Many case-controlled association studies were performed and screened genes based on various hypotheses about the pathogenesis of the disease. As the possible contribution of autoimmune dysfunction in MMD has been suggested, the association of human leukocyte antigen (HLA)-related genes was investigated first. A significant association with HLA B51 and HLA B51-DR4 was shown in Japanese patients and HLA B35 in Korean patients [58–60]. In European patients, the HLA DRB1*03, DRB1*13, DRA*02, DRB*08, and DQB1*03 antigens were more frequent in patients than they were in controls [61].

The next set of genes of interest was based on the increase in the amount of growth factors and cytokines in the blood, CSF, urine, or tissues of patients. Some studies found associations with single-nucleotide polymorphisms (SNPs) in PDGFR- β promoter or TGF- β [62]. Additionally, associations with SNPs in genes related to MMPs and their tissue inhibitors have been demonstrated (TIMP2, MMP2, MMP3 genes or their promoters) [22, 63].

In 2011, two groups reported the RNF213 gene located in chromosome 17q25.3 as the strongest susceptibility gene for MMD in East Asian (Japanese and Korean) patients using two different approaches (genome-wide association

study [GWAS] or whole-exome sequencing [WES]) [64, 65]. A GWAS detected a variant of the RNF213 (p.R4810K) in 95% of familial MMD patients, with 80% in sporadic cases, and demonstrated that the variant strongly increased the risk to develop MMD, with an odds ratio (OR) greater than 190 [65]. Another study utilizing genome-wide linkage analysis and WES in eight multigenerational families revealed that all of the probands had this variant. The variant was also detected in 1.4–2.4% of normal Asian controls, thus providing a possible explanation for the extreme ethnic gradient in disease prevalence [64]. The potentially strong contribution of the variant was further supported by the fact that patients with homozygous mutations of the p. R4810K variant have an earlier age of onset and more severe phenotypes than do those with heterozygous mutations [66]. Recent studies have also revealed novel variants in RNF213 in a small number of Caucasian and Chinese cases [67].

RNF213 encodes for a cytosolic protein with a really interesting new gene (RING) finger domain and a pair of two ATPase associated with a variety of cellular activities (AAA) positive ATPase modules and is speculated to have ubiquitin ligase activity [68]. The functional consequence of the mutation in RNF213 is under intensive evaluation. In an in vitro functional study, the mutation had no effect on the transcription level or ubiquitin ligase activity of the protein [64]. However, in a more recent study, the vascular endothelial cells derived from induced pluripotent stem cells were compared between normal controls and both MMD patients and RNF213 mutant carriers [69]. The latter group showed decreased angiogenic activity compared with the former. Additional experiments have shown the association of RNF213 with securin, the interferon (IFN)-beta signaling pathway, and PI3 kinase-AKT pathway in endothelial cells [69]. A series of in vivo experiments using genetically engineered animals have been performed. Knockdown of RNF213 in zebrafish resulted in abnormal vascular development, showing irregular wall formation and abnormal sprouting vessels [64]. However, RNF213-deficient mice did not show any anatomical or histopathological

evidence of MMD in the brain vasculature under physiological conditions [70]. Another experiment with mice harboring the p.R4810K mutation was not successful in developing MMD under normal conditions [71]. Recently, the environmental setting was added as a crucial component in the experiment by exposing the RNF213-deficient mice to a chronic, ischemic insult. Post-ischemic angiogenesis was found to be enhanced in RNF213-deficient mice in response to chronic hind limb ischemia [72]. Nevertheless, because the role of RNF213 as the causative mutation has not been demonstrated, despite its robustness, the possibility that another causative gene is still to be found should be considered by evaluating the entire genome using whole-genome sequencing.

Investigation of the genes known to be related to other vascular diseases (e.g., coronary artery disease, stroke) or associated diseases in MMS (achalasia) has shown other potential genes. Smooth muscle alpha-actin (ACTA2) is a major component of the contractile apparatus of smooth muscle cells, and mutations of the ACTA2 gene have been known to cause familial thoracic aortic aneurysm and dissections. It was also found to be associated with pseudo-moyamoya angiopathy [73]. Mutations of the guanylate cyclase soluble subunit alpha-3 (GUCY1A3) gene have been found in MMD patients with achalasia. Loss of function mutations of this gene lead to alterations of the nitric oxide pathway in smooth muscle cells, thus supporting the possible pathogenetic mechanism of MMD [74].

Conclusions

MMD is probably caused by a combination of complex etiologies, genetics, and environmental factors. Genetic susceptibility, including RNF213 and many other factors such as the RA pathway, autoimmune process, environmental factors, and hemodynamic stress, may be related to increased levels of growth factors and cytokines. Through mechanisms that are still to be elucidated, the systemic factors and conditions may lead to the proliferation and migration of SMC into the vessel wall, thereby causing thickening of the intima.

The low shear stress in the bifurcation regions may lead to the involvement of only the distal ICA and BA in MMD. Establishing an animal model will shed light on integration of the various, independent factors.

References

1. Suzuki J, Takaku A. Cerebrovascular "moyamoya" disease. Disease showing abnormal net-like vessels in base of brain. *Arch Neurol*. 1969;20:288–99.
2. Lee JY, Choi YH, Cheon JE, et al. Delayed posterior circulation insufficiency in pediatric moyamoya disease. *J Neurol*. 2014;261:2305–13.
3. Fukui M. Guidelines for the diagnosis and treatment of spontaneous occlusion of the circle of Willis ('moyamoya' disease). Research Committee on Spontaneous Occlusion of the Circle of Willis (Moyamoya Disease) of the Ministry of Health and Welfare, Japan. *Clin Neurol Neurosurg*. 1997; 99(Suppl 2):S238–240.
4. Lee M, Zaharchuk G, Guzman R, et al. Quantitative hemodynamic studies in moyamoya disease: a review. *Neurosurg Focus*. 2009;26:E5.
5. Kim SK, Wang KC, Kim IO, et al. Combined encephaloduroarteriosynangiosis and bifrontal encephaloplegio (periosteal) synangiosis in pediatric moyamoya disease. *Neurosurgery*. 2008;62:1456–64.
6. Morioka M, Hamada JI, Kawano T, et al. Angiographic dilatation and branch extension of the anterior choroidal and posterior communicating arteries are predictors of hemorrhage in adult Moyamoya patients. *Stroke*. 2002;34:90–5.
7. Yamashita M, Oka K, Tanaka K. Histopathology of the brain vascular network in moyamoya disease. *Stroke*. 1983;14:50–8.
8. Imaizumi T, Hayashi K, Saito K, et al. Long-term outcomes of pediatric moyamoya disease monitored to adulthood. *Pediatr Neurol*. 1998;18:321–5.
9. Hirotsune N, Meguro T, Kawada S, et al. Long-term follow-up study of patients with unilateral moyamoya disease. *Clin Neurol Neurosurg*. 1997;99(Suppl 2):S178–81.
10. Golby AJ, Marks MP, Thompson RC, et al. Direct and combined revascularization in pediatric moyamoya disease. *Neurosurgery*. 1999;45:50–8.
11. Ozgur BM, Aryan HE, Levy ML. Indirect revascularization for paediatric moyamoya disease: the EDAMS technique. *J Clin Neurosci*. 2006;13:105–8.
12. Rashad S, Fujimura M, Niizuma K, et al. Long-term follow-up of pediatric moyamoya disease treated by combined direct-indirect revascularization surgery: single institute experience with surgical and perioperative management. *Neurosurg Rev*. 2016;39:615–23.
13. Sainte-Rose C, Oliveira R, Puget S, et al. Multiple bur hole surgery for the treatment of moyamoya disease in children. *J Neurosurg*. 2006;105:437–43.

14. Ueki K, Meyer FB, Mellinger JF. Moyamoya disease: the disorder and surgical treatment. *Mayo Clin Proc.* 1994;69:749–57.
15. Lin R, Xie Z, Zhang J, et al. Clinical and immunopathological features of Moyamoya disease. *PLoS One.* 2012;7:e36386.
16. Kuroda S, Houkin K. Moyamoya disease: current concepts and future perspectives. *Lancet Neurol.* 2008;7:1056–66.
17. Takagi Y, Kikuta K, Nozaki K, et al. Histological features of middle cerebral arteries from patients treated for Moyamoya disease. *Neurol Med Chir (Tokyo).* 2007;47:1–4.
18. Czabanka M, Pena-Tapia P, Schubert GA, et al. Characterization of cortical microvascularization in adult moyamoya disease. *Stroke.* 2008;39:1703–9.
19. Kim SJ, Son TO, Kim KH, et al. Neovascularization precedes occlusion in moyamoya disease: angiographic findings in 172 pediatric patients. *Eur Neurol.* 2014;72:299–305.
20. Fujimura M, Watanabe M, Narisawa A, et al. Increased expression of serum matrix Metalloproteinase-9 in patients with moyamoya disease. *Surg Neurol.* 2009;72:476–80.
21. Kang HS, Kim JH, Phi JH, et al. Plasma matrix metalloproteinases, cytokines and angiogenic factors in moyamoya disease. *J Neurol Neurosurg Ps.* 2010;81:673–8.
22. Kang HS, Kim SK, Cho BK, et al. Single nucleotide polymorphisms of tissue inhibitor of metalloproteinase genes in familial moyamoya disease. *Neurosurgery.* 2006;58:1074–80.
23. Kim SK, Yoo JI, Cho BK, et al. Elevation of CRABP-I in the cerebrospinal fluid of patients with Moyamoya disease. *Stroke.* 2003;34:2835–41.
24. Park YS, Jeon YJ, Kim HS, et al. The role of VEGF and KDR polymorphisms in moyamoya disease and collateral revascularization. *PLoS One.* 2012;7:e47158.
25. Kang HS, Wang KC, Kim SK. Circulating vascular progenitor cells in Moyamoya disease. *J Korean Neurosurg Soc.* 2015;57:428–31.
26. Fadini GP, Agostini C, Avogaro A. Endothelial progenitor cells in cerebrovascular disease. *Stroke.* 2005;36:1112–3.
27. Fadini GP, Coracina A, Baesso I, et al. Peripheral blood CD34+KDR+ endothelial progenitor cells are determinants of subclinical atherosclerosis in a middle-aged general population. *Stroke.* 2006;37:2277–82.
28. Ghani U, Shuaib A, Salam A, et al. Endothelial progenitor cells during cerebrovascular disease. *Stroke.* 2005;36:151–3.
29. Jung KH, Roh JK. Circulating endothelial progenitor cells in cerebrovascular disease. *J Clin Neurol.* 2008;4:139–47.
30. Vasa M, Fichtlscherer S, Aicher A, et al. Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. *Circ Res.* 2001;89:E1–7.
31. Asahara T, Murohara T, Sullivan A, et al. Isolation of putative progenitor endothelial cells for angiogenesis. *Science.* 1997;275:964–7.
32. Timmermans F, Plum J, Yoder MC, et al. Endothelial progenitor cells: identity defined? *J Cell Mol Med.* 2009;13:87–102.
33. Kim JH, Jung JH, Phi JH, et al. Decreased level and defective function of circulating endothelial progenitor cells in children with moyamoya disease. *J Neurosci Res.* 2010;88:510–8.
34. Bersano A, Guey S, Bedini G, et al. Research progresses in understanding the pathophysiology of Moyamoya disease. *Cerebrovasc Dis.* 2016;41:105–18.
35. Psaltis PJ, Simari RD. Vascular wall progenitor cells in health and disease. *Circ Res.* 2015;116:1392–412.
36. Lee JY, Moon YJ, Lee HO, et al. Deregulation of Retinaldehyde dehydrogenase 2 leads to defective Angiogenic function of endothelial Colony-forming cells in pediatric Moyamoya disease. *Arterioscler Thromb Vasc Biol.* 2015;35:1670–7.
37. Simper D, Stalboerger PG, Panetta CJ, et al. Smooth muscle progenitor cells in human blood. *Circulation.* 2002;106:1199–204.
38. Urbich C, Heeschen C, Aicher A, et al. Relevance of monocytic features for neovascularization capacity of circulating endothelial progenitor cells. *Circulation.* 2003;108:2511–6.
39. Kang HS, Moon YJ, Kim YY, et al. Smooth-muscle progenitor cells isolated from patients with moyamoya disease: novel experimental cell model. *J Neurosurg.* 2014;120:415–25.
40. Phi JH, Suzuki N, Moon YJ, et al. Chemokine ligand 5 (CCL5) derived from endothelial Colony-forming cells (ECFCs) mediates recruitment of smooth muscle progenitor cells (SPCs) toward critical vascular locations in Moyamoya disease. *PLoS One.* 2017;12:e0169714.
41. Phi JH, Wang KC, Lee JY, et al. Moyamoya syndrome: a window of Moyamoya disease. *J Korean Neurosurg Soc.* 2015;57:408–14.
42. Kim SJ, Heo KG, Shin HY, et al. Association of thyroid autoantibodies with moyamoya-type cerebrovascular disease: a prospective study. *Stroke.* 2010;41:173–6.
43. Lanterna LA, Galliani S, Brembilla C, et al. Association of moyamoya disease with thyroid autoantibodies and thyroid function. *Eur J Neurol.* 2017;24:e9.
44. Hu J, Luo J, Chen Q. The susceptibility pathogenesis of Moyamoya disease. *World Neurosurg.* 2017;101:731–41.
45. Wang Z, Fu Z, Wang J, et al. Moyamoya syndrome with antiphospholipid antibodies: a case report and literature review. *Lupus.* 2014;23:1204–6.
46. Sfikakis PP, Mitsikostas DD, Manoussakis MN, et al. Headache in systemic lupus erythematosus: a controlled study. *Br J Rheumatol.* 1998;37:300–3.
47. Kikuta K, Takagi Y, Nozaki K, et al. Asymptomatic microbleeds in moyamoya disease: T2*-weighted gradient-echo magnetic resonance imaging study. *J Neurosurg.* 2005;102:470–5.

48. Kuroda S, Kashiwazaki D, Ishikawa T, et al. Incidence, locations, and longitudinal course of silent microbleeds in moyamoya disease: a prospective T2*-weighted MRI study. *Stroke*. 2013;44:516–8.
49. Qin Y, Ogawa T, Fujii S, et al. High incidence of asymptomatic cerebral microbleeds in patients with hemorrhagic onset-type moyamoya disease: a phase-sensitive MRI study and meta-analysis. *Acta Radiol*. 2015;56:329–38.
50. Charbel FT, Misra M, Clarke ME, et al. Computer simulation of cerebral blood flow in moyamoya and the results of surgical therapies. *Clin Neurol Neurosurg*. 1997;99(Suppl 2):S68–73.
51. Seol HJ, Shin DC, Kim YS, et al. Computational analysis of hemodynamics using a two-dimensional model in moyamoya disease. *J Neurosurg Pediatr*. 2010;5:297–301.
52. Lee JY, Kim SK, Cheon JE, et al. Posterior cerebral artery involvement in moyamoya disease: initial infarction and angle between PCA and basilar artery. *Childs Nerv Syst*. 2013;29:2263–9.
53. Mineharu Y, Takenaka K, Yamakawa H, et al. Inheritance pattern of familial moyamoya disease: autosomal dominant mode and genomic imprinting. *J Neurol Neurosurg Ps*. 2006;77:1025–9.
54. Ikeda H, Sasaki T, Yoshimoto T, et al. Mapping of a familial moyamoya disease gene to chromosome 3p24.2-p26. *Am J Hum Genet*. 1999;64:533–7.
55. Inoue TK, Ikezaki K, Sasazuki T, et al. Linkage analysis of moyamoya disease on chromosome 6. *J Child Neurol*. 2000;15:179–82.
56. Sakurai K, Horiuchi Y, Ikeda H, et al. A novel susceptibility locus for moyamoya disease on chromosome 8q23. *J Hum Genet*. 2004;49:278–81.
57. Yamauchi T, Tada M, Houkin K, et al. Linkage of familial moyamoya disease (spontaneous occlusion of the circle of Willis) to chromosome 17q25. *Stroke*. 2000;31:930–5.
58. Aoyagi M, Ogami K, Matsushima Y, et al. Human leukocyte antigen in patients with moyamoya disease. *Stroke*. 1995;26:415–7.
59. Han H, Pyo CW, Yoo DS, et al. Associations of Moyamoya patients with HLA class I and class II alleles in the Korean population. *J Korean Med Sci*. 2003;18:876–80.
60. Inoue TK, Ikezaki K, Sasazuki T, et al. Analysis of class II genes of human leukocyte antigen in patients with moyamoya disease. *Clin Neurol Neurosurg*. 1997;99(Suppl 2):S234–7.
61. Kraemer M, Horn PA, Roder C, et al. Analysis of human leukocyte antigen genes in Caucasian patients with idiopathic moyamoya angiopathy. *Acta Neurochir*. 2012;154:445–54.
62. Roder C, Peters V, Kasuya H, et al. Polymorphisms in TGFB1 and PDGFRB are associated with Moyamoya disease in European patients. *Acta Neurochir*. 2010;152:2153–60.
63. Ye S. Polymorphism in matrix metalloproteinase gene promoters: implication in regulation of gene expression and susceptibility of various diseases. *Matrix Biol*. 2000;19:623–9.
64. Liu W, Morito D, Takashima S, et al. Identification of RNF213 as a susceptibility gene for moyamoya disease and its possible role in vascular development. *PLoS One*. 2011;6:e22542.
65. Kamada F, Aoki Y, Narisawa A, et al. A genome-wide association study identifies RNF213 as the first Moyamoya disease gene. *J Hum Genet*. 2011;56:34–40.
66. Miyatake S, Miyake N, Touho H et al (2012) Homozygous c.14576G>A variant of RNF213 predicts early-onset and severe form of moyamoya disease. *Neurology* 78:803–810.
67. Cecchi AC, Guo D, Ren Z, et al. RNF213 rare variants in an ethnically diverse population with Moyamoya disease. *Stroke*. 2014;45:3200–7.
68. Morito D, Nishikawa K, Hoseki J, et al. Moyamoya disease-associated protein mysterin/RNF213 is a novel AAA+ ATPase, which dynamically changes its oligomeric state. *Sci Rep*. 2014;4:4442.
69. Hitomi T, Habu T, Kobayashi H, et al. Downregulation of Securin by the variant RNF213 R4810K (rs112735431, G>A) reduces angiogenic activity of induced pluripotent stem cell-derived vascular endothelial cells from moyamoya patients. *Biochem Biophys Res Commun*. 2013;438:13–9.
70. Sonobe S, Fujimura M, Niizuma K, et al. Temporal profile of the vascular anatomy evaluated by 9.4-T magnetic resonance angiography and histopathological analysis in mice lacking RNF213: a susceptibility gene for moyamoya disease. *Brain Res*. 2014;1552:64–71.
71. Kanoke A, Fujimura M, Niizuma K, et al. Temporal profile of the vascular anatomy evaluated by 9.4-tesla magnetic resonance angiography and histological analysis in mice with the R4859K mutation of RNF213, the susceptibility gene for moyamoya disease. *Brain Res*. 2015;1624:497–505.
72. Fujimura M, Sonobe S, Nishijima Y, et al. Genetics and biomarkers of Moyamoya disease: significance of RNF213 as a susceptibility gene. *J Stroke*. 2014;16:65–72.
73. Guo DC, Papke CL, Tran-Fadulu V, et al. Mutations in smooth muscle alpha-actin (ACTA2) cause coronary artery disease, stroke, and Moyamoya disease, along with thoracic aortic disease. *Am J Hum Genet*. 2009;84:617–27.
74. Wallace S, Guo DC, Regalado E, et al. Disrupted nitric oxide signaling due to GUCY1A3 mutations increases risk for moyamoya disease, achalasia and hypertension. *Clin Genet*. 2016;90:351–60.